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Adrenoceptors

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Preface

Adrenoceptors mediate the physiological effects of the endogenous catecholamines adrenaline and noradrenaline. They consist of nine subtypes grouped into three subfamilies, termed α_1-, α_2-, and β-adrenoceptors, each of which has three subtypes (α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, and β_3). Adrenaline was isolated more than 120 years ago, and adrenoceptors have been targeted by drugs for clinical benefit ever since. Adrenoceptors and their ligands therefore have one of the richest histories, in terms of understanding, drug development, diverse ligand availability, and widespread clinical uses of agonist and antagonist drugs of any of the G protein-coupled receptors (GPCRs). As such, they have been, and remain, prototype GPCRs for new discoveries enabling a better understanding of the concept of G protein-coupled receptors, their function, structure, signal transduction, and regulation. Accordingly, adrenoceptor research has led to several Nobel Prizes including the 1971 prize to Earl E. Sutherland Jr, the 1988 prize to Sir James Black, the 1994 prize to Alfred G. Gilman and Martin Rodbell, and the 2012 prize to Brian Kobilka and Robert J. Lefkowitz.

Today, adrenoceptors remain the molecular targets for worldwide guideline-recommended drugs for the treatment of a wide variety of conditions, and many of these adrenoceptor drugs are the standard of care for their indications. β-antagonists (β-blockers, 4th most commonly prescribed class of drugs) are used for cardiovascular disease (e.g., bisoprolol, carvedilol, metoprolol), glaucoma (e.g., timolol), and migraine (e.g., propranolol). β-agonists (11th most commonly prescribed class of drugs) are used for asthma and COPD (e.g., formoterol, salbutamol, salmeterol, vilanterol). Adrenaline can be lifesaving in anaphylaxis and shock. All of these medications are named on the WHO List of Essential Medicines 2023. α-antagonists are also commonly used (25th most commonly prescribed class of drugs) for hypertension (e.g., doxazosin) and benign prostatic hyperplasia (e.g., alfuzosin, tamsulosin), and α-agonists, e.g. dexmedetomidine, are increasingly used for their sedative properties in intensive care settings. At the other end of the spectrum, highly efficacious inhaled β-agonists (no longer used as inhaled medications – adrenaline, isoprenaline, orciprenaline, and fenoterol) were linked with epidemics of deaths in those with asthma in several areas of the world in the 1960s and 1970s, whilst other β-agonists (clenbuterol) have caused human harm when entering the food chain and

are on the World Anti-Doping Association (WADA) and International Olympic Committee (IOC) list of prohibited drugs.

This volume of the Handbook of Experimental Pharmacology covers a full range of information from in vitro and in vivo studies and understanding to human clinical studies, and on to current and potential future clinical uses, many within each chapter. A short history of adrenoceptor research is provided by Martin C. Michel (Mainz, Germany), and more topic-specific historical aspects are included in the other chapters. Lukas Helfinger and Christopher G. Tate (Cambridge, UK) discuss structures of adrenoceptors identifying details of how ligands bind to orthosteric and allosteric sites to influence receptor activity and transducer coupling, and Andrea Nahles and Stefan Engelhardt (Munich, Germany) review genetic variants of adrenoceptors with in-depth discussion of those posing as significant risk factors. Jillian G. Baker (Nottingham, UK) and Roger J. Summers (Parkville, Australia) provide an in-depth discussion of adrenoceptor ligands, with an overview of their clinical uses, molecular pharmacology, and the assays available to study them. Chantel Mastos, Xiaomeng Xu, Alastair C. Keen, and Michelle L. Halls (Parkville, Australia) review the canonical pathways, new paradigms, and the importance of spatial and temporal control in the signal transduction mechanisms of adrenoceptor subtypes.

Other chapters discuss the roles of adrenoceptors and their subtypes in specific organ systems. Bela Szabo (Freiburg, Germany) reviews the role of neurotransmitter release-modifying adrenoceptors. Three chapters cover cardiovascular aspects, an area that was instrumental in adrenoceptor discovery and has many adrenoceptor-targeted drugs in clinical use. Yee W. Wong, Haris Haqqani, and Peter Molenaar (Chermside, Australia) discuss the role of the three β-adrenoceptor subtypes and drugs acting at β-adrenoceptors in heart failure, tachyarrhythmias, and other cardiovascular disorders. Spoorthy Kulkarni and Ian B. Wilkinson (Cambridge, UK) review the role of adrenoceptors in the pathophysiology and treatment of various forms of arterial hypertension. Erica Langnas and Mervyn Maze (San Francisco, USA) summarize the clinical uses of adrenergic receptor ligands in acute care settings with particular emphasis on the use of α_2-adrenoceptor agonists including dexmedetomidine.

Martin Hennenberg (Munich, Germany) and Martin C. Michel (Mainz, Germany) comprehensively review the role of adrenoceptors, their signal transduction mechanisms, and the use of α_1-adrenoceptor antagonists and β_3-adrenoceptor agonists for the treatment of diseases of the lower urinary tract. Jillian G. Baker and Dominick E. Shaw (Nottingham, UK) discuss the development of drugs for the treatment of asthma and chronic obstructive airways disease with particular emphasis on the role of β_2-adrenoceptors and their agonists and how these could be improved in the future. Yue Ruan, Francesco Buonfiglio, and Adrian Gericke (Mainz, Germany) present an examination of the expression, distribution, and functional roles of α_1-, α_2-, and β-adrenoceptors within various components of the eye and associated structures and how individual receptor subtypes can be targeted to treat ocular conditions including glaucoma.

Two chapters focus on the role of adrenoceptors in the central nervous system. S. Clare Stanford and David J. Heal (London, Nottingham and Bath, UK) review the role of adrenoceptors in psychiatric disorders and their treatments emphasizing the challenges associated with a lack of animal models that recapitulate the human condition as well as a lack of causal links in clinical studies. Actions of drugs used to treat psychiatric disorders on adrenoceptors may contribute to the therapeutic effect or be responsible for side effects. Rachel A. Matt, Renee S. Martin, Andrew K. Evans, Joel R. Gever, Gabrial A. Vargas, Mehrdad Shamloo, and Anthony P. Ford (San Carlos and Palo Alto, USA) discuss the role of noradrenergic pharmacology in the locus coeruleus and identify promising targets for the treatment of neurodegenerative disease.

Finally, Haneen Dwaib (Bethlehem, Palestine) and Martin C. Michel (Mainz, Germany) discuss the role of adrenoceptors in metabolic control, focusing on the role of α_{2A}-adrenoceptors and β-adrenoceptors in the regulation of insulin release from the pancreas. Rosario Amato, Martina Lucchesi, Silvia Marracci, Luca Filippi, and Massimo Dal Monte (Pisa, Italy) examine the role of β-adrenoceptor subtypes in cancer with the β_2-adrenoceptor emerging as important in tumour development and β_1- and β_3-adrenoceptors involved in certain types of cancer.

Taken together, these 16 chapters provide a comprehensive overview of the current state of play for adrenoceptors, their physiological and pathophysiological role, and their ligands as drug treatments for a wide variety of diseases. We trust that this will prove to be a valuable resource to basic science and clinical researchers in both academia and industry and will attract additional investigators to this well-established but still highly active field.

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Contents

Introduction: A Short History of Adrenoceptor Research

Martin C. Michel

Contents

Abstract

This chapter provides a short history of adrenoceptor research starting from the initial discovery of adrenaline. It covers the evolving classification of adrenoceptor subtypes, the cloning of these subtypes from multiple species, and factors such as adrenoceptor regulation, inverse agonism and biased agonism. More details on many of these aspects are provided in other chapters of this volume of Handbook of Experimental Pharmacology.

Keywords

Adrenoceptor · History · Receptor classification

Various reports in the nineteenth century indicated that adrenal glands included a bioactive principle that can increase blood pressure and could be useful in the treatment of asthma. Following experiments with crude extracts by George Oliver

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& E. Schäfer (University College London) (Oliver and Schäfer 1895) and a partial purification by John J. Abel (Johns Hopkins University) that he named epinephrine, Jokichi Takamine (Parke, Davis & Co.) was the first to prepare a pure substance from the adrenals and named it adrenaline (Takami 1902). In the beautiful prose of the time, he wrote "Last summer I began experiments with the object of isolating the active principle, and am pleased to be able to announce that I have succeeded in obtaining a stable crystalline body of constant composition from the gland … The fact that a fraction of a drop of a 1 in 50,000 solution of this body when dropped into the eye blanches the conjunctiva, leaves little doubt that it is the active principle of the gland. The injection of 1 c.c. of a 0.001 per cent. solution of adrenaline into the vein of an 8 kg dog causes the blood-pressure to rise 30 mmHg … The isolation of the active principle of the gland seems to point towards the fact that the wonderful physiological action of the various glands may depend upon the effects of apparently simple chemical substances, and such isolation would naturally give an impetus for the search of active principles of the various organs concerning which we know but little."

Various lines of evidence indicated that certain nerves release an active principle that can increase heart rate and blood pressure. While these effects were mimicked by adrenaline, it was more than 40 years after the discovery of adrenaline that Ulf von Euler (Karolinska Institute) isolated noradrenaline (norepinephrine in American English) as the active principle (von Euler 1945). To this date, American English and the INN classification prefer the epinephrine/norepinephrine terminology, whereas British English and IUPHAR terminology prefer adrenaline/noradrenaline; we will use the latter for reasons discussed elsewhere (Aronson 2000). Similarly, we will use the British English and IUPHAR preferred term adrenoceptor and not its American English synonym of adrenergic receptors. Field-specific aspects of adrenoceptor research will be covered in many corresponding sections of this book.

1 Early Receptor Research

The concept that adrenaline and noradrenaline act on some type of receptor was pioneered by the work of Sir Henry Dale (Wellcome Physiological Research Laboratory) who reported that some ergot alkaloids could reverse the pressor effect of adrenaline (Dale 1906). Testifying to the scientific rigor of Dale, he included control experiments in which the pressor and uterine contraction effects of pituitary extracts were not antagonized by the ergot alkaloids. Various catecholamine derivatives and additional blocking agents including tolazoline and phenoxybenzamine were developed thereafter (see chapter on "Adrenoceptors: receptors, ligands and their clinical uses, molecular pharmacology and assays" in this volume). However, they blocked only the smooth muscle excitatory receptors.

This led to the idea of excitatory and inhibitory adrenoceptors, a concept rejected by Raymond P. Ahlquist (University of Georgia) (Ahlquist 1948). Based on a rank order of potency of various catecholamine derivatives in a large panel of preparations, he found that the same rank order found for most contractile responses

also applied to some relaxant responses. Therefore, he concluded that "because of the opposite effects associated with each type of receptor, the customary signs, E (excitatory) and I (inhibitory), cannot be applied. Therefore, for convenience they have been designated as *alpha* adrenotropic receptors and the *beta* receptors." Realizing the overall complexity of the data, Ahlquist already emphasized that this dual classification was likely to have only interim value (Ahlquist 1967).

Indeed, investigators including Saul Z. Langer (Babraham Institute) and Klaus Starke (University of Essen, later University of Freiburg) proposed in the early 1970s that stimulation of α-adrenoceptors had excitatory effects on smooth muscle but inhibitory effects on transmitter release from neurons. The two receptors appeared to differ in location (post- vs. pre-synaptic) and in ligand recognition profile, leading to the subclassification into postsynaptic α_1- and presynaptic α_2-adrenoceptors, thereby creating a trichotomous classification into α_1-, α_2-, and β-adrenoceptors (Langer 1974; Starke 1987). We meanwhile know that transmitter release modifying adrenoceptors can exist not only on neurons synthesizing and releasing noradrenaline (and adrenaline) but also on those using other neurotransmitters; these can be therefore referred to as presynaptic autoreceptors (modulating the release of noradrenaline and adrenaline) and heteroreceptors (modulating that of other neurotransmitters), respectively (Bennett 1999). Interestingly, presynaptic β-adrenoceptors also exist that in most but not all cases promote neurotransmitter release (Okeke et al. 2017). While imperfect, this classification enabled the discovery of many important drugs that remain in clinical use today. A pioneer in this field was Sir James Black, who discovered among other things propranolol, a discovery rewarded with the 1988 Nobel Prize (Black 1989).

Lands (Sterling Winthrop Research Institute) identified that the effects of adrenaline, noradrenaline, isoprenaline, and several derivatives thereof on the heart and on various smooth muscle preparations could not be reconciled with a homogeneous population of β-adrenoceptors; accordingly, he proposed a further subdivision into β_1- and β_2-adrenoceptors (Lands et al. 1967a, b). Thus, the general agreement in the 1970s became that there were two families of adrenoceptors, i.e., α- and β-adrenoceptors with two subtypes each (α_1, α_2, β_1, and β_2; Fig. 1).

The classification efforts in the 1940s to 1960s occurred with the postulated receptors being a black box. However, it emerged at a similar time to the classification of α- and β-adrenoceptors, that at least some of these receptors conveyed the extracellular signals coming from the catecholamines to intracellular effects via mediators such as cAMP (Rall and Sutherland 1959). The importance of this work was highlighted by the award of the 1971 Nobel Prize to Earl W. Sutherland (Western Reserve University). However, concepts at that time did not yet appreciate that the formation of cAMP resulted from a protein complex, not just the receptor. Subsequently, it became clear that α_2-adrenoceptors primarily signal via inhibition of cAMP formation (Pettinger et al. 1987). Also much later, investigators such as Michael J. Berridge (University of Cambridge) discovered a role for phospholipase C as an effector enzyme in signal transduction including that of phosphatidylinositol hydrolysis (Berridge 1993), that is now considered the primary signaling mechanism for α_1-adrenoceptors (Bylund et al. 1994). Concomitantly it became clear that G

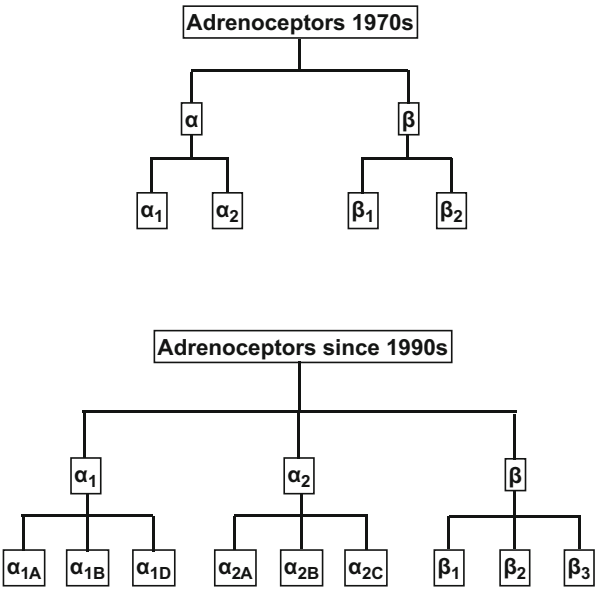

Fig. 1 Classification of adrenoceptors over time

proteins were involved in the signal transduction pathway of adrenoceptors as pioneered by Alfred G. Gilman (University of Texas) (Gilman 1987). Gilman was also pivotal in the discovery of adenylyl cyclase as the effector enzyme of both β- and α_2-adrenoceptors (Tang and Gilman 1992). These discoveries were honored with the 1994 Nobel Prize to Al Gilman and Martin Rodbell (Lefkowitz 1994). They also led to the concept that α_1-adrenoceptors prototypically couple to G proteins of the $G_{q/11}$ family, α_2-adrenoceptors those of the $G_{i/o}$ family, and β-adrenoceptors to G_s proteins (Bylund et al. 1994).

2 Pharmacological Discovery of Additional Adrenoceptor Subtypes

Based on techniques such as radioligand binding and the availability of a greater selection of compounds, the dichotomous classification of adrenoceptors became increasingly challenged in the 1980s. Based on tools such as WB 4101, Leslie Morrow and Ian Creese (University of California San Diego) proposed a further division of α_1-adrenoceptors into the subtypes α_{1A} and α_{1B} (Morrow and Creese 1986). Using a different approach utilizing the alkylating agent chloroethylclonidine, the group of Kenneth Minneman (Emory University) supported this subdivision and expanded the evidence to functional data at the tissue level (Johnson and Minneman 1987). In this scheme, the α_{1A}-adrenoceptor had high affinity for WB 4101 and was less sensitive to inactivation by chloroethylclonidine, whereas the α_{1B}-adrenoceptor had low affinity for WB 4101 and was more sensitive to inactivation by chloroethylclonidine. Other key compounds used to differentiate

these proposed α_1-adrenoceptor subtypes included 5-methyl-urapidil and the stereoisomers of the Ca^{2+}-channel inhibitor niguldipine (Michel et al. 1990). However, even this subdivision could not explain the ligand recognition profile of some α_1-adrenoceptors such as those mediating contraction of rat aorta (Eltze et al. 2001; Oriowo and Ruffolo 1992). Finally, based on a surprisingly low potency of prazosin at some α_1-adrenoceptors, an α_{1L}-subtype was proposed (Kava et al. 1998), although it eventually became clear that this is not a distinct subtype but rather a phenotype of the α_{1A}-adrenoceptor that becomes detectable in some cellular contexts and/or under some experimental conditions (White et al. 2019). The discovery of α_1-adrenoceptor subtypes led to the development of α_{1A}-selective antagonists such as tamsulosin and silodosin that proved useful in the treatment of lower urinary tract symptoms suggestive of benign prostatic hyperplasia and displayed fewer cardiovascular effects compared to those originally introduced for the treatment of arterial hypertension such as doxazosin and terazosin (Michel et al. 2001).

Evidence for heterogeneity of α_2-adrenoceptors emerged in the same period. This included the observations of differences in ligand recognition profiles between tissues and species as pioneered by the groups of Stefan R. Nahorski (University of Leicester) (Cheung et al. 1982; Summers et al. 1983) and David Bylund with the latter being first to formally propose a subdivision into α_{2A}- and α_{2B}-adrenoceptors (Bylund 1985). Interestingly, the pharmacological characterization of these subtypes was based on several ligands that preferentially bind to α_1-adrenoceptors including prazosin and WB 4101.

Soon after the division of β-adrenoceptors into the β_1 and β_2 subtypes, it emerged that the ligand recognition profile in some models was not sufficiently explained by these two subtypes (Furchgott 1972). Specifically, this included lipolysis in rodent adipose tissue as pioneered by the group of Johan Zaagsma (Vrije Universiteit Amsterdam, later Rijksuniversiteit Groningen) (Harms et al. 1974) and relaxation of urinary bladder in some species (Nergardh et al. 1977). While a β_3-adrenoceptor subtype was proposed, this did not find general agreement because these atypical adrenoceptors differed in ligand recognition profile among models and laboratories. Nonetheless, this proposal was sufficient to launch a drug discovery program aimed at the treatment of obesity and type 2 diabetes in various companies (Dwaib and Michel 2023) as pioneered by Jon R. Arch and Mike Cawthorne (Beecham Pharmaceuticals) (Arch et al. 1984). Originally based on experiments with CGP 12177 in the heart, a β_4-adrenoceptor was proposed by the groups of Alberto J. Kaumann (Babraham Institute) and Peter Molenaar (Melbourne University, later University of Queensland) (Kaumann and Molenaar 1997). However, it later become clear that this represents an heterotopic site on the β_1-adrenoceptor (Molenaar 2003).

3 Cloning of Adrenoceptor Subtypes

Many of the above controversies did not get resolved until the various adrenoceptor subtypes were cloned. The first adrenoceptor to be cloned, the β_2-adrenoceptor, was isolated from a hamster smooth muscle cell line by the group of Robert J. Lefkowitz

(Duke University) (Dixon et al. 1986). This was followed in rapid succession by its human ortholog (Kobilka et al. 1987) and by various other adrenoceptor subtypes from humans and other mammalian and non-mammalian species (Table 1). While initial cloning efforts were based on purified protein, later ones were based on homology screening. While most of the encoding genes are intronless, some have introns and/or splice variants in at least some species.

Based on the combined evidence from the pharmacological studies and the receptor cloning, a trichotomous nomenclature of the adrenoceptors was agreed upon in 1994 (Bylund et al. 1994) and slightly updated in 1995 (Hieble et al. 1995), which has stood the test of time (Fig. 1).

Major progress has been made in our understanding of how the adrenoceptors work at the molecular level following their cloning. An important part of this was elucidating their crystal structures. Following that of the human β_2-adrenoceptor (Rasmussen et al. 2007), crystal structures have been determined for other adrenoceptor subtypes including the human α_{1B}-adrenoceptor (Deluigi et al. 2022), the human α_{1D}-adrenoceptor (Janezic et al. 2019), and the turkey β_1-adrenoceptor (Huang et al. 2013). Particularly for the β_2-adrenoceptor, crystal structures have been determined from multiple species and in multiple conformations, i.e., bound to an agonist or an antagonist. Honoring these achievements, the 2012 Nobel Prize in chemistry was awarded to Brian Kobilka (Stanford University) and Robert J. Lefkowitz (Duke University).

4 Additional Adrenoceptor Features

With the advent of radioligand binding studies enabling quantification of adrenoceptor protein density in tissues and cell lines in the 1970s, it became possible to directly determine the regulation of adrenoceptor expression at the protein level. Many groups made major contributions to this research including those of Robert J. Lefkowitz (Duke University), T. Kendall Harden (University of North Carolina), Paul A. Insel (University of California San Diego), and Otto-Erich Brodde (University of Essen). An early review of this field was provided in 1983 (Harden 1983). Following the cloning of the adrenoceptor cDNA, additional investigation of the regulation of adrenoceptor expression at the mRNA level became possible. Thus, prolonged agonist exposure can cause desensitization, whereas prolonged antagonist exposure (although documented in fewer settings) can cause sensitization. The discovery of additional players in the signaling of adrenoceptors revealed that the regulation of adrenoceptor expression and function is complex and differs not only between specific receptor subtypes but also to some degree between the cells expressing them (Gurevich and Gurevich 2008; Kohout and Lefkowitz 2003; Moo et al. 2021). Thus, mechanisms involved in agonist-induced desensitization include a rapid phosphorylation of the receptor by a G protein receptor kinase and uncoupling of the receptor from the G protein and internalization of the receptor; later events include the down-regulation of the mRNA expression, which in turn may include reduced transcription and/or reduced mRNA stability, that leads to decreased

Table 1 Adrenoceptor genes in humans, rats, and mice. aa: number of amino acids; cM: centi-Morgan

	Human			Mouse			Rat		
Subtype	Gene symbol	Chromosome	aa	Gene symbol	Chromosome	aa	Gene symbol	Chromosome	aa
α_{1A}	ADRA1A	8p21.2	466	Adra1a	14D1	466	Adra1a	15p12	466
α_{1B}	ADRA1B	5q33.3	520	Adra1b	11 26.81 cM	514	Adra1b	10q21	515
α_{1D}	ADRA1D	20p13	572	Adra1d	2 63.5 cM	562	Adra1d	3q36	561
α_{2A}	ADRA2A	10q25.2	465	Adra2a	19 49.04 cM	465	Adra2a	1q55	465
α_{2B}	ADRA2B	2q11.2	450	Adra2b	2 61.95 cM	450	Adra2b	3q36	453
α_{2C}	ADRA2C	4p16.3	462	Adra2c	5 18.09 cM	458	Adra2c	14-q21	458
β_1	ADRB1	10q25.3	477	Adrb1	19 51.96 cM	466	Adrb1	1q55	466
β_2	ADRB2	5q32	413	Adrb2	18 35.1 cM	418	Adrb2	18q12.1	418
β_3	ADRB3	8p11.23	408	Adrb3	8 15.94 cM	400	Adrb3	16q12.3	400

expression at the protein level. Moreover, it can include an altered expression of the G proteins a receptor couples to and of the post-receptor events such as the effector enzymes. Many of these regulatory pathways are believed to involve phosphorylation of the receptor, but one of the nine subtypes, the β_3-adrenoceptor lacks the required phosphorylation sites; accordingly, this subtype is less sensitive to agonist-induced regulation but can nonetheless be regulated by other mechanisms in some settings (Okeke et al. 2019).

While the original classification of adrenoceptor ligands included agonists and antagonists that activated the receptor and prevented that, respectively, it emerged early that the efficacy of β-adrenoceptor ligands relative to a reference compound such as isoprenaline covers a wide range from full agonist (i.e., efficacy similar to isoprenaline), partial agonism, and antagonism. This can partly be attributed to the expression density and the cell type in which the receptor is expressed, but also in part to the intrinsic efficacy of the ligand, leading to partial agonism (Jasper and Insel 1992). Historical concepts of adrenoceptor agonism or receptor agonism in general had assumed that an antagonist is a compound that blocks the effects of agonists but lacks direct effects on receptor activity. This was challenged by findings where antagonists could reduce receptor signaling in the absence of agonists. While this was initially found largely in systems with overexpressed and/or constitutively active receptors, it meanwhile has also been observed with natively expressed adrenoceptors and is referred to as inverse agonism (Michel et al. 2020; Schütz and Freissmuth 1992). Whether a ligand exhibits inverse agonism or partial agonism is at least partly dependent on the cell type under investigation.

Moreover, classic concepts of molecular adrenoceptor pharmacology had assumed a single binding pocket for the endogenous catecholamines that is used also by xenobiotic agonists and antagonists. However, most adrenoceptor subtypes and other G protein-coupled receptors exhibit additional (heterotopic) sites outside the pocket that can be used by the xenobiotic ligands. Binding to such heterotopic sites could have direct effects and/or could positively or negatively modulate receptor activation by orthosteric ligands, a phenomenon called allosteric modulation as pioneered, for instance, by Nigel Birdsall (National Institute for Medical Research) or Arthur Christopoulos (Monash University) (Christopoulos and Kenakin 2002; Lazareno et al. 2000).

Finally, investigators such as Terry P. Kenakin (Glaxo Research Laboratories, later University of North Carolina) or Roger Summers (Monash University) found that the rank order of potencies to activate receptors may differ between the cellular responses being measured (Evans et al. 2010, 2013; Kenakin and Morgan 1989). While this phenomenon originally was referred to by many terms, it is now generally called biased agonism. However, similar to partial and inverse agonism, this is strongly affected by the cell type under investigation (tissue/cell type bias) and not only by intrinsic features of the compound. Additional factors such as disease state or prior treatment may also affect how biased agonism can be observed at least quantitatively (Michel et al. 2014). Biased agonism is attractive therapeutically because at least in theory it may allow the discovery of ligands that elicit a desired response while having less potential to cause an adverse reaction mediated by the

same receptor but a different signaling pathway. Whether this is a realistic route in drug discovery and development is being debated (Kenakin 2018; Michel and Charlton 2018).

5 Conclusions on Clinical Implications

This volume of the Handbook of Experimental Pharmacology will discuss the ligands, the signal transduction, and the physiological and therapeutic role of various adrenoceptor subtypes. Overall adrenoceptor research and drug discovery played a major role in our biological understanding and in advances in clinical medicine. Thus, particularly approaches and techniques developed related to the β_2-adrenoceptor have been the prototypes for understanding the function, regulation, and structure of G protein-coupled receptors in general. Adrenoceptors have become the target of many drugs in clinical use. For instance, β-adrenoceptor antagonists alone have 20 FDA-approved indications plus another 11 generally accepted off-label indications (Table 2). Thus, adrenoceptors arguably have shaped modern

Table 2 Indications of β-adrenoceptor antagonists approved by the US Food and Drug Administration (FDA) or established off-label uses. Reproduced with permission from Bond et al. (2022)

FDA-approved indications	Off-label uses
1. Angina	1. Anxiety
2. Hypertension	2. Public speaking
3. Congestive heart failure	3. Post-traumatic stress
4. Myocardial infarction prophylaxis	4. Hypotension induction
5. Atrial fibrillation	5. Portal hypertension
6. Open-angle glaucoma	6. Ethanol withdrawal
7. Migraine prophylaxis	7. Esophageal varices
8. Tremor	8. Hypertensive emergency
9. Thyrotoxicosis	9. Variceal bleeding prophylaxis
10. Atrial flutter	10. Perioperative hypertension+
11. Ventricular arrhythmias (ventricular premature beats)	11. Infantile hemangiomas
12. Myocardial infarction	
13. Pheochromocytoma	
14. Ocular hypertension	
15. Paroxysmal supraventricular tachycardia	
16. Idiopathic hypertrophic subaortic stenosis	
17. Scleroderma renal crisis	
18. Hypertrophic subaortic stenosis	
19. Supraventricular tachycardia or non-compensatory sinus tachycardia	
20. Intraoperative and postoperative tachycardia and hypertension	

medicine more than any other drug target family. Four adrenoceptor-related Nobel Prizes further testify to the groundbreaking role of adrenoceptor research and its impact on biology and human well-being.

References

Ahlquist RP (1948) A study of the adrenotropic receptors. Am J Phys 153:586–600

Ahlquist RP (1967) Development of the concept of alpha and beta adrenotropic receptors. Ann N Y Acad Sci 139:549–552

Arch JR, Ainsworth AT, Cawthorne MA, Piercy V, Sennitt MV, Thody VE, Wilson C, Wilson S (1984) Atypical beta-adrenoceptor on brown adipocytes as target for anti-obesity drugs. Nature 309:163–165

Aronson JK (2000) "Where name and image meet" – the argument for "adrenaline". BMJ 320:506–509

Bennett MR (1999) One hundred years of adrenaline: the discovery of autoreceptors. Clin Auton Res 9:145–159

Berridge MJ (1993) Inositol triphosphate and calcium signalling. Nature 361:315–325

Black J (1989) Drugs from emasculated hormones: the principle of syntopic antagonism. Science 245:486–493

Bond RA, Michel MC, Parra S (2022) ß-Adrenoceptor antagonists. In: Michel MC (ed) Cardiovascular, hematopoietic, urinary and respiratory pharmacology. Elsevier, Amsterdam, pp 497–506

Bylund DB (1985) Heterogeneity of alpha-2 adrenergic receptors. Pharmacol Biochem Behav 22: 835–843

Bylund DB, Eikenberg DC, Hieble JP, Langer SZ, Lefkowitz RJ, Minneman KP, Molinoff PB, Ruffolo RR Jr, Trendelenburg U (1994) IV. International Union of Pharmacology Nomenclature of Adrenoceptors. Pharmacol Rev 46:121–136

Cheung YD, Barnett DB, Nahorski SR (1982) [^{3}H]Rauwolscine and [^{3}H]yohimbine binding to rat cerebral and human platelet membranes: possible heterogeneity of alpha 2-adrenoceptors. Eur J Pharmacol 84:79–85

Christopoulos A, Kenakin T (2002) G protein-coupled receptor allosterism and complexing. Pharmacol Rev 54:323–374

Dale HH (1906) On some physiological actions of ergot. J Physiol 34:163–206

Deluigi M, Morstein L, Schuster M, Klenk C, Merklinger L, Cridge RR, de Zhang LA, Klipp A, Vacca S, Vaid TM, Mittl PRE, Egloff P, Eberle SA, Zerbe O, Chalmers DK, Scott DJ, Plückthun A (2022) Crystal structure of the α_{1B}-adrenergic receptor reveals molecular determinants of selective ligand recognition. Nat Commun 13:382

Dixon RAF, Kobilka BK, Strader DJ, Benovic JL, Dohlman HG, Frielle T, Bolanowski MA, Bennett CD, Rands E, Diehl RE, Mumford RA, Slater EE, Sigal IS, Caron MG, Lefkowitz RJ, Strader CD (1986) Cloning of the gene and cDNA for mammalian ß-adrenergic receptor and homology with rhodopsin. Nature 321:75–79

Dwaib HS, Michel MC (2023) Is the $ß_3$-adrenoceptor a valid target for the treatment of obesity and/or type 2 diabetes? Biomol Ther 13:1714

Eltze M, Boer R, Michel MC, Hein P, Testa R, Ulrich WR, Kolassa N, Sanders KH (2001) In vitro and in vivo uroselectivity of B8805-033, an antagonist with high affinity at prostatic α_{1A}- vs. α_{1B}- and α_{1D}-adrenoceptors. Naunyn Schmiedeberg's Arch Pharmacol 363:649–662

Evans BA, Sato M, Sarwar M, Hutchinson DS, Summers RJ (2010) Ligand-directed signalling at ß-adrenoceptors. Br J Pharmacol 159:1022–1038

Evans BA, Hutchinson DS, Summers RJ (2013) $ß_2$-Adrenoceptor-mediated regulation of glucose uptake in skeletal muscle – ligand-idrected signalling or a reflection of system complexity? Naunyn Schmiedeberg's Arch Pharmacol 386:757–760

Furchgott RF (1972) The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In: Blaschko H, Muecholl E (eds) Catecholamines. Springer, New York, pp 283–335

Gilman AG (1987) G proteins: transducers of receptor-generated signals. Annu Rev Biochem 56: 615–649

Gurevich VV, Gurevich EV (2008) Rich tapestry of G protein-coupled receptor signaling and regulatory mechanisms. Mol Pharmacol 74:312–316

Harden TK (1983) Agonist-induced desensitization of the ß-adrenergic receptor-linked adenylate cyclase. Pharmacol Rev 35:5–32

Harms HH, Zaagsma J, van der Wal B (1974) Beta-adrenoceptor studies. III. On the beta-adrenoceptors in rat adipose tissue. Eur J Pharmacol 25:87–97

Hieble JP, Bylund DB, Clarke DE, Eikenburg DC, Langer SZ, Lefkowitz RJ, Minneman KP, Ruffolo RR Jr (1995) International Union of Pharmacology X. Recommendation for nomenclature of α_1-adrenoceptors: consensus update. Pharmacol Rev 47:267–270

Huang J, Chen S, Zhang JJ, Huang XY (2013) Crystal structure of oligomeric β1-adrenergic G protein-coupled receptors in ligand-free basal state. Nat Struct Mol Biol 20:419–425

Janezic EM, Harris DA, Dinh D, Lee KS, Stewart A, Hinds TR, Hsu PL, Zheng N, Hague C (2019) Scribble co-operatively binds multiple α_{1D}-adrenergic receptor C-terminal PDZ ligands. Sci Rep 9:14073

Jasper JR, Insel PA (1992) Evolving concepts of partial agonism. The ß-adrenergic receptor as a paradigm. Biochem Pharmacol 43:119–130

Johnson RD, Minneman KP (1987) Differentiation of α_1-adrenergic receptors linked to phosphatidylinositol turnover and cyclic AMP accumulation in rat brain. Mol Pharmacol 31: 239–246

Kaumann AJ, Molenaar P (1997) Modulation of human cardiac function through 4 ß-adrenoceptor populations. Naunyn Schmiedeberg's Arch Pharmacol 355:667–681

Kava MS, Blue DR Jr, Vimont RL, Clarke DE, Ford APDW (1998) α_{1L}-Adrenoceptor mediation of smooth muscle contraction in rabbit bladder neck: a model for lower urinary tract tissues of man. Br J Pharmacol 123:1359–1366

Kenakin T (2018) Is the quest for signaling bias worth the effort? Mol Pharmacol 93:266–269

Kenakin TP, Morgan PH (1989) Theoretical effects of single and multiple transducer receptor coupling proteins on estimates of the relative potency of agonists. Mol Pharmacol 35:214–222

Kobilka BK, Dixon RAF, Frielle T, Dohlman HG, Bolanowski MA, Sigal IS, Yang-Feng TL, Francke U, Caron MG, Lefkowitz RJ (1987) cDNA for the human $ß_2$-adrenergic receptor: a protein with multiple membrane-spanning domains and encoded by a gene whose chromosomal location is shared with that of the receptor for platelet-derived growth factor. Proc Natl Acad Sci 84:46–50

Kohout TA, Lefkowitz RJ (2003) Regulation of G protein-coupled receptor kinses and arrestins during receptor desensitization. Mol Pharmacol 63:9–18

Lands AM, Arnold A, McAuliff JP, Luduena FP, Brown TG (1967a) Differentiation of receptor systems activated by sympathetic amines. Nature 214:597–598

Lands AM, Luduena FP, Buzzo HJ (1967b) Differentiation of receptors responsive to isoproterenol. Life Sci 6:2241–2249

Langer SZ (1974) Presynaptic regulation of catecholamine release. Biochem Pharmacol 23:1793–1800

Lazareno S, Popham A, Birdsall NJM (2000) Allosteric interactions of staurosporine and other indolocarbazoles with N-[methyl-^{3}H]scopolamine and acetylcholine at muscarinic receptors subtypes: identification of a second allosteric site. Mol Pharmacol 58:194–207

Lefkowitz RJ (1994) Rodbell and Gilman win 1994 Nobel prize for physiology and medicine. Trends Pharmacol Sci 15:442–444

Michel MC, Charlton SJ (2018) Biased agonism in drug discovery – is it too soon to choose a path? Mol Pharmacol 93:259–265

Michel MC, Hanft G, Gross G (1990) α_{1B}- but not α_{1A}-adrenoceptors mediate inositol phosphate generation. Naunyn Schmiedeberg's Arch Pharmacol 341:385–387
Michel MC, Flannery MT, Narayan P (2001) Worldwide experience with alfuzosin and tamsulosin. Urology 58:508–516
Michel MC, Seifert R, Bond RA (2014) Dynamic bias and its implications for GPCR drug discovery. Nat Rev Drug Discov 13:869–870
Michel MC, Michel-Reher MB, Hein P (2020) A systematic review of inverse agonism at adrenoceptor subtypes. Cells 9:1923
Molenaar P (2003) The 'state' of ß-adrenoceptors. Br J Pharmacol 140:1–2
Moo EV, van Senten JR, Bräuner-Osborne H, Møller TC (2021) Arrestin-dependent and -independent internalization of G protein–coupled receptors: methods, mechanisms, and implications on cell signaling. Mol Pharmacol 99:242–255
Morrow AL, Creese I (1986) Characterization of α_1-adrenergic receptor subtypes in rat brain: a reevaluation of [^{3}H]WB4101 and [^{3}H]prazosin binding. Mol Pharmacol 29:321–330
Nergardh A, Boreus LO, Naglo AS (1977) Characterization of the adrenergic beta-receptor in the urinary bladder of man and cat. Acta Pharmacol Toxicol (Copenh) 40:14–21
Okeke K, Gravas S, Michel MC (2017) Do β_3-adrenoceptor agonists cause urinary bladder smooth muscle relaxation by inhibiting acetylcholine release? Am J Physiol Ren Physiol 313:F859–F861
Okeke K, Angers S, Bouvier M, Michel MC (2019) Agonist-induced desensitisation of β_3-adrenoceptors: where, when and how? Br J Pharmacol 176:2539–2558
Oliver G, Schäfer EA (1895) The physiological effects of extracts of the suprarenal capsules. J Physiol 18:230–276
Oriowo MA, Ruffolo RR Jr (1992) Heterogeneity of postjunctional α_1-adrenoceptors in mammalian aortae: subclassification based on chlorethylclonidine, WB 4101 and nifedipine. J Vasc Res 29: 33–40
Pettinger WA, Umemura S, Smyth DD (1987) Renal α_2-adrenoceptors and the adenylate cyclase-cAMP system: biochemical and physiological interactions. Am J Phys 252:F199–F208
Rall TW, Sutherland EW Jr (1959) Action of epinephrine and norepinephrine in broken cell preparations. Pharmacol Rev 11:464–465
Rasmussen SGF, Choi H-J, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC, Burghammer M, Ratnala VRP, Sanishvili R, Fischetti RF, Schertler GFX, Weis WI, Kobilka BK (2007) Crystal structure of the human β_2 adrenergic G-protein-coupled receptor. Nature 450:383–387
Schütz W, Freissmuth M (1992) Reverse intrinsic activity of antagonists on G protein-coupled receptors. Trends Pharmacol Sci 13:376–380
Starke K (1987) Presynaptic α-autoreceptors. Rev Physiol Biochem Pharmacol 107:74–146
Summers RJ, Barnett DB, Nahorski SR (1983) The characteristics of adrenoceptors in homogenates of human cerebral cortex labelled by ^{3}H-rauwolscine. Life Sci 33:1105–1112
Takami J (1902) The isolation of the active principle of the suprarenal gland. J Physiol 27:xxix–xxx
Tang WJ, Gilman AG (1992) Adenylyl cyclases. Cell 70:869–872
von Euler US (1945) A sympathomometic pressor substance in animal organ extracts. Nature 156: 18–19
White CW, da Silva Junior ED, Lim L, Ventura S (2019) What makes the α_{1A}-adrenoceptor gene product assume a α_{1L}-adrenoceptor phenotype? Br J Pharmacol 176:2358–2365

Structures of Adrenoceptors

Lukas Helfinger and Christopher G. Tate

Contents

Abstract

The first structure of an adrenoceptor (AR), the human β_2-adrenoceptor (hβ_2AR) was published in 2007 and since then a total of 78 structures (up to June 2022) have been determined by X-ray crystallography and electron cryo-microscopy (cryo-EM) of all three βARs (β_1, β_2 and β_3) and four out of six αARs (α_{1B}, α_{2A}, α_{2B}, α_{2C}). The structures are in a number of different conformational states, including the inactive state bound to an antagonist, an intermediate state bound to agonist and active states bound to agonist and an intracellular transducer (G protein or arrestin) or transducer mimetic (nanobody). The structures identify molecular details of how ligands bind in the orthosteric binding pocket (OBP; 19 antagonists, 18 agonists) and also how three different small molecule allosteric modulators bind. The structures have been used to define the molecular details of receptor activation and also the molecular determinants for transducer coupling. This chapter will give a brief overview of the structures, receptor activation, a comparison across the different subfamilies and commonalities of ligand–receptor interactions.

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Keywords

Cryo-EM · Structure · X-ray

Abbreviations

AR	Adrenoceptor
ECL	Extracellular loop
EM	Electron microscopy
GPCR	G-protein coupled receptor
H	Helix
hβ_1AR	Human β_1-adrenoceptor
ICL	Intracellular loop
MD	Molecular dynamics
OBP	Orthosteric binding pocket
RMSD	Root mean square deviation
TM	Transmembrane
tβ_1AR	Turkey β_1-adrenoceptor

1 Structure Determination

G protein-coupled receptors are integral membrane proteins that span biological membranes, thus providing a physical link between the extracellular environment and the cytoplasm of the cell (Pierce et al. 2002; Kobilka and Deupi 2007; Oldham and Hamm 2008). The portion of the receptor embedded in the membrane is highly hydrophobic, which necessitates the use of detergents to extract them from the membrane for subsequent purification and structure determination. X-ray crystallography was the predominant technique for determining protein structures at the end of the twentieth century, but unfortunately the small detergents most suitable for crystallising membrane proteins are very harsh and inevitably inactivated receptors even before they could be purified (Tate 2010). The exception was the light-sensing GPCR, rhodopsin, that in its inactive state in the dark is extremely stable (for a GPCR) and also has the advantage of having extremely low basal activity. The structure of rhodopsin purified from bovine retinas was published in the year 2000 (Palczewski et al. 2000) and showed the canonical GPCR fold of seven transmembrane helices (H1-H7) with a short amphipathic helix (H8) on the intracellular surface (Fig. 1).

Despite the success of determining the structure of rhodopsin, another 7 years elapsed before the first GPCR structure was determined of a receptor that bound a diffusible ligand, the human β_2AR (Cherezov et al. 2007; Rasmussen et al. 2007), with the turkey β_1AR (tβ_1AR) structure published the following year (Warne et al. 2008). The tβ_1AR was used for X-ray structure determination rather than hβ_1AR because it is considerably more stable upon detergent solubilisation than the human

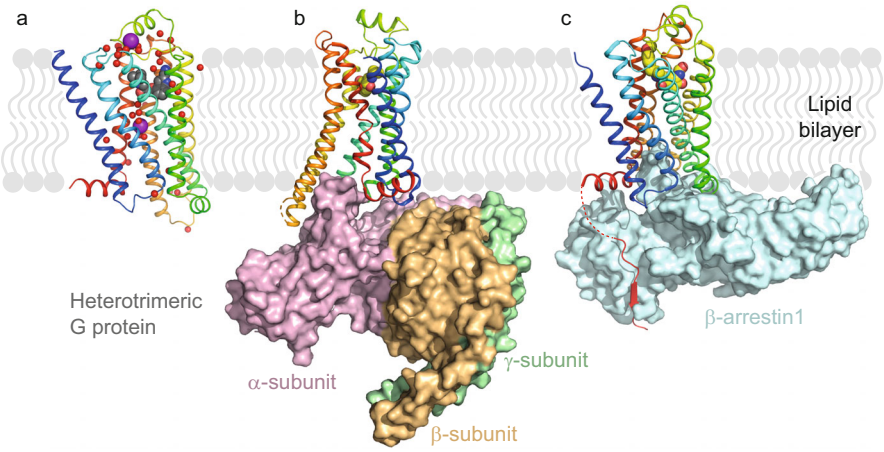
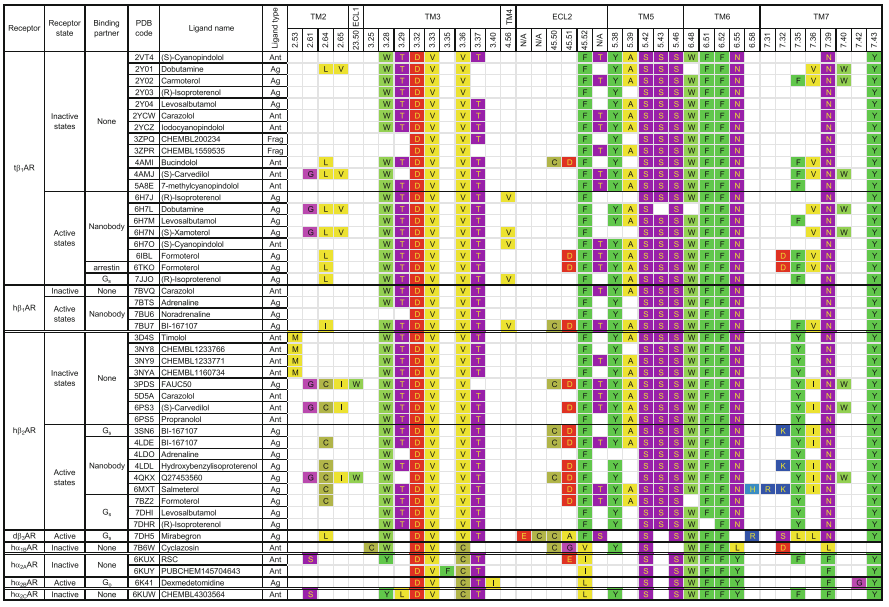


Fig. 1 Structures of the $t\beta_1AR$. (**a**) the inactive state bound to the antagonist cyanopindolol (PDB ID 4BVN; C atoms, grey spheres; water molecules, small red spheres; purple spheres, Na^+ ions), (**b**) the active state bound to the agonist isoproterenol (PDB ID 7JJO; C atoms, yellow spheres) coupled to the G protein G_s, (**c**) the active state bound to formoterol (PDB ID 6TKO; C atoms, yellow spheres) coupled to β-arrestin. In all panels the receptor is in rainbow colouration (N-terminus blue, C-terminus red)

receptor (Serrano-Vega and Tate 2009). The development of a number of generic methodologies was an essential prerequisite to these structures being determined (Tate and Schertler 2009), and these have now enabled the X-ray structure determination of all subsequent GPCRs. These new strategies were necessary because, unlike rhodopsin, β_1AR and β_2AR are unstable in short chain detergents, have higher basal activity and undergo a series of structural transitions between inactive and active states (Nguyen et al. 2017). The three strategies developed were the binding of an antibody (in this case, a F_{ab} fragment: Rasmussen et al. 2007), engineering a fusion protein with T4 lysozyme to facilitate crystal contact formation during crystallogenesis in lipid cubic phase (Rosenbaum et al. 2007), and conformational thermostabilisation through systematic mutagenesis (Serrano-Vega et al. 2008). All three strategies have been used extensively, often in combination, to generate over 600 crystal structures of GPCRs published to date. These include 26 structures of $t\beta_1AR$, 4 structures of $h\beta_1AR$, 37 structures of $h\beta_2AR$, one structure of dog β_3AR and 10 structures of human αARs ($\alpha_{2A}AR$, $\alpha_{2B}AR$, $\alpha_{2C}AR$ and $\alpha_{1B}AR$; see Table 1 for examples). Of note, the first structure determination of a GPCR coupled to a heterotrimeric G protein was in 2011, the β_2AR-G_s complex (Rasmussen et al. 2011), that resulted in the Nobel Prize being awarded to Brian Kobilka in 2012 (Kobilka 2014).

In the last 5 years, there has been a marked change in strategy for structure determination of GPCRs. A series of developments in the field of single-particle cryo-EM over the previous 15 years culminated in better microscopes, faster and more sensitive direct electron detectors, and new algorithms for processing data and

Table 1 Selected structures of adrenoceptors and conserved amino acid residues in the OBP; Ant Antagonist, Ag Agonist, Frag Fragment. Highlighted residues are within 5 Å of the ligand as determined by GPCRdb



determining structures (reviewed in Bai et al. (2015); Fernandez-Leiro and Scheres (2016); McMullan et al. (2016); Vinothkumar and Henderson (2016)). These three factors combined allow structure determination of proteins the size of a GPCR-G protein complex (~120 kDa). This transformed the GPCR field, because it was no longer necessary to spend years engineering a GPCR to improve its stability and/or form well-ordered crystals. Instead, near wild-type receptors can be used and the only protein engineering required may be to improve its production in heterologous expression systems. The first cryo-EM structures of GPCRs coupled to G proteins were published in 2017 (calcitonin receptor, Liang et al. (2017); GLP-1 receptor, Zhang et al. (2017)) and since then over 280 other structures have been determined. These include structures of the $t\beta_1AR$-G_s complex (Su et al. 2020) and $t\beta_1AR$-arrestin complex (Lee et al. 2020), four structures of the $h\beta_2AR$-G_s complex (Zhang et al. 2020; Yang et al. 2021), and the structures of dog β_3AR-G_s (Nagiri et al. 2021) and $h\alpha_{2B}AR$-G_s (Yuan et al. 2020) (see Table 1 for examples). It is interesting to note that the first structures of β_3AR and $\alpha_{2B}AR$ were determined by cryo-EM, whereas previously it would have been easier to determine their inactive state structures by X-ray crystallography. It is also worth noting that structures of GPCRs in lipid nanodiscs have only been determined by cryo-EM and provide a near-native environment for the receptor; this is particularly beneficial in

determining structures in complex with β-arrestin (Lee et al. 2020) that requires lipids for effective association with GPCRs.

To bring the structural biology picture up to the present (July 2022), it is now possible to determine cryo-EM structures of GPCRs in the inactive state using a fusion protein approach and/or when bound to antibodies (Robertson et al. 2021; Bloch et al. 2022; Xu et al. 2022). β_2AR was used as a model Class A receptor for one of the studies and its structure was determined by single-particle cryo-EM in the ligand-free state, and either bound to agonist or antagonist (Xu et al. 2022). It is inevitable that these technologies will generate another surge in GPCR structures over the coming years.

2 Comparison Between Adrenoceptor Structures and How Ligands Bind

Adrenoceptors are divided phylogenetically into two main families, the α-adrenoceptors and β-adrenoceptors, with the α-receptors divided further into the α_1 and α_2 adrenoceptors. Pairwise amino acid sequence analyses show that there is a high conservation in the transmembrane domains (57–87% similarity) suggesting that the overall architecture of the receptors is very similar. This is indeed the case with the active state β-adrenoceptor structures (β_1AR, β_2AR and β_3AR) and the α_{2B}-adrenoceptor (α_{2B}AR) that vary between 0.8 and 1.5 Å RMSD (root mean squared deviation) in all possible pairwise comparisons. The inactive state structures (β_1AR, β_2AR, α_{2A}AR, α_{2C}AR) also show high similarities in structure with RMSDs of pairwise comparisons varying between 0.6 and 1.4 Å. The antagonist-bound α_{1B}AR is a distinct outlier with pairwise comparisons varying between 1.2 and 3.1 Å when compared with the other inactive state adrenoceptor structures. The greatest sequence variation is observed in the loop regions, and the N-terminus and C-terminus, with similarity being as low as 4%. The mechanism of receptor activation is likely to have similarities across the adrenoceptor family. However, both active and inactive state structures are known only for β_1AR and β_2AR, and structures of both conformations are required for elucidating detailed molecular mechanisms of receptor activation; these will be discussed in Sect. 3.

All the adrenoceptors are activated by adrenaline and noradrenaline, and therefore it is perhaps unsurprising that there are some highly conserved residues in the OBP (Table 1). Residues that are within 5 Å of ligands in every adrenoceptor structure determined to date are located in transmembrane helix 3 (H3) ($Asp^{3.32}$, $Val^{3.33}$), H5 ($Ser^{5.42}$) and H6 ($Phe^{6.52}$). Superscripts refer to the Ballesteros-Weinstein numbering system for amino acid residues in GPCRs (Ballesteros et al. 2001). More residues may be included in the list if 44 out of the 47 listed structures (>94%) have a given residue proximal to the ligand; these include residues in H5 ($Ser^{5.46}$), H6 ($Trp^{6.48}$, $Phe^{6.51}$), and H7 ($Tyr^{7.43}$). Another group of residues are those that are also always proximal to the ligand, but they differ in the αARs compared to βARs. For example, residue 3.36 is Cys in αARs and Val in βARs. Similar pairings of residues are 45.52 (Val/Ile/Leu in αARs, Phe in βARs), 6.55 (Leu/Tyr in αARs, Asn in βARs), and 7.39

(Leu/Phe in αARs, Asn in βARs). If all the structures are considered, then the regions that can potentially be proximal to a ligand includes every transmembrane region except H1, and also extracellular loops ECL1 and ECL2. There are very few systematic cases where residues are proximal to a ligand only in one specific conformational state (active or inactive). This is despite the observation that the OBP decreases in volume by up to 41% (Warne et al. 2019) when a G protein mimetic is coupled to the receptor compared to an inactive state (measured in structures bound to the same ligand). Thus, G protein coupling causes an increase in the number of ligand–receptor interactions and/or strength of hydrogen bonds, which increases ligand affinity.

The conservation of adrenoceptor architecture and residues in the OBP suggests that there will be a similarity in how ligands bind. Unfortunately, there is not one ligand that has been used in the structure determination of every adrenoceptor, so a direct comparison between all adrenoceptors cannot be made. In addition, there are currently very few structures of αARs and only very high affinity ligands have been used, because they have been necessary for receptor stabilisation. Comparing the mode of ligand binding between $t\beta_1$AR and $h\beta_2$AR unsurprisingly shows very high similarity, reflecting the high conservation in structure (Fig. 2). Comparisons between other receptors shows more variation, particularly when antagonist-bound structures are compared, although the region where they bind is similar.

The β_1AR (turkey and human) is currently the only GPCR where high-resolution structures have been determined coupled to either a G protein (or G protein mimetic) or β-arrestin (Fig. 3). This allows a direct comparison between the conformation of the receptor and potential changes in the OBP that may illuminate potential mechanisms for ligand bias. Biased ligands signal preferentially through either the G protein or β-arrestin, activating different pathways in the cell and thus have different cellular consequences (Smith et al. 2018; Wootten et al. 2018). It has been suggested that the therapeutic effects of the beta blocker carvedilol are mediated by blocking G protein coupling and allowing arrestin signalling to occur (DeWire and Violin 2011), although this is controversial and has been recently disputed (Benkel et al. 2022). The beneficial effects of salbutamol are thought to be through only the G protein pathway (Nguyen et al. 2017). Comparing the structure of formoterol-bound arrestin-coupled $t\beta_1$AR with the structure of formoterol-bound $t\beta_1$AR coupled to a G protein mimetic, shows a 3 Å shift in the position of ECL3 and also a 1 Å shift of H5 away from the ligand, resulting in decreased ligand-receptor contacts and decreased affinity for the agonist compared to when a G protein is coupled (Lee et al. 2020). Different surfaces at the juxtaposition between the transducer and receptor on the intracellular surface also offers opportunities for the development of novel regulators of signalling. Other structures have been determined with a biased ligand bound, for example carvedilol bound to the inactive state of $t\beta_1$AR (Warne et al. 2012). However, there were no significant differences in conformation when compared to structures bound to other ligands that are not thought to be biased. This really highlights the need of having multiple structures in different conformational states to allow meaningful molecular mechanisms to be established that underpin the structure-activity relationships of ligands.

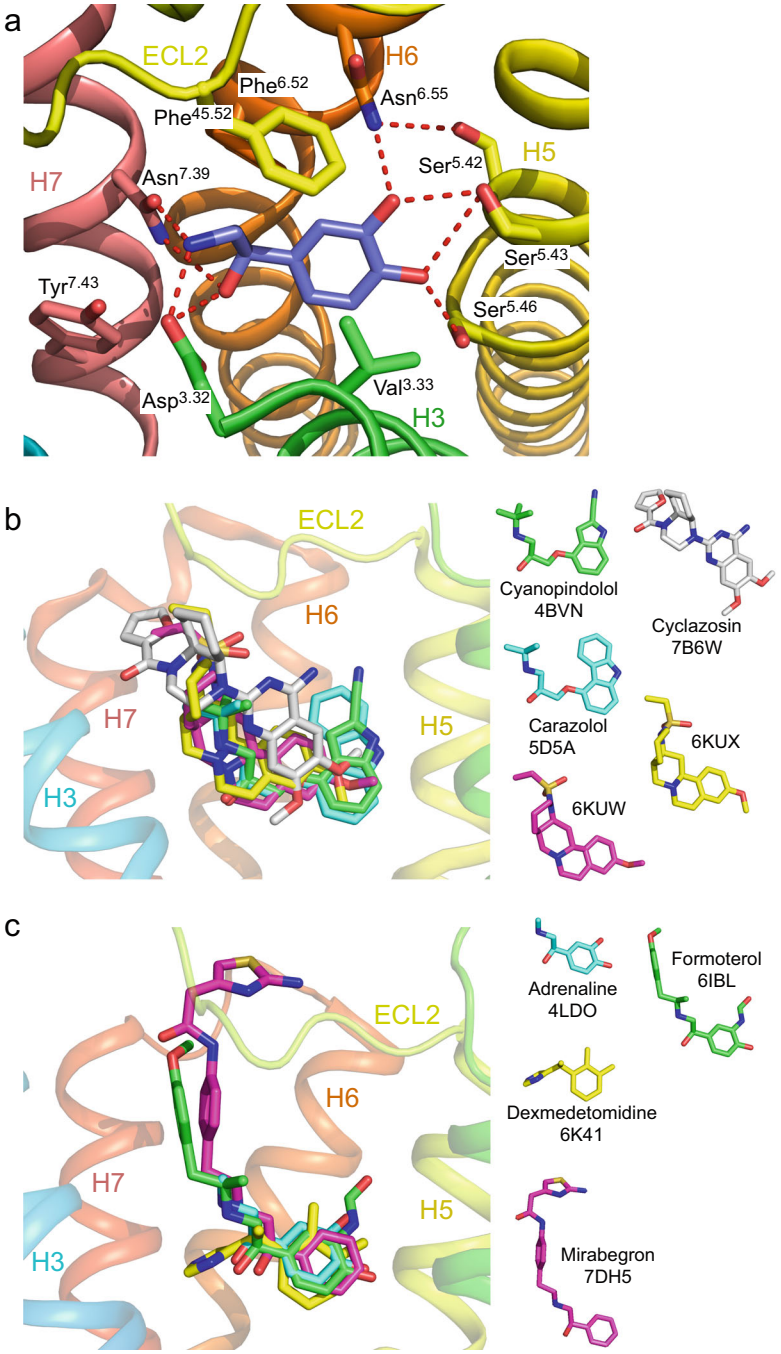


Fig. 2 (**a**) Structure of the orthosteric binding pocket of hβ_1AR bound to noradrenaline, with red dashed lines representing putative hydrogen bonds (PDB code 7BU6), (**b**) binding pose of antagonists in the OBP, (**c**) binding pose of agonists in the OBP. PDB IDs for the structures are given adjacent to the ligand. See Table 1 for species of receptors depicted in (**b**) and (**c**)

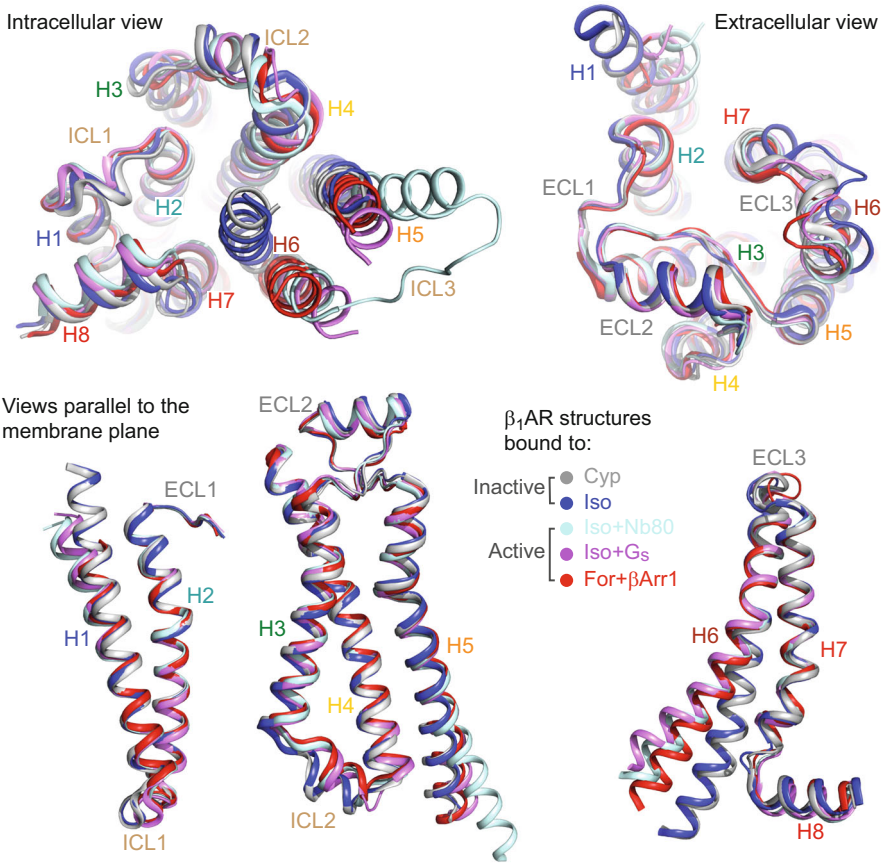


Fig. 3 Different conformational states of $t\beta_1AR$. Overlay of structures in the inactive state bound to cyanopindolol (Cyp) and isoproterenol (Iso), and the active state coupled to the G protein mimetic Nb80, G_s and β-arrestin (For, formoterol)

3 Structure and Activation of β_1AR and β_2AR

The $h\beta_2AR$ and $t\beta_1AR$ were the first two hormone receptor structures determined (Cherezov et al. 2007; Warne et al. 2008). The $h\beta_1AR$ is considerably less thermostable than $t\beta_1AR$ (Serrano-Vega and Tate 2009), so the latter was used extensively for early biochemical work and heterologous expression (Warne et al. 2003). However, even $t\beta_1AR$ was insufficiently stable for crystallisation and therefore it was thermostabilised by the addition of six point mutations (Serrano-Vega et al. 2008). The amino acid sequences of human and turkey β_1AR are 76% identical in the transmembrane regions and there are no significant differences between carazolol-bound structures of $t\beta_1AR$ (PDB code 2YCW; Moukhametzianov et al. (2011)) and the recently determined $h\beta_1AR$ (PDB code 7BVQ; Xu et al. (2021)) structure

(RMSD 0.6 Å, over 1,524 atoms). Thus, conclusions derived from $t\beta_1AR$ structures are applicable to the human receptor, despite there being some differences in pharmacology (Baker 2010), which could arise from kinetic differences in transitions between different conformations or different ligand binding pathways (Xu et al. 2021). There are also no significant structural differences (RMSD 0.6 Å over 1,632 atoms) between carazolol-bound $t\beta_1AR$ (PDB code 2YCW; Moukhametzianov et al. (2011) and $h\beta_2AR$ (PDB code 2RH1; Cherezov et al. (2007)), although intracellular loop 2 (ICL2) contains a short α-helix in $t\beta_1AR$ whereas it is unstructured in $h\beta_2AR$. Structures of $t\beta_1AR$ typically contain a Na^+ ion that appears to stabilise the turn at the end of a short α-helix in extracellular loop 2 (ECL2; (Warne et al. 2008). MD simulations (Dror et al. 2009) of $h\beta_2AR$ resulted in the appearance of an extracellular Na^+ ion and ordering of ICL2 as observed in $t\beta_1AR$. The high-resolution structure of $t\beta_1AR$ at 2.1 Å resolution identified an intramembrane Na^+ ion (Miller-Gallacher et al. 2014) in a similar position to that observed in other receptors (Katritch et al. 2014). However, Na^+ ion concentration does not affect agonist affinity at $t\beta_1AR$ (Miller-Gallacher et al. 2014), unlike in the adenosine A_{2A} receptor ($A_{2A}R$) where Na^+ is an allosteric antagonist (Liu et al. 2012). This is because agonist binding to $A_{2A}R$ results in a transition to an intermediate state very similar to the fully active state (Lebon et al. 2011) where the intramembrane Na^+ ion pocket has collapsed and Na^+ is extruded (presumably down its concentration gradient into the cytoplasm). In contrast, agonist binding to $t\beta_1AR$ does not alter significantly the overall conformation of the receptor (Warne et al. 2011) and so the intramembrane Na^+ binding pocket remains unchanged.

The activation of β_1AR and β_2AR are thought to be essentially identical given the similarities in their overall structures in different conformational states and key amino acid residues are highly conserved. Therefore, in the discussion below residues will be numbered according to the Ballesteros-Weinstein numbering system (Ballesteros et al. 2001), which can be converted conveniently to specific residue numbers using GPCRdb if required (www.gpcrdb.org; Kooistra et al. (2021)). It should be appreciated that structures represent a series of snapshots of selected stable states within the overall conformational landscape of the receptors. However, multiple techniques demonstrate that β_2AR is highly dynamic even in the absence of ligands and appears to access a plethora of different conformations (Manglik et al. 2015). This is consistent with the concept of basal activity, where a receptor in the absence of a ligand can couple functionally to a G protein, implying a structure similar, if not identical, to the structures of agonist-bound receptor-G protein complexes. For clarity, the activation mechanism is given in a linear fashion following a distinct timeline; this might not be the case in reality.

Binding of the full agonist isoproterenol or FAUC50 to $t\beta_1AR$ or $h\beta_2AR$, respectively, resulted in structures showing a 1–2 Å contraction of the OBP and the rotamer change of $Ser^{5.46}$ in comparison with antagonist-bound structures (Rosenbaum et al. 2011; Warne et al. 2011). There were no other significant changes throughout the receptor. When a partial agonist bound, structures showed that there was still the contraction of the OBP, but there was no change in orientation of $Ser^{5.46}$ (Warne et al. 2011). These subtle changes are thought to be sufficient to make the

receptor more likely to transition into an active state capable of coupling to a G protein. The importance of $Ser^{5.46}$ during activation was highlighted through a comparison of the activity and structures of $t\beta_1AR$ bound to cyanopindolol and 7-methylcyanopindolol (7-MeCyp). Cyanopindolol was originally described as an antagonist of β_1AR, but in more sensitive assays it is seen to act as a weak partial agonist (Sato et al. 2015). The cyanopindolol-bound $t\beta_1AR$ structure showed no contraction of the OBP and no rotamer change of $Ser^{5.46}$. However, during activation of the receptor, it would be expected that $Ser^{5.46}$ would have to rotate and therefore modification of cyanopindolol to prevent this, by the addition of a methyl group in the 7 position, would be expected to decrease significantly ligand efficacy. This was indeed the case, and 7-MeCyp acted as a neutral antagonist at $t\beta_1AR$ and a partial inverse agonist at $h\beta_2AR$ (Sato et al. 2015). The rotamer change of $Ser^{5.46}$ reduces the number of van der Waals and polar interactions between transmembrane helices H4, H5 and H6, thus making it more likely that the helices can move into the positions they adopt in active conformations.

No structures of β_1AR or β_2AR intermediates between an agonist-bound inactive state and the G protein-coupled state have been crystallised, although such intermediates are known for other receptors, e.g., $A_{2A}R$ (Lebon et al. 2012). Comparisons between the inactive state and G protein-coupled state shows a number of distinct changes throughout the whole receptor (Rasmussen et al. 2011). There is a closure of the entrance to the orthosteric binding site which reduces the on and off rate of ligands (DeVree et al. 2016). Where structures have been determined in the inactive state and G protein-coupled state bound to the same ligand, it is apparent that there can be up to a 41% decrease in the volume of the OBP due primarily to the inward movement of the extracellular ends of H6 and H7 (Warne et al. 2019). This results in an increase in the number and/or strength of ligand-receptor contacts that are consistent with an increase in ligand affinity when a G protein is coupled. There is a re-arrangement of three residues ($Pro^{5.50}$, $Ile^{3.40}$, $Phe^{6.44}$) at the core of the receptor that is regarded as a key switch in receptor activation (Huang et al. 2015). The contraction of the aqueous cavity off the intramembrane Na^+ binding pocket results in the loss of the Na^+ ion. There are also rearrangements of conserved tyrosine residues $Tyr^{5.58}$ and $Tyr^{7.53}$ that make interactions in the core of the receptor that stabilise the active state (Huang et al. 2015).

The major structural difference between the inactive and active states is on the intracellular surface where the 14 Å outward movement of the intracellular end of H6 (measured at Cα Lys 267 of $h\beta_2AR$) forms a cleft that accommodates the C-terminal α5 helix of the G protein (Rasmussen et al. 2011). There are also additional small changes in the positions of H7 and H5 on G protein coupling. Virtually all the interactions between the heterotrimeric G protein and β_2AR occur via the α-subunit, with only a few minor contacts to the β-subunit (Rasmussen et al. 2011). Of the α-subunit contacts to the receptor, 70% are made by the α5 helix. As is observed in other GPCR-G protein complexes, the majority of contacts are via van der Waals interactions with only a few polar interactions and salt bridges (Garcia-Nafria and Tate 2019).

The structure of tβ_1AR when coupled to β-arrestin is very similar to when it is coupled to either G_s or the G protein mimetic nanobody Nb80 (Lee et al. 2020). However, there are three significant differences. Firstly, the cytoplasmic end of H6 is shifted by only 7 Å when coupled to arrestin compared to 12 Å coupled to G_s (measured at Cα Arg284). Secondly, ECL3 is shifted towards the core of the receptor by 3 Å (measured at Cα Asp318) when arrestin is coupled compared to when G_s is coupled. Thirdly, there is a 1 Å outward movement of H5 away from the ligand in the OBP when arrestin is coupled compared to when nanobody Nb80 is coupled (both structures were determined with formoterol bound). This results in the breakage of hydrogen bonds between the ligand and $Ser^{5.46}$ and $Ser^{5.42}$, which is consistent with a decrease in agonist affinity when arrestin is coupled compared to when G protein is coupled. As mentioned in Sect. 2, these differences are sufficient for the development of novel biased therapeutics to the βARs, but how transferable these findings are to other GPCRs awaits further high-resolution structure determination of cognate pairs of receptors bound to the same ligand and to either a G protein or β-arrestin.

4 Conclusions

The structures of β_1AR and β_2AR have been at the forefront of the GPCR field in understanding receptor conformational changes, efficacy, specificity and transducer coupling. This has been through the determination of multiple structures, bound to different ligands and in multiple conformational states. In comparison, there are only one or two structures of each of the other adrenoceptors and two have yet to have their structures determined. There thus remains considerable work to be done to bring the other receptors up to the level of understanding we have for β_1AR and β_2AR. The recent developments in single-particle cryo-EM will undoubtedly accelerate structure determination of both inactive receptors bound to antagonists and active receptors coupled to G proteins. There are significant technical challenges in determining structures of receptors coupled to β-arrestin, but these are not insurmountable, and many more structures of arrestin-coupled receptors are needed before a detailed understanding of ligand bias can be developed.

References

Bai XC, McMullan G, Scheres SH (2015) How cryo-EM is revolutionizing structural biology. Trends Biochem Sci 40:49–57

Baker JG (2010) The selectivity of beta-adrenoceptor agonists at human beta1-, beta2- and beta3-adrenoceptors. Br J Pharmacol 160:1048–1061

Ballesteros JA, Jensen AD, Liapakis G, Rasmussen SG, Shi L, Gether U, Javitch JA (2001) Activation of the beta 2-adrenergic receptor involves disruption of an ionic lock between the cytoplasmic ends of transmembrane segments 3 and 6. J Biol Chem 276:29171–29177

Benkel T, Zimmermann M, Zeiner J, Bravo S, Merten N, Lim VJY, Matthees ESF, Drube J, Miess-Tanneberg E, Malan D, Szpakowska M, Monteleone S, Grimes J, Koszegi Z, Lanoiselee Y,

O'Brien S, Pavlaki N, Dobberstein N, Inoue A, Nikolaev V, Calebiro D, Chevigne A, Sasse P, Schulz S, Hoffmann C, Kolb P, Waldhoer M, Simon K, Gomeza J, Kostenis E (2022) How carvedilol activates beta(2)-adrenoceptors. Nat Commun 13:7109

Bloch JS, Mukherjee S, Kowal J, Filippova EV, Niederer M, Pardon E, Steyaert J, Kossiakoff AA, Locher KP (2022) Development of a universal nanobody-binding Fab module for fiducial-assisted cryo-EM studies of membrane proteins. Proc Natl Acad Sci U S A 118:e2115435118

Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Kuhn P, Weis WI, Kobilka BK, Stevens RC (2007) High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. Science 318:1258–1265

DeVree BT, Mahoney JP, Velez-Ruiz GA, Rasmussen SG, Kuszak AJ, Edwald E, Fung JJ, Manglik A, Masureel M, Du Y, Matt RA, Pardon E, Steyaert J, Kobilka BK, Sunahara RK (2016) Allosteric coupling from G protein to the agonist-binding pocket in GPCRs. Nature 535: 182–186

DeWire SM, Violin JD (2011) Biased ligands for better cardiovascular drugs: dissecting G-protein-coupled receptor pharmacology. Circ Res 109:205–216

Dror RO, Arlow DH, Borhani DW, Jensen MO, Piana S, Shaw DE (2009) Identification of two distinct inactive conformations of the beta2-adrenergic receptor reconciles structural and biochemical observations. Proc Natl Acad Sci U S A 106:4689–4694

Fernandez-Leiro R, Scheres SH (2016) Unravelling biological macromolecules with cryo-electron microscopy. Nature 537:339–346

Garcia-Nafria J, Tate CG (2019) Cryo-EM structures of GPCRs coupled to Gs, Gi and Go. Mol Cell Endocrinol 488:1–13

Huang W, Manglik A, Venkatakrishnan AJ, Laeremans T, Feinberg EN, Sanborn AL, Kato HE, Livingston KE, Thorsen TS, Kling RC, Granier S, Gmeiner P, Husbands SM, Traynor JR, Weis WI, Steyaert J, Dror RO, Kobilka BK (2015) Structural insights into micro-opioid receptor activation. Nature 524:315–321

Katritch V, Fenalti G, Abola EE, Roth BL, Cherezov V, Stevens RC (2014) Allosteric sodium in class A GPCR signaling. Trends Biochem Sci 39:233–244

Kobilka BK (2014) In: Santesson CG, Holmberg A, Liljestrand G, Granit R, Odelberg W (eds) The Nobel prizes 2012: Les Prix Nobel. Science History Publications

Kobilka BK, Deupi X (2007) Conformational complexity of G-protein-coupled receptors. Trends Pharmacol Sci 28:397–406

Kooistra AJ, Mordalski S, Pandy-Szekeres G, Esguerra M, Mamyrbekov A, Munk C, Keseru GM, Gloriam DE (2021) GPCRdb in 2021: integrating GPCR sequence, structure and function. Nucleic Acids Res 49:D335–D343

Lebon G, Warne T, Edwards PC, Bennett K, Langmead CJ, Leslie AG, Tate CG (2011) Agonist-bound adenosine A2A receptor structures reveal common features of GPCR activation. Nature 474:521–525

Lebon G, Warne T, Tate CG (2012) Agonist-bound structures of G protein-coupled receptors. Curr Opin Struct Biol 22:482–490

Lee Y, Warne T, Nehme R, Pandey S, Dwivedi-Agnihotri H, Chaturvedi M, Edwards PC, Garcia-Nafria J, Leslie AGW, Shukla AK, Tate CG (2020) Molecular basis of beta-arrestin coupling to formoterol-bound beta1-adrenoceptor. Nature 583:862–866

Liang YL, Khoshouei M, Radjainia M, Zhang Y, Glukhova A, Tarrasch J, Thal DM, Furness SGB, Christopoulos G, Coudrat T, Danev R, Baumeister W, Miller LJ, Christopoulos A, Kobilka BK, Wootten D, Skiniotis G, Sexton PM (2017) Phase-plate cryo-EM structure of a class B GPCR-G-protein complex. Nature 546:118–123

Liu W, Chun E, Thompson AA, Chubukov P, Xu F, Katritch V, Han GW, Roth CB, Heitman LH, IJzerman AP, Cherezov V, Stevens RC (2012) Structural basis for allosteric regulation of GPCRs by sodium ions. Science 337:232–236

Manglik A, Kim TH, Masureel M, Altenbach C, Yang Z, Hilger D, Lerch MT, Kobilka TS, Thian FS, Hubbell WL, Prosser RS, Kobilka BK (2015) Structural insights into the dynamic process of beta2-adrenergic receptor signaling. Cell 161:1101–1111

McMullan G, Faruqi AR, Henderson R (2016) Direct electron detectors. Methods Enzymol 579:1–17

Miller-Gallacher JL, Nehme R, Warne T, Edwards PC, Schertler GF, Leslie AG, Tate CG (2014) The 2.1 A resolution structure of cyanopindolol-bound beta1-adrenoceptor identifies an intramembrane Na+ ion that stabilises the ligand-free receptor. PloS One 9:e92727

Moukhametzianov R, Warne T, Edwards PC, Serrano-Vega MJ, Leslie AG, Tate CG, Schertler GF (2011) Two distinct conformations of helix 6 observed in antagonist-bound structures of a beta1-adrenergic receptor. Proc Natl Acad Sci U S A 108:8228–8232

Nagiri C, Kobayashi K, Tomita A, Kato M, Kobayashi K, Yamashita K, Nishizawa T, Inoue A, Shihoya W, Nureki O (2021) Cryo-EM structure of the beta3-adrenergic receptor reveals the molecular basis of subtype selectivity. Mol Cell 81:3205–3215 e3205

Nguyen LP, Al-Sawalha NA, Parra S, Pokkunuri I, Omoluabi O, Okulate AA, Windham Li E, Hazen M, Gonzalez-Granado JM, Daly CJ, McGrath JC, Tuvim MJ, Knoll BJ, Dickey BF, Bond RA (2017) beta2-Adrenoceptor signaling in airway epithelial cells promotes eosinophilic inflammation, mucous metaplasia, and airway contractility. Proc Natl Acad Sci U S A 114:E9163–E9171

Oldham WM, Hamm HE (2008) Heterotrimeric G protein activation by G-protein-coupled receptors. Nat Rev Mol Cell Biol 9:60–71

Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M (2000) Crystal structure of rhodopsin: a G protein-coupled receptor. Science 289:739–745

Pierce KL, Premont RT, Lefkowitz RJ (2002) Seven-transmembrane receptors. Nat Rev Mol Cell Biol 3:639–650

Rasmussen SG, Choi HJ, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC, Burghammer M, Ratnala VR, Sanishvili R, Fischetti RF, Schertler GF, Weis WI, Kobilka BK (2007) Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. Nature 450:383–387

Rasmussen SG, DeVree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS, Thian FS, Chae PS, Pardon E, Calinski D, Mathiesen JM, Shah ST, Lyons JA, Caffrey M, Gellman SH, Steyaert J, Skiniotis G, Weis WI, Sunahara RK, Kobilka BK (2011) Crystal structure of the beta2 adrenergic receptor-Gs protein complex. Nature 477:549–555

Robertson MJ, He F, Meyerowitz JG, Seven AB, Panova O, Peroto M-C, Che T, Skiniotis G (2021) Structure determination of inactive-state GPCRs with 1 a universal nanobody. BiorXiv

Rosenbaum DM, Cherezov V, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Yao XJ, Weis WI, Stevens RC, Kobilka BK (2007) GPCR engineering yields high-resolution structural insights into beta2-adrenergic receptor function. Science 318:1266–1273

Rosenbaum DM, Zhang C, Lyons JA, Holl R, Aragao D, Arlow DH, Rasmussen SG, Choi HJ, Devree BT, Sunahara RK, Chae PS, Gellman SH, Dror RO, Shaw DE, Weis WI, Caffrey M, Gmeiner P, Kobilka BK (2011) Structure and function of an irreversible agonist-beta(2) adrenoceptor complex. Nature 469:236–240

Sato T, Baker J, Warne T, Brown GA, Leslie AG, Congreve M, Tate CG (2015) Pharmacological analysis and structure determination of 7-methylcyanopindolol-bound beta1-adrenergic receptor. Mol Pharmacol 88:1024–1034

Serrano-Vega MJ, Tate CG (2009) Transferability of thermostabilizing mutations between beta-adrenergic receptors. Mol Membr Biol 26:385–396

Serrano-Vega MJ, Magnani F, Shibata Y, Tate CG (2008) Conformational thermostabilization of the beta1-adrenergic receptor in a detergent-resistant form. Proc Natl Acad Sci U S A 105:877–882

Smith JS, Lefkowitz RJ, Rajagopal S (2018) Biased signalling: from simple switches to allosteric microprocessors. Nat Rev Drug Discov 17:243–260

Su M, Zhu L, Zhang Y, Paknejad N, Dey R, Huang J, Lee MY, Williams D, Jordan KD, Eng ET, Ernst OP, Meyerson JR, Hite RK, Walz T, Liu W, Huang XY (2020) Structural basis of the activation of heterotrimeric Gs-protein by isoproterenol-bound beta1-adrenergic receptor. Mol Cell 80:59–71 e54

Tate CG (2010) Practical considerations of membrane protein instability during purification and crystallisation. Methods Mol Biol 601:187–203

Tate CG, Schertler GF (2009) Engineering G protein-coupled receptors to facilitate their structure determination. Curr Opin Struct Biol 19:386–395

Vinothkumar KR, Henderson R (2016) Single particle electron cryomicroscopy: trends, issues and future perspective. Q Rev Biophys 49:e13

Warne T, Chirnside J, Schertler GF (2003) Expression and purification of truncated, non-glycosylated Turkey beta-adrenergic receptors for crystallization. Biochim Biophys Acta 1610:133–140

Warne T, Serrano-Vega MJ, Baker JG, Moukhametzianov R, Edwards PC, Henderson R, Leslie AG, Tate CG, Schertler GF (2008) Structure of a beta1-adrenergic G-protein-coupled receptor. Nature 454:486–491

Warne T, Moukhametzianov R, Baker JG, Nehme R, Edwards PC, Leslie AG, Schertler GF, Tate CG (2011) The structural basis for agonist and partial agonist action on a beta(1)-adrenergic receptor. Nature 469:241–244

Warne T, Edwards PC, Leslie AG, Tate CG (2012) Crystal structures of a stabilized beta1-adrenoceptor bound to the biased agonists bucindolol and carvedilol. Structure 20:841–849

Warne T, Edwards PC, Dore AS, Leslie AGW, Tate CG (2019) Molecular basis for high-affinity agonist binding in GPCRs. Science 364:775–778

Wootten D, Christopoulos A, Marti-Solano M, Babu MM, Sexton PM (2018) Mechanisms of signalling and biased agonism in G protein-coupled receptors. Nat Rev Mol Cell Biol 19:638–653

Xu X, Kaindl J, Clark MJ, Hubner H, Hirata K, Sunahara RK, Gmeiner P, Kobilka BK, Liu X (2021) Binding pathway determines norepinephrine selectivity for the human beta1AR over beta2AR. Cell Res 31:569–579

Xu J, Chen G, Wang H, Cao S, Heng J, Deupi X, Du Y, Kobilka BK (2022) Calcineurin-fusion facilitates Cryo-EM structure determination of a family A GPCR. BioRxiv

Yang F, Ling S, Zhou Y, Zhang Y, Lv P, Liu S, Fang W, Sun W, Hu LA, Zhang L, Shi P, Tian C (2021) Different conformational responses of the β2-adrenergic receptor-Gs complex upon binding of the partial agonist salbutamol or the full agonist isoprenaline. Natl Sci Rev 8: nwaa284

Yuan D, Liu Z, Kaindl J, Maeda S, Zhao J, Sun X, Xu J, Gmeiner P, Wang HW, Kobilka BK (2020) Activation of the alpha(2B) adrenoceptor by the sedative sympatholytic dexmedetomidine. Nat Chem Biol 16:507–512

Zhang Y, Sun BF, Feng D, Hu HL, Chu M, Qu QH, Tarrasch JT, Li S, Kobilka TS, Kobilka BK, Skiniotis G (2017) Cryo-EM structure of the activated GLP-1 receptor in complex with a G protein. Nature 546:248–253

Zhang Y, Yang F, Ling S, Lv P, Zhou Y, Fang W, Sun W, Zhang L, Shi P, Tian C (2020) Single-particle cryo-EM structural studies of the beta2AR-Gs complex bound with a full agonist formoterol. Cell Discov 6:45

Genetic Variants of Adrenoceptors

Andrea Ahles and Stefan Engelhardt

Contents

Abstract

Adrenoceptors are class A G-protein-coupled receptors grouped into three families (α_1-, α_2-, and β-adrenoceptors), each one including three members. All nine corresponding adrenoceptor genes display genetic variation in their coding and adjacent non-coding genomic region. Coding variants, i.e., nucleotide exchanges within the transcribed and translated receptor sequence, may result

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in a difference in amino acid sequence thus altering receptor function and signaling. Such variants have been intensely studied in vitro in overexpression systems and addressed in candidate-gene studies for distinct clinical parameters. In recent years, large cohorts were analyzed in genome-wide association studies (GWAS), where variants are detected as significant in context with specific traits. These studies identified two of the in-depth characterized 18 coding variants in adrenoceptors as repeatedly statistically significant genetic risk factors – p. Arg389Gly in the β_1- and p.Thr164Ile in the β_2-adrenoceptor, along with 56 variants in the non-coding regions adjacent to the adrenoceptor gene loci, the functional role of which is largely unknown at present. This chapter summarizes current knowledge on the two coding variants in adrenoceptors that have been consistently validated in GWAS and provides a prospective overview on the numerous non-coding variants more recently attributed to adrenoceptor gene loci.

Keywords

Adrenoceptor · Genetic variation · GWAS · Non-coding · Polymorphism

1 Introduction

Genetic variation is defined as the difference in the DNA sequence among individuals. More than 300 million variants have been identified in the human genome, distributed throughout protein-coding and non-coding regions (Lettre 2022). These variants either consist of single nucleotide exchanges (also known as single nucleotide polymorphisms, SNPs), insertion/deletion mutations or tandem repeat polymorphisms. Current classifications define variants occurring at a minor allelic frequency of >5% as common, and a frequency of 0.5–5% and <0.5% determines a low-frequency and rare variant, respectively (Abecasis et al. 2012). Together, several thousand variants associated with adrenoceptor genes have been identified, the largest fraction residing in non-coding genomic regions. Annotated to one reference genome (Morales et al. 2022), these are listed in common databases (Ensembl, NCBI) with univocal genomic location and dbSNP ("rs") number.

All nine adrenoceptor genes contain single nucleotide variations in their coding regions. Except for the α_{1B}- and α_{1D}-adrenoceptor (ADRA1B and ADRA1D), such non-synonymous adrenoceptor variants have been intensely studied both in vitro upon overexpression in cell systems and in vivo in candidate-gene studies for certain disease traits or drug treatment. A detailed overview of all these variants and the corresponding studies published is given in (Ahles and Engelhardt 2014). However, many of these early studies are – due to the nature of their candidate gene-driven approach – necessarily biased toward distinct genomic loci and did not replicate in later genome-wide association studies (GWAS). For GWAS, genotyping is performed using microarrays, whole exome and whole genome sequencing. They are summarized in the GWAS Catalog (Buniello et al. 2019) which to date (Nov

2022) comprises >6,000 studies published within the last decades. GWAS represent an unbiased method to determine the genetic background of complex human diseases and to uncover potentially causative (poly)genetic variants (Duncan and Brown 2018; Tam et al. 2019). They often comprise cohorts of >100,000 individuals, allowing for high statistical power and providing reliable numbers on variant frequency in different populations and disease conditions. With this technological progress, GWAS have become the standard for evaluating the association of a certain genetic variant with physiological and pathological phenotypes. Even rare variants and common variants with relatively small effect sizes (i.e., a rather slight difference between the two allelic variants on the respective phenotypic parameter) can be detected in an appropriately sized cohort. Yet, a GWAS identifies hundreds of associated variants for one trait each alone typically conferring rather little risk. These studies are further limited to phenotypes whose characteristics can be analyzed in a systematic way, while rare or hard to study phenotypes remain underrepresented in GWAS. Statistical analysis of GWAS data and stratification is complex and rather incomprehensive, a problem that is aggravated in meta-analysis of different GWAS, which vastly lack statistical traceability as differently analyzed cohorts are combined (Tam et al. 2019). To circumvent the report of false positive GWAS hits, we do not mention adrenoceptor-associated variants that were found statistically significant in a single cohort for an unrelated trait throughout this chapter. We relate to the GWAS Catalog and further require a GWAS cohort of >1,000 individuals.

Accordingly, two of the 18 previously characterized non-synonymous adrenoceptor variants in the coding region were repeatedly reported as associated with a specific (disease) trait: the β_1-adrenoceptor (ADRB1) variation p.Arg389Gly (rs1801253) and p.Thr164Ile (rs1800888) within the β_2-adrenoceptor (ADRB2) ("protein" nomenclature: prefix p. for protein – major amino acid – position of variant amino acid – minor amino acid). Genetic analysis for GWAS also includes non-coding regions, and non-coding genomic variants are receiving increased attention as they comprise about 90% of all reported associations (Maurano et al. 2012). These non-coding variants are typically mapped to the two protein-coding genes they are located in between and are defined as "regulatory region variant" or "intergenic variant." Regulatory variants induce sequence changes in regulatory DNA elements, such as enhancers, transcription factor binding sites, or methylated DNA regions (Rojano et al. 2019). These alterations may, in turn, affect the binding affinity of transcription factors and subsequently the expression of neighboring genes. In addition, epigenetic patterns intersect with genetic information, potentially fine-tuning the functional properties of a non-coding variant in a cell- and tissue-specific manner (Oh and Petronis 2021; Vohra et al. 2021). A proof of concept for non-coding adrenoceptor variants acting on receptor expression is still lacking. Besides, with the recent release of the complete sequence of a human genome (Nurk et al. 2022), several long-noncoding RNAs (lncRNAs) were newly identified, some of which are even located within an adrenoceptor gene locus. lncRNA sequences are typically transcribed into RNA of >200 bases, and these RNA molecules are reported to modulate the expression of protein-coding genes, yet

their detailed function remains to be determined (Statello et al. 2021). With these novel annotations, some of the non-coding variants are now located within the exon or the intron of an lncRNA. The specific trait associations reported in GWAS for such a variant might refer to the lncRNA's function. To date, the interpretation of GWAS results on non-coding variants necessarily remains somewhat speculative. The detailed and unbiased knowledge of non-coding variants demands new studies for in-depth characterization of these variants and their effect on gene expression.

Structured by adrenoceptor subtypes, this chapter aims to provide a comprehensive summary of non-coding variants attributed to adrenoceptor genes and reports current knowledge on the two coding variants ADRB1-p.Arg389Gly and ADRB2-p.Thr164Ile, which reveal whether these coding variants alter the expression and/or function of the receptor protein or whether the variants just represent markers for the reported traits in GWAS.

2 Variants Associated with α_1-Adrenoceptors

α_1-adrenoceptors are robustly expressed in cardiac and smooth muscle. Their activation by catecholamines contributes to contraction and subsequently controls blood pressure, pupil width, bladder, and prostate tone (O'Connell et al. 2014; Akinaga et al. 2019) (for details on expression, see Chapter "Expression Pattern and Species Differences"). As postsynaptic receptors in the central nervous system, α_1-adrenoceptors stimulate transmitter release (Perez 2020). Three α_1-adrenoceptor subtypes are encoded in the human genome: α_{1A}, α_{1B}, and α_{1D} (*ADRA1A, ADRA1B, ADRA1D*). The *ADRA1A* and the *ADRA1B* locus are subject to alternative splicing resulting in different protein-coding isoforms, while the *ADRA1D* is expressed as one single isoform. For none of these three receptors, a coding variant showed significant association with a specific parameter in a GWAS. Non-coding variants were detected for all three α_1-adrenoceptor subtypes (Table 1).

The *ADRA1A* gene is located on the reverse strand of chromosome 8. The adjacent protein-coding genes for dihydropyrimidinase like 2 (*DPYSL2*) and stathmin-4 (*STMN4*) are about 0.1 and 0.7 Mb distant, respectively. In addition, lncRNAs are located in the *ADRA1A* intronic region (both on the reverse, i.e., *ADRA1A* strand, and the forward strand) and upstream of the *ADRA1A* gene locus. The four non-coding variants associated with *ADRA1A* detected in GWAS are depicted in Fig. 1a, along with adjacent coding genes and annotated lncRNAs. These genomic variants are related to traits of the central nervous system. Two of these variants were found to be associated with total PHF-tau, which is implicated in the pathogenesis of Alzheimer's disease (Wang et al. 2020): rs6998591 located within the intronic region of the *ADRA1A* gene and the intergenic variant rs13273959 attributed to both the *ADRA1A* and the *DPYSL2* gene. The latter encodes a brain enriched protein involved in microtubule assembly and synaptic signaling that might play a role in the development of Alzheimer's disease (Williamson et al. 2011).

Table 1 α_1-adrenoceptor genetic variation. Each variant is defined with its unique dbSNP number and listed with its minor allelic frequency (MAF, minor allele in brackets) and the reported GWAS association

Location	dbSNP	MAF	GWAS association	References
ADRA1A				
Regulatory	rs7845740	0.40 (T)	Bitter alcoholic beverage consumption	Zhong et al. (2019)
Intergenic Intronic	rs13273959 rs6998591	0.08 (G) 0.32 (T)	PHF-tau measurement	Wang et al. (2020)
Intergenic	rs145140079	0.001 (T)	Periventricular white matter hyperintensities	Armstrong et al. (2020)
ADRA1B				
Intergenic	rs148871069	0.0004 (G)	SBP, DBP, pulse pressure Hematocrit, red blood cell count, hemoglobin	Hoffmann et al. (2017) Chen et al. (2020); Sakaue et al. (2021)
ADRA1D				
Regulatory	rs297690	0.29 (T)	DBP	Plotnikov et al. (2022)
Intergenic	rs190933	0.30 (T)		Sakaue et al. (2021)
Intergenic	rs6037811	0.50 (A)	Schizophrenia	Trubetskoy et al. (2022)
Intergenic Intronic	rs3859671 rs835879	0.50 (G) 0.48 (C)	Mononucleosis	Tian et al. (2017)
Intronic	rs835875	0.46 (C)	Heel bone mineral intensity	Kim (2018); Kichaev et al. (2019)

Abbreviations: *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *PHF-tau* paired helical filaments (main constituent: tau protein)

The regulatory region variant rs7845740 was found to be associated with bitter alcoholic beverage consumption in two independent cohorts (Zhong et al. 2019), a complex trait that presumes a central nervous contribution. This variant is located within a binding site of the transcription factor CCTC-binding factor (CTCF), which promotes or represses gene expression and can affect enhancer–promoter interaction (Abecasis et al. 2012). It is thus imaginable that rs7845740 interferes with the expression of the two adjacent protein-coding genes (*ADRA1A* and *DPYSL2*) and of the lncRNAs which were identified within the *ADRA1A* intronic sequence (Fig. 1a). Finally, the rare variant rs145140079 (minor allelic frequency: 0.1%), located upstream of the *ADRA1A* start codon, was associated with white matter hyperintensities in two cohorts (Armstrong et al. 2020). These are supposed to be caused by cerebral small vessel disease and to increase stroke mortality and cognitive and functional impairment, including Alzheimer's disease (Tubi et al. 2020).

The *ADRA1B* gene is located on the forward strand of chromosome 5. Figure 1b depicts the main *ADRA1B* isoform and transcript variant X6. While the distance to the adjacent protein-coding gene 5′ of the gene locus (*IL12B*) is 0.7 MB, the *TTC1*

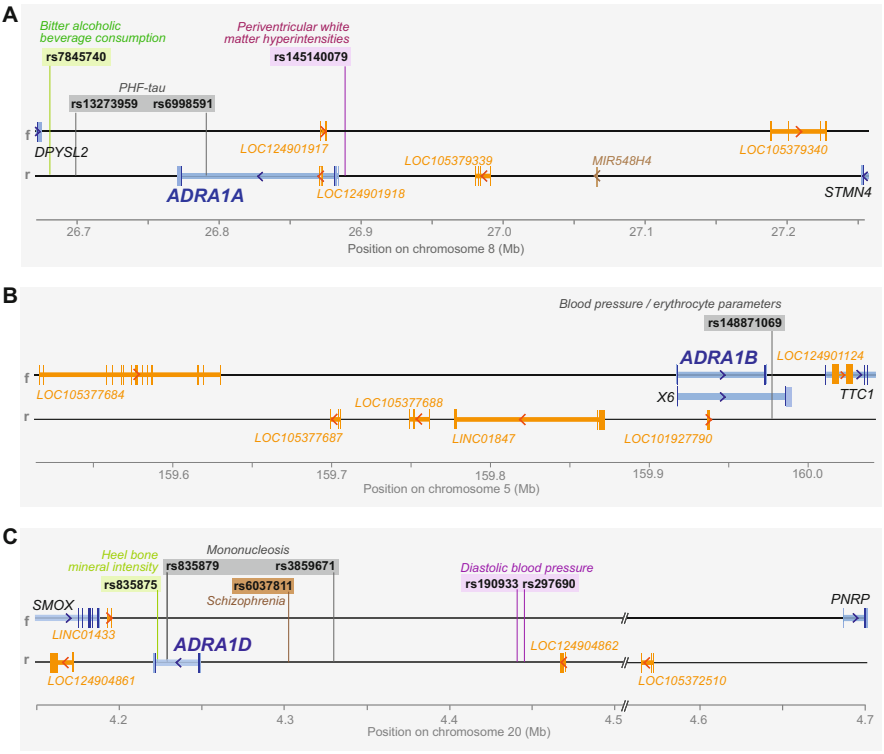

Fig. 1 α_1-adrenoceptor genetic variation. Genomic loci of (**a**) *ADRA1A* (transcript variant 1), (**b**) *ADRA1B* and (**c**) *ADRA1D*. 3′- and 5′-untranslated regions of protein-coding genes are marked in light blue, exons in dark blue. lncRNAs are shown in orange. Genetic variants are depicted according to their genomic location and grouped by reported traits

gene encoding for tetratricopeptide repeat domain 1 is only 36 Kb 3′ of *ADRA1B*. The genomic region further contains several not yet characterized lncRNAs.

For the *ADRA1B*, only one non-coding variant was reported repeatedly in GWAS. rs148871069 displays a rare variant with a minor allelic frequency of 0.04% and is located about 4 kb 3′ of the *ADRA1B* gene (regarding the main *ADRA1B* isoform) in an intergenic region and within the intron of the *ADRA1B* transcript variant X6, respectively (Fig. 1b). The variant was repeatedly detected in GWAS, statistically significant for different traits (Table 1): First, the minor G allele was associated with decreased erythrocyte density which went along with decreased hematocrit and less hemoglobin content (Chen et al. 2020; Sakaue et al. 2021). Second, the same SNP was associated with blood pressure, the minor G allele showing a decrease in both systolic and diastolic blood pressure as well as in pulse pressure (Hoffmann et al. 2017), a physiologic parameter that might be related to α_1-adrenoceptor-mediated contraction of arteries (Akinaga et al. 2019). Whether the non-coding variant rs148871069 affects receptor expression and function, and

whether there is a functional impact of ADRA1B on the modulation of red blood cell composition and pulse pressure, is unclear to date.

The *ADRA1D* gene is located on the reverse strand of chromosome 20, flanked by the genes coding for spermine oxidase (SMOX) and prion protein (PNRP), as well as four lncRNA genes. Six non-coding variants are associated with the *ADRA1D* (Fig. 1c): The intergenic variant rs190933 and the regulatory region variant rs297690 were found to be associated with diastolic blood pressure. The latter variant is located within an enhancer element and thus can potentially affect the expression of *ADRA1D* or the lncRNA *LOC124904862*, which is situated 24 kb upstream of the variant. In addition, an association of the intronic variant rs835875 with heel bone mineral density is reported (Kim 2018; Kichaev et al. 2019). The intergenic variant rs6037811 has been associated with schizophrenia (Trubetskoy et al. 2022), a trait that is linked to alterations in the sympathetic nervous system (Perez 2020). Finally, one single GWAS reported an association with "susceptibility to mononucleosis measurement" with two hits associated with the *ADRA1D*: the intergenic variant rs3859671 located 5′ of the *ADRA1D* gene, and rs835879 in the intronic region of the *ADRA1D* (Tian et al. 2017). The potential link of the latter traits with the *ADRA1D* gene remains to be determined.

In summary, eleven SNPs located in the non-coding regions adjacent to or within α_1-adrenoceptor genes have been validated in GWAS. Although some traits reported directly relate to the function of the respective adrenoceptor, a direct link of one of the nucleotide alterations to altered receptor expression and signaling remains undetermined to date.

3 Variants Associated with α_2-Adrenoceptors

α_2-adrenoceptors are expressed in multiple organs, including adipose tissue, smooth muscles, and the brain (for details on expression, see Chapter "Expression Pattern and Species Differences"). They are involved in regulating blood pressure, pain, and neurotransmitter release. α_2-adrenoceptor signaling further stimulates platelet aggregation and inhibits lipolysis and insulin release from the pancreas (Giovannitti et al. 2015). All three α_2-adrenoceptor subtypes (*ADRA2A, ADRA2B*, and *ADRA2C*) contain variants in their coding and non-coding regions. The insertion/deletion mutation c.901_909del (p.Glu301_Glu303del, resp. rs28365031) located in the third intracellular loop of the ADRA2B protein was found to be associated with diastolic blood pressure in one GWAS (Sakaue et al. 2021). Due to the lack of confirmation by a second cohort, this coding variant is not discussed in detail in this chapter. GWAS hits in non-coding regions attributed to α_2-adrenoceptor loci are limited to the *ADRA2A* (Table 2).

The *ADRA2A* gene is located on the forward strand of chromosome 10. The adjacent protein-coding gene 5′ of the *ADRA2A* is located at a distance of about 63 kb (SHOC2, leucine-rich repeats scaffold protein), while 3′ the distance to the following protein-coding gene comprises nearly 1 Mb.

Table 2 Genetic variation of the α_2-adrenoceptor *ADRA2A*. Each variant is identified by its unique dbSNP number and listed with its minor allelic frequency (MAF, minor allele in brackets) and the reported GWAS association

Location	dbSNP	MAF	GWAS association	References
Regulatory Intergenic	rs869244 rs4545476	0.38 (A) 0.48 (T)	Platelet aggregation	Johnson et al. (2010); Chen et al. (2019)
Intergenic Intergenic	rs12775580 rs12244654	0.08 (T) 0.17 (T)	Glucose levels	Richardson et al. (2022) Sakaue et al. (2021)
Intergenic	rs11195508	0.11 (G)	Hemoglobin A1c levels Glycated hemoglobin levels	Sakaue et al. (2021) Sinnott-Armstrong et al. (2021)

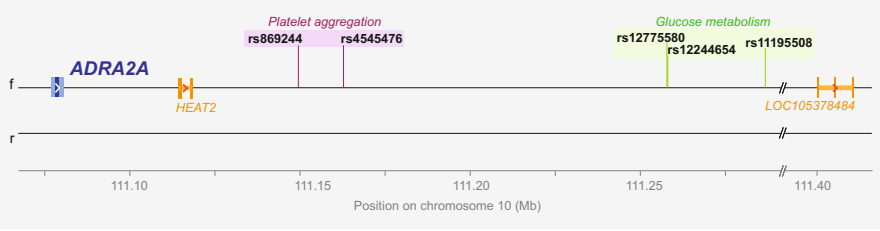

Fig. 2 Variants attributed to the human α_{2A}-adrenoceptor. Genomic locus of *ADRA2A*. 3′- and 5′-untranslated regions of protein-coding genes are marked in light blue, exons in dark blue. lncRNAs are shown in orange. Genetic variants are depicted according to their genomic location and grouped by reported traits

All five detected variants are located 3′ of the *ADRA2A* gene and of the gene encoding lncRNA HEart disease-Associated Transcript 2 (*HEAT2*) (Fig. 2). The traits they are associated with coincide with α_2-adrenoceptor function. Both rs869244 and rs4545476 are associated with differences in platelet aggregation (Johnson et al. 2010; Chen et al. 2019). While the latter is defined as an intergenic variant, rs869244 is localized in an enhancer sequence, allowing for variant-specific regulation of ADRA2A expression and the expression of the immune cell-enriched lncRNA *HEAT2* (Boeckel et al. 2019) (Fig. 2). In addition, an association with glucose regulation has been reported for three intergenic non-coding variants (rs12775580, rs12244654, and rs11195508) that are attributed to the *ADRA2A* gene (Table 2). Although an impact of intergenic variants on the expression of neighboring genes is not described to date, these associations match the reported function of the ADRA2A, namely the inhibition of insulin secretion from the pancreas by ADRA2A (Fagerholm et al. 2011).

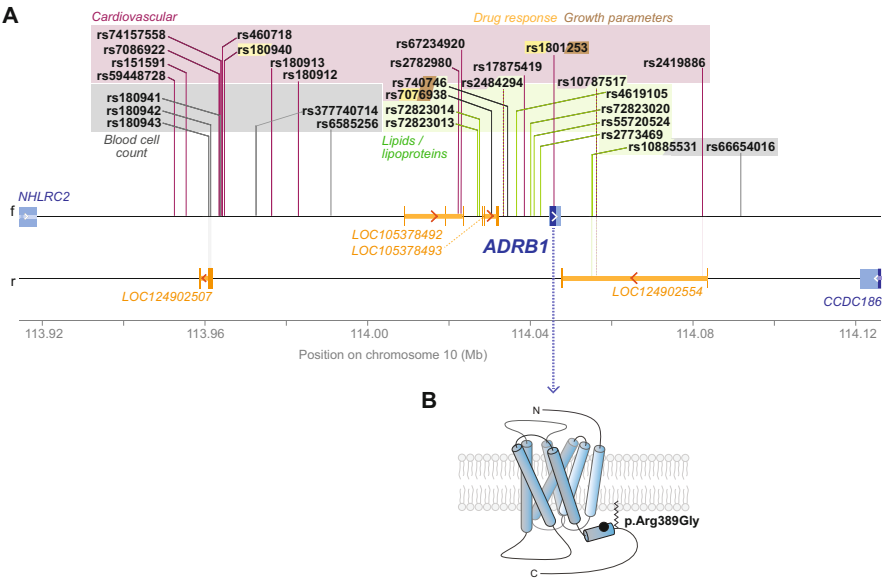

Fig. 3 β_1-adrenoceptor genetic variation. (**a**) *ADRB1* gene locus and adjacent protein-coding genes (blue), lncRNAs (orange) and variants repeatedly associated with a certain trait in GWAS (grouped by reported traits). (**b**) ADRB1 protein and location of the coding variant p.Arg389Gly (rs1801253)

4 Variants Associated with the β_1-Adrenoceptor

The β_1-adrenoceptor (ADRB1) stimulates cardiac output and renin release and thereby maintains blood pressure (Dorn 2010) (for details on ADRB1 signaling, see Chapter "Signal Transduction, Canonical and Alternative Pathways"; for its implication in cardiovascular disease, see Chapters "Cardiovascular: Heart Failure, Ischemic Heart Disease, Arrhythmia" and "Cardiovascular: Hyper- and Hypotension, Shock"). Apart from its robust expression in cardiac myocytes, *ADRB1* expression is high in adipose tissue, where it has been reported to stimulate lipolysis in adipocytes (Riis-Vestergaard et al. 2020) (See also Chapter "Expression Pattern and Species Differences"). With the coding variant p.Arg389Gly and 29 associated non-coding variants, the *ADRB1* locus contains by far the most GWAS hits of the nine adrenoceptor subtypes (Fig. 3a), underscoring its essential role in various physiological systems and diseases.

4.1 The ADRB1 Coding Variant p.Arg389Gly

The intron-less *ADRB1* gene encodes a receptor protein of 477 amino acids. Position 389 is located in helix 8, which is formed between the distal end of transmembrane domain 7 and the C-terminal palmitoylation site (Fig. 3b). Here, arginine is

substituted by glycine at a mean minor allelic frequency of 30%. Evidence toward a functional role of this common variation has been detected in vitro, and there is strong indication for its relevance from multiple clinical studies, including GWAS.

In early in vitro studies conducted in cell lines, the Arg389-variant displayed higher basal and agonist-induced adenylyl cyclase-mediated cAMP formation compared to the Gly389-variant (Mason et al. 1999; Joseph et al. 2004; Ahles et al. 2015). The beating frequency of isolated rat cardiac myocytes was likewise higher when expressing the human Arg389-variant. Using fluorescence resonance energy transfer to monitor conformational changes within the receptor proteins upon application of different ligands, the β-blocker carvedilol induced larger changes in the conformation of the Arg389-variant compared to the Gly389-variant (Rochais et al. 2007).

Alternative signaling, i.e., the interaction of the ADRB1 with arrestins and subsequent receptor desensitization, has been investigated in a variant-specific manner. Here, norepinephrine-induced receptor phosphorylation at intracellular serine and threonine residues by G protein-coupled receptor kinases (GRKs) was stronger for the Arg389-variant, which subsequently led to enhanced recruitment of β-arrestin (Ahles et al. 2015; McCrink et al. 2016). In agreement with its more potent interaction with arrestins, the Arg389-ADRB1 exhibited greater agonist-promoted desensitization than the Gly389-variant (Liggett et al. 2006). Upon cardiomyocyte-specific transgenic overexpression of the human ADRB1 variants in mice, increased basal and dobutamine-induced contractility levels have been reported for the Arg389- compared to the Gly389-variant (Mialet Perez et al. 2003). In addition, desensitization was enhanced for the Arg389-variant in older animals pointing toward a "hyperfunctionality" of the Arg389-variant, and vice versa a "hypofunctionality" for the Gly389-variant.

The elucidation of the crystal structure of the turkey ADRB1 then provided a structural basis for the increased functionality of the Arg389-variant. In these structures, helix 8 is well resolved and contains an arginine at the respective conserved site, whose side chain is oriented toward helix 1 (Warne et al. 2012) (for details on ADRB1 structure, see Chapter "Structures of Adrenoceptors"). In the corresponding model of the human ADRB1, the polar side chains of residues Lys85 and Thr86 in helix 1 are in juxtaposition with Arg389. While Thr86 could interact with Arg389 via hydrogen bonding, repulsion forces are prominent between the helix1-Lys85 and the helix8-Arg389. These electrostatic interactions are different in the Gly389-ADRB1 and might increase the dynamics of the receptor protein, thereby providing a structural basis for the variant-dependent functionality of the ADRB1 (Ahles et al. 2015). Experimentally, the speed of receptor activation has been assessed in dependence on the helix1/helix8 interface by fluorescence resonance energy transfer. Here, the Arg389-ADRB1, expressed in HEK293 cells, showed a faster activation than the Gly389-variant when repeatedly stimulated. This difference was not detected anymore when the helix1/helix8 interface was disrupted by mutating Lys85 and Thr86 to unpolar residues (Ahles et al. 2015).

The functional impact of p.Arg389Gly has finally been validated in GWAS in recent years. These studies typically comprise >100,000 individuals of different

ethnic backgrounds. The first GWAS in which p.Arg389Gly appeared as a hit was on association with birth weight in an analysis of nearly 70,000 Europeans (Horikoshi et al. 2013), with Gly389 being linked to a lower birth weight compared to Arg389. This association has been confirmed in two further GWAS (Warrington et al. 2019; Plotnikov et al. 2020). The authors link a lower birth weight to higher blood pressure in adulthood. The underlying mechanism of this proposed relation remains elusive. Yet, the p.Arg389Gly variation has subsequently been detected as associated with the trait systolic and/or diastolic blood pressure in five GWAS (Surendran et al. 2016; Hoffmann et al. 2017; Feitosa et al. 2018; Sung et al. 2018; Giri et al. 2019). This association with blood pressure is not limited to the coding variation, as it has also been detected for non-coding variants attributed to the *ADRB1* gene (Table 3), the latter presumably acting through modulation of ADRB1 expression. p.Arg389Gly was further found to be associated with cardiovascular disease; the pathologic details were not further specified by the authors (Kichaev et al. 2019). In contrast, p.Arg389Gly was not associated with heart failure risk in any of the >30 GWAS investigating this trait.

These unbiased results from GWAS are reflected by the general outcome of clinical studies that have been conducted in the pre-GWAS era to assess the relevance of the ADRB1 variation p.Arg389Gly in hypertension and heart failure: In the three candidate-gene association studies on hypertension comprising the largest cohorts (n > 1,000), the Arg389-variant was associated with a higher risk for hypertension (Gjesing et al. 2007; Tikhonoff et al. 2008; Johnson et al. 2011), while studies on variant-specific prevalence in heart failure did not result in a univocal association (Ahles and Engelhardt 2014). Importantly, candidate-gene studies show evidence that p.Arg389Gly affects the response to β-blockers: On the one hand, the effect of β-blockers has been studied in healthy individuals under conditions of increased heart rate and blood pressure (by exercise or dobutamine infusion). While basal and maximal hemodynamics did not differ between Arg389 and Gly389 homozygotes, the relative decrease evoked by β-blockers was greater for Arg389 in four out of five study groups (Ahles and Engelhardt 2014). On the other hand, candidate-gene studies on patients treated with β-blockers comprising cohort sizes >1,000, demonstrated improved survival for Arg389 with heart failure (Liggett et al. 2006; O'Connor et al. 2012; Aleong et al. 2013) or coronary artery disease (Pacanowski et al. 2008) compared to Gly389 carriers. Also, a meta-analysis of three smaller studies (in total 504 heart failure patients) revealed a significantly greater improvement of left ventricular ejection fraction for Arg389 homozygotic individuals when treated with β-blockers compared to Gly389 carriers (Muthumala et al. 2008). The five large published GWAS that tested for genetic variation associated with β-blocker response did not report any association – in contrast to the candidate-gene studies mentioned above. A definite statement on the impact of p.Arg389Gly on drug response awaits further GWAS and ideally a subgrouping for different β-blockers. Comedication with additional antihypertensive agents might be considered for analysis, as p.Arg389Gly was associated with treatment with diuretics and drugs acting at the renin-angiotensin-aldosterone-system in two independent GWAS (Wu et al. 2019; Sakaue et al. 2021).

Table 3 β_1-adrenoceptor genetic variants associated with cardiovascular traits. Each variant is defined with its unique dbSNP number and listed with its minor allelic frequency (MAF, minor allele in brackets) and the reported GWAS association

Location	dbSNP	MAF	Reported trait	References
5' of ADRB1				
Intergenic	rs59448728	0.25 (A)	SBP	Sakaue et al. (2021)
Regulatory	rs151591	0.28 (A)	SBP, DPB	Plotnikov et al. (2022)
Intergenic	rs7086922	0.07 (T)	DPB	Plotnikov et al. (2022)
Intergenic	rs74157558	0.07 (A)	SBP	Plotnikov et al. (2022)
Intergenic	rs460718	0.39 (A)	SBP	Sakaue et al. (2021)
Intergenic	rs180940	0.39 (A)	SBP, DBP (+ smoking)	Sung et al. (2018)
Intergenic	rs180913	0.41 (T)	SBP, DBP, MAP	Takeuchi et al. (2018)
Intergenic	rs180912	0.46 (T)	Hypertension	Takeuchi et al. (2018)
lncRNA LOC105378492, intron Regulatory	rs2782980	0.28 (T)	SBP, DBP (± smoking) MAP	Wain et al. (2011, 2017a); Sung et al. (2018); Kichaev et al. (2019); Sakaue et al. (2021)
lncRNA LOC105378492, intron	rs67234920	0.17 (A)	PR interval	Ntalla et al. (2020)
lncRNA LOC105378493, intron	rs7076938	0.30 (C)	MAP	Liu et al. (2016)
Intergenic	rs2484294	0.29 (G)	SBP, DBP, MAP (± alcohol)	Feitosa et al. (2018); Plotnikov et al. (2022)
Regulatory	rs740746	0.29 (G)	SBP, DBP, MAP (± alcohol) Electrocardiography	Ehret et al. (2016); Feitosa et al. (2018); Plotnikov et al. (2022) Verweij et al. (2020)
Intergenic	rs17875419	nd	DBP	Warren et al. (2017)

(continued)

Table 3 (continued)

Location	dbSNP	MAF	Reported trait	References
ADRB1 exon				
	rs1801253 p.Arg389Gly	0.30 (G)	SBP (± smoking) DPB (± alcohol/ smoking) Cardiovascular disease	Surendran et al. (2016); Hoffmann et al. (2017); Giri et al. (2019) Feitosa et al. (2018); Sung et al. (2018) Kichaev et al. (2019)
3' of ADRB1				
lncRNA LOC124902554, intron	rs10787517	nd	SBP	Wain et al. (2017b)
lncRNA LOC124902554, intron	rs2419886	0.24 (T)	Serum calcium measurement	Sakaue et al. (2021); Young et al. (2021)

Abbreviations: *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *MAP* mean arterial pressure, *nd* not determined

4.2 Non-coding Variants Attributed to the ADRB1

The *ADRB1* gene is located on the forward strand of chromosome 10, flanked by lncRNAs, with the adjacent 3′ and 5′ protein-coding genes NHL repeat-containing protein 2 (*NHLRC2*), a not yet fully characterized protein, and coiled-coil domain containing 186 (*CCDC186*, also known as CTCL tumor-associated antigen) about 120 and 70 kb distant, respectively. Both 3′ and 5′ of the *ADRB1* locus, lncRNAs are annotated (Fig. 3a), whose role in physiology and disease is vastly unknown to date. The traits reported for *ADRB1*-associated variants can be subdivided into four major groups: cardiovascular, blood cell count, lipids/lipoproteins, and growth parameters, with some variants associated with multiple of these groups.

In line with ADRB1 function and the coding variant p.Arg389Gly, an association with a cardiovascular trait was reported for 16 non-coding *ADRB1* variants. The majority of these are linked to the traits systolic and/or diastolic blood pressure (Table 3). In particular, the regulatory region variants are suggestive of influencing ADRB1 expression and function, thereby resulting in variant-specific differences in blood pressure: rs151591 is part of a CTCF binding site (Plotnikov et al. 2022), rs2782980 (Wain et al. 2011, 2017a; Sung et al. 2018; Kichaev et al. 2019; Sakaue et al. 2021) and rs740746 (Ehret et al. 2016; Feitosa et al. 2018; Plotnikov et al. 2022) localize in enhancer regions. Moreover, five SNPs are located within the intron of lncRNAs (rs2782980 and rs67234920 in *LOC105378492*; rs7076938, rs10787517, and rs2419886 in *LOC124902554*), hypothesizing a relation of the reported cardiovascular trait and the (patho)physiological function of the respective lncRNA.

Table 4 β_1-adrenoceptor genetic variants associated with drug treatment. Each variant is defined with its unique dbSNP number and listed with its minor allelic frequency (MAF, minor allele in brackets) and the reported GWAS association

Location	dbSNP	MAF	Reported trait	References
5' of ADRB1				
Intergenic	rs180940	0.39 (A)	Beta-blocking agent use	Sakaue et al. (2021)
lncRNA LOC105378493, intron	rs7076938	0.30 (C)	Diuretics use	Wu et al. (2019)
ADRB1 exon				
	rs1801253 p.Arg389Gly	0.30 (G)	Medication use (diuretics, RAAS agents)	Wu et al. (2019); Sakaue et al. (2021)

Of note, two variations 5′ of the *ADRB1* have been additionally found associated with drug treatment (Table 4). First, the intergenic variant rs1800940 was associated with β-blocker use (Sakaue et al. 2021), presuming an influence of the variant on receptor function. Second, rs7076938, located in the intronic region of lncRNA *LOC105378493,* was detected as one determinant of diuretics treatment (Wu et al. 2019), a first-line medication for hypertension.

Besides the multiple associations with cardiovascular traits, seven non-coding variants were reported in GWAS for blood cell count. Five of these were linked to alterations in the number of white blood cells (in general or specifically neutrophils, leukocytes, lymphocytes), in line with the immunomodulatory action of ADRB1, if indeed a genomic link can once be attested between non-coding variants and the receptor gene (Table 5). Among these, rs6585256 displays a regulatory region variant as it is annotated in an enhancer element, modulating both lymphocyte count and platelet crit (i.e., the proportion of blood volume occupied by platelets) (Vuckovic et al. 2020). The variants rs180943 and rs180942 are located within an exon of the lncRNA LOC124902507, which is annotated 5′ of the *ADRB1* gene. These represent nucleotide substitutions that are transcribed and alter the sequence of the lncRNA, which might, in turn, alter lncRNA expression or folding and subsequent interaction with specific proteins or RNA molecules. The link to white blood cell composition regarding the GWAS results (Kichaev et al. 2019; Chen et al. 2020; Vuckovic et al. 2020; Sakaue et al. 2021) remains to be determined. Also, rs10885531, located in the intron of lncRNA *LOC124902554* 3′ of the *ADRB1* gene and associated with reticulocyte count (Dastani et al. 2012), might determine the (unknown) function of this lncRNA.

Furthermore, 11 non-coding variants are associated with traits on lipids (triglycerides, cholesterol), (apo)lipoproteins (very low density, low density, and high density lipoprotein), and the respective ratios (Table 5). As β_1-adrenoceptor activation induces lipolysis, such GWAS hits appear logical. Here, rs740746, located in an enhancer element and already a hit for cardiovascular traits

Table 5 β_1-adrenoceptor genetic variants associated with blood cell count, lipid metabolism, and growth parameters. Each variant is defined with its unique dbSNP number and listed with its minor allelic frequency (MAF, minor allele in brackets) and the reported GWAS association

Location	dbSNP	MAF	Reported trait	References
			Blood cell count	
5' of ADRB1				
lncRNA LOC124902507, exon	rs180943	0.38 (G)	Neutrophil count Leukocyte count	Kichaev et al. (2019); Vuckovic et al. (2020); Sakaue et al. (2021)
lncRNA LOC124902507, exon	rs180942	0.46 (T)	White blood cell count	Chen et al. (2020)
Intergenic	rs180941	0.44 (A)	Neutrophil count White blood cell count	Chen et al. (2020)
Intergenic	rs377740714	nd	Lymphocyte count Leukocyte count	Sakaue et al. (2021)
Regulatory	rs6585256	0.41 (A)	Lymphocyte count Platelet crit	Vuckovic et al. (2020)
3' of ADRB1				
lncRNA LOC124902554, intron	rs10885531	0.45 (T)	Reticulocyte count	Vuckovic et al. (2020)
Intergenic	rs66654016	0.12 (C)	Lymphocyte count	Chen et al. (2020)
			Lipids and lipoproteins	
5' of ADRB1				
Intergenic	rs72823013	0.05 (A)	Lipoproteins / cholesterol, aspartate aminotransferase	Klarin et al. (2018); Klimentidis et al. (2020); Sakaue et al. (2021); Richardson et al. (2022)
Intergenic	rs72823014	0.06 (A)	(Apo) lipoproteins/ cholesterol, aspartate aminotransferase	Richardson et al. (2020, 2022); Chen et al. (2021)
lncRNA LOC105378493, intron	rs7076938	0.30 (C)	Lipoproteins/ cholesterol	Liu et al. (2017)
Intergenic	rs2484294	0.29 (G)	Lipoproteins/ triglycerides	Richardson et al. (2022)

(continued)

Table 5 (continued)

Location	dbSNP	MAF	Reported trait	References
Regulatory	rs740746	0.29 (G)	Lipoproteins/ triglycerides	Qi and Chatterjee (2018); Huang et al. (2021)
Intergenic	rs4619105	0.05 (A)	Lipoproteins	Hoffmann et al. (2018)
Intergenic	rs72823020	0.07 (A)	(Apo) lipoproteins	Richardson et al. (2020, 2022)
Intergenic	rs55720524	0.05 (T)	Lipoproteins / cholesterol	Richardson et al. (2022)
Regulatory	rs2773469	0.29 (A)	Triglycerides	Richardson et al. (2020)
3' of ADRB1				
lncRNA LOC124902554, intron	rs10885531	0.45 (T)	Adiponectin	Dastani et al. (2012)
lncRNA LOC124902554, intron	rs10787517	nd	Lipoproteins/ cholesterol	Ripatti et al. (2020); Sakaue et al. (2021); Richardson et al. (2022)
			Birth/growth parameters	
5' of ADRB1				
lncRNA LOC105378493, intron	rs7076938	0.30 (C)	Birth weight Body height	Horikoshi et al. (2016); Warrington et al. (2019); Sakaue et al. (2021)
Regulatory	rs740746	0.29 (G)	Birth weight/ body height, infant head circumference	Yang et al. (2019)
ADRB1 exon				
	rs1801253 p.Arg389Gly	0.30 (G)	(offspring) Birth weight Body height	Horikoshi et al. (2013); Kichaev et al. (2019); Warrington et al. (2019); Plotnikov et al. (2020)

Abbreviation: *nd* not determined

(Table 3), is associated with lipid traits (levels of cholesterol, triglycerides, LDL, and HDL) (Qi and Chatterjee 2018; Huang et al. 2021). A second regulatory region variant is solely associated with triglyceride levels (Richardson et al. 2020): rs2773469 locates to an open chromatin region, i.e., a region that can be assessed by DNA regulatory elements and thus is important for transcriptional regulation of neighboring genes. Next to six intergenic variants, the intron of lncRNA *LOC105378493* (located 5′ of *ADRB1*) and of lncRNA *LOC124902554* (3′ of *ADRB1*) both contain variants associated with lipid metabolism traits (Dastani et al. 2012; Chen et al. 2020; Ripatti et al. 2020; Sakaue et al. 2021; Richardson et al. 2022).

Finally, the complex traits of body height and birth weight, the coding *ADRB1* variant p.Arg389Gly is associated with, were repeatedly reported for two non-coding variants (Table 5): again, the regulatory region variant rs740746 (Yang et al. 2019) and rs7076938 (Horikoshi et al. 2016; Warrington et al. 2019; Sakaue et al. 2021) located in the intron of lncRNA *LOC105378493*, which already was detected in studies on lipid traits, mean arterial pressure, and diuretics use (see Tables 3, 4, and 5).

In summary, the *ADRB1* genetic region is subject to numerous GWAS reports, including the common coding polymorphism p.Arg389Gly. These unbiased results acknowledge the established critical role of the β_1-adrenoceptor in blood pressure regulation, and in addition suggest major implications of the *ADRB1* genetic region on blood cell composition, lipid metabolism, and body growth. These associations should especially be considered when elucidating the function of the lncRNAs located adjacent to the *ADRB1* gene.

5 Variants Associated with the β_2-Adrenoceptor

β_2-adrenoceptors (ADRB2) are expressed throughout various cell types including smooth muscle and immune cells (for details on expression, see Chapter "Expression Pattern and Species Differences"). Their activation induces vasodilation and relaxation of bronchial and uterine smooth muscle. β_2-Agonists are applied to treat broncho-constrictive diseases and preterm labor. Next to the rare *ADRB2* coding variant p.Thr164Ile (rs1800888), 11 non-coding variants attributed to the *ADRB2* were repeatedly reported to be significantly associated with specific traits in GWAS (Fig. 4a).

5.1 The ADRB2 Coding Variant p.Thr164Ile

The intron-less *ADRB2* gene contains three non-synonymous variations in its open reading frame. The two common coding variants p.Gly16Arg and p.Gln27Glu – both located in the extracellular N-terminus of the ADRB2 – were reported to affect the functional properties of the ADRB2 in some but not all in vitro studies (Ahles and Engelhardt 2014). Subsequently, these variants have to date not been found

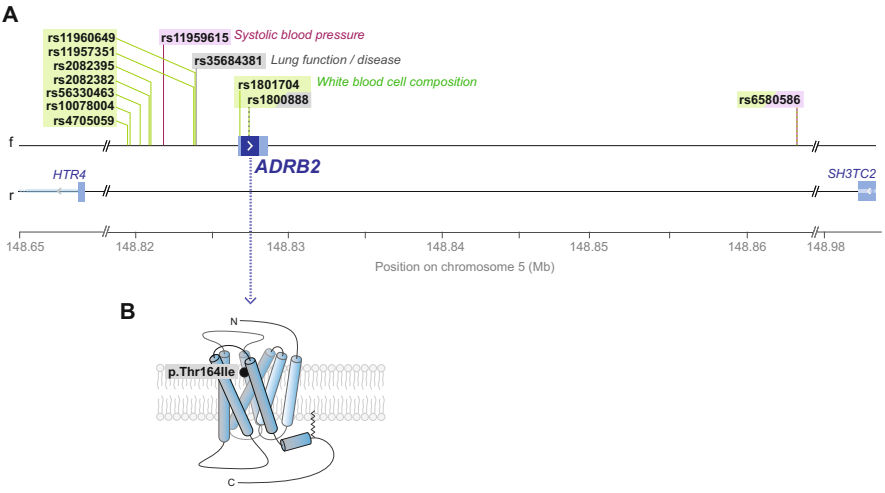

Fig. 4 β_2-adrenoceptor genetic variation. (**a**) *ADRB2* gene locus and adjacent protein-coding genes. Light blue – 3′-and 5′-untranslated regions, dark blue- exons. Variants are depicted according to their genomic location and grouped by reported traits. (**b**) ADRB2 protein and location of the coding variant p.Thr164Ile (rs1800888)

associated with any trait in a GWAS. In contrast, the p.Thr164Ile variant is rare, with isoleucine occurring at an allelic frequency of 2% and in a heterozygous state. This variation locates within the lipid bilayer in transmembrane helix 4 (Fig. 4b), a well-conserved region that has been resolved in the numerous crystal structures published (see Chapter "Structures of Adrenoceptors"). As these structures typically contain threonine at position 164 and an effort to crystallize an Ile164-ADRB2 protein has not been undertaken to date, conclusions arising from structures of the ADRB2 protein are limited to modeling studies and considerations on chemical charges, i.e., the exchange of the polar threonine residue to hydrophobic isoleucine. Position 164 is located near the ligand binding pocket of the ADRB2 and a determinant of the helix4/helix5 interface with Thr164 putatively forming hydrogen bonds with two conserved serines (Ser203 and Ser207) in transmembrane domain 5 (Archala et al. 2022). Hence, p.Thr164Ile is suggested to modulate ligand binding affinities and to influence ADRB2 activation dynamics (Warne et al. 2008, 2011).

Indeed, signaling defects of the Ile164-ADRB2 have already been observed in vitro. When overexpressed in CHW-1102 cells, the Ile164-variant displayed a three- to fourfold lower binding affinity for catecholamines and the β-agonist isoproterenol compared to the Thr164-variant. Consequently, the interaction of the agonist-stimulated ADRB2 with the stimulatory G protein Gs was also decreased for Ile164 (Green et al. 1993), in line with a decrease in basal and agonist-stimulated adenylyl cyclase activity as determined in CHW-1102 cells and transgenic mice overexpressing the different ADRB2 variants (Turki et al. 1996). The loss of function of p.Thr164Ile was further confirmed for the endogenous receptor by analyzing isoproterenol-stimulated cAMP formation in lymphocytes, comparing

Ile164 carriers and Thr164 homozygotes (Büscher et al. 2002). The potency of β-agonist-induced lipolysis (Hoffstedt et al. 2001) and inhibition of IgE-mediated histamine release (Kay et al. 2003, 2007) were reduced in Ile164 carriers.

The effects of the p.Thr164Ile variation observed in vitro were confirmed in large cohorts (Table 6): two GWAS on lung function found the Ile164 variant to be associated with decreased forced expiratory volume (FEV1) (Wain et al. 2017a; Shrine et al. 2019). Consequently, the FEV/FEC ratio was reduced, and peak expiratory flow was significantly decreased (Kichaev et al. 2019; Shrine et al. 2019). Moreover, in a study comprising different ethnic groups, the variant was associated with chronic obstructive pulmonary disease (Moll et al. 2021). The eosinophil percentage of neutrophils was increased for Ile164 (Vuckovic et al. 2020), a finding that might be associated with the severity of asthma and COPD exacerbations (Barnes 2019). Of note, p.Thr164Ile was not detected as a relevant contributor in GWAS for asthma or any cardiovascular disease.

GWAS on treatment efficacy of β-agonists to prevent bronchoconstriction in asthmatic or COPD patients or on their effect on cardiovascular parameters are lacking to date, yet small studies on the response to β_2-agonists predict a significantly reduced response in Ile164 carriers: Both in healthy volunteers (Dishy et al. 2004; Bruck et al. 2005) and patients with congestive heart failure (Barbato et al. 2007) the dilatative effect and the cardiac response, respectively, to β-agonists were less pronounced in Ile164 carriers compared to non-carriers.

5.2 Non-coding Variants Attributed to the ADRB2

The *ADRB2* gene is located on the forward strand of chromosome 5. The genes 5′ and 3′ adjacent to the *ADRB2* locus are >150 kb distant and encode HTR4 (5-hydroxytryptamine receptor) and SH3TC2 (SH3 domain and tetratricopeptide repeat-containing protein 2). Not a single lncRNA is annotated in the intergenic regions 5′ and 3′ of the receptor sequence. Eleven non-coding variants are associated with a trait related to either blood pressure, lung function/disease, or white blood cell composition with only one of these variants located 3′ of the *ADRB1* gene locus (Fig. 4a). The two intergenic variants rs11959615 and rs6580586 are associated with systolic blood pressure (Kulminski et al. 2018; Kichaev et al. 2019), a trait that coincides β_2-adrenoceptor expression in smooth muscles of blood vessels and the vasodilatory effect of ADRB2 activation. In line with ADRB2 function in the lung, the intergenic variant rs35684381 was found associated with chronic obstructive pulmonary disease (Sakornsakolpat et al. 2019), a trait to which the coding variant p.Thr164Ile has also been linked. The majority of GWAS hits associated with the *ADRB2*, however, are related to the composition of white blood cells, consistent with the receptor's expression in immune cells and the established concept of sympathetic control of immune responses by norepinephrine secreted from sympathetic nerves activating immune cell ADRB2 (Udit et al. 2022). This obvious relation suggests that the nine non-coding variants associated with immune cell count alter ADRB2 expression and thereby modulate immune cell composition. This regulation is an

Table 6 β_2-adrenoceptor genetic variants. Each variant is defined with its unique dbSNP number and listed with its minor allelic frequency (MAF, minor allele in brackets) and the reported GWAS association

Location	dbSNP	MAF	Reported trait	References
			Blood pressure	
5' of ADRB2				
Intergenic	rs11959615	0.35 (T)	SBP	Kichaev et al. (2019)
3' of ADRB2				
Intergenic	rs6580586	0.28 (C)	SBP	Kulminski et al. (2018)
			Lung function/disease	
5' of ADRB2				
Intergenic	rs35684381	0.25 (C)	COPD	Sakornsakolpat et al. (2019)
ADRB2 exon	rs1800888 p.Thr164Ile	0.004 (T)	Lung function COPD	Wain et al. (2017a); Kichaev et al. (2019); Shrine et al. (2019); Moll et al. (2021)
			White blood cells	
5' of ADRB2				
Intergenic	rs4705059	0.35 (C)	Leucocyte/ eosinophil count	Astle et al. (2016); Sakaue et al. (2021)
Intergenic	rs10078004	0.35 (G)	Neutrophil % of leucocytes	Vuckovic et al. (2020)
Regulatory	rs56330463	0.35 (T)	White blood cell count and composition (e.g., eosinophils, neutrophils)	Astle et al. (2016); Kichaev et al. (2019); Chen et al. (2020); Vuckovic et al. (2020); Kachuri et al. (2021)
Regulatory	rs2082382	0.15 (G)	White blood cell count (e.g., leucocytes, neutrophils, granulocytes)	Astle et al. (2016); Kichaev et al. (2019); Vuckovic et al. (2020); Kachuri et al. (2021); Sakaue et al. (2021)
Regulatory	rs2082395	0.35 (A)	Monocyte % of leucocytes	Vuckovic et al. (2020)
Intergenic	rs11957351	0.35 (C)	Eosinophil count	Höglund et al. (2022)
Intergenic	rs11960649	0.35 (A)	Neutrophil/ white blood cell count	Chen et al. (2020)
ADRB2 - 5'-UTR	rs1801704	0.21 (C)	Neutrophil-to-lymphocyte ratio	Kachuri et al. (2021)
ADRB2 - exon	rs1800888 p.Thr164Ile	0.004 (T)	Eosinophil % of leukocytes	Vuckovic et al. (2020)
3' of ADRB2				
Intergenic	rs6580586	0.28 (C)	Eosinophil count Eosinophil % of leucocytes	Vuckovic et al. (2020)

Abbreviations: *SBP* systolic blood pressure, *COPD* chronic obstructive pulmonary disease

established mechanism for 5' UTR variants as they induce mutations in transcription factor binding sites. Here, rs1801704 is located within the *ADRB2* 5' UTR and leads to a nucleotide exchange in the binding motives for the transcription factors Myb-like protein 1 (MYBL1, variant position: 2 of 17) and glial cells missing transcription factor 1 (GCM1, variant position: 8 of 28). Altered transcription factor binding and activation might alter ADRB2 expression in a cell-type specific manner and be an explanation for a variant-dependent neutrophil-to-lymphocyte ratio as determined by GWAS (Kachuri et al. 2021).

Furthermore, variants in regulatory regions adjacent to the *ADRB2* gene locus might also modify ADRB2 expression by altering the sequence of a CTCF binding site (rs2082382, rs56330463, and rs2082395) (Table 6). Particularly for rs2082382 and rs56330463 an association with blood cell composition has been independently reported in several cohorts: rs2082382 determines neutrophil, leukocyte, and myeloid white blood cell count and is significantly associated with the traits "sum of neutrophil and eosinophil counts" as well as "sum of basophil and neutrophil counts" (Astle et al. 2016; Kichaev et al. 2019; Vuckovic et al. 2020; Kachuri et al. 2021; Sakaue et al. 2021). Likewise, rs56330463 has been repeatedly associated with eosinophil count and the percentage distribution of different immune cell types (Astle et al. 2016; Kichaev et al. 2019; Chen et al. 2020; Vuckovic et al. 2020; Kachuri et al. 2021). Additionally, four intergenic variants 5′ of the *ADRB2* gene locus and one variant 3′ have reports on association with different white blood cells (Table 6), underlining the importance of the *ADRB2* gene and genomic region concerning interindividual immune responses.

In summary, GWAS hits within the *ADRB2* gene and adjacent to the gene locus are coherent with receptor function on vaso-/bronchodilation and immune responses. Results from GWAS have confirmed the importance of the rare coding polymorphism p.Thr164Ile, while the functional impact of the intensely studied N-terminal coding variants p.Arg16Gly and p.Gln27Glu remains uncertain as they were not associated with any trait in any GWAS published to date.

6 Variants Associated with the β_3-Adrenoceptor

The β_3-adrenoceptor (ADRB3) is expressed in human bladder, parts of the gastrointestinal tract and female genital (for details on expression, see Chapter "Expression Pattern and Species Differences"). The relaxation effect of β_3-agonists is therapeutically exploited for the treatment of overactive bladder syndrome (Michel and Korstanje 2016; Schena and Caplan 2019).

Within the *ADRB3* gene, four non-synonymous variations have been characterized in vitro. In particular the common variation p.Trp64Arg, located at the intracellular end of transmembrane domain 1, was investigated in various candidate-gene studies on cardiac disease, obesity, diabetes, and hyperuricemia. Overall, these studies presume the Arg64-allele as a risk factor specifically for overactive bladder syndrome/hyperuricemia and type 2 diabetes (Ahles and Engelhardt 2014; Michel 2023). However, this conclusion was not validated by

GWAS, which also have been published for the trait "hyperuricemia" and to a great extent for "diabetes": The ADRB3 was not associated with any trait in a single GWAS, i.e., none of the *ADRB3* coding variants or attributed non-coding variants has been reported as a hit in a large cohort.

7 Conclusions

GWAS test thousands of genetic variants across many individual human genomes (and ethnic groups) to identify those statistically associated with a specific trait or disease (Duncan and Brown 2018). Due to the effective suppression of bias and the inherent statistical power, this approach has largely replaced earlier candidate-gene studies. As a result of this paradigmatic change and the rapidly increasing number and scope of GWAS, also much of the earlier literature on adrenoceptor variants has to be scrutinized (Ahles and Engelhardt 2014) leaving us with a sobering number of two trustworthy adrenoceptor coding variants. These two variants are p.Arg389Gly in the β_1-adrenoceptor and the rare p.Thr164Ile variation in the β_2-adrenoceptor.

The genome-wide approach has however added another layer of complexity through the identification of numerous non-coding genetic variants, many of them within regulatory regions of adrenoceptor genes. While the mechanistic basis of the effects assigned to the coding variants appears largely resolved, we are only at the very beginning to understand how non-coding genetic variation determines traits and disease risk. Regarding the adrenoceptor-associated non-coding variants, a first layer of investigation will need to define whether their effect is indeed related to the regulation of expression of the adjacent receptor gene. If so, multiple candidate mechanisms come into play, ranging from altered binding of the transcriptional machinery to the evolving world of non-coding RNAs, many of which are also transcribed from adrenoceptor loci and which we have reported in this chapter.

References

Abecasis GR, Auton A, Brooks LD et al (2012) An integrated map of genetic variation from 1,092 human genomes. Nature 491:56–65. https://doi.org/10.1038/nature11632

Ahles A, Engelhardt S (2014) Polymorphic variants of adrenoceptors: pharmacology, physiology, and role in disease. Pharmacol Rev 66:598–637. https://doi.org/10.1124/pr.113.008219

Ahles A, Rodewald F, Rochais F et al (2015) Interhelical interaction and receptor phosphorylation regulate activation kinetics of different human beta1-adrenoceptor variants. J Biol Chem 290: 1760–1769. https://doi.org/10.1074/jbc.M114.607333

Akinaga J, García-Sáinz JA, Pupo AS (2019) Updates in the function and regulation of α1-adrenoceptors. Br J Pharmacol 176:2343–2357. https://doi.org/10.1111/bph.14617

Aleong RG, Sauer WH, Davis G et al (2013) Prevention of atrial fibrillation by Bucindolol is dependent on the Beta1389 Arg/Gly adrenergic receptor polymorphism. JACC Heart Fail 1: 338–344. https://doi.org/10.1016/j.jchf.2013.04.002

Archala A, Plazinski W, Plazinska A (2022) The Val34Met, Thr164Ile and Ser220Cys polymorphisms of the β2-adrenergic receptor and their consequences on the receptor conformational features: a molecular dynamics simulation study. Int J Mol Sci 23. https://doi.org/10.3390/ijms23105449

Armstrong NJ, Mather KA, Sargurupremraj M et al (2020) Common genetic variation indicates separate causes for periventricular and deep white matter hyperintensities. Stroke 51:2112–2121. https://doi.org/10.1161/STROKEAHA.119.027544

Astle WJ, Elding H, Jiang T et al (2016) The allelic landscape of human blood cell trait variation and links to common complex disease. Cell 167:1415–1429.e19. https://doi.org/10.1016/j.cell.2016.10.042

Barbato E, Penicka M, Delrue L et al (2007) Thr164Ile polymorphism of beta2-adrenergic receptor negatively modulates cardiac contractility: implications for prognosis in patients with idiopathic dilated cardiomyopathy. Heart 93:856–861. https://doi.org/10.1136/hrt.2006.091959

Barnes PJ (2019) Inflammatory endotypes in COPD. Allergy Eur J Allergy Clin Immunol 74:1249–1256. https://doi.org/10.1111/all.13760

Boeckel JN, Perret MF, Glaser SF et al (2019) Identification and regulation of the long non-coding RNA Heat2 in heart failure. J Mol Cell Cardiol 126:13–22. https://doi.org/10.1016/j.yjmcc.2018.11.004

Bruck H, Leineweber K, Park J et al (2005) Human beta2-adrenergic receptor gene haplotypes and venodilation in vivo. Clin Pharmacol Ther 78:232–238. https://doi.org/10.1016/j.clpt.2005.06.002

Buniello A, Macarthur JAL, Cerezo M et al (2019) The NHGRI-EBI GWAS catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. Nucleic Acids Res 47:D1005–D1012. https://doi.org/10.1093/nar/gky1120

Büscher R, Eilmes KJ, Grasemann H et al (2002) beta2 adrenoceptor gene polymorphisms in cystic fibrosis lung disease. Pharmacogenetics 12:347–353

Chen MH, Yanek LR, Backman JD et al (2019) Exome-chip meta-analysis identifies association between variation in ANKRD26 and platelet aggregation. Platelets 30:164–173. https://doi.org/10.1080/09537104.2017.1384538

Chen MH, Raffield LM, Mousas A, et al (2020) Trans-ethnic and ancestry-specific blood-cell genetics in 746,667 individuals from 5 global populations

Chen VL, Du X, Chen Y et al (2021) Genome-wide association study of serum liver enzymes implicates diverse metabolic and liver pathology. Nat Commun 12:1–13. https://doi.org/10.1038/s41467-020-20870-1

Dastani Z, Hivert MF, Timpson NJ et al (2012) Novel loci for adiponectin levels and their influence on type 2 diabetes and metabolic traits: a multi-ethnic meta-analysis of 45,891 individuals. PLoS Genet 8. https://doi.org/10.1371/journal.pgen.1002607

Dishy V, Landau R, Sofowora GG et al (2004) Beta2-adrenoceptor Thr164Ile polymorphism is associated with markedly decreased vasodilator and increased vasoconstrictor sensitivity in vivo. Pharmacogenetics 14:517–522

Dorn GW (2010) Adrenergic signaling polymorphisms and their impact on cardiovascular disease. Physiol Rev 90:1013–1062. https://doi.org/10.1152/physrev.00001.2010

Duncan EL, Brown MA (2018) Genome-wide association studies. Genet Bone Biol Skelet Dis Second Ed:33–41. https://doi.org/10.1016/B978-0-12-804182-6.00003-4

Ehret GB, Ferreira T, Chasman DI et al (2016) The genetics of blood pressure regulation and its target organs from association studies in 342,415 individuals. Nat Genet 48:1171–1184. https://doi.org/10.1038/ng.3667

Fagerholm V, Haaparanta M, Scheinin M (2011) A 2-adrenoceptor regulation of blood glucose homeostasis. Basic Clin Pharmacol Toxicol 108:365–370. https://doi.org/10.1111/j.1742-7843.2011.00699.x

Feitosa MF, Kraja AT, Chasman DI et al (2018) Novel genetic associations for blood pressure identified via gene-alcohol interaction in up to 570K individuals across multiple ancestries

Giovannitti JA, Thoms SM, Crawford JJ (2015) Alpha-2 adrenergic receptor agonists: a review of current clinical applications. Anesth Prog 62:31–38. https://doi.org/10.2344/0003-3006-62.1.31

Giri A, Hellwege JN, Keaton JM et al (2019) Trans-ethnic association study of blood pressure determinants in over 750,000 individuals. Nat Genet 51:51–62. https://doi.org/10.1038/s41588-018-0303-9

Gjesing AP, Andersen G, Albrechtsen A et al (2007) Studies of associations between the Arg389Gly polymorphism of the beta1-adrenergic receptor gene (ADRB1) and hypertension and obesity in 7677 Danish white subjects. Diabet Med 24:392–397. https://doi.org/10.1111/j.1464-5491.2006.02031.x

Green SA, Cole G, Jacinto M et al (1993) A polymorphism of the human beta 2-adrenergic receptor within the fourth transmembrane domain alters ligand binding and functional properties of the receptor. J Biol Chem 268:23116–23121

Hoffmann TJ, Ehret GB, Nandakumar P et al (2017) Genome-wide association analyses using electronic health records identify new loci influencing blood pressure variation. Nat Genet 49:54–64. https://doi.org/10.1038/ng.3715

Hoffmann TJ, Theusch E, Haldar T et al (2018) A large electronic-health-record-based genome-wide study of serum lipids. Nat Genet 50:401–413. https://doi.org/10.1038/s41588-018-0064-5

Hoffstedt J, Iliadou A, Pedersen NL et al (2001) The effect of the beta(2) adrenoceptor gene Thr164Ile polymorphism on human adipose tissue lipolytic function. Br J Pharmacol 133:708–712. https://doi.org/10.1038/sj.bjp.0704125

Höglund J, Hadizadeh F, Ek WE et al (2022) Gene-based variant analysis of whole-exome sequencing in relation to eosinophil count. Front Immunol 13:1–13. https://doi.org/10.3389/fimmu.2022.862255

Horikoshi M, Yaghootkar H, Mook-Kanamori DO et al (2013) New loci associated with birth weight identify genetic links between intrauterine growth and adult height and metabolism. Nat Genet 45:76–82. https://doi.org/10.1038/ng.2477

Horikoshi M, Beaumont RN, Day FR et al (2016) Genome-wide associations for birth weight and correlations with adult disease. Nature 538:248–252. https://doi.org/10.1038/nature19806

Huang LO, Rauch A, Mazzaferro E et al (2021) Genome-wide discovery of genetic loci that uncouple excess adiposity from its comorbidities. Nat Metab 3:228–243. https://doi.org/10.1038/s42255-021-00346-2

Johnson AD, Yanek LR, Chen MH et al (2010) Genome-wide meta-analyses identifies seven loci associated with platelet aggregation in response to agonists. Nat Genet 42:608–613. https://doi.org/10.1038/ng.604

Johnson AD, Newton-Cheh C, Chasman DI et al (2011) Association of hypertension drug target genes with blood pressure and hypertension in 86,588 individuals. Hypertension 57:903–910. https://doi.org/10.1161/HYPERTENSIONAHA.110.158667

Joseph SS, Lynham JA, Grace AA et al (2004) Markedly reduced effects of (−)-isoprenaline but not of (−)-CGP12177 and unchanged affinity of beta-blockers at Gly389-beta1-adrenoceptors compared to Arg389-beta1-adrenoceptors. Br J Pharmacol 142:51–56. https://doi.org/10.1038/sj.bjp.0705753

Kachuri L, Jeon S, DeWan AT et al (2021) Genetic determinants of blood-cell traits influence susceptibility to childhood acute lymphoblastic leukemia. Am J Hum Genet 108:1823–1835. https://doi.org/10.1016/j.ajhg.2021.08.004

Kay LJ, Chong LK, Rostami-Hodjegan A, Peachell PT (2003) Influence of the thr164ile polymorphism in the beta2-adrenoceptor on the effects of beta-adrenoceptor agonists on human lung mast cells. Int Immunopharmacol 3:91–95

Kay LJ, Rostami-Hodjegan A, Suvarna SK, Peachell PT (2007) Influence of beta2-adrenoceptor gene polymorphisms on beta2-adrenoceptor-mediated responses in human lung mast cells. Br J Pharmacol 152:323–331. https://doi.org/10.1038/sj.bjp.0707400

Kichaev G, Bhatia G, Loh PR et al (2019) Leveraging polygenic functional enrichment to improve GWAS power. Am J Hum Genet 104:65–75. https://doi.org/10.1016/j.ajhg.2018.11.008

Kim SK (2018) Identification of 613 new loci associated with heel bone mineral density and a polygenic risk score for bone mineral density, osteoporosis and fracture. PloS One 13:1–20. https://doi.org/10.1371/journal.pone.0200785

Klarin D, Damrauer SM, Cho K et al (2018) Genetics of blood lipids among ~300,000 multi-ethnic participants of the million veteran program. Nat Genet 50:1514–1523. https://doi.org/10.1038/s41588-018-0222-9

Klimentidis YC, Arora A, Newell M et al (2020) Phenotypic and genetic characterization of lower LDL cholesterol and increased type 2 diabetes risk in the UK biobank. Diabetes 69:2194–2205. https://doi.org/10.2337/db19-1134

Kulminski AM, Huang J, Loika Y et al (2018) Strong impact of natural-selection-free heterogeneity in genetics of age-related phenotypes. Aging (Albany NY) 10:492–514. https://doi.org/10.18632/aging.101407

Lettre G (2022) One step closer to linking GWAS SNPs with the right genes. Nat Genet 11–12. https://doi.org/10.1038/s41588-022-01093-0

Liggett SB, Mialet-Perez J, Thaneemit-Chen S et al (2006) A polymorphism within a conserved beta(1)-adrenergic receptor motif alters cardiac function and beta-blocker response in human heart failure. Proc Natl Acad Sci U S A 103:11288–11293. https://doi.org/10.1073/pnas.0509937103

Liu C, Kraja AT, Smith JA et al (2016) Meta-analysis identifies common and rare variants influencing blood pressure and overlapping with metabolic trait loci. Nat Genet 48:1162–1170. https://doi.org/10.1038/ng.3660

Liu DJ, Peloso GM, Yu H et al (2017) Exome-wide association study of plasma lipids in >300,000 individuals. Nat Genet 49:1758–1766. https://doi.org/10.1038/ng.3977

Mason DA, Moore JD, Green SA, Liggett SB (1999) A gain-of-function polymorphism in a G-protein coupling domain of the human beta1-adrenergic receptor. J Biol Chem 274:12670–12674

Maurano MT, Humbert R, Rynes E et al (2012) Systematic localization of common disease-associated variation in regulatory DNA. Science (80-) 337:1190–1195. https://doi.org/10.1126/science.1222794

McCrink KA, Brill A, Jafferjee M et al (2016) β1-adrenoceptor Arg389Gly polymorphism confers differential β-arrestin-binding tropism in cardiac myocytes. Pharmacogenomics 17:1611–1620. https://doi.org/10.2217/pgs-2016-0094

Mialet Perez J, Rathz DA, Petrashevskaya NN et al (2003) Beta 1-adrenergic receptor polymorphisms confer differential function and predisposition to heart failure. Nat Med 9:1300–1305. https://doi.org/10.1038/nm930

Michel MC (2023) Are β3-adrenoceptor gene polymorphisms relevant for urology? NeurourolUrodyn 42:33–39. https://doi.org/10.1002/nau.25082

Michel MC, Korstanje C (2016) β3-adrenoceptor agonists for overactive bladder syndrome: role of translational pharmacology in a repositioning clinical drug development project. Pharmacol Ther 159:66–82. https://doi.org/10.1016/j.pharmthera.2016.01.007

Moll M, Jackson VE, Yu B et al (2021) A systematic analysis of protein-altering exonic variants in chronic obstructive pulmonary disease. Am J Physiol Lung Cell Mol Physiol 321:L130–L143. https://doi.org/10.1152/AJPLUNG.00009.2021

Morales J, Pujar S, Loveland JE et al (2022) A joint NCBI and EMBL-EBI transcript set for clinical genomics and research. Nature 604:1–6. https://doi.org/10.1038/s41586-022-04558-8

Muthumala A, Drenos F, Elliott PM, Humphries SE (2008) Role of beta adrenergic receptor polymorphisms in heart failure: systematic review and meta-analysis. Eur J Heart Fail 10:3–13. https://doi.org/10.1016/j.ejheart.2007.11.008

Ntalla I, Weng LC, Cartwright JH et al (2020) Multi-ancestry GWAS of the electrocardiographic PR interval identifies 202 loci underlying cardiac conduction. Nat Commun 11:1–12. https://doi.org/10.1038/s41467-020-15706-x

Nurk S, Koren S, Rhie A et al (2022) The complete sequence of a human genome. Science 376:44–53. https://doi.org/10.1126/science.abj6987

O'Connell TD, Jensen BC, Baker AJ, Simpson PC (2014) Cardiac alpha1-adrenergic receptors: novel aspects of expression, signaling mechanisms, physiologic function, and clinical importance. Pharmacol Rev 66:308–333. https://doi.org/10.1124/pr.112.007203

O'Connor CM, Fiuzat M, Carson PE et al (2012) Combinatorial pharmacogenetic interactions of bucindolol and β1, α2C adrenergic receptor polymorphisms. PloS One 7:e44324. https://doi.org/10.1371/journal.pone.0044324

Oh ES, Petronis A (2021) Origins of human disease: the chrono-epigenetic perspective. Nat Rev Genet 22:533–546. https://doi.org/10.1038/s41576-021-00348-6

Pacanowski MA, Gong Y, Cooper-Dehoff RM et al (2008) Beta-adrenergic receptor gene polymorphisms and beta-blocker treatment outcomes in hypertension. Clin Pharmacol Ther 84:715–721. https://doi.org/10.1038/clpt.2008.139

Perez DM (2020) α1-Adrenergic receptors in neurotransmission, synaptic plasticity, and cognition. Front Pharmacol 11:1–22. https://doi.org/10.3389/fphar.2020.581098

Plotnikov D, Williams C, Guggenheim JA (2020) Association between birth weight and refractive error in adulthood: a Mendelian randomisation study. Br J Ophthalmol 104:214–219. https://doi.org/10.1136/bjophthalmol-2018-313640

Plotnikov D, Huang Y, Khawaja AP et al (2022) High blood pressure and intraocular pressure: a Mendelian randomization study. Invest Ophthalmol Vis Sci 63. https://doi.org/10.1167/iovs.63.6.29

Qi G, Chatterjee N (2018) Heritability informed power optimization (HIPO) leads to enhanced detection of genetic associations across multiple traits. PLoS Genet 14:1–21. https://doi.org/10.1371/journal.pgen.1007549

Richardson TG, Sanderson E, Palmerid TM et al (2020) Evaluating the relationship between circulating lipoprotein lipids and apolipoproteins with risk of coronary heart disease: a multivariable Mendelian randomisation analysis. PLoS Med 17:1–22. https://doi.org/10.1371/JOURNAL.PMED.1003062

Richardson TG, Leyden GM, Wang Q et al (2022) Characterising metabolomic signatures of lipid-modifying therapies through drug target mendelian randomisation. PLoS Biol 20:1–17. https://doi.org/10.1371/journal.pbio.3001547

Riis-Vestergaard MJ, Richelsen B, Bruun JM et al (2020) Beta-1 and not Beta-3 adrenergic receptors may be the primary regulator of human brown adipocyte metabolism. J Clin Endocrinol Metab 105:E994–E1005. https://doi.org/10.1210/clinem/dgz298

Ripatti P, Rämö JT, Mars NJ et al (2020) Polygenic hyperlipidemias and coronary artery disease risk. Circ Genomic Precis Med:59–65. https://doi.org/10.1161/CIRCGEN.119.002725

Rochais F, Vilardaga J, Nikolaev VO et al (2007) Real-time optical recording of beta1-adrenergic receptor activation reveals supersensitivity of the Arg389 variant to carvedilol. J Clin Invest 117:229–235. https://doi.org/10.1172/JCI30012

Rojano E, Seoane P, Ranea JAG, Perkins JR (2019) Regulatory variants: from detection to predicting impact. Brief Bioinform 20:1639–1654. https://doi.org/10.1093/bib/bby039

Sakaue S, Kanai M, Tanigawa Y et al (2021) A cross-population atlas of genetic associations for 220 human phenotypes. Nat Genet 53:1415–1424. https://doi.org/10.1038/s41588-021-00931-x

Sakornsakolpat P, Prokopenko D, Lamontagne M et al (2019) Genetic landscape of chronic obstructive pulmonary disease identifies heterogeneous cell-type and phenotype associations. Nat Genet 51:494–505. https://doi.org/10.1038/s41588-018-0342-2

Schena G, Caplan MJ (2019) Everything you always wanted to know about β3-AR * (* but were afraid to ask). Cells 8:357. https://doi.org/10.3390/cells8040357

Shrine N, Guyatt AL, Erzurumluoglu AM et al (2019) New genetic signals for lung function highlight pathways and chronic obstructive pulmonary disease associations across multiple ancestries. Nat Genet 51:481–493. https://doi.org/10.1038/s41588-018-0321-7

Sinnott-Armstrong N, Tanigawa Y, Amar D et al (2021) Genetics of 35 blood and urine biomarkers in the UK Biobank. Nat Genet 53:185–194. https://doi.org/10.1038/s41588-020-00757-z

Statello L, Guo CJ, Chen LL, Huarte M (2021) Gene regulation by long non-coding RNAs and its biological functions. Nat Rev Mol Cell Biol 22:96–118. https://doi.org/10.1038/s41580-020-00315-9

Sung YJ, Winkler TW, de las Fuentes L et al (2018) A large-scale multi-ancestry genome-wide study accounting for smoking behavior identifies multiple significant loci for blood pressure. Am J Hum Genet 102:375–400. https://doi.org/10.1016/j.ajhg.2018.01.015

Surendran P, Drenos F, Young R et al (2016) Trans-ancestry meta-analyses identify rare and common variants associated with blood pressure and hypertension. Nat Genet 48:1151–1161. https://doi.org/10.1038/ng.3654

Takeuchi F, Akiyama M, Matoba N et al (2018) Interethnic analyses of blood pressure loci in populations of east Asian and European descent. Nat Commun 9. https://doi.org/10.1038/s41467-018-07345-0

Tam V, Patel N, Turcotte M et al (2019) Benefits and limitations of genome-wide association studies. Nat Rev Genet 20:467–484. https://doi.org/10.1038/s41576-019-0127-1

Tian C, Hromatka BS, Kiefer AK et al (2017) Genome-wide association and HLA region fine-mapping studies identify susceptibility loci for multiple common infections. Nat Commun 8. https://doi.org/10.1038/s41467-017-00257-5

Tikhonoff V, Hasenkamp S, Kuznetsova T et al (2008) Blood pressure and metabolic phenotypes in relation to the ADRB1 Arg389Gly and ADRA2B I/D polymorphisms in a White population. J Hum Hypertens 22:864–867. https://doi.org/10.1038/jhh.2008.73

Trubetskoy V, Pardiñas AF, Qi T et al (2022) Mapping genomic loci implicates genes and synaptic biology in schizophrenia. Nature 604:502–508. https://doi.org/10.1038/s41586-022-04434-5

Tubi MA, Feingold FW, Kothapalli D et al (2020) White matter hyperintensities and their relationship to cognition: effects of segmentation algorithm. Neuroimage 206:116327. https://doi.org/10.1016/j.neuroimage.2019.116327

Turki J, Lorenz JN, Green SA et al (1996) Myocardial signaling defects and impaired cardiac function of a human beta 2-adrenergic receptor polymorphism expressed in transgenic mice. Proc Natl Acad Sci U S A 93:10483–10488

Udit S, Blake K, Chiu IM (2022) Somatosensory and autonomic neuronal regulation of the immune response. Nat Rev Neurosci 23:157–171. https://doi.org/10.1038/s41583-021-00555-4

Verweij N, Benjamins JW, Morley MP et al (2020) The genetic makeup of the electrocardiogram. Cell Syst 11:229–238.e5. https://doi.org/10.1016/j.cels.2020.08.005

Vohra M, Sharma AR, Prabhu BN, Rai PS (2021) SNPs in sites for DNA methylation, transcription factor binding, and miRNA targets leading to allele-specific gene expression and contributing to complex disease risk: a systematic review. Public Health Genomics 23:155–170. https://doi.org/10.1159/000510253

Vuckovic D, Bao EL, Akbari P et al (2020) The polygenic and monogenic basis of blood traits and diseases. Cell 182:1214–1231.e11. https://doi.org/10.1016/j.cell.2020.08.008

Wain LV, Verwoert GC, O'reilly PF et al (2011) Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure. Nat Genet 43:1005–1012. https://doi.org/10.1038/ng.922

Wain LV, Shrine N, Artigas MS et al (2017a) Genome-wide association analyses for lung function and chronic obstructive pulmonary disease identify new loci and potential druggable targets. Nat Genet 49:416–425. https://doi.org/10.1038/ng.3787

Wain LV, Vaez A, Jansen R et al (2017b) Novel blood pressure locus and gene discovery using genome-wide association study and expression data sets from blood and the kidney. Hypertension 70:e4–e19. https://doi.org/10.1161/HYPERTENSIONAHA.117.09438

Wang H, Yang J, Schneider JA et al (2020) Genome-wide interaction analysis of pathological hallmarks in Alzheimer's disease. Neurobiol Aging 93:61–68. https://doi.org/10.1016/j.neurobiolaging.2020.04.025

Warne T, Serrano-Vega MJ, Baker JG et al (2008) Structure of a beta1-adrenergic G-protein-coupled receptor. Nature 454:486–491. https://doi.org/10.1038/nature07101

Warne T, Moukhametzianov R, Baker JG et al (2011) The structural basis for agonist and partial agonist action on a β(1)-adrenergic receptor. Nature 469:241–244. https://doi.org/10.1038/nature09746

Warne T, Edwards PC, Leslie AGW, Tate CG (2012) Crystal structures of a stabilized β1-adrenoceptor bound to the biased agonists bucindolol and carvedilol. Structure 20:841–849. https://doi.org/10.1016/j.str.2012.03.014

Warren HR, Evangelou E, Cabrera CP et al (2017) Genome-wide association analysis identifies novel blood pressure loci and offers biological insights into cardiovascular risk. Nat Genet 49: 403–415. https://doi.org/10.1038/ng.3768

Warrington NM, Beaumont RN, Horikoshi M et al (2019) Maternal and fetal genetic effects on birth weight and their relevance to cardio-metabolic risk factors. Nat Genet 51:804–814. https://doi.org/10.1038/s41588-019-0403-1

Williamson R, Van Aalten L, Mann DMA et al (2011) CRMP2 hyperphosphorylation is characteristic of Alzheimer's disease and not a feature common to other neurodegenerative diseases. J Alzheimers Dis 27:615–625. https://doi.org/10.3233/JAD-2011-110617

Wu Y, Byrne EM, Zheng Z et al (2019) Genome-wide association study of medication-use and associated disease in the UK Biobank. Nat Commun 10:1–10. https://doi.org/10.1038/s41467-019-09572-5

Yang XL, Zhang SY, Zhang H et al (2019) Three novel loci for infant head circumference identified by a joint association analysis. Front Genet 10. https://doi.org/10.3389/fgene.2019.00947

Young WJ, Warren HR, Mook-Kanamori DO et al (2021) Genetically determined serum calcium levels and markers of ventricular repolarization: a Mendelian randomization study in the UK Biobank. Circ Genomic Precis Med 14:E003231. https://doi.org/10.1161/CIRCGEN.120.003231

Zhong VW, Kuang A, Danning RD et al (2019) A genome-wide association study of bitter and sweet beverage consumption. Hum Mol Genet 28:2449–2457. https://doi.org/10.1093/hmg/ddz061

Adrenoceptors: Receptors, Ligands and Their Clinical Uses, Molecular Pharmacology and Assays

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Abstract

The nine G protein-coupled adrenoceptor subtypes are where the endogenous catecholamines adrenaline and noradrenaline interact with cells. Since they are important therapeutic targets, over a century of effort has been put into developing drugs that modify their activity. This chapter provides an outline of how we have arrived at current knowledge of the receptors, their physiological roles and the methods used to develop ligands. Initial studies in vivo and in vitro with isolated organs and tissues progressed to cell-based techniques and the use of cloned adrenoceptor subtypes together with high-throughput assays that allow close examination of receptors and their signalling pathways. The crystal structures of many of the adrenoceptor subtypes have now been determined opening up new possibilities for drug development.

Keywords

α-Adrenoceptor · β-adrenoceptor · Adrenoceptor ligands · Affinity · Agonist · Antagonist · Efficacy · Pharmacology · Pharmacological assays

Abbreviations

ADHD	Attention deficit/hyperactivity disorder
AF	Atrial Fibrillation
AR	Adrenoceptor
Bmax	Maximum number of binding sites
BPH	Benign prostatic hyperplasia
cAMP	Cyclic adenosine monophosphate
COPD	Chronic obstructive pulmonary disease
CRE	cAMP response element
CREB	cAMP response element binding protein
Cryo-EM	Cryo-electron microscopy
DAG	Diacylglycerol
DARPin	Designed ankyrin repeat protein
EC_{50}	Concentration required to stimulate a half maximum response in that system
ECAR	Extracellular acidification rate
EGFR	Epidermal growth factor receptor
EL	Extracellular loop
Epac	Exchange protein directly regulated by cAMP
FA	Full agonist
GDP	Guanosine diphosphate
GPCR	G protein-coupled receptor
GRK	G protein receptor kinase
GTP	Guanosine trisphosphate
IA	Inverse agonist
IC	Intracellular loop
IP_3	Inositol trisphosphate
ISA	Intrinsic sympathomimetic activity
ISH	In situ hybridisation
JGA	Juxta glomerular apparatus
Jnk	Jun N-terminal kinase
K_b	Dissociation constant for an antagonist as calculated from parallel shift of an agonist ligand concentration response curve in the absence and presence of a known concentration of the antagonist (e.g. Gaddum equation)
K_d	Dissociation constant = concentration required to bind half of the receptors
K_i	Dissociation constant as calculated from inhibition of another ligand e.g. in a radioligand binding assay (e.g. Cheng-Prusoff equation)

Mab	Monoclonal antibody
MAPK	Mitogen-activated protein kinase
mTORC2	Mammalian target of rapamycin complex 2
NA	Neutral antagonist
NAM	Negative allosteric modulator
nLuc	Nano luciferase
PA	Partial agonist
PAM	Positive allosteric modulator
PIP2	Phosphatidylinositol 4,5-bisphosphate
PK	Pharmacokinetic
PKA	Protein kinase A
PTSD	Post-traumatic stress disorder
SPA	Scintillation proximity assay
TM	Transmembrane domain

1 General Introduction

Adrenoceptors (AR) comprise a group of nine G protein-coupled receptors that are the targets of the endogenous catecholamines adrenaline and noradrenaline. They are divided into 3 subgroups, α_1, α_2 and β that have 3 subtypes in each, namely α_{1A}, α_{1B}, α_{1D}; α_{2A}, α_{2B}, α_{2C}; and β_1, β_2 and β_3 (Altosaar et al. 2019). The canonical signalling pathway utilised by α_1-AR is $G_{q/11}$ coupling to phospholipase C to cause hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to inositol trisphosphate (IP_3) and diacylglycerol (DAG) with a consequent increase in intracellular Ca^{2+} and activation of protein kinase C (PKC). The α_2-AR subgroup are G_i-coupled to inhibit adenylyl cyclase and reduce the production of cAMP, whereas β-AR are G_s-coupled to stimulate adenylyl cyclase and increase cAMP production (Altosaar et al. 2019). However, many of the AR subtypes can couple to multiple G proteins and may have G protein independent actions and display complex signalling profiles although the physiological and clinical roles of some of these are currently uncertain (Littmann et al. 2015; da Silva Junior et al. 2017; Woo et al. 2019; Proudman and Baker 2021; De Pascali et al. 2022; Proudman et al. 2022a). The receptors are expressed in a very wide variety of cell types, and the pattern of expression determines the type of response observed following stimulation of the sympathetic nervous system. The targeting of AR has produced antagonist and agonist drugs useful for the treatment of many diseases which are currently used for coronary artery disease, hypertension, cardiac arrythmias, heart failure, portal hypertension, hyperthyroidism, migraine, glaucoma, anxiety, benign prostatic hyperplasia (BPH), overactive bladder, post-traumatic stress disorder (PTSD), asthma and chronic obstructive pulmonary disease (COPD), hypotension and shock, anaphylaxis, sedation, drug (e.g. opiate, alcohol, benzodiazepine) withdrawal, attention deficit hyperactivity disorder (ADHD), delirium, nasal decongestion, rosacea and muscle spasm and may have roles in

depression. The drugs currently in use are generally agonists, antagonists or inverse agonists, but there is great interest in the development of biased agonists and allosteric modulators that potentially promise even more selective actions associated with minimalisation of side effects. Most of the data presented in this chapter relates to human receptors (unless otherwise stated) and whilst there are examples highlighted, this is not an exhaustive review of all the published literature.

2 History of Drugs Acting at Adrenoceptors

By the turn of the twentieth century, the pharmacological properties of catecholamines were being described. Oliver, experimenting with organ extracts, discovered that ingestion of sheep adrenal gland caused constriction of the radial artery (Oliver and Schäfer 1894) and subsequently in animal experiments showed that adrenal medullary extract caused vasoconstriction and increased blood pressure and heart rate (Oliver and Schafer 1895). There then began a series of attempts to isolate the active compound culminating in the isolation of crystalline adrenaline by Takamine in 1901 (Yamashima 2003). The history of the development of drugs acting selectively at ARs really began with the work of Sir Henry Dale (Dale 1906). He recognised that stimulation of the sympathetic nervous system could produce a variety of effects including vasoconstriction in some regions and vasodilation in others, contraction or relaxation of smooth muscle, positive inotropic and chronotropic effects in the heart and metabolic changes. Dale showed that the actions of adrenaline were altered by preexposure to ergotoxine, and he interpreted that the change from a vasoconstrictor to a vasodilator response indicated a mixed response under normal conditions and that ergotoxine caused a selective paralysis of myoneural junctions responsible for the vasoconstrictor response. Later Cannon and Rosenblueth (1937) suggested an alternative explanation, namely that nerve terminals released two transmitters sympathin E (excitatory) and sympathin I (inhibitory) an idea that gained support in the 1930s/40s.

However, a landmark publication from Raymond Ahlquist in 1948 (Ahlquist 1948) adopted an approach based on the rank order of potency of a series of natural and synthetic agonists leading to the suggestion that the actions of the sympathetic neurotransmitter adrenaline (later corrected to noradrenaline) were mediated by two groups of receptors – α and β. He postulated that these receptor groups could not be classified purely on the basis of excitatory or inhibitory actions and used the rank order of potency of a series of 6 sympathomimetic amines in a number of assay systems – vasoconstriction, contraction of the uterus and ureters, contraction of the nictitating membrane of the eye, dilatation of the pupil, inhibition of the gut and stimulation of the heart – to define his receptors. He concluded that *most* of the excitatory functions – vasoconstriction, uterine contraction, nictitating membrane contraction, and dilator pupillae were mediated by α receptors – whereas *most* of the inhibitory functions – vasodilation, relaxation of uterine and bronchial smooth muscle – and *one* excitatory function – cardiac stimulation – were mediated by β receptors. This classification also provided an explanation for the actions of

ergotoxine and β-haloalkylamine antagonists that blocked α- but not β-AR-mediated responses (Bylund et al. 1994). More evidence was provided with the publication in 1957 of the properties of the first selective β-AR antagonist (partial agonist) dichloroisoprenaline (Powell and Slater 1958) which lowered heart rate. Since increased sympathetic drive caused by exercise, stress or emotion can in individuals with atherosclerosis result in angina due to myocardial ischaemia, Sir James Black and his colleagues at ICI Pharmaceuticals surmised that blockade of the effects of catecholamines would prevent angina by reducing the cardiac workload. This led to the development of pronethalol (later withdrawn due to CNS toxicity and potential carcinogenic effects) and propranolol, both effective β-blockers with little sympathomimetic activity (Black et al. 1964, 1965). Propranolol remains an important β-blocker in clinical use today.

Refinement of the approach pioneered by Ahlquist led Lands et al. to conclude that there were two types of β-AR, β_1 – that predominated in heart, small intestine and adipose tissues and β_2 – that produced relaxation in uterine, vascular and bronchial smooth muscle (Lands et al. 1967a, b). As early as 1900 it had been recognised that adrenal extracts and later adrenaline were useful for the treatment of asthma. However, adrenaline was short acting being metabolically unstable and broken down by catechol-O-methyltransferase and also caused tachycardia, high blood pressure and muscle tremor. Even before the subclassification of adrenoceptors, isoprenaline was discovered and found to have many of the beneficial effects of adrenaline without increasing blood pressure. Although adrenaline and isoprenaline (and later drugs such as orciprenaline) were effective bronchodilators, their use was associated with an excessive death rate in asthmatics possibly due to cardiac arrythmias and/or tachyphylaxis and desensitisation, properties that were associated with the full agonist properties of these drugs (see chapter on "Asthma and COPD" in this volume). The introduction of salbutamol (Brittain et al. 1968; Cullum et al. 1969) and terbutaline (Wetterlin and Svensson 1968; Bergman et al. 1969; Persson and Olsson 1970) in the late 1960s solved many of the problems associated with these earlier bronchodilators. Both compounds are partial agonists with some selectivity for β_2-ARs, and both are metabolically stable. This ensured that they were less likely to cause desensitisation and cardiac arrythmias and to have a longer duration of action.

Subclassification of the α-AR also followed: it was recognised that prejunctional (pre-synaptic) and post-junctional α-ARs had different pharmacological characteristics, and it was suggested that they be subdivided into α_2 and α_1-ARs, respectively (Langer 1974; Starke et al. 1974). However, this anatomically based subdivision of α-ARs was soon superseded by a pharmacologically based subdivision that recognised that there were situations where receptors with the properties of α_2-AR were located post-junctionally (Berthelsen and Pettinger 1977). The subsequent development of more sophisticated pharmacological tools such as receptor binding techniques soon led to the further subdivision of both α_1- (Morrow and Creese 1986; Han et al. 1987; Johnson and Minneman 1987) and α_2-ARs (Bylund 1985, 1988; Michel et al. 1989a). In addition, the identification of β-AR mediated responses resistant to blockade by propranolol (which blocked both β_1 and β_2-AR)

suggested that there could be at least a third β-AR subtype (Kaumann and Blinks 1980; Arch et al. 1984; Bond and Clarke 1988). In recent years, the advent of molecular biology techniques has facilitated measurement of the characteristics of particular receptor subtypes when transfected into suitable mammalian cells. The subclassification of receptors, whether based on tissue or cell responses, radioligand binding, and molecular biology techniques, is now largely in agreement that there are α_1, α_2 and β-ARs with each subgroup having 3 subtypes – α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2 and β_3.

3 Receptor Subclassification

Two major technical advances in the 1980–1990s provided the tools that facilitated the subclassification of the adrenoceptor subtypes – radioligand binding and molecular biology. Radioligand binding facilitated the detailed characterisation of compounds and receptor populations in tissues (U'Prichard et al. 1977; Jarrott et al. 1979), whereas the cloning of the β_2-AR (Dixon et al. 1986) opened the door to an approach which led to the identification of receptor subtypes in tissues by a variety of techniques and the accurate determination of agonist and antagonist selectivity, affinity and efficacy using recombinant systems expressing a single predominant receptor subtype.

An example of the radioligand binding approach was the evidence for the subdivision of α_1-ARs into α_{1A}- and α_{1B}-AR by contrasting the binding properties of two radioligands, ^{3}H prazosin and ^{3}H WB4101, in homogenates of rat cerebral cortex (Morrow and Creese 1986). Using another binding approach, it was observed that about half of ^{125}I-HEAT binding to α_1-AR in rat cerebral was blocked by the alkylating agent chloroethylclonidine with these sites corresponding to α_{1B}-ARs that demonstrated a low affinity for WB4101 (Minneman 1988). Functional studies gave rise to the suggestion that there was a fourth α_1-AR subtype designated α_{1L} based on its low affinity for prazosin (Oshita et al. 1991). This was supported by studies in several tissues, but evidence was subsequently produced that showed that α_{1L}-AR represent a conformational state of the α_{1A}-AR (Ford et al. 1997) supported by the finding that α_{1L}-AR mediated responses in mouse prostate were abolished in α_{1A}-AR knockout mice (Gray et al. 2008).

In a similar approach using ^{3}H clonidine and ^{3}H yohimbine, α_2-AR were initially subdivided into α_{2A}- and α_{2B} AR subtypes based on their pharmacological properties (Bylund 1985). Eventually three pharmacologically distinct α_2-AR subtypes were defined with some species orthologs exhibiting distinct ligand recognition profiles. The α_{2A}-AR subtype, which has low affinity for prazosin and high affinity for oxymetazoline, is found in human platelets and HT29 cells (Bylund 1988). The α_{2B}-AR is found in neonatal rat lung and NG108 cells (Bylund et al. 1988) and has relatively high affinity for prazosin and a low affinity for oxymetazoline. α_{2C}-ARs were identified in opossum kidney (OK) cells and have relatively high affinity for prazosin and low affinity for oxymetazoline but is pharmacologically distinct from α_{2B}-AR (Blaxall et al. 1991). The α_{2D}-AR, originally identified in rat salivary gland

(Michel et al. 1989a) and bovine pineal (Simonneaux et al. 1991), proved to be a species orthologue of human α_{2A}-AR.

Adopting a similar approach to Ahlquist, Lands et al. (1967b) used the rank order of potency of a series of agonists to show that β-AR could be divided into at least 2 subtypes, β_1- and β_2-AR, with β_1-AR being defined as the predominant subtype in heart and adipose tissue and displaying similar sensitivity to both adrenaline and noradrenaline, whereas β_2-ARs predominated in the smooth muscle of lung, blood vessels and uterus and displayed higher sensitivity to adrenaline. It subsequently became apparent that there were responses mediated by β-AR that could not be explained by the presence of just two β-AR subtypes. In particular, β-AR mediated responses in both brown and white adipose tissue displayed atypical characteristics distinct from those in many other tissues, being resistant to blockade by then existing β-AR antagonists. This pharmacological property was exploited by Jon Arch and Mike Cawthorne and the Beecham's group in the 1980s who developed novel atypical β-AR agonists such as BRL37344 that were effective anti-obesity agents in rodents but unfortunately had poor efficacy in humans (for a review, see Arch and Kaumann 1993). Later the β-AR subtypes present in tissues could be identified and quantitated using radioligands such as ^{125}I hydroxybenzylpindolol (Minneman et al. 1979a). Although many of the characteristics of atypical β-ARs could be explained by the presence of the β_3-AR, this did not apply in all cases, and hence, the possibility of additional subtypes could not be excluded.

3.1 Affinity, Efficacy, and Adrenoceptor Secondary Conformations

Whilst many of the differences between drugs acting on ARs could be explained by actions at particular receptor subtypes, there are other mechanisms such as the presence of sites on the receptor in addition to the orthosteric binding sites that when occupied can modify activity. As well as the α_{1A}-AR "high" and "low" affinity state mentioned above, it has been recognised for several decades that the molecular pharmacological properties of certain ligands acting at the β_1-AR cannot be described by action at a conventional single orthosteric site on the receptor. This has been studied in much more detail at the β_1 than the α_{1A}-AR. The β-ligands, pindolol (Lubawski and Wale 1969), CGP12177 (Staehelin et al. 1983) and similar derivatives were found to stimulate partial agonist responses (in cells, tissues and animals) at concentrations substantially higher than the concentrations needed to block the effects of catecholamines and so were considered to be "non-conventional partial agonists" (Kaumann and Birnbaumer 1973; Kaumann and Blinks 1980; Lowe et al. 2002).

Initial observations were in vivo (e.g. with rodent and cat heart, measuring heart rate) and demonstrated that the β-blocker pindolol inhibited the agonist actions of catecholamines, but at higher concentrations pindolol had partial agonist actions of its own. Also, these responses to pindolol (and other "non-conventional β-blockers") were relatively resistant to antagonism by "conventional" β-blockers (Kaumann and

Blinks 1980; Hicks et al. 1987; Malinowska and Schlicker 1996). Furthermore, the agonist actions of pindolol had also been noted in rodent, cat and human myocardium preparations to be biphasic (Walter et al. 1984; Kaumann and Lobnig 1986). Once receptors were cloned and experiments in transfected cell lines expressing single receptor subtypes became possible, observations with CGP12177 and rat and human transfected β_1-AR (Pak and Fishman 1996) found similar unusual pharmacological actions that were difficult to reconcile with a single site of action. But to really understand these observations, it is first necessary to remind ourselves of the basic pharmacology concepts of affinity and efficacy.

Affinity is the ability of a ligand to bind to a given receptor and is usually measured as a K_d value or the concentration required to bind half of the receptors (no matter how it is measured). If this value has been determined by the inhibition of another ligand, for example in a radioligand binding assay, and calculated by e.g. the Cheng–Prusoff equation, the term K_i may be used. If the value has been determined in a functional assay, from the parallel shift of an agonist concentration–response curve in the absence and presence of a known concentration of antagonist and calculated by, e.g., the Gaddum equation, the term K_b can be used. Thus, K_i and K_b both represent the concentration required to bind half of the receptors but delineate the method of how this was measured. Propranolol binds to and blocks agonist responses at the β_1-AR with an affinity (K_d value) of about 4 nM (Baker 2005a). This K_d value for propranolol at the β_1-AR should be ~4 nM whatever the agonist or radioligand present (assuming no other changes such as receptor phosphorylation that affects affinity), and this should be true for all transfected cell-based assays, experiments in native cells, tissue preparations and in vivo studies (assuming same species throughout). Indeed, the affinity pattern of several antagonists (including selective antagonists, i.e. those that bind to one subtype much better than others) is often used to determine which receptor subtypes are present in a given tissue. However, in β_1-AR studies, responses to some agonists (e.g. catecholamines) are readily blocked (K_d for propranolol ~4 nM) whilst agonist responses to other ligands such as pindolol and CGP12177 appeared "resistant" to antagonism (requiring 100-fold greater antagonist present for a similar blockade giving K_d values of propranolol ~400 nM) (Kaumann and Blinks 1980; Hicks et al. 1987; Malinowska and Schlicker 1996). This is not consistent with all ligands competing at a single site.

Efficacy (or intrinsic efficacy) is the ability of a ligand to stimulate a response. Clearly all ligands that stimulate a response must have affinity (although this can vary widely), but there is also a wide range in the intrinsic efficacy of ligands, from the most efficacious full agonists to partial agonists, neutral antagonists and inverse agonists. For high efficacy agonists such as adrenaline, the coupling efficiency is so high that they only need to occupy a few receptors to stimulate a significant response. Thus, their EC_{50} value (concentration required to stimulate half the maximum response) in a given system may be 1 nM, whilst their affinity (K_d value, concentration required to bind to half of the receptors) may be much lower reflected in a much higher concentration and subsequent K_d e.g. 1,000 nM required to bind half the receptors (giving an intrinsic efficacy ratio of 1,000 in that system). If the intrinsic efficacy of a ligand is less, this ratio becomes less such that a full agonist

may have an EC_{50} of 100 nM and an affinity of 1,000 nM (efficacy ratio of 10). If the intrinsic efficacy of the ligand is less still, it becomes a partial agonist (an agonist that occupies all of the receptors present in that system but is still unable to stimulate a maximum response), and the EC_{50} and K_d become the same. Thus, if the affinity is 1,000 nM (half of the receptors are bound = K_d value), with this half of the receptors occupied, this will stimulate half the maximum response that this ligand can stimulate (and the EC_{50} will also be 1,000 nM). Therefore, for full agonists the EC_{50} is smaller (left-shifted) than the K_d, and the two become equal for partial agonists. It is not possible for a ligand to have an EC_{50} value greater than its K_d, because once the binding sites are occupied, any response that the ligand can stimulate will be generated. This is true for all agonists and inverse agonists if this ligand is interacting with a single site on the receptor. Thus, "non-conventional agonists" that block catecholamines readily (with low K_d values) yet stimulate an agonist response with substantially higher concentrations (higher EC_{50}) are not compatible with a single site of action on the β_1-AR (Kaumann and Birnbaumer 1973; Kaumann and Blinks 1980; Kaumann et al. 2001). Neither can biphasic concentration response curves (e.g. those observed for pindolol in some systems) occur via a single site of receptor activation.

The initial explanation for all of these unusual findings was the presence of a fourth β-AR and hence the suggestions of a "putative β_4-AR" (Kaumann and Blinks 1980; Galitzky et al. 1997; Molenaar et al. 1997a; Kaumann et al. 1998; Preitner et al. 1998; Sarsero et al. 1998, 1999; Cohen et al. 2000; Lowe et al. 2002). However, studies in knockout mice demonstrated that whilst this "non-conventional" pharmacology remained in β_2-AR and β_3-AR knockout mice (Kaumann et al. 1998, 2001; Preitner et al. 1998; Cohen et al. 2000), in β_1-AR knockout mice the cardiostimulant effects of CGP12177 were lost, and thus the "non-conventional or β-blocker resistant" agonist responses required the presence of the β_1-AR (Konkar et al. 2000a; Granneman 2001; Kaumann et al. 2001). Others reported parallels in the β_1-AR and β_4-AR pharmacology leading them to suggest that CGP12177 was acting via a low-affinity state of the β_1-AR (Kompa and Summers 1999; Lewis et al. 2004).

At about the same time as the β_1-AR knockout studies were being conducted, the ARs were cloned and studies with transfected cell lines, with a single receptor subtype present, became possible. These studies, looking at the β_1-AR in isolation, clearly demonstrated that all of these pharmacological responses could be seen with just the one AR subtype present – the β_1-AR. Pak and Fishman, using CHW cells transfected with the human or rat β_1-AR (Pak and Fishman 1996), reported that CGP12177 inhibited isoprenaline at concentrations of about 1 nM, yet stimulated agonist responses within the same system with an EC_{50} of about 30 nM (see Fig. 5 in Pak and Fishman 1996) and Fig. 1c in this chapter. This again is not compatible with CGP12177 interacting at a single site on the receptor.

Several groups have examined this subsequently and found that the "non-conventional" pharmacology is entirely dependent on the presence of the β_1-AR, and therefore must be occurring at a different active conformations of this receptor (Pak and Fishman 1996; Konkar et al. 2000a, b; Baker et al. 2003a; Joseph et al. 2004a;

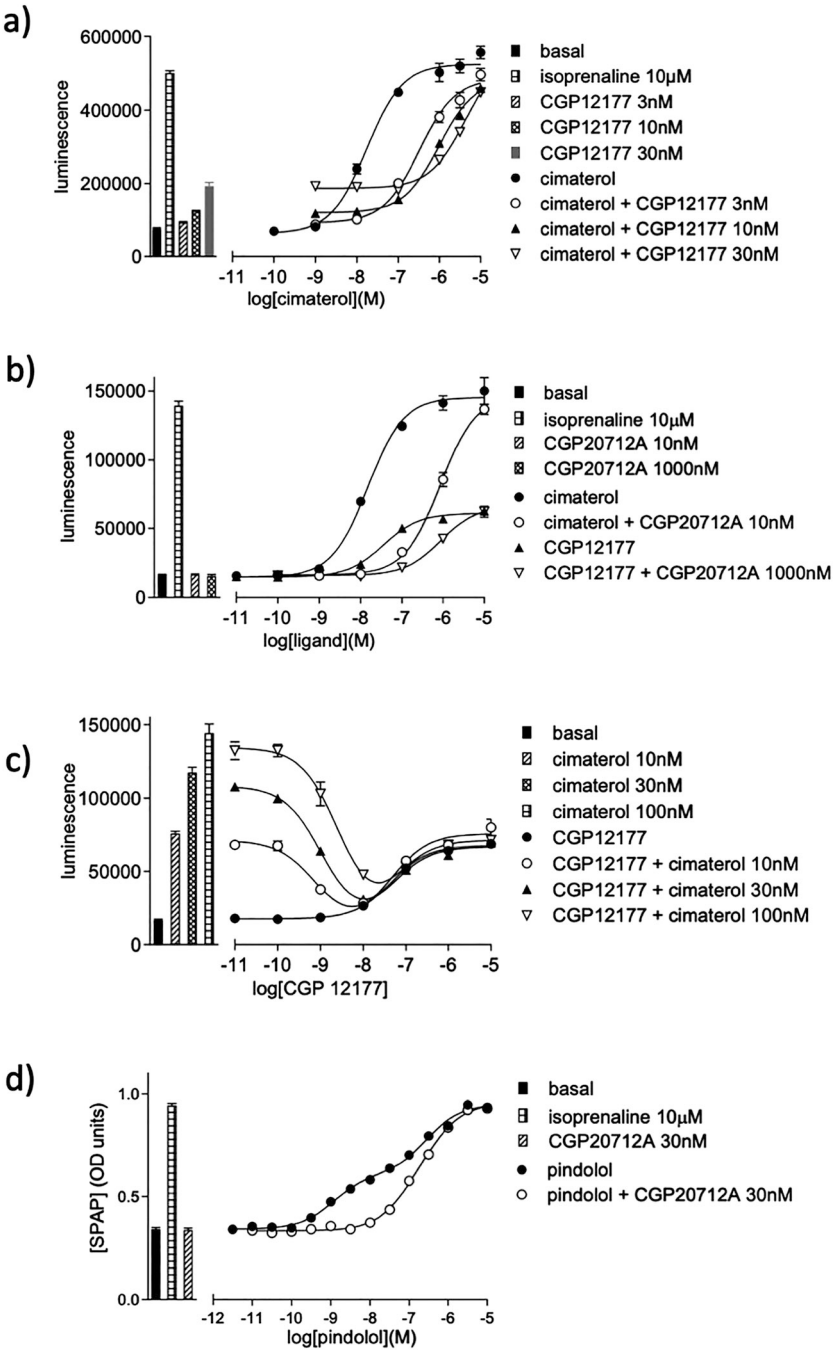

Fig. 1 Experimental data illustrating examples of the properties of the different conformations of the β_1-AR. (**a**) CGP12177 inhibits the CRE-luciferase response to cimaterol with high affinity in CHO cells stably expressing the human β_1-AR at physiological levels (79 fmol/mg protein).

Baker 2005b). In transfected cells, the affinity of CGP12177 (obtained from ^{3}H CGP12177 binding, or from CGP12177 inhibition of a more efficacious agonist) is reported to be 0.2–0.4 nM, yet it has an EC_{50} value of about 20–30 nM in the same system. This resembles the finding for pindolol that was 200–400 times more effective at inhibiting catecholamine responses in human atria than causing cardiostimulatory effects (Joseph et al. 2003).

Thus, it is accepted that there are at least two agonist conformations of the β_1-AR – the catecholamine conformation, the site of action of catecholamines and many agonists and where antagonists bind with high affinity, and a secondary (CGP12177) conformation where some ligands (including CGP12177 and pindolol) have agonist actions that are relatively resistant to antagonists (Granneman 2001; Molenaar 2003; Arch 2004; Kaumann and Molenaar 2008). Studies in animals, human tissues and transfected cells have expanded knowledge about the conformations at which agonists and partial agonists act (Konkar et al. 2000b; Lowe et al. 2002; Baker et al. 2003a; Joseph et al. 2003, 2004a; Baker 2005b; Baker 2010a). Thus, the ligands that have been reported to stimulate the secondary conformation include alprenolol, bucindolol, carazolol, carvedilol, CGP12177, cyanopindolol, LY362884, oxprenolol, pindolol, SDZ21009 and SR59230A.

The size of response to a partial agonist depends on several factors including receptor reserve and efficacy of effector coupling. Thus, in systems with low coupling efficiency or few receptors, pindolol may appear as a neutral antagonist. In systems with better coupling or more receptors, only the secondary response may be seen and therefore the pindolol response is relatively resistant to β-AR antagonists. In well-coupled systems or those with more receptor reserve, a biphasic pindolol agonist response may be seen, with the first component occurring via the

Fig. 1 (continued) Cimaterol is a known catecholamine conformation agonist (Baker 2005b). The K_d value for CGP12177 is 0.18 nM (calculated from a parallel shift associated with neutral antagonism of the catecholamine conformation as the partial agonism occurs via the secondary conformation). If the K_d is calculated by the partial agonism method of Stephenson (that assumes cimaterol and CGP12177 are acting at the same conformation) the K_d is actually similar at 0.16 nM. The K_d for ^{3}H CGP12177 determined from saturation binding in these cells is again similar 0.15 nM (Baker et al. 2003a). (**b**) CRE-luciferase response in a single 96-well plate of CHO cells stably expressing the human β_1-AR where the cimaterol response at the catecholamine conformation is inhibited by 10 nM CGP20712A with a K_d value of 0.2 nM, whereas the partial agonist response to CGP12177 (EC_{50} = 36 nM i.e. different from K_ds above) requires 1,000 nM CGP20712A to produce a similar shift, giving a K_d value for CGP20712A at the secondary conformation of 46 nM. (**c**) CGP12177 concentration–response curve alone, and in the presence of fixed concentrations of cimaterol (10, 30 and 100 nM) in a single 96-well plate of CHO cells stably expressing the human β_1-AR. Low concentrations of CGP12177 show no agonist activity but inhibit the cimaterol response, whereas higher concentrations of CGP12177 display agonist activity. There is therefore a "high affinity inhibitory dip" in the cimaterol response. (**d**) Biphasic concentration–response curve to pindolol in the absence and presence of 30 nM CGP20712A in CHO cells stably expressing the human β_1-AR (at 1146fmol/mg protein). The first component is inhibited more readily by CGP20712A (K_d for CGP20712A of 0.63 nM) than the second component, suggesting the first component is occurring via the catecholamine conformation and the second component via the secondary conformation

primary catecholamine site. CGP12177 may also display biphasic responses in some high expressing/well-coupled systems, but agonist stimulation of the catecholamine site seems less efficient than pindolol, with CGP12177 showing "cleaner" or more pure secondary site agonist responses. In most systems, CGP12177 is a neutral antagonist of the primary catecholamine conformation and thus has become the main ligand for examining secondary site responses.

Thus, there are now 4 pharmacological lines of evidence that support the existence of at least 2 active pharmacological recognition sites on the β_1-AR (Fig. 1), observed in transfected cell systems, tissue preparations (including human) and in vivo:

1. *Discrepancies in* K_d *and* EC_{50} *for a given partial agonist* – For example, the affinity of CGP12177 (K_d 0.15–0.18 nM) is substantially different from the concentration required to stimulate a response (EC_{50} 36 nM) in the same cells (Fig. 1a, b) (Baker 2005b); pindolol was 200–400 times more effective at inhibiting catecholamine responses in human atria than stimulating its cardiostimulatory effects (Joseph et al. 2003).
2. *Antagonist affinity measurements*. Agonist actions at the primary high affinity catecholamine site produced by agonists such as cimaterol are antagonised with high affinity by β-antagonists such as CGP20712A (i.e. K_d value 0.2 nM), whereas agonist responses occurring at the secondary conformation require much higher concentrations of antagonist (Fig. 1b). Thus, partial agonist responses to CGP12177 at the secondary site are antagonised by CGP20712A with a K_d value of 46 nM. Furthermore, K_d values for inhibition of the secondary site are not universally 100-fold lower than the K_d values for the catecholamine site. For atenolol the K_d values for catecholamine and secondary site are 130 nM and 150,000 nM, a >1,000-fold difference in affinities for the 2 sites, whereas with ICI118551 (250 nM and 1,500 nM) the affinities differ by only sixfold (Baker 2005b). This has been reported in cells transfected with human receptors (Joseph et al. 2004a) and in the ferret heart (Lowe et al. 2002) and is further evidence that the secondary conformation is a separate pharmacological entity (Fig. 1b).
3. *The "high affinity inhibitory dip"*. Within a single experiment it is sometimes possible to demonstrate both inhibition of the high affinity catecholamine conformation and activation of the low affinity secondary conformation (Fig. 1c). Thus, at low concentrations, CGP12177 inhibits fixed concentrations of a catecholamine site agonist such as cimaterol, adrenaline or noradrenaline, but as the CGP12177 concentration increases, stimulation of the secondary conformation occurs. Thus, there is an initial dip in the curve as increasing concentrations of CGP12177 (or similar compound) inhibit the agonist, before the direct agonist response to CGP12177 is seen (Pak and Fishman 1996; Baker et al. 2003a, 2014; Baker 2005b, 2010a). This "dip" would not be possible if the ligands were interacting at a single receptor conformation (Fig. 1c).
4. *Biphasic responses.* Some ligands display a biphasic concentration–response curve (Fig. 1d). In many cases the first component (at lower agonist

concentrations) is readily blocked by an antagonist (suggesting catecholamine site activation), whereas the second component (occurring at higher agonist concentrations) requires higher concentrations of antagonist to shift the curve (suggesting secondary site activation). Again this has been observed in both cellular studies (Baker et al. 2003a; Baker 2010a) and heart preparations (Kaumann and Lobnig 1986). Ligands displaying two component responses at the β_1-AR include alprenolol, bucindolol, carazolol, carvedilol, cyanopindolol, oxprenolol, pindolol (Fig. 1d) and SDZ21009.

Interestingly, the β_1-AR secondary conformation is present in many species and there does not appear to be an equivalent secondary site present in the human β_2-AR (or turkey equivalent = $t\beta_{3C}$). An equivalent secondary agonist conformation is present in the human β_3-AR (and turkey equivalent = $t\beta_{4C}$; (Baker et al. 2002; Baker 2005c, 2010a, b). The secondary component is associated with amino acids in the extracellular end of TM4 and appears conserved across several species, including the β_1-AR of rat, mouse, guinea pig, turkey, cat and human (Baker et al. 2014).

Although the secondary conformation has been clearly demonstrated in the human heart (Kaumann 1996; Joseph et al. 2003; Sarsero et al. 2003) and blood vessels (Kozlowska et al. 2006), and would likely be accessed by concentrations of carvedilol used in humans (100 ng/ml ≈ 300 nM, (Sawangkoon et al. 2000)), the physiological relevance remains unknown. Interestingly, the agonist actions of pindolol were found helpful in managing orthostatic hypertension (Man In't Veld and Schalekamp 1981). Studies involving β_1-AR polymorphisms have arrived at different conclusions with one study suggesting that the naturally occurring polymorphisms have no effect on ligand affinity, efficacy or secondary conformation (Baker et al. 2013), whereas another suggested that the Gs-cAMP secondary messenger coupling was reduced for isoprenaline-induced responses more than that to CGP12177 in the Gly389 polymorphism compared to the wild-type Arg389 receptor (Joseph et al. 2004b).

3.2 Cloning of Adrenoceptor Subtypes

The cloning of AR subtypes eventually solved many of the mysteries surrounding the pharmacological properties, localisation and function of these receptors. Three α_1-AR subtypes were identified by cloning, starting with the α_{1B}-AR from DDT cells (hamster smooth muscle) (Cotecchia et al. 1988) and then a novel α_1-AR from bovine brain initially nominated as the α_{1C}-AR (Schwinn et al. 1990). This was subsequently shown to correspond to the pharmacologically defined α_{1A} subtype (Ford et al. 1994; Hieble et al. 1995). A subtype cloned from rat cortex was originally designated the α_{1A}- or $\alpha_{1A/C}$ -AR but later identified as a novel subtype the α_{1D}-AR (Lomasney et al. 1991; Piascik et al. 1995). This subtype was characterised functionally in tissues (Piascik et al. 1995; Kenny et al. 1995) but signals less effectively upon agonist stimulation due to its primarily intracellular localisation (Hein and Michel 2007).

Three distinct α_2-AR subtypes have been identified by cloning. The α_{2A}- and α_{2B}-AR subtypes that correspond to the subtypes characterised in pharmacological studies were cloned from man (Kobilka et al. 1987a; Weinshank et al. 1990). The third subtype corresponding to the α_{2C} in opossum OK cells was cloned from the human kidney (Murphy and Bylund 1988; Regan et al. 1988). On the basis of the predicted amino acid sequence, the α_{2D} was shown to be a species orthologue of the human α_{2A}-AR and therefore not considered to be a separate subtype. Many additional α_2 subtypes have been identified in other species, including five receptor genes in zebrafish and eight in the pufferfish (Ruuskanen et al. 2004). In the zebrafish, three of the subtypes are similar to those found in mammals (orthologs, the same gene in different species), whereas the other two are not found in mammals, but are paralogs (duplicated genes in the same species). The significance of many receptor subtypes in these species is not well understood (Bylund 2005).

The first β_2-AR to be cloned was the hamster β_2-AR (Dixon et al. 1986) that provided probes that enabled the cloning of the human subtype (Chung et al. 1987; Kobilka et al. 1987b). Due to sequence differences the same approach could not be used to clone the β_1-AR and instead a β_2-AR cDNA was used to identify and clone a related receptor the 5-HT_{1A} receptor (Fargin et al. 1988) that in turn was used to identify and clone the β_1-AR (Frielle et al. 1987). In yet another approach the β_3-AR was cloned from a human genomic library using the entire coding regions of the turkey β_1-AR and human β_2-AR (Emorine et al. 1989). In addition to positive clones containing the β_1- and β_2-AR, a novel clone was identified that proved to be the β_3-AR. In contrast to the β_1- and β_2-AR genes that are intronless, the human β_3-AR gene has two exons and a single intron whilst the mouse has three exons and two introns (Schena and Caplan 2019). Despite the presence of introns, no splice variants have been reported in humans and only two functional receptor splice variants have been reported in mice (Evans et al. 1999; Hutchinson et al. 2002).

3.3 Adrenoceptor Structure

The β_2-AR was the first GPCR for a hormone or neurotransmitter to have its structure solved by X-ray crystallography. The wild-type receptor in a lipid environment in complex with carazolol and a Fab that bound to the third intracellular loop was solved at 3.4 Å/3.7 Å (Rasmussen et al. 2007) and an engineered carazolol-bound β_2-AR in combination with a T4 lysozyme fusion protein at 2.4 Å (Cherezov et al. 2007). The structure of a turkey β_1-AR modified to improve thermostability and in the antagonist conformation, bound to cyanopindolol, was solved at 2.7 Å the following year (Warne et al. 2008). These pioneering studies laid out approaches that could be adopted to solve GPCR structures, but progress on the other AR subtypes has been slow. However, structures for the α_{1B}, α_{2A}, α_{2B}, α_{2C} and β_3-AR have appeared only relatively recently. The stabilised variant of the α_{1B}-AR was solved bound to (+) cyclazosin and fused to a crystallisation chaperone DARPin (designed ankyrin repeat protein D12 crystallisation chaperone) (Deluigi et al. 2022). The α_{2A}-AR was crystallised from an engineered receptor containing a modified thermostabilised

apocytochrome in the third intracellular loop (IC3) and a truncated N- and C-terminal in combination with a partial agonist RES and an antagonist RS79948 (Qu et al. 2019). The structure of an IC3 modified α_{2B}-AR in complex with dexmedetomidine and Go at a resolution of 2.9 Å was determined by single particle cryo-EM (Yuan et al. 2020). The α_{2C}-AR structure in combination with RS79948 was solved at 2.8 Å but also provided insights into factors that determine drug selectivity at α_{2A}-/α_{2C}-AR (Chen et al. 2019). More recently, the cryo-EM structure of the dog β_3-AR in complex with the β_3-AR selective agonist mirabegron has been described suggesting a rationale for selectivity of drugs at this AR (Nagiri et al. 2021). This work was further expanded to examine a wider range of β-AR agonists and identified a narrow exosite more suitable for accommodating agonists with elongated shapes such as mirabegron and solabegron (Nureki et al. 2022). There are now numerous active, inactive and biased conformation structures available for AR subtypes (Wu et al. 2021) with those available for β_1- and β_2-AR providing information that informs ligand recognition and activation mechanisms (Wu et al. 2021).

It should be recognised however that not all of the crystallised receptors are from structures that are functionally active or have the same pharmacological characteristics as the wild-type receptors. For example, the original two β_2-AR structures (IL3 FAB fragment and t4 lysozyme fusions (Rasmussen et al. 2007; Cherezov et al. 2007) were not functionally active, and the initial turkey β_1-AR had point mutations that increased its stability so making crystallisation possible, but at the expense of significantly reduced agonist function (Warne et al. 2008; Baker et al. 2011a).

4 Physiological Roles of Adrenoceptors, Distribution, Clinical Uses and Side Effects of Drugs

All AR subtypes (α_1, α_2 and β) are found throughout the cardiovascular system (blood vessels and heart) and are important in blood pressure regulation (via vasoconstriction and vasodilation) and cardiac output (heart rate (chronotropy), force of contraction (inotropy), rate of conduction through the AV node (dromotropy) and relaxation during diastole (lusitropy)). As the hormones mediating the fight or flight response, adrenaline and noradrenaline cause vasoconstriction and vasodilatation, redirecting blood to organs such as the heart and skeletal muscle essential for this response and away from non-essential tissues such as skin and gut (hence "white with fright" and feeling queasy if nervous) with a net result of increased blood pressure. They also cause an increase in cardiac output and bronchodilation, in order to maximise oxygen delivery to skeletal muscle, as well as metabolic effects such as increased blood glucose (by glycogenolysis and gluconeogenesis) and increased glucose uptake into skeletal muscle. In cases of septic and anaphylactic shock associated with a life-threatening drop in blood pressure due to profound vasodilation, adrenaline and noradrenaline are used clinically by infusion in intensive care settings as non-selective agonists of all ARs to mimic the fight or flight response and increase blood pressure and cardiac output. They are used with caution in cardiogenic shock. Adrenaline injections are used routinely in cardiac arrest and as first line in anaphylaxis (e.g. the "Epipen" carried by those with

previous anaphylaxis used immediately to prevent a fall in blood pressure and maintain bronchodilation, en route to hospital care).

Specific AR subtypes are also targets for clinical use, underpinned by knowledge of the location and function of the receptor and its role in pathological conditions, leading to the development of subtype selective ligands.

The α_1-AR subgroup are largely involved in smooth muscle contraction in blood vessels, particularly in the skin and gut, smooth muscle of the iris and the bladder (Graham et al. 1996; Ford et al. 1997; Piascik and Perez 2001; O'Connell et al. 2014). A summary table of the clinical uses of α_1-AR drugs is found in Table 1. Oxymetazoline and xylometazoline, working as vasoconstrictors, are available as over-the-counter nasal decongestants. Selective α_{1A}-AR agonists such as dabuzalgron also have some potential for the treatment of heart failure due to doxorubicin toxicity (Beak et al. 2017). α_1-AR antagonists are used to treat hypertension (including in phaeochromocytoma), benign prostatic hyperplasia (BPH) and PTSD (Proudman et al. 2022b). Several antidepressants and antipsychotics have significant α_{1A}-AR affinity (Proudman et al. 2022b) that may explain their hypotensive effects. Since there are also α_1-AR in the brain, compounds that combine high affinity for the α_{1A}-AR and blood–brain barrier penetration may include α_{1A}-AR antagonism in their spectrum of action.

The α_2-AR are also present in blood vessels, and α_2-AR agonists such as clonidine and moxonidine were introduced as antihypertensive agents. However, it was soon recognised that they also acted in the CNS where they have both pre- and post-junctional actions at α_2-AR (Langer 2015). Noradrenaline activating prejunctional α_2-AR autoreceptors inhibits further noradrenaline release from the same neuron. However, α_2-AR can also act as prejunctional heteroreceptors to inhibit the release of other neurotransmitters as well as having post-junctional effects. Many of the effects of α_2-AR agonists on blood pressure were due to CNS effects, but central α_2-AR are now the main target for α_2-AR agonists such as dexmedetomidine that are now used for sedation and managing delirium, nausea, agitation and opiate consumption in both intensive and palliative care. Furthermore, dexmedetomidine has a unique property of facilitating cooperative or arousal sedation, allowing neurosurgery to be undertaken in awake patients. Some ~90% of mammalian (including human) brain α_2-AR are of the α_{2A}-AR subtype and are widespread in the prefrontal cortex where activation increases cognitive function (Erdozain et al. 2019; Proudman et al. 2022a). The remaining 10% are α_{2C}-AR and mainly found in the striatum and hippocampus. α_2-AR agonists are also used to treat ADHD, glaucoma, rosacea (due to vasoconstricting effects) and muscle spasms (Proudman et al. 2022a). α_2-AR antagonists are generally not used therapeutically although yohimbine was touted as an aphrodisiac. However α_2-AR antagonism is associated with the mechanism of action of many anti-depressants and anti-psychotic drugs (Langer 2015). The α_2-AR antagonist idazoxan has anti-depressant properties and the anti-psychotic drugs risperidone and paliperidone have nM affinity for α_{2C}-AR (Proudman et al. 2022b). Clozapine has somewhat lower affinity, but its α_2-AR antagonist properties have been suggested to have a role in its clinical efficacy (Brosda et al. 2014; Langer 2015). However, it is interesting to note that all of the second-generation antipsychotic drugs have even higher potency at α_1-AR compared

Table 1 Localisation and major clinical uses of ligands acting on α_1-adrenoceptors

Signalling	α_1-AR – $G_{q/11}$-coupled, inositol phosphate production, Ca^{2+} release and phospholipase C activation. Secondary transduction mechanisms include $G_{12/13}$ to cause stimulation of phospholipases A2/D and with lower coupling efficiency to G_s to activate adenylyl cyclase and increase cAMP (α_{1A}- and α_{1B}-)		
Subtype	α_{1A}	α_{1B}	α_{1D}
Main sites of receptor localisation	Blood vessels, urogenital tract, brain	Blood vessels, brain	Blood vessels, brain
Antagonists			
Medical condition and example drug used	Hypertension – doxazosin		
	Benign prostatic hyperplasia – tamsulosin PTSD – doxazosin, prazosin Phaeochromocytoma – phenoxybenzamine, phentolamine	None	None
High affinity non-selective antagonists	Cyclazosin, doxazosin, terazosin, prazosin		
Subtype-selective antagonists (selectivity)	SNAP5089 (>1,000-fold) Niguldipine (1,000-fold) Silodosin (500–1,000-fold) RS100329 (100–1,000-fold)	None	BMY7378 (100–200-fold)
Other compounds of note with antagonist properties	Other classes of compounds that have high α_{1A}-AR affinity include tri-cyclic antidepressants, e.g. amitriptyline, clomipramine Anti-psychotics(neuroleptics), e.g. chlorpromazine, risperidone Carvedilol and labetalol that are primarily β-AR antagonists have significant α_{1A}-AR affinity Labetalol is used in pregnancy for hypertension, pre-eclampsia, eclampsia		
Agonists			
Medical condition and example drug used	Hypotension in shock – adrenaline, noradrenaline		
	Nasal congestion – oxymetazoline, xylometazoline	None	None
Non-selective agonists	Adrenaline, noradrenaline, phenylephrine		
Subtype selective agonists	A61603 (>500-fold greater α_{1A}-AR affinity)	None	None

to α_2-AR (Proudman et al. 2022b). Thus, α_2-AR antagonism may have a role to play in the treatment of schizophrenia, bipolar disorder and dementia. Although present in platelets where activation is involved in platelet aggregation, this is not a clinical target. Table 2 summarises the clinical uses of drugs acting at α_2-AR and their clinical uses.

Table 3 summarises drugs acting at β-AR and current clinical uses. Activation of β_1-AR produces positive inotropic and chronotropic as well as dromotropic and lusitropic effects on the heart (Molenaar et al. 2000). Their other main physiological

Table 2 Localisation and major uses of ligands acting on α_2-adrenoceptors

Signalling	α_2-AR are Gi-coupled to inhibit adenylyl cyclase and reduce cAMP, also opening of K^+ channels and inhibition of Ca^{2+} channels. Activation of phospholipase A_2		
Subtype	α_{2A}	α_{2B}	α_{2C}
Main sites of receptor localisation	Blood vessels, brain, platelets	Blood vessels	Blood vessels, brain, adrenal chromaffin cells
Antagonists			
Medical condition and example drug used	None	None	None
High affinity non-selective antagonists	Yohimbine (rauwolscine), RX821002, atipamezole, RS79948		
Subtype-selective antagonists (selectivity)	BRL44408 (10–60-fold)	None	MK912 (10–40-fold) JP1302 (40–60-fold)
Other compounds with antagonist properties	Yohimbine (from tree bark) has been used as an aphrodisiac. A number of antidepressants such as amitriptyline and mirtazapine and antipsychotics such as risperidone, paliperidone and aripiprazole have significant antagonist properties at α_2-AR		
Agonists			
Medical condition and example drug used	Hypertension – clonidine, moxonidine		
	Hypnosis/sedation, anxiolysis and analgesia – dexmedetomidine ADHD – guanfacine Glaucoma and facial erythema in rosacea – brimonidine Muscle spasm – tizanidine	None	None
Non-selective agonists	Adrenaline, noradrenaline, brimonidine		
Subtype-selective agonists (selectivity)	Guanabenz (Yamashima 2003), guanfacine (Powell and Slater 1958) and brimonidine (Brittain et al. 1968) display low α_{2A}-AR selectivity; oxymetazoline and related imidazolines have higher (Frang et al. 2003) α_{2A}-AR selectivity but also high affinity for α_1-AR and 5HT receptors	None	
Other agonist compounds of note	Lofexidine has been used to treat opiate withdrawal		

There are substantial species differences in the molecular pharmacology of α_2-adrenoceptor ligands

Table 3 Localisation and major uses of ligands acting on β-adrenoceptors

Signalling	β-AR – Gs-coupled to activate adenylyl cyclase and increase cAMP. Also reported to couple to Gi/o to inhibit adenylyl cyclase reduce cAMP, activate guanylyl cyclase and promote ERK1/2 phosphorylation		
Subtype	β_1	β_2	β_3
Main sites of receptor localisation	Heart, blood vessels, kidney, brain	Lungs, blood vessels, skeletal muscle, heart, brain, bone marrow, spleen	Brown and white fat in rodents, gut, urinary and gall bladder
Antagonists			
Medical condition and example drug used	Heart failure – bisoprolol, metoprolol, carvedilol, nebivolol Arrhythmias – bisoprolol, sotalol Ischaemic heart disease – bisoprolol, timolol Hypertension – bisoprolol, atenolol	None	None
	Glaucoma – timolol, betaxolol Anxiety – propranolol Migraine – propranolol Benign essential tremor – propranolol Thyrotoxicosis – propranolol Portal hypertension and variceal bleeding – propranolol		
High affinity non-selective antagonists	Propranolol, carvedilol, bupranolol, timolol, CGP12177, carazolol, cyanopindolol		
Subtype-selective antagonists (selectivity)	CGP20712A (>500-fold) NDD825 (1,000-fold) NDD713 (>500-fold)	ICI118551 (300-fold)	L755507 (50–200-fold) L748337 (37–400-fold)
Other antagonists of note	Esmolol is a short-acting, esterase-sensitive β-blocker used intravenously in arrhythmias particular in peri-operative or ITU settings Carvedilol and labetalol have some α_{1A}-AR affinity as well as higher β-AR affinity Labetalol is used in pregnancy for hypertension, pre-eclampsia, eclampsia		

Subtype	β_1	β_2	β_3
Agonists			
Medical condition and example drug used[a]	Shock – adrenaline and noradrenaline Anaphylaxis – adrenaline Cardiac arrest – adrenaline		
	Bradycardia – isoprenaline as bridge to pacemaker	Asthma/COPD: short-acting rescue – salbutamol, terbutaline Long acting – formoterol, salmeterol Ultra-long acting (COPD only) – vilanterol, olodaterol, indacaterol	Overactive bladder syndrome – mirabegron, solabegron and vibegron
Non-selective agonists	Adrenaline, noradrenaline, cimaterol		
Subtype-selective agonists (selectivity)[b]		Salmeterol (>1,000-fold) Formoterol (300-fold) Fenoterol (10-fold)	Mirabegron (>500-fold) Solabegron(>200-fold) Vibegron (>1,000-fold)
Other agonists of note	Noradrenaline has some but minimal β_1-AR selectivity Denopamine is marginally β_1-AR selective and may have β_1-AR-selective intrinsic efficacy	Adrenaline has some but minimal β_2-AR selectivity	

Some antagonists that block both β_1- and β_2-AR (e.g. propranolol) may owe some of their actions to blockade of β_2-AR (e.g. for migraine and portal hypertension β_2-AR-mediated vasodilatation in brain and liver blood vessels, benign essential tremor β_2-AR in skeletal muscle. Other conditions (e.g. thyrotoxicosis, anxiety) may benefit from both β_1-AR inhibition of tachycardia and β_2-AR inhibition of skeletal muscle tremor). Antagonists with some β_1-AR selectivity (e.g. atenolol, bisoprolol) may be somewhat more effective antihypertensives due to β_1-AR-mediated blockade of renin release from the kidney. The eye has both β_1 and β_2-AR

[a] Adrenaline and noradrenaline may also act via α_1- and α_2-AR to cause vasoconstriction to increase blood pressure in shock

[b] β_3-AR pharmacology displays marked species variation. The selective agonists shown display high activity at the human β_3-AR

function is to promote renin release from the juxtaglomerular cells in the kidney (do Vale et al. 2019). The non-selective β-AR agonist isoprenaline is used to increase heart rate in acute bradyarrhythmias as a short-term bridge until a permanent pacemaker can be inserted. β_1-AR agonists such as dobutamine were used acutely to stimulate the failing heart. It was thought that partial agonists (drugs with intrinsic sympathomimetic activity or "ISA") would be helpful in heart failure, but longer-term β_1-AR activation increases myocardial fibrosis, apoptosis and remodelling, worsening heart failure and resulting in a higher incidence of myocardial infarction

and adverse cardiovascular outcomes. Even activation from partial agonists is associated with worse outcomes (The Xamoterol in Severe Heart Failure Study Group 1990; Cruickshank 1993). β-AR antagonists were originally developed in the 1960s for angina and hypertension (Black et al. 1964, 1965) and are still used for these purposes. Neutral β-antagonists (β-blockers) used in the acute MI period reduce mortality 39%, 36% and 26% for timolol, metoprolol and propranolol, respectively. Metoprolol, bisoprolol, carvedilol and nebivolol reduce mortality from heart failure by 34–35% (see references in Baker and Wilcox 2017). β-AR antagonists are first-line treatment for arrhythmias including bisoprolol for AF and tachyarrhythmias and sotalol (which also has class II and class III anti-arrhythmic properties) for paroxysmal AF. β-AR antagonists remain important for the management of hypertension, portal hypertension, thyrotoxicosis and hypertension in pregnancy (labetalol, which also displays some α_{1A}-AR antagonism), glaucoma (β_1 and/or β_2-AR antagonism), migraine, anxiety and benign essential tremor (some effects may be β_2-AR related). Several β-AR antagonists cross the blood–brain barrier and affect sleep quality and can cause nightmares (most pronounced with pindolol, alprenolol, metoprolol and lipid-soluble β-antagonists).

The β_2-AR subtype is very widely distributed in the body and when activated causes smooth muscle relaxation in the lung bronchi, in arteries and veins and in the gastrointestinal tract, (Billington et al. 2017; Proudman et al. 2022b) uterus (Liu et al. 1998) and bladder. The main clinical β_2-AR uses are inhaled β_2-AR agonists for bronchodilation in asthma and COPD. Short-acting (rescue) β_2-AR agonists are used during acute exacerbations (salbutamol, the well-known "blue inhaler", or as a nebuliser when needed). Longer acting, twice daily inhaled β_2-AR agonists such as salmeterol or formoterol are used for long-term maintenance (in conjunction with inhaled corticosteroids), and the newer ultra-long-acting inhaled agonists are used once a day in COPD (see chapter on "Asthma and COPD" in this volume for detail). β_2-AR agonists have many other actions such as lipolysis in adipose tissue, tremor, glucose uptake and anabolic effects in skeletal muscle (Sato et al. 2014; Mukaida et al. 2019), glycogenolysis and gluconeogenesis in the liver as well as reduced hyperkalaemia in life-treatening situations by promoting cellular potassium uptake (Beta-2 Adrenergic Agonists 2012). Recent developments show promise for the treatment of type 2 diabetes by increasing skeletal muscle glucose uptake (Sato et al. 2014; Mukaida et al. 2019). In the past, they were used to delay delivery in the treatment of threatened abortion (Beta-2 Adrenergic Agonists 2012). There are selective β_2-AR antagonists but none currently in therapeutic use, although the use of non-selective β-AR antagonists (mainly aimed at β_2-AR antagonism) has the potential to decrease tumour growth and metastatic spread in cancer (Choy et al. 2016; Albinana et al. 2022; Zhang et al. 2023) (see "β-adrenoceptors in cancer" chapter in this volume for more details).

The β_3-AR subtype has roles in promoting lipolysis in adipose tissue in rodents and relaxation of the bladder in various species including humans. There have been many attempts to target this subtype as a potential treatment for obesity but with limited success (Arch et al. 1984; Arch and Kaumann 1993). On the other hand, β_3-AR selective agonists such as mirabegron are now widely used to treat overactive

bladder syndrome (Michel et al. 2010; Michel and Korstanje 2016). There are few β_3-AR selective antagonists available, e.g. L748,337, and currently these are mostly utilised in laboratory studies.

5 The Molecular Pharmacology of Drugs Interacting with Adrenoceptors

5.1 Orthosteric Interactions

5.1.1 Full Agonists

Agonists are defined by two properties, affinity, or the ability to bind to the receptor, and efficacy, the ability to produce a response (Strange 2008; Proudman et al. 2022a). High efficacy agonists may have to occupy only a small fraction of the total receptor population to achieve a maximum response (Fig. 2a). The selectivity of agonists can be challenging to measure as it reflects both the affinity and efficacy for a particular AR subtype (Kenakin 1982, 1999; Clarke and Bond 1998; Baker 2010a). An agonist with higher affinity for one subtype but similar efficacy across subtypes will exhibit selectivity as will an agonist with similar affinity across subtypes but higher efficacy at one subtype (Kenakin 1982, 1999; Clarke and Bond 1998; Baker 2010a). In addition the response to an agonist will be influenced by the expression levels of receptors, signalling proteins and their location, efficiency of coupling, the response measured and desensitisation during the assay (Baker 2010a). The affinity of agonists (K_d values) can be determined from binding studies, but values will be influenced by the properties of the radioligand or fluorescent ligand used to label the receptor. Very few ligands used to label receptors are neutral antagonists so for example ^{125}I cyanopindolol and ^{3}H CGP12177, widely used to label β-AR, are high affinity partial agonists at all 3 subtypes (Pietri-Rouxel and Strosberg 1995; Cohen et al. 1999; Baker et al. 2003a, 2014; Joseph et al. 2004a; Baker 2010a; Sykes and Charlton 2012; Sato et al. 2015; Soave et al. 2016). The potency of agonists can be measured (EC_{50} value) from second messenger or other downstream responses. Various mathematical receptor modelling techniques have been applied to determine intrinsic efficacy, but the simplest is the ratio between affinity and potency (efficacy ratio). Although the actual values obtained are very system dependent, useful comparisons can be made if a number of compounds are measured in parallel in the same system, as these can be directly compared (Strange 2008; Baker 2010a). Utilising this system a high efficacy full agonist will have a larger efficacy ratio (K_d/EC_{50}) than a full agonist with lower efficacy. This can have high predictive value for the behaviour of compounds in different systems. Provided responses are all measured in the same system, the efficacy ratio can be compared across agonists to achieve a rank order of agonist intrinsic efficacy (Furchgott 1967). Whilst adrenaline and noradrenaline are highly efficacious agonists at all 9 ARs, other highly efficacious more selective agonists include phenylephrine and methoxamine at α_1-AR; brimonidine, moxonidine and α-methylnoradrenaline at α_2-AR; and isoprenaline, fenoterol and orciprenaline at β-AR.

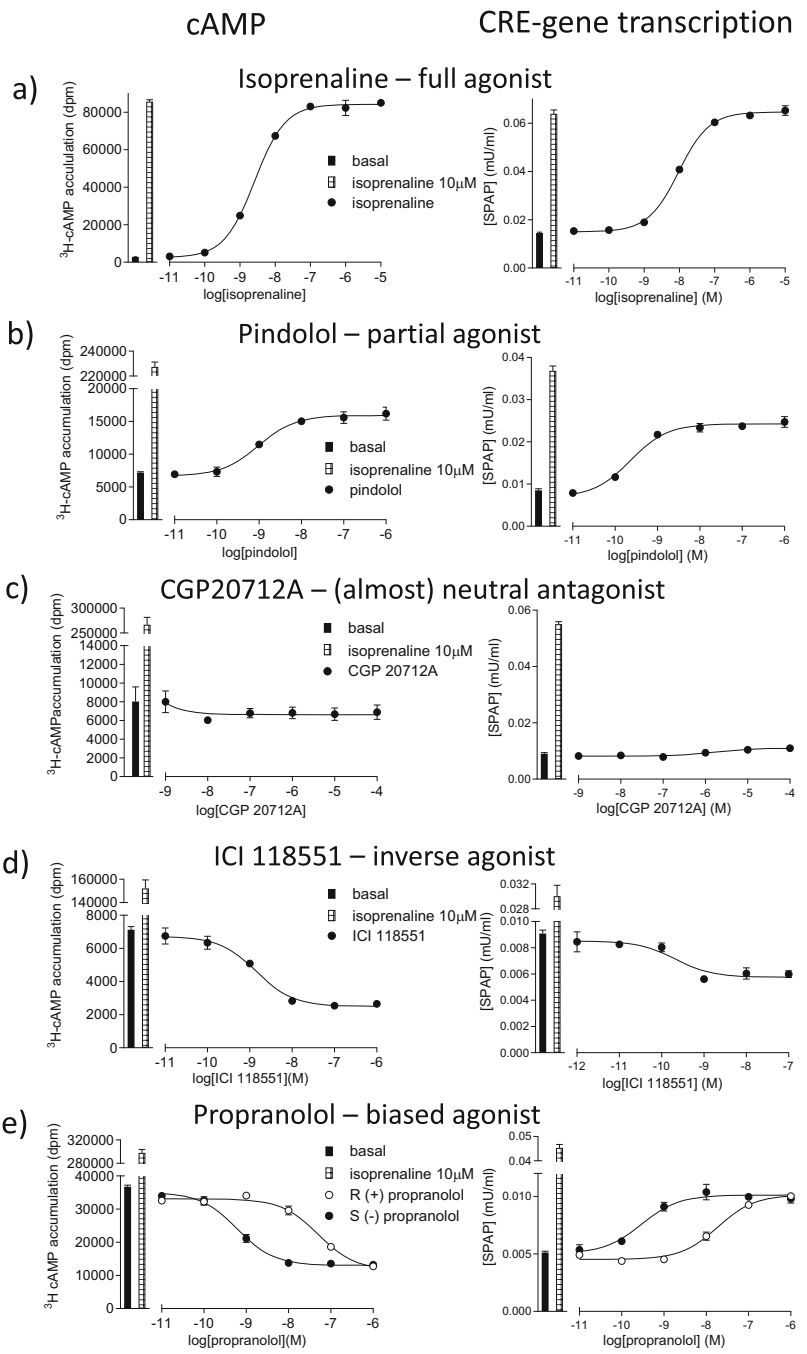

Fig. 2 Examples of different orthosteric responses occurring at β_2-adrenoceptors. ^{3}H cAMP accumulation and CRE-reporter gene (SPAP) production in living CHO cells stably expressing

5.1.2 Partial Agonists

Partial agonists are by definition agonists that produce a sub-maximal response irrespective of the fraction of the receptor population that is occupied (Fig. 2b). The maximal response to a partial agonist is associated with complete occupation of the receptor population. The response to a partial agonist is very system dependent so in a system with high receptor expression and efficient coupling to downstream signalling a partial agonist may produce the maximum response of the system. In contrast, in a system expressing low levels of receptors or with poor coupling to the signalling pathway the partial agonist may exhibit little if any response and may even behave as an antagonist. Thus, partial agonism can be determined by the efficacy ratio that approaches 1 when half the receptors are bound and the half maximal response EC_{50} corresponds to the K_d. However, if the efficacy of the ligand is less than that of a full agonist, partial agonists can be ranked in order of intrinsic efficacy by comparing the maximum response to a known standard or full agonist measured in parallel in the same assay. Measurements of the intrinsic efficacy provide useful information about how a drug is likely to behave in other systems. Partial agonists (or lower efficacy agonists) include naphazoline, clonidine, dexmedetomidine and guanfacine at α_1 and α_2-AR and salbutamol, salmeterol, xamoterol, bucindolol and pindolol at β-AR.

Partial agonism in β-ligands was referred to as intrinsic sympathomimetic activity (ISA). In the past, it was thought that partial agonists (or β-blockers with ISA) would be helpful in heart failure and ischaemic heart disease in providing some increased cardiac output and blood pressure whilst inhibiting the main effects of adrenaline. β-blockers with ISA such as pindolol (Fig. 2b), alprenolol, xamoterol and bucindolol were not found beneficial (and in some cases were harmful), whereas β-blockers without ISA (neutral antagonists in physiologically relevant systems) resulted in 26–40% reductions in mortality for both ischaemic heart disease and heart failure (Baker et al. 2011b; Baker and Wilcox 2017).

Fig. 2 (continued) the human β_2-AR and a CRE-SPAP reporter gene (Baker et al. 2003b, d). (**a**) Isoprenaline is a full agonist of both upstream cAMP production and downstream CRE-SPAP production in both assays (forskolin stimulates the same maximum response in both assays in these cells). The affinity (pK_d) of isoprenaline determined from radioligand binding in these cells is 6.64 (=229 nM). (**b**) Pindolol is a partial agonist in both assays. The CRE-SPAP assay is more efficiently coupled than the cAMP response so that the partial agonist response represents a greater proportion of the overall response. The affinity (pK_d) of pindolol determined by radioligand binding in these cells is 9.23 (=0.59 nM). (**c**) CGP20712A has no agonist action in the ^{3}H cAMP accumulation assay (neutral antagonist) and very little agonism in the amplified CRE-gene transcription assay. The affinity (pK_d) of CGP20712A determined by radioligand binding in these cells is 6.11 (776 nM). (**d**) ICI118551 is an inverse agonist in both assays. The affinity (pK_d) of ICI118551 determined by radioligand binding in these cells is 9.26 (0.55 nM). (**e**) Propranolol is a biased agonist. It acts as an inverse agonist to decrease ^{3}H cAMP accumulation below basal levels, but, in the same cells, acts as an agonist to stimulate an increase in the CRE-gene transcription response. The affinity (pK_d) of propranolol determined by radioligand binding in these cells is 9.08 (0.83 nM)

5.1.3 Antagonists

Antagonists are somewhat easier to define and characterise than agonists. The affinity with which they interact with the receptor (K_d value) is often comparable across a wide range of assays including assays from direct measures of binding to antagonism of an agonist in functional assays, in systems endogenously expressing receptors (tissue or whole animal) and in recombinant systems (assuming the same species). However, there may be some variation across assays dependent on conditions used, with species, level of receptor expression (physiological or highly expressed), whole cell vs homogenate and buffer composition all as potential factors (Proudman et al. 2022b). True neutral antagonists are classified as drugs that occupy the receptor but have no intrinsic efficacy (Fig. 2c) and block the effects of full, partial or inverse agonists (Urban et al. 2007). This type of antagonist forms only a comparatively small fraction of those acting at ARs because of the special characteristics required – i.e. the receptor activation state stabilised by the ligand is the same as that of the basal state of the receptor (see below) (Kenakin 2004), and prazosin (α_1), yohimbine and RX821002 (α_2) and CGP20712A (β_1 and β_2) come close to meeting this requirement.

5.1.4 Inverse Agonists

Many antagonists are actually inverse agonists and clearly exhibit this property when examined in systems that display constitutive activity or high receptor expression. Inverse agonists inhibit activity, display negative efficacy and stabilise a receptor conformation that generates less second messenger interaction than the basal equilibrium state of the receptor (Fig. 2d) (Kenakin 2004). Several β-AR antagonists are actually inverse agonists, but the clinical relevance of this is unknown (Baker et al. 2003b, 2011b).

5.1.5 Biased Agonists

Whilst ARs classically mediate responses to the endogenous ligands adrenaline and noradrenaline, drugs designed as agonists or antagonists for these receptors can activate alternative cell signalling pathways, with the potential to influence clinical efficacy. Some drugs acting at ARs have differential capacity for pathway activation, described as stimulus trafficking, biased agonism, functional selectivity or ligand-directed signalling (Azzi et al. 2003; Baker et al. 2003b; Kenakin 2007; Evans et al. 2010). These terms refer to responses where one drug has higher efficacy than another for one signalling pathway, but a lower efficacy (or inverse agonism) for a second pathway measured in a system where other agonists measured in parallel are behaving as "normal" ligands (Fig. 2e). The accepted explanation for such responses is that the drugs have the capacity to induce or stabilise distinct active conformations of the receptor that in turn display altered coupling efficiency to different effectors (Kenakin 2007; Evans et al. 2010). This is consistent with biophysical studies showing that drugs can indeed promote distinct conformational states (Qu et al. 2019; Ma et al. 2020). Thus, in its purest form biased agonism is associated with compounds producing different conformational states of a receptor that in turn couple with different efficiency to individual signalling pathways. However, the

physicochemical properties of a drug can also influence its distribution and pattern of signalling. For instance if ARs are located and functional intracellularly as well as at the cell surface (Wei and Smrcka 2022), the pattern of signalling observed with a polar ligand that sees only cell surface receptors may well differ from that of a lipophilic ligand that can interact with both cell surface and intracellular receptors. There is currently significant interest in biased agonism (whatever the mechanism) for its potential to maintain therapeutically relevant responses and minimise unwanted side effects.

Biased signalling can also be seen if there is a reversal in the rank order of intrinsic efficacy (or potency) of agonists measured in 2 different signalling cascades, provided that agonists are measured simultaneously alongside a common standard, all conditions/cell lines/tissue preparations being used are identical and all potential for off-target activation have been investigated and ruled out (Sato et al. 2007).

5.2 Allosteric Interactions

5.2.1 Positive Allosteric Modulators (PAMs)

Allosteric modulators interact with sites on GPCRs that are topographically distinct from those identified by classical agonists and antagonists that act at the usual "orthosteric" site on the receptor (Christopoulos 2002). Allosteric modulators bind to the receptor, producing a change in the receptor conformation, such that there is an increase in the affinity or the intrinsic efficacy (or both) of the orthosteric agonists (or increase in affinity of the antagonists). In the case of a full orthosteric agonist, the effect of a PAM is to shift the concentration–response curve to the left, and it may do this by either increasing the affinity or intrinsic efficacy of the agonist (Fig. 3a). With a partial agonist an increase in affinity in response to the PAM causes a parallel shift to the left of the concentration–response curve (Fig. 3b), whereas an increase in intrinsic efficacy in response to a PAM causes an increase in the maximum response (Fig. 3c). The main advantages of allosteric modulators include a self-limiting effect, a selectivity profile quite distinct from affinity and efficacy at the orthosteric site, and they have no effect on their own, but augment the response to the orthosteric agonist or antagonist (Christopoulos 2002). A PAM selective for one receptor subtype would therefore augment a response at that receptor alone and would have the effect of increasing the selectivity of the orthosteric ligand. This would mean that, given together with the PAM, a lower dose of orthosteric ligand would be required for the same effect, thus minimising off-target side effects. There are currently few positive allosteric modulators (PAMs) acting at ARs. Cmpd-6 was shown to display properties of a PAM with orthosteric agonists, G proteins and β-arrestins but also reduces antagonist binding to β_2-AR (Ahn et al. 2018; Liu et al. 2019). The polar derivative Cmpd-6FA, which has similar pharmacology, was used to determine the structure of the PAM-bound β_2-AR, suggesting that it stabilised the active form of the receptor (Liu et al. 2019). Cmpd-6 also acts as a PAM of carvedilol, increasing its binding affinity and ability to competitively antagonise orthosteric agonists (Pani

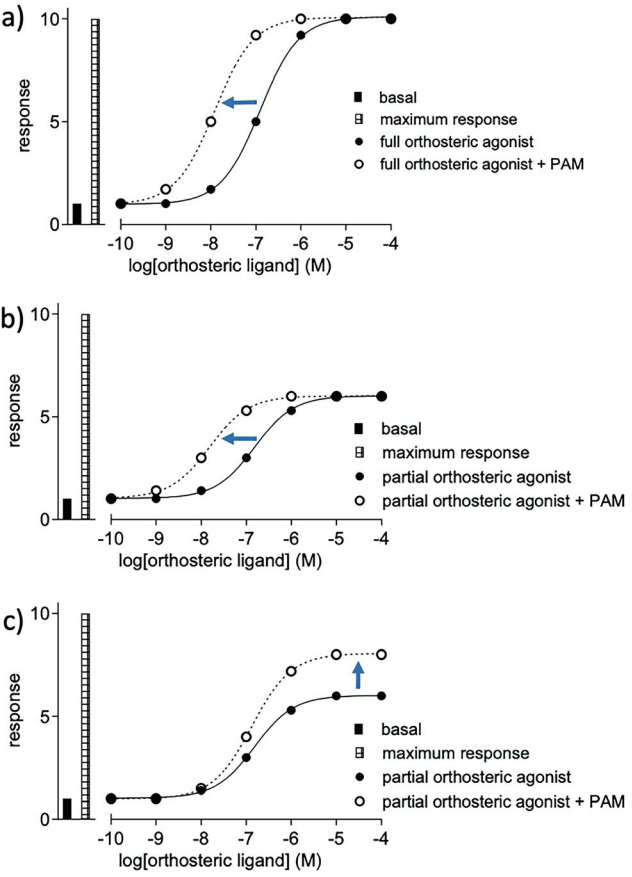

Fig. 3 Effect of allosteric modulation on responses to agonists acting at an orthosteric site. Theoretical data showing the effect of a positive allosteric modulator (PAM) on responses to a full and a partial agonist (**a**) The effect of a PAM on responses to a full orthosteric agonist. The shift to the left of the concentration–response curve would be seen if the PAM was increasing the affinity or if it was increasing the intrinsic efficacy of the orthosteric agonist. (**b**) The presence of a PAM that increases the affinity of the orthosteric partial agonist causes a parallel shift to the left of the concentration–response curve. (**c**) The presence of a PAM that increases the efficacy of the orthosteric partial agonist causes an increased maximum response of the concentration–response curve. A negative allosteric modulator (NAM) would cause the agonist responses to move in the opposite direction

et al. 2021). It potentiates β-arrestin-1 binding and functions but not Gαs-mediated binding of carvedilol at the β_2-AR (Pani et al. 2021).

5.2.2 Negative Allosteric Modulators (NAMs)

Negative allosteric modulators, like positive allosteric modulators, bind to the receptor at a site distinct from the orthosteric site but in contrast stabilise a conformation of the receptor that decreases the stimulatory activity of orthosteric ligands

and therefore reduces responses to agonists, partial agonists and inverse agonists. NAMs also have the advantages of self-limiting actions, a different selectivity profile to ligands acting at the orthosteric site, require the presence of an orthosteric ligand to have an effect, and offer a way of increasing the selectivity of antagonists and agonists. NAMs have been developed for α_{1A}-AR (Campbell et al. 2017; Chen et al. 2022), β_1-AR (Abiko et al. 2022) and β_2-AR (Ahn et al. 2017; Liu et al. 2017, 2020), but none are currently available for clinical use.

5.2.3 Basal State, Ligand Efficacy Classification and Clinical Relevance

Finally, it is important to remember that whilst the affinity of a ligand is fixed and should not change regardless of the system in which it is being measured, the classification of the efficacy of the ligand (agonist, partial agonist, antagonist etc.) will depend on the system in which it is being measured (Michel et al. 2020). Thus, a ligand of given efficacy stabilises a certain activated state of the receptor. If the basal state of system is very low (basal state 1 in Fig. 4), that ligand will appear as a substantial agonist. If the basal state is higher, but still below the activated state stabilised by the ligand, then the ligand will appear more of as a partial agonist (basal state 2, Fig. 4). If the basal state is equal to that of the ligand-stabilised state, the ligand will appear as a neutral antagonist (basal state 3, Fig. 4). If the basal state of the system is high, then the ligand will appear as an inverse agonist (basal state 4, Fig. 4). An analogy in vivo would be that an animal given a ligand that stabilised a medium efficacy state of the receptor induces a heart rate of ~80 bpm. In an asleep animal with a heart rate of

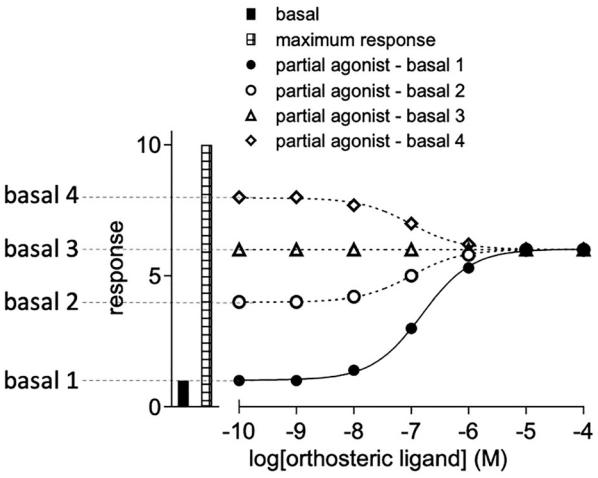

Fig. 4 Effect of basal activity on responses to a partial agonist. Theoretical data for an orthosteric partial agonist ligand that stabilises a conformation capable of stimulating 60% of the maximum response as measured from varying levels of basal response. At a low basal level (basal 1) the response to the ligand is ~60% of the maximal response; at a higher level (basal 2) the partial agonist would stimulate a response 33% of the maximum response; if the basal level was the same as that stimulated by the ligand, it would appear as a neutral antagonist (basal 3); and if the basal level was higher than the conformation stabilised by the ligand, it would appear as an inverse agonist (basal 4)

~50 bpm, the drug would increase the heart rate to ~80 bpm so it would be an agonist. However, in an awake state with a heart rate of ~80 bpm, the ligand would appear as a neutral antagonist. In a nervous state with an adrenaline-stimulated heart rate of ~120 bpm, the ligand would reduce the heart rate to ~80 bpm, and thus behave as an inverse agonist of that high baseline state. Thus, in theory, every ligand except the most efficacious full agonist and most efficacious inverse agonist could appear as a partial, neutral or inverse agonist depending on the system in which they are investigated (at one time these were known as protean agonists, but as this applies in theory to virtually all ligands the term is now rarely used (Kenakin 2001; Gbahou et al. 2003; Baker 2008)). In reality, full agonists behave as such in almost all systems, but the degrees of partial agonism vary between systems, with inverse agonism hard to detect. However, the rank order of ligand intrinsic efficacy should remain the same. To make predictions of physiological or clinical responses from responses measured in cell systems, it is important to understand the relation between the two. For example, there was an excellent correlation between the degree of partial agonism of a range of β-AR ligands examined in CHO cells expressing the β_1-AR and the increase in heart rate measured in conscious rats (Baker et al. 2011b). In this study, ligands inducing cAMP responses greater than 20% of the maximum measured in transfected CHO cells provided a linear correlation with heart rate in the animals so that the effect on the heart rate of the rat could be predicted from knowledge of the cAMP response in a transfected cell. Also, it demonstrated that 20% cAMP activation in that CHO cell system was equivalent to, or lower than, basal activation of the animal heart rate and predicted that ligands stimulating less than 20% cAMP in the transfected CHO cells would not cause an increase in heart rate.

6 Screening Techniques: Assays Enabling the Study of Adrenoceptors

A brief description of screening techniques is included here as the approach and the model used may have an important influence on the characteristics of the ligand reported. Originally ARs were studied in in vitro tissue preparation and in animal studies and the pharmacology of ligands and receptor subtypes determined from these studies. However, the major disadvantage of this approach is the complexity of the systems (multiple AR subtypes present within a given tissue) that often make interpretation of results difficult. Other disadvantages include the significant skills required to conduct animal experiments, their relative slowness compared to cellular techniques, the cost of animal facilities and labour and ethical considerations associated with animal studies. The use of laboratory animals and their tissues also runs the risk of species differences in the expression of the ARs present in a given tissue, species differences in the structure/pharmacology of the receptor and species differences in the expression of signalling molecules, all of which can result in significant differences in pharmacology/clinical effectiveness of ligands between the animal versus human studies (Arch et al. 1984; Arch and Kaumann 1993). Many disease states have to be artificially induced in animal models, whilst others

(e.g. asthma) are hard to mimic, again making extrapolation from animal model to diseased human state potentially trickier. However, these animal models still have an important role in drug development, from proof-of-concept to late-stage studies, as the receptors are studied in their endogenous environment and expressed at physiological levels. The use of other animal models such as AR subtype knockout mice, conditional knockouts and transgenic animals expressing human AR subtypes can also provide very useful information about drug selectivity and actions.

Much of the more recent and detailed receptor subtype pharmacological information has been obtained in recombinant systems where a single AR subtype (generally the human isoform) has been transfected into a heterologous cell type (e.g. CHO or HEK cells Pak and Fishman 1996; Konkar et al. 2000b; Hoffmann et al. 2004; Baker 2005a, 2010a; Proudman et al. 2020, 2022a, b; Proudman and Baker 2021) with the receptor of interest expressed at higher levels than in most physiological systems in order to examine ligand binding, cell signalling, desensitisation, regulation and localisation following the administration of an agonist, antagonist or compound with other pharmacological properties. Whilst these models undoubtedly provide useful information, they do not recapitulate the physiological environment experienced by endogenously expressed receptors in native tissues. In particular, the stoichiometry of signalling components and their localisation are likely to differ from those present in native cells and tissues. The design of these assays needs to take into consideration factors such as post-translational modification by glycosylation or palmitoylation, the range of G proteins expressed in the cells, desensitisation and internalisation and the formation of receptor homo- or hetero-oligomers and signalling complexes with other proteins that may affect the pharmacology observed. High expression levels of receptors as often observed in these recombinant systems influence pharmacology and can enhance factors such as constitutive activity and efficacy of agonists as well as increasing the potential for interaction with multiple G proteins, all of which may result in interesting pharmacological phenomena but having no physiological or clinical relevance. This said, some responses (for affinity, selectivity and intrinsic efficacy) do translate well from responses measured in model cell systems to those measured in whole animals (Baker et al. 2011b, 2017, 2020). Finally, responses in tissues in the normal "well" state may differ considerably from that in a diseased state, and/or a diseased tissue that has been subjected to significant drug treatment. For example, the relative proportions of β_1- vs β_2-AR in the human heart change with disease (from 80:20 in normal heart to 60:40 in failing heart), and in addition, the overall expression of β_1-AR is reduced (by 62%) in failing compared to non-failing heart (Bristow et al. 1986). Thus, studies in diseased states cannot always be assumed to be representative of non-diseased physiology or response to drugs, and vice versa. Many studies in human tissue are from organs removed at surgery (e.g. diseased heart removed during transplant) and will have been subjected to significant (likely high dose) drug exposure for weeks to months beforehand which in themselves could cause significant alterations in the pharmacological and physiological function of the organ, beyond just the disease state.

Much more recently, emphasis has shifted to high-throughput techniques, multiplexing and the use of biosensors, often in transfected cell systems, with increasing miniaturisation in the methods. In many cases, the receptors are altered (expressed

as tagged or fusion proteins with signalling molecules), or signalling molecules are altered (tagged or fused), taking a further step away from the native environment. Although these approaches offer advantages of huge volume of output and therefore cost-effectiveness, attention needs to be paid to design as ligand depletion in tiny volumes and equilibrium conditions can become compromised and many of the assumptions built into the data analysis and calculations may no longer hold.

6.1 Molecular Pharmacology Assays

6.1.1 Receptor Binding: Radioligands and Fluorescent Ligands

Given the considerations mentioned above, high-throughput assays can be very useful in identifying and characterising compounds active at AR subtypes. One of the first high-throughput assays to be developed and still widely used is the radioligand binding assay (Jarrott et al. 1979; Dooley et al. 1986; Halme et al. 1995; Horie et al. 1995; Ford et al. 1997; Candelore et al. 1999; Louis et al. 1999; Joseph et al. 2004a; Baker 2005a, 2010a; Maiga et al. 2013; Flanagan 2016; Proudman et al. 2020, 2022a, b; Proudman and Baker 2021) or the more recent fluorescence-based ligand binding assays (Baker et al. 2003c; Daly and McGrath 2011; Soave et al. 2020) that are particularly useful for the measurement of receptor expression levels, affinity and kinetic parameters. Important features include the choice of a suitable unlabelled ligand to define non-specific binding to non-receptor cell constituents or filters used to separate bound and free radioligand. Binding to other components of the system including tubes or plates used for incubation can also influence the effective ligand concentration. The best definition of non-specific binding is usually achieved using an excess of competitor that has similar pharmacology but different chemistry to the radioligand. Another consideration that applies to the use of whole cells to determine binding is the use of hydrophilic ligands (e.g. ^{3}H CGP12177) to minimise uptake into the cell (Baker 2005a, 2010) that could otherwise manifest as over-estimation of binding. Fluorescent tags (fluorophores) often added to ligands are often large lipophilic substituents that can alter non-specific binding/labelling and cellular distribution of the parent ligand. Caution is required with some fluorescent ligands that are highly lipophilic and over time may partition into the cell membrane or seep into the cell (Baker et al. 2003c). Furthermore, the addition of a fluorophore to a ligand is likely to alter the molecular pharmacology of the parent. Thus, every new ligand–fluorophore pairing needs a full pharmacological evaluation as a novel molecule of its affinity, efficacy, non-specific binding, kinetics and intracellular uptake. Saturation binding experiments allow the determination of the maximum number of binding sites (Bmax) and affinity of the radioligand or fluorescent ligand. Binding studies are most often carried out using high affinity antagonist ligands (that usually have slow dissociation rates) that allow for simple separation of bound and free ligand. They are most useful for the development and characterisation of antagonist ligands although they have specific uses for characterising agonists (Baker 2010a; Proudman and Baker 2021; Proudman et al. 2022a). Membrane binding assays, unlike whole cell assays, can be conducted in the absence or presence of GTP. An increase in affinity seen as a shift of the competition

curve of an agonist measured in the presence of GTP (that causes the G protein to dissociate from the receptor, thus measuring, "inactive receptor") compared to that measured in the absence of GTP (where the receptor is coupled to a G protein and unable to dissociate, thus measuring an "active state") can give an indirect indication of agonist-stabilised G protein coupling and thus agonism/ligand efficacy (Strange 2008). However, this should not be relied on as a measure of ligand intrinsic efficacy. That said, very few ligands used to label receptors are neutral antagonists; e.g. ^{125}I cyanopindolol and ^{3}H CGP12177 that are widely used to label β-AR are high affinity partial agonists at all 3 subtypes (Baker 2010a; Sykes and Charlton 2012; Sato et al. 2015). Allosteric ligands will often alter the characteristics of ligand binding at orthosteric sites that makes binding a useful technique for identifying allosteric interactions (Wilson et al. 1991; Leppik et al. 1998, 2000; Flanagan 2016; Ahn et al. 2017; Campbell et al. 2017; Chen et al. 2022).

The often time-consuming step of separation of bound and free radioligand can be circumvented by the use of scintillation proximity assays (SPA) in which the membrane containing the receptor is immobilised to a plate containing a scintillant so that binding of the radioligand to the receptor produces scintillation that can be detected in a β-counter (Flanagan 2016). This method is particularly useful being quick and avoiding the potentially distorting step of separation of bound and free ligand and washing. It can also be used to measure kinetics of binding and to identify binding of relatively low affinity ligands. The choice between radioligand and fluorescent ligand binding techniques may be in part due to availability of radioisotopes and readers, the availability of fluorescent ligands and specialised fluorescence readers and differences between molecular pharmacological parameters between parent and fluorescent ligands. Fluorescent ligands have the added advantage of being able to visualise the location of receptors in the cell or tissue an approach that is more complicated with radioligands. Fluoroligands can also be used indirectly with transfected SNAP-tagged or NLuc-tagged receptors where the interaction between receptor and fluorescent ligand can be measured. These methods have the advantage of high signal to noise ratio and low background (Flanagan 2016; Soave et al. 2016), but require receptor tagging that then has to be evaluated to understand the effects the tag has on receptor behaviour (expression, internalisation) and pharmacology (ligand affinity, efficacy, kinetics) and downstream signalling, and are clearly not applicable to the study of native receptors.

6.1.2 GTPγs Binding

The next step following agonist occupation of an AR subtype is guanine nucleotide exchange that can be examined by the measurement of [^{35}S] GTPγs to cell membranes containing the receptor of interest (Thomsen et al. 2005). It is particularly useful as it immediately follows receptor activation and is not subject to amplification or regulation by other cellular processes (Milligan 2003). It is most useful for characterising $G_{\alpha i/o}$ linked ARs as $G_{\alpha i/o}$ has a rapid GDP/GTP turnover rate and often high levels of expression, but the assay requires a filtration and washing step that limits throughput. This disadvantage can be circumvented by the use of SPA beads and the poor signal obtained with $G_{\alpha s}$ and $G_{\alpha q}$ linked ARs by

immunoprecipitation of the ^{35}S GTPγs-bound G protein α subunit (Milligan 2003). There are also non-radioactive GTPγs binding assays available (Frang et al. 2003).

6.1.3 cAMP Accumulation

These are particularly important assays used for characterising ARs as the β-AR subtypes all activate adenylyl cyclase, the α_2-AR subtypes inhibit adenylyl cyclase and the α_1-AR subtypes have a small but significant effect on cAMP production. Bioassay of cAMP produced following stimulation of β-AR subtypes is generally straightforward, and there is a wide choice of assays available including radiometric, luminescence, fluorescence polarisation and time-resolved fluorescence assays (Wang et al. 2004). ^{3}H cAMP accumulation assays preload the cells with ^{3}H adenine that is then converted into ^{3}H cAMP by the cell following agonist activation. The ^{3}H cAMP produced by the cells is therefore measured directly (e.g. by column chromatography) (Minneman et al. 1979b). PDE inhibitors are often present, meaning that ^{3}H cAMP accumulation can be measured over time, including experiments over hours allowing equilibrium between receptor and ligand to be reached. Most other assays are based on competition between biologically produced unlabelled cAMP and a labelled cAMP for highly specific cAMP monoclonal antibody (Mab). In the radiometric SPA (GE Healthcare) and Flashplate (Perkin Elmer) assays, the Mab is conjugated to either SPA beads or scintillant-coated plate wells, and cAMP competes with ^{125}I cAMP for binding. AlphaScreen is a luminescence-based assay in which cAMP competes with biotinyl cAMP for binding to streptavidin-coated beads. There are several fluorescence polarisation assays available that utilise competition between cAMP and fluor-cAMP for binding to cAMP Mab. In homogeneous time-resolved fluorescence (HTRF – Cisbio), cAMP competes with acceptor-labelled cAMP to europium-labelled cAMP Mab. In a slightly different approach utilised by the HitHunter cAMP assay, cAMP competes with ED-cAMP for cAMP Mab with the remaining ED-cAMP free to complement EA to form an active β-galactosidase that hydrolyses a substrate to produce a fluorescent or luminescent product. All methods are sensitive and generally have a low signal to noise ratio, so the choice of assay method often boils down to cost and availability of a suitable instrument to measure the signal. For all these competition assays, it is essential when measuring the signal to ensure that the amounts of cAMP in the sample fall on the linear part of the concentration–response curve for the standard curve.

Like all G_i-coupled receptors, studying cAMP levels following α_2-AR stimulation is somewhat more complicated. The same assay methods can be used, but what is usually measured is the inhibition of cAMP production against a background of adenylyl cyclase stimulation by forskolin. There is no standardised technique for this procedure, and each system has to be set up and optimised before reliable results can be achieved. Factors that should be considered include the concentration of forskolin used, time of exposure before addition of α_2-AR agonists and time of agonist exposure. These factors vary with the cell system and α_2-AR subtype being studied (see HitHunter protocols). Characterisation of α_2-AR antagonists involves the addition of antagonist and equilibration before the addition of forskolin and α_2-AR agonist.

Some cell systems respond to α_1-AR stimulation with an increase in cAMP in addition to the canonical signalling pathways of phosphatidylinositol hydrolysis and increases in intracellular Ca^{2+} (Proudman and Baker 2021). The responses are generally small compared to those emanating from β-AR stimulation and occur with higher agonist concentrations than those required to activate the canonical G_q-coupled pathway. The cAMP generated can be determined using the assays described above.

6.1.4 Phosphatidylinositol Hydrolysis and Intracellular Calcium (Ca^{2+}) Assays

α_1-AR are G_q-coupled and the canonical signalling pathway involves phosphatidylinositol (PI) hydrolysis and increases in intracellular Ca^{2+} concentration (Cotecchia et al. 1988; Minneman 1988; Minneman et al. 1994; Schwinn et al. 1995; Theroux et al. 1996; Ford et al. 1997; Zhong and Minneman 1999; Eglen et al. 2007). The original methods measuring IP hydrolysis involve pre-loading the cells with ^3H myoinositol, which is then converted into ^3H inositol phosphates by the cell following agonist activation. ^3H inositol phosphates are then separated using ion exchange resins (Berridge et al. 1982) and if required ^3H phosphatidylinositol is separated from cell membranes. Again, inhibitors can be present (e.g. lithium to prevent inositol-1-phosphate activity) meaning that an accumulation of ^3H inositol phosphates over time, including at receptor–ligand equilibrium, can be reached. Just as with cAMP, more recent methods involve competition between cell-generated inositol phosphates and exogenous labelled inositol phosphates (e.g. IP_1 accumulation in cells by competition between cell-generated and acceptor-labelled IP_1 binding to europium-labelled Mab). Widely used techniques such as the fluorescent imaging plate reader technique (FLIPR – Molecular Devices) examine G_q-coupled responses by changes in intracellular Ca^{2+}, and pre-load cells with Ca^{2+}-sensitive dyes, which following agonist stimulation respond to Ca^{2+} release with a fluorescent signal (Shibata et al. 1995; Theroux et al. 1996; Zhong and Minneman 1999; da Silva Junior et al. 2017). These assays and the related AequoScreen are particularly useful in allowing the detailed kinetics of reactions to be followed in real time together with the characteristics of agonists, antagonists and allosteric modulators. A drawback of Ca^{2+} measurements is that they occur rapidly (over seconds) meaning assays are not at equilibrium, and pharmacological analysis of agonist and agonist–antagonist interactions (and calculations of parameters, most of which assume equilibrium conditions) is more difficult to analyse.

6.1.5 Reporter Gene Assays

Reporter gene assays examine the production of a protein in response to a change in DNA transcription and translation, following activation of a signalling cascade. The cells first need to be transfected with the appropriate reporter gene. For useful assay development, the novel protein should have low basal activity and a good response to activation of the signalling pathway (Hill et al. 2001; Baker et al. 2003a, b, 2004). The reporter is usually an enzyme that confers new properties to the cell and generates a product that is measurable by fluorescence, or bioluminescence. For example β-AR stimulation causes activation of adenylyl cyclase and generation of

cAMP that activates protein kinase A (PKA) that moves to the nucleus to phosphorylate cAMP binding protein (CREB) that binds to a cAMP response element (CRE) in a target gene, e.g. CRE-luciferase (Hill et al. 2001; Baker et al. 2003a). As with direct cAMP assays G_i-coupled receptors such as α_2-AR can be measured by inhibition of forskolin-stimulated cAMP generation (Hill et al. 2001; Proudman et al. 2022a). Reporter gene assays have some advantages including characterisation of pharmacological properties of agonists, antagonists, biased agonism, inverse agonists and allosteric modulation. Measurements require several hours for the production of the reporter protein so are usually at receptor equilibrium, although the long incubation times can lead to receptor phosphorylation, desensitisation or other alterations of the pharmacological properties observed. There is often a degree of signal amplification that can be highly beneficial in identifying low efficacy partial agonists but may be misleading with regard to determining the degree of partial agonism at the level of the second messenger. The timing of the gene transcription vs second messenger response may also be important – there is some evidence that long-term low second messenger activation results in gene transcription, whereas large but short-lived second messenger changes do not (Baker et al. 2004).

6.1.6 RNAseq

Of the GPCRomic approaches currently available, RNAseq is one of the most useful and powerful although it can be somewhat challenging due to the amount of data generated. It involves the extraction of mRNA, testing and validation of quality, reverse transcription to produce a cDNA followed by alignment of reads to the AR subtype and signalling pathway components being studied. This allows changes in expression of the receptor and signalling components to be followed following drug treatment (Insel et al. 2019). This approach has the advantage of study of receptors in cells and tissues that endogenously express them at physiological levels and in locations where they are normally found (Insel et al. 2019). This can be important given the increasing recognition of compartmentalisation of GPCR signalling (Eichel and von Zastrow 2018).

6.1.7 Label-Free Technologies

Label-free technologies add another dimension to characterisation of GPCRs in intact cells. Rather than examining particular components of cellular signalling, they ask the question of whether the cell responds to a change in environment. This can be particularly useful for the examination of responses of orphan GPCRs where the signalling pathway is unknown or in the case of ARs identification of signalling pathways that operate in addition to the canonical pathways. One of the first approaches used was to measure extracellular acidification rate (ECAR) that detects lactic and carbonic acid output from cells during metabolism (Hutchinson et al. 2005; Sato et al. 2007, 2008; Evans et al. 2011). More recently, this has been refined to allow the simultaneous measurement of ECAR and oxygen consumption rate to provide time-resolved information on mitochondrial respiration and glycolysis (Merlin et al. 2018). Additional information on compounds that influence mitochondrial respiration can be obtained by subjecting cells to the well-defined

mitochondrial stress test. Other label-free approaches include resonant waveguide grating that utilise gratings built into the base of micro-titre plates that reflect light that alters to detect changes in the section of the cell closest to the grating surface following changes in signalling. Electrical biosensors examine whole-cell changes across a substrate, an electrode and a cellular layer growing over the electrode. These systems have high-throughput capability and can be used to examine GPCR activity over extended periods of time (Grundmann and Kostenis 2015). Results from label-free approaches are often very comparable to those obtained using conventional high-throughput screening assays such as cAMP or Ca^{2+} but do have the advantage that on occasions they can identify additional signalling pathways and can be used in native cells.

6.2 Other Assays for Studying Adrenoceptors

Less frequently encountered assays for ARs include receptor phosphorylation, β-arrestin recruitment, GTPase activity and ERK1/2 phosphorylation (Michel et al. 2020). Assays may be manipulated to better detect activity for example treating cells with forskolin to increase cAMP levels in order to measure inhibition by $G_{\alpha i/o}$-coupled receptors. Increases in basal levels are also useful for detecting inverse agonism, and this can be achieved by over-expressing receptors, G proteins or adenylyl cyclase, expression of receptor/G protein fusion proteins or expression of constitutively active receptor mutants (that may be naturally occurring receptor mutants) (Michel et al. 2020). Whilst readily detected in these model systems, inverse agonism is much more difficult to observe in physiological systems and may be absent (Michel et al. 2020).

Several newer assays involve complementation of two proteins that generate a protein with unique properties (e.g. nano-luc). These have advantages that they have a good signal to noise ratio. However, whereas one activated receptor can generate several second messenger effector molecules (e.g. cAMP or calcium increase), the stoichiometry of some complementation assays requires a 1:1 receptor to effector coupling, which can make agonists appear lower efficacy.

Some complementation assays are irreversible meaning an accumulation (rather than equilibrium) of signal occurs. So it is possible that the same agonist can appear to have very different intrinsic efficacy, even if measured within the same cells – low efficacy in a complementation assay, moderate efficacy in a second messenger assay and high efficacy in a gene transcription assay.

Finally, it must be mentioned that there are many long-standing in vitro (e.g. organ bath) and in vivo assays (e.g. heart rate monitoring) that have been used extensively over many years. They have the advantage of being generally well understood and are systems in which physiological levels of receptors and signal transduction systems are present. Responses in animals and animal tissues may suffer from the disadvantage of not truly reflecting the pharmacology of human systems (e.g. species differences in receptor subtype pharmacology and /or tissue receptor expression) or the disease process being targeted (e.g. induced animal

disease states not always being representative of human disease). Studies in human tissues (tissue often being obtained from explants of diseased tissue at the time of organ transplant and therefore usually after extensive drug treatment) are technically highly demanding and tissues may display a variety of disease processes and/or reflect drug treatment. However, studies in vitro and in vivo remain a vital link in the drug development process.

7 α_1-Adrenoceptor Ligands

7.1 α_1-Adrenoceptor Location, Function and Signalling

The α_1-AR subgroup of ARs are expressed in blood vessels, heart, urinary tract, brain, kidney and liver (Table 1) (Minneman 1988; Bylund et al. 1994; Graham et al. 1996; Ford et al. 1997; Piascik and Perez 2001; O'Connell et al. 2014; Proudman and Baker 2021). The individual subtypes have some clearly defined roles in the cardiovascular system, in the genitourinary system and in the brain. α_{1A}-ARs are present in the smooth muscle of blood vessels (particularly in small densely innervated arteries). Agonist activation causes smooth muscle contraction, narrowing the blood vessel, leading to an increase in blood pressure (Akinaga et al. 2019). In the rodent heart, α_{1A}-AR activation appears protective and involves a number of factors including physiological hypertrophy, prevention of myocyte death, augmentation of positive inotropism, and induction of ischaemic preconditioning (O'Connell et al. 2014; Akinaga et al. 2019). α_{1A}-AR are present throughout the urogenital tract where activation mediates contraction in the ureter, bladder, urethra and prostate, and this subtype is also involved in the control of fertility in the male (White et al. 2013). α_{1A}-AR are also abundant in the brain where stimulation increases neurogenesis, learning and memory and improves mood (Akinaga et al. 2019). Although present in blood vessels, the physiological role of α_{1B}-AR is less clear. In the heart, α_{1B}-AR activation appears to cause maladaptive hypertrophy (O'Connell et al. 2014; Akinaga et al. 2019). Activation of brain α_{1B}-AR improves memory consolidation and exploratory activity and causes behavioural activation (Akinaga et al. 2019). α_{1D}-AR are present in blood vessels where activation causes contraction of large poorly innervated arteries, raising blood pressure and causing vessel hypertrophy (Akinaga et al. 2019).

Activation of α_1-ARs increases phospholipase C (PLC) activity to cause hydrolysis of phosphatidylinositol 4,5 bisphosphate (PIP_2) to diacylgycerol (DAG) and inositol 1,4,5 trisphosphate (IP_3). Whilst DAG remains associated with the cell membrane, IP_3 is released into the cytoplasm where it activates IP_3 receptors in the smooth endoplasmic reticulum to release Ca^{2+} to cause changes in cellular activity (Akinaga et al. 2019). Phospholipase A_2 is also activated by α_1-AR leading to increases in hydrolysis of phospholipids to arachidonic acid and lysophosphatidic acid. Arachidonic acid may be further processed to eicosanoids that are powerful modulators of inflammation. There are marked differences between α_1-AR subtypes in the efficacy with which they activate Ca^{2+} signalling with the rank order of

efficiency being α_{1A}- > α_{1B}- > α_{1D}-AR (Theroux et al. 1996). In addition to their effects on Ca^{2+} signalling α_1-ARs also activate MAP kinases including ERK, Jnk and p38 MAPK to cause increased DNA synthesis and promote growth and proliferation of cells. As with Ca^{2+} signalling the efficiency of coupling of the α_1-AR subtypes to MAP kinases varies from α_{1A}-AR that couple to ERK, Jnk and p38 MAPKs to α_{1B}-AR that couple to ERK and p38 MAPK whereas α_{1D}-AR appear to couple only to ERK (Zhong and Minneman 1999). α_1-AR agonists also act at α_{1A}- and α_{1B}-AR that couple to G_s to activate adenylyl cyclase to increase intracellular cAMP levels although the potency of all currently known compounds is much lower for this signalling pathway than for responses measured for Ca^{2+} or MAPK signalling in the same cells suggesting less efficient α_1-AR-G_s coupling (Horie et al. 1995; Obika et al. 1995; Proudman and Baker 2021).

7.2 Non-selective α_1-Adrenoceptor Agonists

Table 4 lists several α_1-AR agonists with their relative affinity (K_i) and potency (EC_{50}) measurements by receptor subtype. Non-selective agonists acting at α_1-AR have clinical uses (Table 1) notably in the management of hypotension associated with shock. The most widely used intravenous agents are the endogenous AR agonists noradrenaline and adrenaline and the non-selective α_1-AR agonist phenylephrine (Akinaga et al. 2019). Phenylephrine is also a common constituent of orally and nasally administered over-the-counter medicines for symptomatic relief of colds, coughs, flu, allergies, sinusitis and bronchitis, where vasoconstriction reduces mucosal oedema. However, they can produce many side effects including increased blood pressure, dizziness, headache and tachycardia (Akinaga et al. 2019), and there are significant problems of rebound hypercongestion if used for prolonged periods. Noradrenaline, adrenaline and phenylephrine are all high efficacy full agonists for Ca^{2+}, MAPK and cAMP signalling but do display some minor differences in profile. Generally, all are more efficacious at activating Ca^{2+} signalling than MAPK activation and show lower efficacy for increasing cAMP (Table 2). All three agonists display lower potency for Ca^{2+} signalling at the α_{1D}-AR (and no cAMP responses) although this may at least be partly explained by a lower cell surface expression of this subtype (Piascik and Perez 2001; Proudman and Baker 2021). Table 4 lists other agonists, roughly in order of ligand intrinsic efficacy.

7.3 Selective α_1-Adrenoceptor Agonists

Selective agonists exist for the α_{1A}-AR but currently not for the other two subtypes (Table 4). Oxymetazoline and xylometazoline are widely used topically as nasal decongestants, in eyedrops to cause mydriasis and to treat erythema in rosacea (Akinaga et al. 2019). Both compounds display significant α_{1A}-AR selectivity (driven by higher α_{1A}-AR selective affinity) with lower intrinsic efficacy than the catecholamines; they also have significant agonist activity at α_2-AR and $5HT_{1A/B/D}$

Table 4 α_1-AR agonists. Affinity (pK_i, measured from receptor binding) and potency of agonists (pEC_{50}) acting at primarily Gq-coupled human α_{1A}-,α_{1B}- and α_{1D}-adrenoceptors expressed in recombinant systems. Indications are given for efficacy although this will vary with the system being studied. Particular features of each agonist are noted in comments. FA = full agonist; PA = partial agonist; NR = no response

Ligand	Subtype	pK_i	pEC_{50} Ca^{2+}	pEC_{50} Erk1/2	pEC_{50} cAMP	Efficacy	Comments	Reference
Adrenaline	α_{1A}-AR	5.1–6.5	9.1	7.7	5.6	FA	Endogenous agonist	(Horie et al. 1995; Schwinn et al. 1995; Shibata et al. 1995; Proudman and Baker 2021)
	α_{1B}-AR	3.9–6.5	9.4	7.6	5.4	FA		
	α_{1D}-AR	5.3–7.3	7.7	6.7	NR	FA		
Noradrenaline	α_{1A}-AR	4.8–6.4	7.7–8.6	6.3–7.5	5.5–5.8	FA	Endogenous agonist	(Horie et al. 1995; Shibata et al. 1995; Taniguchi et al. 1999; Evans et al. 2011; da Silva Junior et al. 2017; Proudman and Baker 2021)
	α_{1B}-AR	3.8–6.2	9.2	7.6	5.5	FA		
	α_{1D}-AR	5.5–7.4	7.8	6.6	NR	FA		
Phenylephrine	α_{1A}-AR	4.9–5.3	7.0–8.3	6.6–7.9	4.9–5.6	FA	Non-selective α_1-AR	(Taniguchi et al. 1999; Evans et al. 2011; da Silva Junior et al. 2017; Proudman and Baker 2021)
	α_{1B}-AR	3.9	9.0	7.8	6.1	FA		
	α_{1D}-AR	4.7	7.2	6.2	NR	FA		
A61603	α_{1A}-AR	6.8	9.5–10.3	7.5–9.9	7.6–8.1	FA	Highly selective α_{1A}-AR	(Evans et al. 2011; da Silva Junior et al. 2017; Proudman and Baker 2021)
	α_{1B}-AR	<4	6.5	5.8	5.6	PA		
	α_{1D}-AR	3.9	5.3	<4	NR	PA		
Cirazoline	α_{1A}-AR	6.2–6.9	8.5–9.2	9.0	6.9	FA	Selective α_{1A}-AR	(Evans et al. 2011; Proudman and Baker 2021)
	α_{1B}-AR	5.1–6.0	8.1	6.9	6.9	PA		
	α_{1D}-AR	5.5–6.2	6.9	5.4	NR	PA		
Methoxamine	α_{1A}-AR	4.6–5.4	6.4–8.1	7.1–7.6	4.4–5.3	FA	Selective α_{1A}-AR	(Evans et al. 2011; Taniguchi et al. 1999; da Silva Junior et al. 2017; Proudman and Baker 2021)
	α_{1B}-AR	<3–4	6.6	5.6	<4	FA		
	α_{1D}-AR	3.8–5.0	5.4	5.1	NR	PA		

Oxymetazoline	α_{1A}-AR	7.2–8.2	8.9–9.3	7.2–9.0	NR,7.2	PA	Selective α_{1A}-AR, also agonist at α_2-AR, and at endogenous 5HT receptors expressed in CHO cells	(Horie et al. 1995; Obika et al. 1995; Shibata et al. 1995; Taniguchi et al. 1999; Evans et al. 2011; da Silva Junior et al. 2017; Proudman and Baker 2021)
	α_{1B}-AR	5.2–6.5	7.4	7.3	6.7	PA		
	α_{1D}-AR	5.3–6.4	5.6	7.3	NR	PA		
Xylometazoline	α_{1A}-AR	6.9	8.6	8.4	6.9	FA	Selective α_{1A}-AR, also agonist at α_2-AR, and at endogenous 5HT receptors expressed in CHO cells	(Proudman and Baker 2021)
	α_{1B}-AR	5.2	<4	6.7	NR	PA		
	α_{1D}-AR	5.2	<4	6.9	NR	PA		
Dobutamine	α_{1A}-AR	6.3	8.4	7.4	6.1	PA	Some selectivity α_{1A}-AR, β-AR agonist	(Baker 2010; Proudman and Baker 2021)
	α_{1B}-AR	5.4	<4	<4	NR	PA		
	α_{1D}-AR	5.4	7.6	6.1	NR	PA		
Dabuzalgron	α_{1A}-AR	7.4	7.5			PA	Selective α_{1A}-AR	(Blue et al. 2004)
	α_{1B}-AR	5.8	<5			PA		
	α_{1D}-AR	5.2	<5			PA		
NS-49	α_{1A}-AR	6.2	6.4			PA	Some selectivity α_{1A}-AR, higher efficacy α_{1A}-AR	(Obika et al. 1995)
	α_{1B}-AR	5.1	5.5			PA		
	α_{1D}-AR	5.4	5.4			PA		

receptors (da Silva Junior et al. 2017; Proudman and Baker 2021). A61603 is a highly selective α_{1A}-AR full highly efficacious agonist (again largely due to higher α_{1A}-affinity) that acts as a lower efficacy partial agonist at α_{1B}- and α_{1D}-AR (Evans et al. 2011; da Silva Junior et al. 2017; Proudman and Baker 2021). Dabuzalgron is a partial agonist with significant selectivity for α_{1A}-AR that has been suggested as a possible treatment for doxorubicin-induced cardiac failure (Beak et al. 2017).

7.4 Non-selective α_1-Adrenoceptor Antagonists

Table 5 lists several α_1-AR antagonists with their relative affinity measurements by receptor subtype. Non-selective α_1-AR antagonists were first used to control hypertension associated with catecholamine secretion from phaeochromocytoma (Table 1) (Spear and Griswold 1948). They became standard treatment and, together with β-blockers, greatly reduced the cardiovascular peri-operative mortality (Ross et al. 1967). Drugs used include the competitive antagonist phentolamine and the longer acting antagonist phenoxybenzamine (Ross et al. 1967; Proudman et al. 2020). Phentolamine is reasonably selective for α_1-AR, but phenoxybenzamine also blocks prejunctional α_2-AR and neuronal and extraneuronal uptake of catecholamines (Langer 1974). The effectiveness of these compounds for lowering blood pressure led to the development of α_1-AR antagonists with nanomolar affinity and fewer side effects including prazosin, terazosin and the more commonly used doxazosin that block all 3 subtypes with similar affinity (Table 5) (Proudman et al. 2020). Non-selective antagonists, e.g. prazosin, terazosin, doxazosin and indoramin, may be used to treat BPH but, as expected, also cause a reduction in blood pressure (particularly postural hypotension). Prazosin and increasingly doxazosin have been found useful in the management of PTSD (Raskind et al. 2000; Bajor et al. 2022), presumably by blocking CNS noradrenaline actions (Table 1). Carvedilol is a β-AR antagonist that is used to treat congestive heart failure but is also a non-selective α_1-AR antagonist (Table 5) (Proudman et al. 2020). This property has the potential to contribute to its therapeutic effectiveness by lowering peripheral resistance and the work load on the heart (O'Connell et al. 2014). However, clinical trials involving doxazosin suggest that other agents (e.g. thiazide diuretics) reduce cardiovascular deaths more and doxazosin appeared to increase heart failure (ALLHAT Collaborative Research Group 2000). Carvedilol has a similar beneficial effect to other β-antagonists in heart failure, suggesting that its β-antagonist properties rather than α-blockade are important in preventing deaths in cardiovascular disease (Baker and Wilcox 2017). Labetalol also has α_{1A}-affinity and is used in hypertension, particular in pregnancy.

The non-selective α_1-AR antagonists ^{125}I HEAT (BE2254) and ^{3}H prazosin are used to label α_1-AR (Schwinn et al. 1995; Shibata et al. 1995).

Table 5 α_1-AR antagonists. Affinity (pK_i, measured from receptor binding and pK_b measured from parallel shift of an agonist concentration–response curve) of antagonists acting at human α_{1A}-,α_{1B}- and α_{1D}-adrenoceptors expressed in recombinant systems. Indications are given for efficacy although this will vary with the system being studied. Particular features of each antagonist are noted in comments. NA = neutral antagonist; IA = inverse agonist; PA = partial agonist; NCA = non-competitive antagonist; (W) weak effect

Ligand	Subtype	pK_i	pK_b Ca^{2+}	pK_b IP	Efficacy	Comments	Reference
HEAT (BE2254)	α_{1A}-AR	8.6–9.9			IA	2-site binding to α_{1D}-AR	(Obika et al. 1995; Shibata et al. 1995; Maiga et al. 2013; da Silva Junior et al. 2017; Proudman et al. 2020)
	α_{1B}-AR	8.0–10.2			IA		
	α_{1D}-AR	8.1–9.5					
Prazosin	α_{1A}-AR	9.0–9.9		8.7	IA	Non-subtype-selective antagonist	(Shibata et al. 1995; Wetzel et al. 1995; Ford et al. 1997; Leonardi et al. 1997; Daniels et al. 1999; Williams et al. 1999; Hieble 2000; Proudman et al. 2020)
	α_{1B}-AR	8.7–9.9		9.6	NA		
	α_{1D}-AR	9.1–10.2		9.6	IA		
Silodosin	α_{1A}-AR	9.4–10.4	9.5		NA	Selective α_{1A}-AR	(Shibata et al. 1995; Quaresma et al. 2019; Proudman et al. 2020)
	α_{1B}-AR	6.5–7.7					
	α_{1D}-AR	6.9–8.7					
SNAP 5089	α_{1A}-AR	8.9–9.7			IA	Highly selective α_{1A}-AR	(Wetzel et al. 1995; Leonardi et al. 1997; Hieble 2000; Proudman et al. 2020)
	α_{1B}-AR	5.6–7.1			IA(W)		
	α_{1D}-AR	5.7–6.7					
Niguldipine	α_{1A}-AR	9.1–9.9		8.4	IA	Selective α_{1A}-AR	(Shibata et al. 1995; Wetzel et al. 1995; Ford et al. 1997; Proudman et al. 2020)
	α_{1B}-AR	6.3–7.7		6.6	IA(W)		
	α_{1D}-AR	5.9–7.4		6.7			
Tamsulosin	α_{1A}-AR	9.7–10.7	10.0	10.5	IA	2-site binding to α_{1D}-AR	(Shibata et al. 1995; Ford et al. 1997; Leonardi et al. 1997; Williams et al. 1999; Hieble 2000; Proudman et al. 2020)
	α_{1B}-AR	8.1–9.7	8.2	9.4	IA		
	α_{1D}-AR	9.2–10.2	9.8	9.8			
Terazosin	α_{1A}-AR	7.9–8.2			IA	Non-subtype-selective antagonist	(Wetzel et al. 1995; Leonardi et al. 1997; Hieble 2000; Proudman et al. 2020)
	α_{1B}-AR	8.0–8.7			IA		
	α_{1D}-AR	7.7–8.6					

(continued)

Table 5 (continued)

Ligand	Subtype	pK_i	pK_b Ca^{2+}	pK_b IP	Efficacy	Comments	Reference
RS-100329	α_{1A}-AR	9.6		9.6		Selective α_{1A}-AR, 2-site binding to α_{1D}-AR	(Williams et al. 1999; Proudman et al. 2020)
	α_{1B}-AR	6.7–7.5		7.8			
	α_{1D}-AR	7.6–7.9		7.9			
Ro 70-0004	α_{1A}-AR	8.9		8.6		Selective α_{1A}-AR	(Williams et al. 1999; Hieble 2000)
	α_{1B}-AR	7.1		6.7			
	α_{1D}-AR	7.2		7.1			
ρ-Da1a	α_{1A}-AR	9.2–9.3			NCA	Toxin – unsurmountable antagonist of binding. α_{1A}-AR selective	(Quinton et al. 2010; Maiga et al. 2013)
	α_{1B}-AR	7.3					
	α_{1D}-AR	6.0					
BMY-7378	α_{1A}-AR	6.4–6.6			IA/PA	Selective α_{1D}-AR, 2-site binding α_{1D}	(Hieble 2000; Proudman et al. 2020; Proudman and Baker 2021)
	α_{1B}-AR	6.2–7.0			IA		
	α_{1D}-AR	8.6–9.2			IA		
Carvedilol	α_{1A}-AR	8.4–8.6				β-blocker with α_1-activity	(Hieble 2000; Proudman et al. 2020)
	α_{1B}-AR	7.8–9.0					
	α_{1D}-AR	7.9–9.0					
Cyclazosin	α_{1A}-AR	7.5–8.9			IA	Off-target on α_2, D2, $5HT_{1A}$. 2-site binding to α_{1D}-AR	(Hieble 2000; Proudman et al. 2020)
	α_{1B}-AR	8.7–9.2			IA		
	α_{1D}-AR	7.6–9.9					
WB4101	α_{1A}-AR	9.0–9.8		8.9	IA	2-site binding α_{1D}	(Ford et al. 1997; Proudman et al. 2020)
	α_{1B}-AR	7.4–9.6			IA		
	α_{1D}-AR	8.6–9.0			NA		
Phentolamine	α_{1A}-AR	8.2–8.3			IA	2-site binding α_{1D}	(Leonardi et al. 1997; Proudman et al. 2020)
	α_{1B}-AR	6.6–7.6			IA		
	α_{1D}-AR	6.8–7.8			IA		
5-methyl urapidil	α_{1A}-AR	8.2–9.2		8.2	IA(W)	Selective α_{1A}-AR, weak IA	(Leonardi et al. 1997; Daniels et al. 1999; Ford et al. 1997; Proudman et al. 2020)
	α_{1B}-AR	6.1–7.7			IA(W)		
	α_{1D}-AR	5.6–8.0			IA		

7.5 Selective α_1-Adrenoceptor Antagonists

In an attempt to reduce the side effect of postural hypotension associated with the use of α_1-AR antagonists to treat BPH, subtype selective antagonists were developed that have also proved useful in obtaining a better understanding of the physiological role of individual subtypes. Compounds used mainly in laboratory studies include the potent and highly selective α_{1A}-AR antagonists SNAP 5089, Ro-70-0004 and RS-100329 (Table 5) (Proudman et al. 2020). Silodosin also has high α_{1A}-selectivity but has other unwanted side effects (Jung et al. 2017; Proudman et al. 2020). Tamsulosin was introduced as a prostate-selective antagonist but displays similar potency at α_{1A}- and α_{1D}-AR (Table 5). There are few selective antagonists for the α_{1D}-AR subtype. BMY-7378 displays some selectivity for α_{1D}-AR (Jung et al. 2017; Proudman et al. 2020) and has been used experimentally to examine the role of α_{1D}-AR (Table 3). There are no α_{1B} selective antagonists. In addition, many antidepressants and antipsychotics have significant α_{1A}-AR affinity, which may partly underlie their CNS actions, but also reflect their effects on blood pressure (particularly postural hypotension) that may explain the higher incidence of falls and fractures with these drugs (Proudman et al. 2020).

7.6 Irreversible or Non-competitive α_1-Adrenoceptor Antagonists

Phenoxybenzamine is an *N,N*-disubstituted-2-chloroethylamine containing a nitrogen mustard group that in aqueous solution at physiological pH cyclises to form ethyleniminium ions that covalently bind to a cysteine in TM3 of α-AR in a non-reversible reaction (Proudman et al. 2020). It has a multitude of off-target actions in addition to its interaction with α_1-AR (see Sect. 8.1). In contrast, ρ-Da1a is a three-finger fold toxin from green mamba venom that is a potent non-competitive antagonist showing some selectivity for the α_{1A}-AR (Quinton et al. 2010; Maiga et al. 2013). The toxin interacts with the human α_{1A}-AR orthosteric pocket and shares receptor interaction points with both antagonist ($F86^{2.64}$, $F288^{6.51}$ and $F312^{7.39}$) and agonist ($F288^{6.51}$ and $F312^{7.39}$) ligands.

7.7 Allosteric Modulators of α_1-Adrenoceptors

A number of compounds have been identified as negative allosteric modulators (NAMs) of α_1-AR. These include amiloride and a number of analogues such as 5-(N,N-hexamethylene) amiloride (HMA) (Leppik et al. 2000), 9-aminoacridines (Campbell et al. 2017) and bis(4-aminoquinolines) (Chen et al. 2022). All of these compounds increase the rate of dissociation of ^{3}H prazosin from α_{1A}-AR and act as non-competitive antagonists of noradrenaline (Leppik et al. 2000; Campbell et al. 2017; Chen et al. 2022). The 9-aminoacridines have similar effects on the α_{1B}-AR (Campbell et al. 2017).

8 α_2-Adrenoceptor Ligands

8.1 α_2-Adrenoceptor Location, Function and Signalling

All three α_2-AR subtypes are widely distributed in the periphery being present in the heart, blood vessels, lung, kidney, pancreas, gastrointestinal tract, adrenal gland, spleen and platelets but also in the brain (Table 2) (Perala et al. 1992; Eason and Liggett 1993; Proudman et al. 2022b). α_2-AR are important for control of blood pressure, analgesia, sedation, platelet aggregation and hypothermia (Proudman et al. 2022b). α_2-AR were known to be present in peripheral blood vessels where activation causes vasoconstriction; however, their major role in the CNS was discovered by accident in 1962 with clonidine (an α_2-AR agonist) that was developed originally as a nasal decongestant and topical vasoconstrictor. The trial physician allowed his secretary to self-administer a few drops of clonidine intranasally to alleviate her cold symptoms, and she became hypotensive and bradycardic and fell asleep for 24 h (Stahle 2000). Clonidine went on to be developed as an antihypertensive but also highlighted the CNS effects of α_2-AR agonists.

In the CNS, although originally identified and regarded as prejunctional autoreceptors, it rapidly became evident that α_2-AR are located both pre- and postjunctionally (Berthelsen and Pettinger 1977). A variety of techniques have been used to map the distribution of α_2-AR subtypes including autoradiography, in situ hybridisation (ISH) and immunohistochemistry. Autoradiography has the advantage of locating receptor protein but has limited resolution and is dependent on the selectivity of the ligands used. In situ hybridisation has the advantage of specificity, but mRNA does not necessarily co-locate with protein. Immunohistochemistry has the potential to provide high resolution, but the specificity of the antibodies used has to be carefully tested. ISH and autoradiographic studies point to α_{2A}-AR located in the locus coeruleus and other noradrenergic cell bodies involved in controlling sympathetic outflow, brainstem, cerebral cortex, hippocampus, septum, hypothalamic and amygdaloid nuclei and spinal cord (MacDonald et al. 1997).

More recent detailed studies in human prefrontal cortex show that the dominant receptors are α_{2A}-AR (87%) and are located post-synaptically, with the remaining α_{2C}-AR (13%) being located more evenly pre- and post-synaptically (60/40) (Erdozain et al. 2019). α_{2C}-AR are largely located in caudate putamen, olfactory tubercle, hippocampus and cerebral cortex (MacDonald et al. 1997). α_{2B}-AR are weakly expressed solely in the thalamus (Erdozain et al. 2019).

Activation of α_2-AR causes coupling to $G_{\alpha i/o}$ proteins and inhibition of adenylyl cyclase, inhibition of voltage-gated Ca^{2+} channels, increased Na^+/H^+ exchange and opening of K^+ channels (Limbird 1988; MacDonald et al. 1997). In some circumstances, α_2-AR can also couple to $G_{\alpha s}$ to produce increased adenylyl cyclase activity and cAMP accumulation (Eason et al. 1992; Eason and Liggett 1995). Coupling to $G_{\alpha s}$ is associated with high receptor expression and high efficacy agonists (Proudman et al. 2022a).

8.2 Non-selective α_2-Adrenoceptor Agonists

Although the endogenous agonists noradrenaline and adrenaline are full agonists acting at all three α_2-AR subtypes with similar potency, they do not have any known clinical actions associated with these effects (Jasper et al. 1998; Peltonen et al. 1998; Pihlavisto et al. 1998). Table 6 lists α_2-AR agonists. The non-selective partial agonist clonidine was one of the first α_2-AR agonists to be developed and is a centrally acting antihypertensive that alters baroreflex control to cause hypotension and bradycardia (Jasper et al. 1998; Peltonen et al. 1998; Pihlavisto et al. 1998). These properties are shared by two other non-selective α_2-AR partial agonists dexmedetomidine and xylazine, but these compounds are mainly used for their hypnotic, anxiolytic and analgesic effects. Dexmedetomidine is increasingly used in intensive care settings for sedation (without causing respiratory depression, including "awake cooperative sedation" enabling brain surgery in awake patients) and to reduce opiate consumption. In both palliative care and intensive care, it is used to reduce delirium, agitation and nausea (Nelson et al. 2003; Giovannitti et al. 2015; Weerink et al. 2017; Lee 2019; Gaertner and Fusi-Schmidhauser 2022). Xylazine has been used in veterinary medicine for >50 years for its analgesic and sedative effects in a variety of species including cats, dogs and horses and has been superseded to some extent by dexmedetomidine which is more potent (Table 6). An important advantage conferred by these compounds is that the sedation associated with their use is easily reversible (Barends et al. 2017). The selectivity of dexmedetomidine for α_2-AR is mainly associated with its high affinity for these subtypes (Proudman et al. 2022a). Brimonidine (UK14304) is a non-subtype-selective full agonist that has vasoconstrictor and anti-inflammatory properties that make it useful for the treatment of facial erythema in rosacea and glaucoma where it reduces aqueous humour production whilst increasing its outflow (Adkins and Balfour 1998; Jasper et al. 1998; Peltonen et al. 1998; Pihlavisto et al. 1998; Piwnica et al. 2014). Tizanidine is used for muscle spasm and muscle cramps and helps with spasticity (Giovannitti et al. 2015).

8.3 Selective α_2-Adrenoceptor Agonists

There are no truly subtype-selective α_2-AR agonists. A few compounds show marginal selectivity for one or more subtypes, but it is not clear how selectivity translates into therapeutic use. Guanabenz is a partial agonist that displays some α_{2A}-AR selectivity but also activates α_{2B}-AR and has no agonist actions in cell lines expressing low levels of α_{2C}-AR although it has affinity for this subtype (Table 6) (Jasper et al. 1998). It is used mainly as a centrally acting antihypertensive perhaps reflecting its actions on the two dominant α_2-AR subtypes in the CNS. Guanfacine is somewhat α_{2A}-AR selective and is mainly used in ADHD (Newcorn et al. 2022). Lofexidine is now used predominantly to treat the symptoms of opiate withdrawal (Urits et al. 2020). It has an interesting pharmacological profile appearing in GTPγs binding assays to be a selective agonist at α_{2B}-AR yet in binding studies displaying

Table 6 α_2-AR agonists. Affinity (pK_i, measured from receptor binding) and potency of agonists (pEC_{50}) acting at primarily Gi-coupled human α_{2A}-,α_{2B}- and α_{2C}-adrenoceptors expressed in recombinant systems. Indications are given for efficacy although this will vary with the system being studied. Particular features of each agonist are noted in comments. FA = full agonist; PA = partial agonist; PAM = positive allosteric modulator; NR = no response; BBB = blood brain barrier

Ligand	Subtype	pK_i	pEC_{50} GTPγS	pIC_{50} cAMP	pEC_{50} cAMP	pEC_{50} Erk	Efficacy	Comments	Reference
Adrenaline	α_{2A}-AR	3.7–5.8	6.8	6.5	5.5	8.0	FA	Endogenous agonist	(Jasper et al. 1998; Proudman et al. 2022a)
	α_{2B}-AR	3.6–5.2	6.2	7.6	6.3	7.5	FA		
	α_{2C}-AR	4.9–5.8	6.2	6.7	NR	7.6	FA		
Noradrenaline	α_{2A}-AR	3.6–5.7	6.0–6.7	6.6	5.3	7.7	FA	Endogenous agonist	(Jasper et al. 1998; Peltonen et al. 1998; Proudman et al. 2022a)
	α_{2B}-AR	3.5–6.0	6.1–6.6	7.8	6.9	7.8	FA		
	α_{2C}-AR	4.5–5.9	4.9–6.4	6.5	NR	7.7	FA		
Clonidine	α_{2A}-AR	6.7–7.2	7.6	8.2	6.4	9.0	PA	Non-subtype-selective partial agonist. Passes BBB	(Jasper et al. 1998; Peltonen et al. 1998; Proudman et al. 2022a)
	α_{2B}-AR	6.3–7.2	7.3	8.6	7.4	8.0	PA		
	α_{2C}-AR	6.6–6.9	6.0	7.5	NR	7.8	PA		
Dexmedetomidine	α_{2A}-AR	7.7–7.9	7.6–8.5	9.3	7.6	9.5	PA	Non-subtype-selective partial agonist. Passes BBB	(Jasper et al. 1998; Peltonen et al. 1998; Proudman et al. 2022a)
	α_{2B}-AR	7.5–7.7	8.1–8.5	10.9	9.9	9.2	FA		
	α_{2C}-AR	7.0–7.5	7.5–7.6	9.2	NR	9.6	PA-FA		
Xylazine	α_{2A}-AR	4.9–5.2	5.7	6.5	5.1	7.5	PA	Non-subtype-selective partial agonist. Passes BBB	(Jasper et al. 1998; Proudman et al. 2022a)
	α_{2B}-AR	5.2–5.5	5.7	7.7	6.4	7.4	PA		
	α_{2C}-AR	4.8–5.2	5.9	6.8	NR	7.1	PA		
Guanabenz	α_{2A}-AR	7.0–7.7	8.3	8.4	NR	9.1	PA	Somewhat α_{2A}-selective partial agonist. Passes BBB	(Jasper et al. 1998; Proudman et al. 2022a)
	α_{2B}-AR	6.0–6.6	7.0	9.0	7.9	8.5	PA		
	α_{2C}-AR	6.4	<5	7.5	NR	8.4	PA		

Guanfacine	α_{2A}-AR	6.6–7.3	7.3	8.0	6.5	9.0	PA	Somewhat α_{2A}-selective	(Uhlen et al. 1994; Jasper et al. 1998; Proudman et al. 2022a)
	α_{2B}-AR	5.6–6.0	6.5	8.7	7.6	8.5	PA		
	α_{2C}-AR	5.4–6.0	6.2	7.2	NR	8.0	PA		
Lofexidine	α_{2A}-AR	7.8–8.4	<5, 8.2[a]				NR/FA	Somewhat α_{2A}-selective	(Jasper et al. 1998; Diamanti et al. 2012)
	α_{2B}-AR	6.9–7.2	6.9–7.4				PA		
	α_{2C}-AR	6.8–7.2	<5, 8.9[a]				NR/FA		
Oxymetazoline	α_{2A}-AR	7.3–8.1	8.0	8.4	NR	#	PA	α_{2A}-selective, # = off-target agonist affects on α_1-AR and at endogenous 5HT receptors expressed in CHO cells	(Uhlen et al. 1994; Jasper et al. 1998; Proudman et al. 2022a)
	α_{2B}-AR	5.0–5.8	6.3	8.8	7.7	#	PA		
	α_{2C}-AR	6.4–6.7	6.7	7.4	NR	#	PA		
UK14304 (Brimonidine)	α_{2A}-AR	6.4–6.7	7.3–8.1	8.9	7.1	9.1	FA	α_{2A}-AR-selective full agonist	(Jasper et al. 1998; Peltonen et al. 1998; Proudman et al. 2022a)
	α_{2B}-AR	5.6–6.0	6.2–6.6	8.4	7.2	7.8	PA		
	α_{2C}-AR	5.7–6.1	6.2	8.0	NR	8.2	PA		
Naphazoline	α_{2A}-AR	7.0–7.7	<5	7.8	NR	8.7			(Jasper et al. 1998; Proudman et al. 2022a)
	α_{2B}-AR	5.8–6.4	6.7	8.8	7.6	8.3	PA		
	α_{2C}-AR	6.4	<5	7.1	NR	7.9	PA		
Xylometazoline	α_{2A}-AR	7.6–7.8	<5	8.1	NR	#	PA	# = off target agonist affects on α_1-AR and at endogenous 5HT receptors expressed in CHO cells	(Jasper et al. 1998; Proudman et al. 2022a)
	α_{2B}-AR	5.4–6.0	6.8	8.8	7.8	#	PA		
	α_{2C}-AR	6.9–7.0	<5	7.2	NR	#	PA		
Moxonidine	α_{2A}-AR	5.0		7.5	5.8	8.5	FA	High efficacy passes BBB	(Proudman et al. 2022a)
	α_{2B}-AR	4.6		7.5	6.5	7.1	FA		
	α_{2C}-AR	4.8		6.3	NR	6.9	FA		

(continued)

Table 6 (continued)

Ligand	Subtype	pK_i	pEC_{50} GTPγS	pIC_{50} cAMP	pEC_{50} cAMP	pEC_{50} Erk	Efficacy	Comments	Reference
Tizanidine	α_{2A}-AR	6.0		7.6	5.8	8.4	PA	High efficacy PA	(Proudman et al. 2022a)
	α_{2B}-AR	5.8		7.8	6.3	7.0	PA		
	α_{2C}-AR	5.8		6.5	NR	6.8	PA		
C10 – homobivalent 4-aminoquinoline	α_{2A}-AR	7.5					PAM?	Allosteric agonist. α_{2A}-AR selective. Biphasic effect on cAMP production	(Li et al. 2020)
	α_{2B}-AR	6.0							
	α_{2C}-AR	5.3							

[a] ECAR

high affinity for all 3 subtypes (Table 6) (Jasper et al. 1998; Diamanti et al. 2012). However when examined in another assay system, using the cytosensor microphysiometer to measure cell activity by extracellular acidification rate, lofexidine was a potent activator of all three α_2-AR subtypes (Diamanti et al. 2012). This suggested that lofexidine could produce its therapeutic actions by selective activation of α_{2B}-AR or alternatively by stimulating novel signal transduction pathways in all 3 subtypes. Oxymetazoline, a partial agonist, has α_{2A}-AR selectivity (compared to α_{2B} or α_{2C}) due to higher α_{2A}-AR affinity (Table 6), a property that may play a role in its use as a nasal decongestant and vasoconstrictor. However, as reported in Sect. 7.3, its affinity for α_{2A}-ARs is very similar to that for α_{1A}-ARs, and it also has significant 5HT receptor interaction. Xylometazoline is chemically related to oxymetazoline and is also used as a nasal decongestant and vasoconstrictor. It has α_{1A}- and α_{2A}-AR selectivity in receptor binding studies but when examined for effects on GTPγs binding, activity was only seen at α_{2B}-AR and not at the other α_2-AR subtypes (Table 6) (Jasper et al. 1998). Similar observations were made for cAMP responses (CRE-gene transcription) where oxymetazoline and xylometazoline appeared to have higher intrinsic efficacy at α_{2B}- compared to α_{2A}- and α_{2C}-AR compared to other α_2-AR agonists (Proudman et al. 2022a).

It is interesting to note that until recently most of the information available on α_2-AR agonists has been pK_i values obtained in binding studies with radiolabelled antagonists and pEC_{50} values obtained using GTPγs binding (Table 5). Such an approach may distort the apparent selectivity of the compounds studied. Determination of pK_i values of agonists in competition studies with labelled antagonists may be affected by the efficacy of the agonists, with low efficacy partial agonists being more effective competitors than comparable full agonists. The efficacy of an agonist at a particular subtype can therefore influence the pK_i value determined and therefore the apparent selectivity of the compound. This effect can be clearly observed from earlier studies in which an agonist ^{3}H clonidine was used to label α_2-AR where agonists compete for binding with much higher pK_i values than those obtained using antagonist ligands (U'Prichard et al. 1977; Jarrott et al. 1979). The potency of agonists in studies that determine pEC_{50} values will depend on both the affinity and efficacy of the agonist and is very system dependent (Proudman and Baker 2021). The pEC_{50} values provided in Table 6 give a practical guide to likely selectivity and potency, but it should be borne in mind that many of these figures have been obtained using almost exclusively GTPγs binding. Comprehensive comparisons of a wide range of α_2-AR agonists using a range of assay systems including modulation of cAMP levels, inhibition of Ca^{2+} channels, increased Na^+/H^+ exchange and opening of K^+ channels have until recently been largely lacking although viable high-throughput screening methods are now available (Storch et al. 2017). A recent study utilising human α_2-AR subtypes and examining pK_i in binding studies, inhibition of cAMP generation, increases in cAMP generation and ERK1/2 phosphorylation over a wide range of compounds has filled many of these knowledge gaps (Proudman et al. 2022a).

8.4 Non-selective α_2-Adrenoceptor Antagonists

Yohimbine, from the tree bark of the African Corynanthe yohimbe tree (*Pausinystalia johimbe*), is probably the oldest α_2-AR antagonist in "clinical" use – it has been used as an aphrodisiac for over a century and increases sexual behaviours and improves erectile dysfunction acting via both brain and penile α_2-AR (Proudman et al. 2022b; Morales 2001; Tam et al. 2001). Idazoxan was developed in the 1970s and whilst being selective for α_2-over α_1-AR had limited use due to substantial imidazoline receptor affinity (Brown et al. 1990; Michel et al. 1989b). However, its 2-methyl congener, RX821002, retained high α_2-AR affinity without binding to imidazoline sites (although some 5-HT affinity remains) (Clarke and Harris 2002; Miralles et al. 1993). Most α_2-AR antagonists currently available do not display marked subtype selectivity (Table 7) and yohimbine, its stereoisomer rauwolscine, RX821002 and MK912 are used experimentally often in ^{3}H labelled form to study α_2-AR (Proudman et al. 2022b; Jasper et al. 1998; Pihlavisto et al. 1998; MacLennan et al. 1997; Uhlen et al. 1994). Yohimbine, rauwolscine, RX821002, RS79948 and atipamezole all bind with high affinity to all 3 α_2-AR subtypes (Table 7) (Proudman et al. 2022b; Halme et al. 1995; Jasper et al. 1998; Pihlavisto et al. 1998; MacLennan et al. 1997; Uhlen et al. 1998; Haapalinna et al. 1997; Laurila et al. 2011). Atipamezole has high α_2-AR selectivity and is used to reverse the sedative and analgesic actions of dexmedetomidine in veterinary medicine (Table 7) (Haapalinna et al. 1997). Lisuride has high affinity for all 3 subtypes but also for α_{1A}- and α_{1D}-AR as well as many dopamine and 5HT receptor subtypes (Millan et al. 2002). Likewise, bromocriptine has reasonably high affinity for α_2-AR subtypes but even higher affinity for α_1-AR subtypes and significant activity at some dopamine and 5HT receptor subtypes (Millan et al. 2002). Although α_2-AR antagonism appears to be an important property of a number of drugs used to treat bipolar disorder and schizophrenia (Langer 2015) the affinity of these drugs for α_2-ARs is not as high as for α_{1A}-AR (Proudman et al. 2020, 2022b). The α_2-AR antagonist idazoxan has been shown to have beneficial effects both as monotherapy and as an add-on treatment, but this property has not been widely utilised (Langer 2015).

8.5 Selective α_2-Adrenoceptor Antagonists

BRL44408 is the most selective α_{2A}-AR ligand available being some 60-fold selective for α_{2A}- vs α_{2B}-AR but only ninefold selective for α_{2C}-AR (Table 7) (Proudman et al. 2022b). ARC239 displays some selectivity for α_{2B}-AR, but this is modest vs the α_{2A}-AR and marginal for the α_{2C}-AR (Proudman et al. 2022b). MK912 displays some selectivity for α_{2C}-AR (Uhlen et al. 1994), but this is only 13-fold for the α_{2A}- and 46-fold for the α_{2B}-AR (Table 7) (Proudman et al. 2022b). None of the antagonist ligands have a degree of selectivity that makes them useful for determining the physiological roles of α_2-AR subtypes in tissues that contain a

Table 7 α_2 -AR antagonists. Affinity (pK_i, measured from receptor binding) of antagonists acting at human α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors expressed in recombinant systems. Particular features of each antagonist are noted in comments. NA = neutral antagonist; IA = inverse agonist; PA = partial agonist; NCA = non-competitive antagonist; (W) weak effect

Ligand	Subtype	pK_i	Efficacy	Comments	Reference
Rauwolscine	α_{2A}-AR	8.4–9.0	IA	Antagonist with similar affinity for all 3 subtypes. Stereoisomer of yohimbine with similar uses. ^{3}H rauwolscine used to label α_2-AR	(Uhlen et al. 1994; MacLennan et al. 1997; Jasper et al. 1998; Laurila et al. 2011; Proudman et al. 2022b)
	α_{2B}-AR	8.3–9.0	IA		
	α_{2C}-AR	9.1–9.3			
Yohimbine	α_{2A}-AR	8.4–8.7	IA	Antagonist with similar affinity for all 3 subtypes. CNS stimulant	(Uhlen et al. 1994; MacLennan et al. 1997; Proudman et al. 2022b)
	α_{2B}-AR	7.7–8.4	IA		
	α_{2C}-AR	8.0–8.8	IA		
RX821002	α_{2A}-AR	8.1–9.4	IA	Antagonist with similar affinity for all 3 subtypes. ^{3}H RX821002 used to label α_2 -AR	(Uhlen et al. 1994; MacLennan et al. 1997; Pihlavisto et al. 1998; Proudman et al. 2022b)
	α_{2B}-AR	7.5–8.7	IA		
	α_{2C}-AR	8.1–8.8	IA		
MK 912	α_{2A}-AR	8.7–9.1		^{3}H MK 912 used to label α_2 -AR. Somewhat selective α_{2C}-AR antagonist	(Uhlen et al. 1994; Jasper et al. 1998; Proudman et al. 2022b)
	α_{2B}-AR	8.2–9.1	NA		
	α_{2C}-AR	9.8–10.2			
BRL44408	α_{2A}-AR	7.2–8.2		Selective α_{2A}-AR	(Uhlen et al. 1994; Proudman et al. 2022b)
	α_{2B}-AR	5.4–6.2			
	α_{2C}-AR	6.2–6.8			
Lisuride	α_{2A}-AR	9.0–10.3		Anti-Parkinsons drug with dopamine and 5HT receptor effects	(Millan et al. 2002; Proudman et al. 2022b)
	α_{2B}-AR	8.5–9.9			
	α_{2C}-AR	9.3–9.9			
Idazoxan	α_{2A}-AR	7.2–8.0	NA/IA	Antagonist of α_2 and imidazoline receptors	(MacLennan et al. 1997; Jasper et al. 1998; Laurila et al. 2011; Proudman et al. 2022b)
	α_{2B}-AR	6.4–7.6			
	α_{2C}-AR	6.6–7.7			
Phentolamine	α_{2A}-AR	7.3–7.7	IA	Non-selective α antagonist	(MacLennan et al. 1997; Jasper et al. 1998; Proudman et al. 2022b)
	α_{2B}-AR	6.7–7.5	NA		
	α_{2C}-AR	6.6–6.9			

(continued)

Table 7 (continued)

Ligand	Subtype	pK_i	Efficacy	Comments	Reference
Bromocriptine	α_{2A}-AR	8.0		Off-target dopamine receptor agonist	(Millan et al. 2002)
	α_{2B}-AR	7.5			
	α_{2C}-AR	7.6			
Atipamezole	α_{2A}-AR	8.5–8.7	NA	High affinity for all 3 subtypes. Main use to reverse dexmedetomidine	(Laurila et al. 2011; Proudman et al. 2022b)
	α_{2B}-AR	7.9–8.6	PA(W)		
	α_{2C}-AR	8.4–8.5			
RS79948	α_{2A}-AR	8.9–9.2		Antagonist with similar high affinity for all 3 subtypes	(Uhlen et al. 1998; Proudman et al. 2022b)
	α_{2B}-AR	8.6–9.3			
	α_{2C}-AR	9.1–9.4			
ARC239	α_{2A}-AR	5.8–6.0			(Uhlen et al. 1994; Laurila et al. 2011; Proudman et al. 2022b)
	α_{2B}-AR	6.8–7.7	NA		
	α_{2C}-AR	6.9–7.5			

mixture of subtypes. Although the use of α_2-AR subtype knockout mice may provide some answers to their roles, the problem of adaptation to the knockout often poses problems.

8.6 Allosteric Modulators of α_2-Adrenoceptors

The dissociation rate of antagonist radioligands such as ^{3}H yohimbine, ^{3}H rauwolscine and ^{3}H RX821002 from α_{2A}-AR (Leppik et al. 1998) or α_{2B}-AR (Wilson et al. 1991) is increased by amiloride and analogues. There is evidence that C10-homo bivalent 4-aminoquinoline acts as an allosteric agonist with some α_{2A}-AR-selective properties (Li et al. 2020), but a comprehensive profile has yet to be described.

9 β-Adrenoceptor Ligands

9.1 β-Adrenoceptor Location, Function and Signalling

β-AR subtypes are important therapeutic targets and are widely distributed both in peripheral organs and tissues and in the CNS (Table 3). They have major roles in the catecholamine-driven "fight or flight" response – including in the heart to increase cardiac output, lungs to maximise oxygen uptake and blood vessels to redistribute blood and essential metabolites to essential "fight or flight" organs such as skeletal muscle whilst minimising it to skin and bowels.

β_1-AR are the predominant β-AR in the human heart where they represent 59–86% of total β-AR (Heitz et al. 1983; Stiles et al. 1983; Vago et al. 1984; Golf et al. 1985; Bristow et al. 1986; Buxton et al. 1987). As well as being expressed in the myocardium, β_1-AR (and β_2-AR) are expressed in the conducting system in AV node, AV bundle and Purkinje tissue where they make up 50–80% of total β-AR present (Summers et al. 1987, 1989; Elnatan et al. 1994). β_1-AR activation appears responsible for increases in rate (chronotropy) and force of contraction (inotropy) of the heart as well as increased conduction through the AV node (dromotropy) and relaxation of the myocardium during diastole (lusitropy) (Kaumann 1997). In the coronary arteries activation of β_1-AR causes relaxation (Bylund et al. 1994). Other peripheral tissues with significant populations of β_1-AR include the kidney, where β_1-AR stimulation increases renin release by activating receptors on the JGA (see chapter on "Adrenoceptors in the Lower Urinary Tract" in this volume), adipose tissue and salivary gland. In the brain, β_1-AR are present in the cerebral cortex, hippocampus, amygdala, pineal, putamen and accumbens (see chapter on "Locus Coeruleus and Noradrenergic Pharmacology in Neurodegenerative Disease" in this volume).

β_2-AR have a wide distribution in the body. In the periphery, β_2-AR are expressed in the lung, arteries and tongue together with the heart (Summers et al. 1989), adipose tissue, bone marrow, spleen, gall bladder, skeletal muscle, liver and adipose

tissue (Summers et al. 1987; Kim et al. 1991; Nevzorova et al. 2002; Evans et al. 2019). In the lung, catecholamine release causes stimulation of β_2-AR that produces marked bronchodilation, a property mimicked by the β-AR agonists used in the treatment of asthma and COPD. In arteries and veins, β_2-AR stimulation causes vasodilatation and, in the heart, positive inotropic and chronotropic responses. Activation also increases glucose uptake and anabolic effects in skeletal muscle and glycogenolysis and gluconeogenesis in the liver (Rizza et al. 1980; Barth et al. 2007; Sato et al. 2014). In bone marrow and spleen, β_2-AR are associated with modulation of immune functions and in adipose tissue influence lipolysis (Evans et al. 2019; Sharma and Farrar 2020). High expression of β_2-AR also occurs in reproductive tissues in the male with significant expression in the penis, prostate and epididymis and in the female in breast, vagina, placenta and uterus (see chapter on "Adrenoceptors in the Lower Urinary Tract" in this volume) where functions are more obscure but likely involve relaxation of smooth muscle. In the brain, β_2-AR are highly expressed in the substantia nigra and hippocampus with more modest expression in the amygdala, thalamus, locus coeruleus and spinal cord (see chapter on "Locus Coeruleus and Noradrenergic Pharmacology in Neurodegenerative Disease" in this volume). In the hippocampus, β_2-AR located on astrocytes promote glucose uptake and glutamate production and stimulation promotes memory consolidation (Gibbs and Summers 2005; Catus et al. 2011).

β_3-AR have a localised distribution in humans being present in urinary and gall bladder, with lower expression in fat, intestine and brain (Table 3) (Uhlen et al. 2015). In the urinary bladder, β_3-AR activation relaxes the detrusor muscle and increases bladder capacity, whereas in gastrointestinal tissues β_3-AR mediate relaxation (Roberts et al. 1997). In females, high concentrations of β_3-AR are expressed in the ovary, fallopian tubes, uterine endometrium and placenta (Uhlen et al. 2015). In rodents, β_3-AR have other important roles and are highly expressed in rodent white (WAT) and brown (BAT) adipose tissue where they mediate lipolysis and thermogenesis, respectively (Cannon and Nedergaard 2004; Nedergaard et al. 2007; Evans et al. 2019), but the expression and function of β_3-AR in these tissues in humans is less important (Schena and Caplan 2019). There have been many attempts to target β_3-AR as a potential treatment for human obesity but so far without success (Arch 2011).

Agonist binding to β-AR causes increased coupling to $G_{\alpha s}$, release of $G_{\beta\gamma}$ subunits, activation of adenylyl cyclase with generation of cAMP and activation of PKA. With continued stimulation, β_1 and β_2-AR are phosphorylated by G protein receptor kinases (GRKs) leading to recruitment of β-arrestins, uncoupling from G proteins and receptor internalisation, causing inhibition of cAMP signalling and desensitisation. In contrast, β_3-AR largely lack the GRK phosphorylation sites on the C-terminus and are resistant to desensitisation. β-arrestin recruitment activates other signalling pathways including ERK1/2, activation of Ca^{2+}/CAMKII and EGFR transactivation (Wang et al. 2017). In addition to coupling to $G_{\alpha s}$ all three subtypes can also couple to $G_{\alpha i/o}$ proteins (Li et al. 2004) to modulate cAMP generation and produce smooth muscle relaxation via cGMP (Li et al. 2004). Coupling of β-AR to $G_{\alpha i/o}$ proteins also releases $G_{\beta\gamma}$ subunits that activate ERK1/2 independently of

β-arrestins (Collins 2011) and the β_3-AR can activate ERK1/2 by recruitment of Src-kinase to prolines in the third intracellular loop and C-terminus (Collins 2011). In some tissues where this has been studied, subcellular distribution of β_1- and β_2-AR is also important in terms of the response observed and the effect of drugs. In cardiomyocytes, β_2-AR are confined to caveolae and lipid rafts, whereas β_1-AR are also found in intracellular compartments (Xiang 2011). Stimulation of β_2-AR produces cAMP that is confined to t-tubules, whereas β_1-AR stimulation also produces cAMP at the cell surface (Nikolaev et al. 2006). The cell permeability of agonists and antagonists therefore influences the response observed (Wang et al. 2021).

There are also other examples of β-AR subtypes coupling to other pathways by mechanisms that are yet to be fully resolved. In some cells, β_2-AR stimulation leads to Ca^{2+} release from intracellular stores following activation of PLC and IP_3 receptors but not the canonical pathways involving Gαs, $G_{\alpha i/o}$ or cAMP (Galaz-Montoya et al. 2017). In skeletal muscle, the increase in glucose uptake caused by β_2-AR activation involves activation of mTORC2 that can be achieved with only minor increases in cAMP and without desensitisation (Mukaida et al. 2019).

9.2 Non-selective β-Adrenoceptor Agonists

Table 8 lists β-agonists with their affinity (pK_i) and potency (pEC_{50}) measurements at the different β-AR subtypes. The endogenous agonists noradrenaline and adrenaline are full agonists at all 3 β AR subtypes (Table 8). Noradrenaline is somewhat selective for β_1-AR (due to slightly increased β_1-AR affinity) and is used clinically by slow intravenous infusion to increase blood pressure associated with shock mainly utilising its actions on α_1-AR although actions on β-AR help maintain cardiac output. Adrenaline has a similar pharmacological profile but with slightly higher β_2-AR affinity is somewhat selective for β_2-AR. It is also used by infusion in shock to support blood pressure, by intravenous bolus during cardio-pulmonary resuscitation following cardiac arrest and intramuscularly for the treatment of anaphylaxis in order to reduce throat swelling, cause bronchodilation and maintain heart function and blood pressure.

Isoprenaline is a non-selective β-AR agonist (Table 8), with little effect on α-AR. Although originally developed for use in asthma, its clinical use is now restricted to infusion in cases of bradycardia or heart block as a bridge prior to insertion of a permanent pacemaker. Zinterol is active across all 3 subtypes but most potent at β_2- and β_3-AR (due to β_2-AR selective affinity). Carazolol is a potent partial agonist in most experimental situations that is used to reduce stress during transportation of animals where it is often characterised as an antagonist.

Table 8 β-AR agonists. Affinity (pK_i, measured from receptor binding) and potency of agonists (pEC_{50}) acting at primarily Gs-coupled human β_1-, β_2- and β_3-adrenoceptors expressed in recombinant systems. Indications are given for efficacy although this will vary with the system being studied. Particular features of each agonist are noted in comments

Ligand	Subtype	pK_i	pEC_{50} cAMP	pEC_{50} β-arr2	Efficacy	Comments	Reference
Noradrenaline	β_1-AR	5.4–6.0	7.9		FA	Endogenous agonist	(Frielle et al. 1988; Isogaya et al. 1999; Hoffmann et al. 2004; Baker 2010a; van Wieringen et al. 2013; Littmann et al. 2015; Woo et al. 2019; De Pascali et al. 2022)
	β_2-AR	3.5–5.4	5.4–6.4	4.7–5.2	FA		
	β_3-AR	4.4–5.5	7.2		FA		
Adrenaline	β_1-AR	5.2–6.0	7.6		FA	Endogenous agonist	(Frielle et al. 1988; Hoffmann et al. 2004; Baker 2010a; van Wieringen et al. 2013; Littmann et al. 2015; Woo et al. 2019; De Pascali et al. 2022)
	β_2-AR	5.4–6.1	6.8–7.9	6.6–6.9	FA		
	β_3-AR	3.9–4.7	6.5		FA		
Isoprenaline	β_1-AR	6.1–7.0	8.0–8.6		FA	Non-subtype-selective β-AR agonist	(Frielle et al. 1988; Hoffmann et al. 2004; Takasu et al. 2007; Baker 2010a; Procopiou et al. 2010; Littmann et al. 2015; Woo et al. 2019; De Pascali et al. 2022)
	β_2-AR	6.2–6.7	7.2–8.7	7.2–7.3	FA		
	β_3-AR	5.4–5.8	7.2–7.4		FA		
RO 363	β_1-AR	7.7–8.0			FA	β_1-AR selective	(Molenaar et al. 1997b; Sugimoto et al. 2002)
	β_2-AR	5.8–6.1					
	β_3-AR	4.5	5.5		PA		
Xamoterol	β_1-AR	7.0–7.2			PA	β_1-AR selective	(Isogaya et al. 1999; Baker 2005a)
	β_2-AR	5.9–6.1			PA		
	β_3-AR	4.5					
Denopamine	β_1-AR	6.1	7.7		FA	Somewhat β_1-AR selective	(Isogaya et al. 1999; Baker 2010)
	β_2-AR	5.8	5.7		PA		
	β_3-AR	5.3	6.3		PA		

Dobutamine	β_1-AR	5.2–5.5	6.8		FA		(Isogaya et al. 1999; Baker 2010a; De Pascali et al. 2022)
	β_2-AR	5.3–5.9	6.3	–	PA		
	β_3-AR	5.1	6.4		FA		
Fenoterol	β_1-AR	4.9–5.0	7.5		FA	β_2-AR selective	(Baker 2010a; Baker et al. 2015; Littmann et al. 2015; Woo et al. 2019; De Pascali et al. 2022)
	β_2-AR	5.5–7.0	6.1–8.9	6.9–7.3	FA		
	β_3-AR	5.4	7.6		FA		
Formoterol	β_1-AR	5.6–6.5	7.0–8.3		PA/FA	β_2-AR selective, long acting	(Isogaya et al. 1999; Battram et al. 2006; Baker 2010a; Beattie et al. 2010; Aparici et al. 2012; Baker et al. 2015; Littmann et al. 2015)
	β_2-AR	7.6–8.6	8.6–10.1	8.0	FA		
	β_3-AR	5.0–5.8	7.6–9.2		FA		
Indacaterol	β_1-AR	6.2–6.9	6.6–8.7		PA	β_2-AR selective, ultra long acting	(Battram et al. 2006; Aparici et al. 2012; Slack et al. 2013)
	β_2-AR	7.4–7.9	8.1–9.5		FA/PA		
	β_3-AR	5.4–5.5	6.7–8.8		FA		
Salbutamol	β_1-AR	4.7–5.6	5.9–6.3		PA	Somewhat β_2-AR selective	(Isogaya et al. 1999; Battram et al. 2006; Hutchinson et al. 2006; Baker 2010a; Slack et al. 2013; Baker et al. 2015; Littmann et al. 2015; De Pascali et al. 2022)
	β_2-AR	5.6–6.3	6.3–7.7	6.3–7.4	FA/PA		
	β_3-AR	4.0–4.6	4.8–6.3		FA		
Terbutaline	β_1-AR	3.9–4.5	5.8		FA	Somewhat β_2-AR selective	(Baker et al. 2003d; Hoffmann et al. 2004; Baker 2010a)
	β_2-AR	4.8–5.5	7.1–7.3		FA		
	β_3-AR	3.7–4.1	5.9		FA		
Salmeterol	β_1-AR	5.4–6.1	6.9–7.2		FA	β_2-AR selective, long acting	(Baker et al. 2003d; Hoffmann et al. 2004; Baker 2005a; Battram et al. 2006; Baker 2010a; Beattie et al. 2010; Aparici et al. 2012; Woo et al. 2019)
	β_2-AR	6.8–9.3	8.2–9.9	7.6–8.1	FA		
	β_3-AR	5.1–6.3	6.0–7.6		FA		

(continued)

Table 8 (continued)

Ligand	Subtype	pK_i	pEC_{50} cAMP	pEC_{50} β-arr2	Efficacy	Comments	Reference
Clenbuterol	β_1-AR	6.6–6.7	7.3		FA	β_2-AR selective, banned by International Olympic Committee and World Anti-Doping Agency	(Hutchinson et al. 2006; Baker 2010a; Baker et al. 2015)
	β_2-AR	7.4–7.9	9.2		FA		
	β_3-AR	5.4	4.5–6.2		PA		
Ractopamine	β_1-AR	7.0	8.7		FA	Promoter of lean muscle mass used in farming	(Kern et al. 2009; Baker 2010a; De Pascali et al. 2022)
	β_2-AR	6.6–6.9	7.6–7.8	4.4	PA		
	β_3-AR	5.8	7.0		PA		
Zinterol	β_1-AR	6.0	7.2		FA	β_2-AR selective but active across all 3 subtypes	(Hutchinson et al. 2006; Sato et al. 2008; Baker 2010a; Littmann et al. 2015)
	β_2-AR	8.0	9.5	7.7	FA		
	β_3-AR	6.3–7.1	8.1–8.6		FA		
Carazolol	β_1-AR	9.7	9.2		PA	Active across all 3 subtypes	(Gerhardt et al. 1999; Baker 2010a)
	β_2-AR	10.5	9.8		PA/A		
	β_3-AR	8.4–8.7	8.2–8.8		FA/PA		
Solabegron	β_1-AR		<5–5.9			Selective β_3-AR agonist	(Hicks et al. 2007; Takasu et al. 2007; Igawa and Michel 2013; Michel and Korstanje 2016)
	β_2-AR		<5–5.4				
	β_3-AR		7.6–8.4				
Mirabegron	β_1-AR		4.9			Selective β_3-AR agonist	(Takasu et al. 2007; Vrydag et al. 2009; Hatanaka et al. 2013; Igawa and Michel 2013; Dehvari et al. 2020)
	β_2-AR		5.2				
	β_3-AR	7.3	7.7–9.0				
Vibegron	β_1-AR		<5			Selective β_3-AR agonist	(Edmondson et al. 2016; Di Salvo et al. 2017; Brucker et al. 2022)
	β_2-AR		<5				
	β_3-AR		8.7–9.0				

CGP12177	β_1-AR	8.8–9.6	7.6–8.2[a]		PA	β_3-AR agonist and partial agonist at β_1-/β_2-AR (activates secondary confomation of β_1-AR)	(Pietri-Rouxel and Strosberg 1995; Cohen et al. 1999; Baker et al. 2003a; Joseph et al. 2004a; Baker 2005a; Baker et al. 2014; Soave et al. 2016)
	β_2-AR	8.4–10.0	9.4		PA		
	β_3-AR	7.0	5.9–6.9		PA		
BRL37344	β_1-AR	5.2–5.8	4.9–7.0		FA	Somewhat rodent selective β_3-AR agonist. Non-selective at human β-AR	(Blin et al. 1994; Strosberg 1997; Hoffmann et al. 2004; Takasu et al. 2007; Baker 2010a; Littmann et al. 2015)
	β_2-AR	5.0–6.5	6.4–6.9	6.3	FA/PA		
	β_3-AR	6.4–6.5	6.3–7.8		PA		
CL316243	β_1-AR	3–3.1			A	Rodent-selective β_3-AR agonist	(Strosberg 1997; Gerhardt et al. 1999; Yanagisawa et al. 2000; Baker 2005a; Hutchinson et al. 2006)
	β_2-AR	3.7–4.1			A		
	β_3-AR	4.9–5.2	4.3–7.2		PA		
L755507	β_1-AR	6.2	7.6		FA	Selective β_3-AR agonist	(Sato et al. 2008; Baker 2010a)
	β_2-AR	6.8	7.1		PA/A		
	β_3-AR	7.9–8.6	10.1–12.3		FA		
L748337	β_1-AR	5.4	6.2		PA	Selective β_3-AR partial agonist / antagonist	(Sato et al. 2008; Baker 2010a; van Wieringen et al. 2013)
	β_2-AR	6.5	6.4		PA		
	β_3-AR	8.0–8.7	8.4–9.1		PA/A		

FA full agonist, *PA* partial agonist, *PAM* positive allosteric modulator, *NR* no response

[a] Agonist response at higher doses at secondary site

9.3 Selective β-Adrenoceptor Agonists

An enormous amount of research has gone into developing subtype-selective β-AR agonists as the receptors are high value therapeutic targets in the treatment of asthma and COPD. Because of the important role the β_1-AR plays in controlling the rate and force of the heart a great deal of effort initially went into developing β_2-selective agonists. Compounds such as RO363 are used experimentally to examine the physiological roles of β_1-AR but do not have the pharmacokinetic properties suitable for clinical use. Xamoterol has more appropriate PK properties and was trialled for the treatment of heart failure being a β_1-AR partial agonist that provided cardiac stimulation yet could block the deleterious effects of high plasma levels of endogenous catecholamines associated with this condition. Unfortunately, prolonged stimulation of β_1-AR with this compound clearly worsened heart failure and increased mortality so this approach was not successful (The Xamoterol in Severe Heart Failure Study Group 1990; Cruickshank 1993). Xamoterol, denopamine and dobutamine have varying degrees of β_1-AR selectivity (Table 8) and were previously used over short periods to maintain cardiac function.

Selective β_2-AR agonists on the other hand are one of the most successful groups of drugs so far developed and are used to treat asthma and COPD. Prior to their development, sympathomimetics or catecholamines with actions at β_2-AR were used but side effects such as anxiety, tachycardia, tremor and sweating were all too apparent (Billington et al. 2017). The first β_2-AR-selective agonist to be developed, salbutamol, although still short acting (4–6 h) was an improvement on the catechol-O-methyltransferase-sensitive catecholamines and due to its selectivity displayed fewer side effects. In most systems, salbutamol is a partial agonist but has good efficacy in the lung due to high β_2-AR expression in the bronchial smooth muscle. Salbutamol and similar short-acting β_2-AR agonists such as terbutaline (Table 8) are usually given by inhalation as required to provide rapid but short-term relief and their partial agonist properties and intermittent nature of use make desensitisation of the bronchodilator response unlikely.

In attempts to improve nocturnal symptoms (without the need for repeat nocturnal dosing), the longer acting compounds salmeterol and formoterol with actions up to 12 h were developed. Salmeterol (salbutamol headgroup with a long hydrocarbon chain) was found to be extremely β_2-selective due to high selective affinity for the β_2-AR. The hydrocarbon chain binds into a unique exosite on the β_2-AR (at top of TM6 and EL3) whilst the headgroup then enters the orthosteric site (Baker et al. 2015; Masureel et al. 2018). The molecular reasons for the β_2-AR-selective affinity and longer duration of action of formoterol remain unknown. Subsequently, even longer acting compounds such as indacaterol, olodaterol and vilanterol were introduced with a duration of action of up to 24 h and are used in COPD (Battram et al. 2006). For a full history of the development of β_2-AR ligands, their different pharmacological features and their clinical uses and side effects, see chapter on "Asthma and COPD" in this volume.

For all selective β_2-AR agonists, generation of cAMP and activation of PKA is believed to play an important role in bronchodilation. However, the cell types

involved may not be confined to smooth muscle as β_2-AR in lung are also located on epithelial cells, submucosal glands, vascular endothelium and inflammatory cells including mast cells, macrophages and eosinophils (Billington et al. 2017). Since PKA phosphorylates a wide variety of substrates that influence smooth muscle tone, many other mechanisms may be involved in the response to β_2-AR agonists (for details, see chapter on "Asthma and COPD" in this volume and Billington et al. 2017). CREB also regulates cell growth so β_2-AR agonists may also reduce the muscle hypertrophy associated with asthma. There is also evidence suggesting that Epac (exchange protein directly regulated by cAMP) plays a role independently of PKA and could represent another pathway (Billington et al. 2017). Long-term exposure of β_2-AR to full agonists such as isoprenaline causes recruitment of GRKs, phosphorylation of the receptor and interaction with β-arrestins to activate other pathways and receptor internalisation (Nobles et al. 2011), but there is little evidence that the inhaled β_2-agonists used today for asthma and COPD have any clinical issues with desensitisation or tachyphylaxis.

Another effect of β-AR agonists is promotion of the growth of skeletal muscle (Kim et al. 1992) that is utilised to promote increased weight and leanness in livestock utilising compounds such as ractopamine. However, this compound shows little in the way of selectivity for β_2-AR (Table 8). Clenbuterol, which displays some β_2-AR selectivity (Table 8), was popular amongst body-builders as well as in the farming industry. However, significant human harm from ingesting meat from clenbuterol-treated animals has been observed. Clenbuterol is now only used to treat COPD in horses and is listed as a banned substance by the International Olympic Committee and World Anti-Doping Agency (WADA), and meat monitoring programmes are in place. Given these issues, β_2-AR agonist use in elite athletes remains restricted to inhaled salbutamol, salmeterol and formoterol even though there is evidence to suggest that clenbuterol when used under controlled conditions may have potential for the treatment of type II diabetes (Sato et al. 2014; Kalinovich et al. 2020). The relationship between glucose and β_2-AR agonists is complex. Whilst β_2-AR activation increases hepatic gluconeogenesis and glycogenolysis to increase plasma glucose as part of the fight or flight response, it also promotes glucose uptake into skeletal muscle, as part of the same response. More recent studies show that the dual β_2-/β_3-AR agonist BRL37344 that is a weak partial agonist for cAMP accumulation when acting at β_2-AR in skeletal muscle acts as a full agonist for glucose uptake without recruitment of β-arrestin or desensitisation (Mukaida et al. 2019). In vivo BRL37344 (or its esterified pro-drug BRL35135) acting at β_2-AR also improved glucose tolerance in humans and in rats and in β_3-AR knockout mice (Mitchell et al. 1989; Cawthorne et al. 1992; Mukaida et al. 2019).

The β_3-AR was originally known as the atypical β-AR and was characterised by low affinity for most β-AR antagonists (Arch and Kaumann 1993). This characteristic was matched by the recognition that in tissues that displayed this property, functional responses were selectively produced by atypical β-AR agonists. This led to the atypical β-AR being identified as a target for the treatment of obesity by compounds such as BRL37344 developed by the team at Beecham Pharmaceuticals (Arch et al. 1984). However, although this compound and congeners were very

effective in treating obesity in rodent models, they proved to have very low efficacy in humans. Subsequently, it became clear that many of the properties of the atypical β-AR could be explained by the β_3-AR (Emorine et al. 1989), and it was recognised that human and rodent receptors displayed distinct differences in pharmacology. Agonists displaying high affinity and selectivity for the human β_3-AR such as L755507 and L748337 were developed (Table 8) (Sato et al. 2008; Baker 2010a) and have proved very useful in laboratory studies. The failure of the β_3-AR to present as a viable target for obesity led to examination of other possibilities, and the identification of high levels of β_3-AR mRNA in bladder together with a relaxation response to β_3-AR agonists suggested that they could be useful for the treatment of overactive bladder syndrome (Michel and Korstanje 2016). Mirabegron, solabegron and vibegron are all highly selective agonists at the human β_3-AR (Table 8) and have been successfully introduced as treatments for this condition (Michel and Korstanje 2016). The development of these human β_3-AR selective compounds has also recreated interest in the possible metabolic effects of these compounds (Dehvari et al. 2020).

9.4 Biased Agonism at the β_2-Adrenoceptor

Biased agonism (or ligand-directed signalling) has been reported at the β_2-AR such that the responses observed cannot be explained by a linear signalling cascade. Many ligands originally considered as β-AR antagonists have been found to have inverse agonist or partial agonist activity. These compounds display their inverse or partial agonist properties across all signalling cascades studied. Propranolol, however, has been shown to reduce cAMP signalling, thus acting as an inverse agonist whilst at the same time stimulating other signalling responses such as ERK1/2 phosphorylation or cAMP response element (CRE)-gene transcription (Azzi et al. 2003; Baker et al. 2003b). Several other ligands acting at β-AR, such as carvedilol, have been reported to be biased agonists at the β_2-AR, but there is significant controversy and heterogeneity between studies (Wisler et al. 2007; Benkel et al. 2022). It may be that for some compounds, signalling bias is a tissue-dependent phenomenon and will depend on the precise scaffold and signalling molecules expressed in that tissue. The possible clinical implications of biased signalling at the β-AR are currently uncertain as biased and unbiased β-AR antagonists appear equally effective in clinical settings (e.g. bisoprolol, metoprolol and carvedilol in heart failure).

9.5 β-Adrenoceptor Antagonists: Classification – Cardioselectivity and Intrinsic Sympathomimetic Activity

β-AR antagonists (β-blockers) were first developed in the 1960s in a focused programme to generate drugs for hypertension and heart disease (Baker et al. 2011c). Ligands that activated β-AR were modified until compounds were discovered that bound but were unable or less able to generate agonist responses

(antagonists). Propranolol was one of the first drugs to be developed and remains in clinical use today (Black et al. 1965). Cardioselectivity is a term frequently used but its meaning varies between users. Firstly, cardioselective has been used in the past to describe ligands with more cardiac (β) than vasodilatory (α) effects. Secondly, when β-agonists were developed for asthma (1940s +) and β-AR antagonists developed for hypertension and heart disease (1960s +) they were often referred to as "bronchoselective" or "cardioselective" respectively (Harms 1976; Prichard 1988). Thus, bronchoselective referred to a greater bronchodilatation relative to cardiac stimulation. These terms came into being before the recognition that different populations of β-AR existed (first proposed in 1967). Although β_1-AR make up the majority of β-AR in the heart, there is also a significant population of β_2-AR (Summers et al. 1987, 1989; Elnatan et al. 1994; Kaumann 1997; Molenaar et al. 2000). In this context, cardioselectivity means heart vs lung selectivity and therefore does not equate to β_1-AR selectivity, and indeed, many β-blockers in clinical use have poor β_1-AR selectivity (Table 9). Finally, others have used the term cardioselectivity to mean β_1-selectivity. However even if highly β_1-selective clinical antagonists were available, they would not block all cardiac β-AR given the significant number of β_2-AR present in the heart. However, such drugs would be useful in producing a degree of cardiac β-AR blockade without antagonism of the effects of β_2-AR agonists in the lung (Baker et al. 2017). In contrast, non-selective β-blockers are effective in antagonising all cardiac β-AR but have the unwanted side effect of inhibition of the bronchodilator actions of β_2-AR agonists in the lung. As the different subtypes are now well known, and subtype selectivity well documented, it may be time to retire the confusing term "cardioselectivity" and use subtype selectivity instead.

ISA is another term that was widely used in conjunction with clinical β-AR antagonists. The term was originally used to describe drugs that inhibited the β-AR agonist actions of high intrinsic efficacy agonists such as adrenaline and noradrenaline but still provided some "sympathetic activity" in relation to a small increase in heart rate or blood pressure when given alone. ISA (a description at the animal level) and partial agonism as described in molecular pharmacological studies are closely related (Kenakin 1982; Joseph et al. 2003; Baker et al. 2011b). In clinical studies in heart failure and ischaemic heart disease, drugs with ISA, including xamoterol, bucindolol, alprenolol and pindolol, were not found to beneficial and in some cases were actually harmful (The Xamoterol in Severe Hart Failure Study Group 1990; Cruickshank 1993). It seems that β-AR antagonists lacking agonist actions are the most beneficial in heart failure.

9.6 Non-selective β-Adrenoceptor Antagonists

Table 9 lists several β-antagonists together with their affinity values at the different human β-AR subtypes. The classification of β-AR antagonists was originally made on the basis of their actions at β_1- and β_2-AR, and almost all non-selective $\beta_{1/2}$-AR antagonists have low affinity for the β_3-AR. They also have a variety of

Table 9 Affinity (pK_i, measured from receptor binding) antagonists (and pEC_{50} for partial agonists) acting at human β_1-, β_2- and β_3-adrenoceptors expressed in recombinant systems. Many of these ligands (originally developed as antagonists) have significant partial agonist activity as denoted by PA. Indications are given for efficacy where appropriate although this will vary with the system being studied. Particular features of each agonist are noted in comments. NA = neutral antagonist; IA = inverse agonist; PA = partial agonist; (W) weak effect

Ligand	Subtype	pK_i	pEC_{50} cAMP	Efficacy	Comments	Reference
Cyanopindolol	β_1-AR	10.1–10.4	9.6	PA	Non-selective -widely used as a ^{125}I radioligand for β-ARs. Biphasic β_1-AR agonist response	(Hoffmann et al. 2004; Baker 2010a; Sykes and Charlton 2012; Sato et al. 2015)
	β_2-AR	10.3–11.4	10.0	PA		
	β_3-AR	8.4–9.5	8.8	PA		
CGP12177	β_1-AR	8.8–9.6	7.6–8.2[a]	NA/PA	Non-selective for β_1–/β_2-AR, hydrophilic ^{3}H radioligand used for labelling intact cells. β_3-AR agonist. β_1-AR and human β_3-AR secondary conformation agonist	(Pietri-Rouxel and Strosberg 1995; Cohen et al. 1999; Baker et al. 2003a; Joseph et al. 2004a; Baker 2005a, b, c; Baker et al. 2014; Soave et al. 2016; Baker et al. 2002)
	β_2-AR	8.4–10.0	9.4	NA/PA		
	β_3-AR	7.0	5.9–6.9	PA		
Carazolol	β_1-AR	9.7–10.2	9.2	PA	High potency at all subtypes. Marked partial agonist properties especially at β_3-AR. Biphasic β_1-AR agonist response	(Pietri-Rouxel and Strosberg 1995; Strosberg 1997; Baker 2010a; Sato et al. 2015)
	β_2-AR	9.9–10.5	9.8–10.1	NA/PA		
	β_3-AR	8.4–8.7	7.9–8.8	PA/FA		
Labetalol	β_1-AR	7.6–8.2	6.4	PA	Combined α_1- and β-AR antagonist	(Baker et al. 2003a; Baker et al. 2003b; Baker 2005a)
	β_2-AR	8.0	8.1	PA		
	β_3-AR	6.2				
Pindolol	β_1-AR	8.6–9.7	8.8	PA	Biphasic β_1-AR agonist response	(Baker et al. 2003b; Joseph et al. 2004a; Baker 2010a)
	β_2-AR	9.2	9.0–9.1	PA		
	β_3-AR	7.1	7.4	PA		
SR59230A	β_1-AR	7.5–8.4	8.0	PA	Non-selective but reasonable potency at β_3-AR	(Candelore et al. 1999; Baker 2010a; Michel et al. 2010)
	β_2-AR	8.5–9.3	8.1	NA/PA		
	β_3-AR	7.4–8.4	7.6	PA		
Carvedilol	β_1-AR	8.8–9.8	7.6	PA/IA	Non-selective for β_1-/β_2-AR, also α_1-AR antagonist. Biphasic β_1-AR agonist response	(Candelore et al. 1999; Baker et al. 2003a; Baker et al. 2003b; Joseph et al. 2004a; Baker 2005a; Baker et al. 2017)
	β_2-AR	9.4–10.6	9.1	PA		
	β_3-AR	8.3–9.4				

S(−) propranolol	β_1-AR	7.9–8.7	7.1	NA/PA/ IA(W)	Marginally selective for β_2−/β_1-AR, low affinity for β_3-AR. Biased agonist at β_2-AR	(Pietri-Rouxel and Strosberg 1995; Baker et al. 2003a; Baker et al. 2003b; Joseph et al. 2004a; Baker 2005a; Soave et al. 2016)
	β_2-AR	9.1–9.2	NR/9.5	NA/IA		
	β_3-AR	6.8–6.9	5.8	NA/PA		
Timolol	β_1-AR	8.3–9.0				(Baker et al. 2003b; Joseph et al. 2004a; Baker 2005a)
	β_2-AR	8.9–9.7		IA		
	β_3-AR	6.8				
Bupranolol	β_1-AR	7.3–9.4	8.8	NA/PA		(Pietri-Rouxel and Strosberg 1995; Candelore et al. 1999; Baker et al. 2003a; Baker et al. 2003b; Joseph et al. 2004a; Baker 2005a)
	β_2-AR	8.3–9.9	NR/9.6	NA/IA		
	β_3-AR	6.8–7.3	NR	NA		
Practolol	β_1-AR	6.1			Marginally selective for β_1- vs β_2-AR	(Baker 2005a)
	β_2-AR	5.0				
	β_3-AR	<4				
Nebivolol	β_1-AR	9.1–9.2	9.0	NA/PA	Marginally selective for β_1- vs β_2-AR	(Baker 2010a; Frazier et al. 2011; Baker et al. 2017)
	β_2-AR	7.9–8.0	NR	A		
	β_3-AR	5.7–7.0	NR	A		
Metoprolol	β_1-AR	7.0–7.9		IA	Marginally selective for β_1- vs β_2-AR	(Candelore et al. 1999; Baker et al. 2003b; Hoffmann et al. 2004; Joseph et al. 2004a; Baker 2005a)
	β_2-AR	5.2–7.2	7.2	IA		
	β_3-AR	5.0–5.2				
Atenolol	β_1-AR	6.4–7.6	6.7	NA/PA	Marginally selective for β_1- vs β_2-AR	(Baker et al. 2003a; Baker et al. 2003b; Hoffmann et al. 2004; Joseph et al. 2004a; Baker 2005a)
	β_2-AR	5.1–6.6	6.5	IA		
	β_3-AR	4.1–4.2				
7-methyl cyanopindolol	β_1-AR	10.4			Inverse agonist used to determine β_1-AR structure	(Sato et al. 2015)
	β_2-AR	10.4				
	β_3-AR					

(continued)

Table 9 (continued)

Ligand	Subtype	pK_i	pEC_{50} cAMP	Efficacy	Comments	Reference
CGP20712A	β_1-AR	7.8–9.6		IA	Highly selective (1,000-fold) for β_1- vs β_2-AR	(Candelore et al. 1999; Baker et al. 2003b; Baker et al. 2017; Hoffmann et al. 2004; Baker 2005a; Sato et al. 2015; Soave et al. 2016)
	β_2-AR	5.4–6.1	NR	NA		
	β_3-AR	5.2–5.7				
NDD-713	β_1-AR	7.8–8.5			β_1-AR selective, devoid of ISA, off-target, and toxicology issues – good distribution, metabolism and elimination	(Baker et al. 2017)
	β_2-AR	5.1–5.4				
	β_3-AR	5.5				
NDD-825	β_1-AR	8.3–9.0			β_1-AR selective, devoid of ISA, off-target, and toxicology issues – good distribution, metabolism and elimination	(Baker et al. 2017)
	β_2-AR	5.3–5.8				
	β_3-AR	5.7				
ICI118551	β_1-AR	6.5–7.2			Most selective β_2-AR antagonist available	(Baker et al. 2003b; Baker 2005a; Sato et al. 2015; Baker et al. 2017)
	β_2-AR	9.1–9.4	9.1	IA		
	β_3-AR	6.4				
Butoxamine	β_1-AR	4.9	5.6	PA	Somewhat β_2-AR selective	(Baker 2010a)
	β_2-AR	6.2	6.4	NA/PA		
	β_3-AR	<4	NR	NA		
L-748337	β_1-AR	5.4–6.4	6.2	PA	β_3-AR selective, 2-site binding at β_3-AR	(Candelore et al. 1999; Baker 2010a; van Wieringen et al. 2013)
	β_2-AR	6.5–6.7	6.4	PA		
	β_3-AR	8.0–8.6	8.4	PA		

characteristics that make them useful in particular experimental or clinical situations. Thus, cyanopindolol has extremely high affinity at β_1- and β_2-AR and about 100-fold lower (but still high) affinity for β_3-AR (Table 9). It acts as a partial agonist at all three subtypes (Baker 2010a) and is widely used in radioiodinated form as a radioligand to study β-ARs in homogenate binding (Isogaya et al. 1999; Louis et al. 1999; Joseph et al. 2004a; Sykes and Charlton 2012; Sato et al. 2015) and autoradiographic studies (Summers et al. 1989; Molenaar et al. 1990; Elnatan et al. 1994; Roberts et al. 1995). The derivative 7-methyl cyanopindolol is an inverse agonist that has been used to determine the β_1-AR structure (Sato et al. 2015). CGP12177 has a similar selectivity profile but, at the β_1-AR and human β_3-AR, acts as an antagonist at the catecholamine conformation whilst stimulating an agonist response at the secondary conformation (see Sect. 3.1). At the β_2-AR, it is a weak partial agonist of the single catecholamine conformation (Kaumann 1997; Kaumann et al. 1998; Baker et al. 2003a; Joseph et al. 2004b). It has partial agonist actions at the human β_3-AR, apparently via a secondary conformation (Pietri-Rouxel and Strosberg 1995; Cohen et al. 1999; Baker 2005c;). ^{3}H CGP12177 is used as a radioligand and is hydrophilic making it particularly useful for labelling cell surface receptors in intact cells (Baker 2005a, 2010a). Propranolol, bupranolol and timolol have similar pharmacological profiles having nanomolar affinity at β_1- and β_2-AR and 2–3 orders of affinity lower at β_3-AR (Pietri-Rouxel and Strosberg 1995; Candelore et al. 1999; Louis et al. 1999; Baker et al. 2003a; Joseph et al. 2004a; Baker 2005a; Soave et al. 2016). Carazolol has a similar selectivity profile and is often classified as a high affinity antagonist but displays quite marked partial agonist properties in many systems (Pietri-Rouxel and Strosberg 1995; Sabio et al. 2008; Baker 2010a; Sato et al. 2015) (Tables 8 and 9). SR59230A is widely described in the literature as a β_3-AR-selective antagonist but in fact displays little selectivity. Whilst it has relatively high affinity for β_3-AR compared to other β-AR antagonists, it has similar or somewhat higher affinity for β_1- and β_2-AR (Table 9) and also displays partial agonist properties at the β_3-AR (Hutchinson et al. 2005; Sato et al. 2007; Michel et al. 2010). Studies using this compound and particularly those carried out in tissues that contain mixed populations of subtypes need to be carefully evaluated (Michel et al. 2010). Carvedilol and labetalol also display the pattern of relatively high affinity for β_1- and β_2-AR and lower affinity for β_3-AR (Table 9). However, they also have α_1-AR affinity (Table 5).

9.7 Subtype-Selective β-Adrenoceptor Antagonists

β-AR antagonists that selectively blocked β_1-AR were developed on the basis that this subtype was mainly involved in controlling the rate and force of the heart (Black et al. 1965) and renin release from the JGA in the kidney (do Vale et al. 2019). They were initially found to be useful for the treatment of high blood pressure and angina and subsequently for migraine, anxiety, benign essential tremor, glaucoma and hyperthyroidism (Baker 2005a). β_1-AR blockade may also be useful in ameliorating stroke-associated neuroinflammation by blocking neutrophil β_1-ARs (Clemente-

Moragon et al. 2022). As mentioned above, the β_1-AR-selective affinity of most clinically used β-antagonists is poor. This translates into the likelihood of a degree of β_2-AR blockade when used clinically, and bronchospasm is a common side effect of these drugs (Baker 2005a); hence they remain contraindicated in those with asthma. Given the high cardiovascular risk in people with COPD, and the poor airway reversibility in COPD compared to asthma, β-AR antagonists are used with caution as secondary prevention in those who have already had a cardiac event and have COPD (Quint et al. 2013; Lipworth et al. 2016; Baker and Wilcox 2017; Rasmussen et al. 2020; Davis et al. 2023). Highly selective β_1-AR antagonists such as CGP20712A (Table 9) (Dooley et al. 1986) have been very useful for laboratory studies but interact with other receptors and have never been developed for clinical use (Dooley et al. 1986; Baker et al. 2017). More recently, β_1-AR antagonists such as NDD-713 and NDD-825 with good selectivity and PK, no partial agonist, off-target effects or toxicology issues have been developed that may eliminate cardiovascular and lung problems associated with current drugs (Baker et al. 2017) (Table 9).

Selective β_2-AR antagonists have no current clinical uses but are useful in laboratory studies of the role of this β-AR subtype. Butoxamine was the first to be identified but displays only modest selectivity for β_2-AR. ICI118551 (O'Donnell and Wanstall 1980) has proved to be a much more useful tool with selectivity for β_2-AR compared to β_1- and β_3-AR of 200–500 and has been widely used (Table 9) (Summers et al. 1987, 1989; Molenaar et al. 1990; Elnatan et al. 1994; Roberts et al. 1995, 1997; Kompa and Summers 1999; Hoffmann et al. 2004; Baker 2005a). Its selectivity is also evident in human studies (Tattersfield and Cragg 1983). In cancer, there is increasing interest in the potential of β_1- and β_2-AR antagonists to reduce both the growth of the primary tumour and reduce metastatic spread with evidence from laboratory and epidemiological studies that propranolol and carvedilol may be effective, particularly with regard to β_2-AR antagonism (Sloan et al. 2010; Kim-Fuchs et al. 2014; Udumyan et al. 2017; Wagner et al. 2018; Gillis et al. 2021; Lofling et al. 2022).

The only β_3-AR-selective antagonist for which reasonable evidence exists is L 748337 that displays 100–400-fold selectivity vs β_1-AR and 40–50-fold selectivity vs β_2-AR (Table 9) (Candelore et al. 1999; Baker 2010a). It has been used as the radioligand ^{3}H L 748337 to identify and characterise β_3-AR in tissues (van Wieringen et al. 2013). An interesting characteristic is that it displays higher affinity for human than rat β_3-AR putting it in a range that is promising for the identification of β_3-AR protein expression in human tissues (van Wieringen et al. 2013). In recombinant systems, L 748337 displays partial agonist properties (Baker 2010a).

9.8 Irreversible β-Adrenoceptor Antagonists

Irreversible (slowly reversible) antagonists such as the bromoacetylated derivative of pindolol (BIM) and alprenolol (BAAM) have been used to covalently bind amino acids and block β-ARs (Jasper et al. 1988; Molenaar et al. 1988). BIM is selective for β-AR vs α_1- and α_2-AR and has nM affinity with preincubation with 10 nM BIM

inactivating about 70% of receptors whilst not affecting the properties of the remainder (Jasper et al. 1988). In guinea pig left atria, BIM produced rightward shifts of concentration–response curves to isoprenaline with a reduction in maximal response and in guinea pig left atrial membranes reduced the number of ^{125}I CYP binding sites without changes in the K_d (Molenaar et al. 1988). BAAM likewise has long-lasting effects and "irreversibly" blocked 90% β-AR within 4 h injection, with receptor levels taking up to a month to recover (Pitha et al. 1982; Kuenzel et al. 1983) and causing a reduction in blood pressure and heart rate that lasted for longer than 48 h, but with little evidence for blood–brain barrier permeability.

10 Conclusions and Future Developments

The nine ARs are one of the most studied groups of GPCRs. They are important targets for a wide variety of drugs used to treat many different diseases. The development of techniques to study adrenoceptors including functional studies (in vivo and in vitro), radioligand binding techniques and receptor cloning have often been adapted to study other GPCRs. The β_2-AR in particular is often regarded as the prototypical GPCR. These techniques initially led to the identification of the 3 subgroups of ARs α_1, α_2 and β and to the exploration of the therapeutic possibilities of targeting an individual subgroup. Agonists acting at α and β-AR, including catecholamines, are used in the treatment of hypotension associated with shock. Compounds with more α_1-AR activity such as phenylephrine, oxymetazoline and xylometazoline are used as nasal decongestants. Amongst antagonists, non-selective α_1-AR antagonists remain important in the management of blood pressure and BPH and have important roles in PTSD and phaeochromocytoma. The identification of three α_1-AR subtypes, α_{1A}, α_{1B}, α_{1D}, led to the development of some compounds selective for the α_{1A}-AR subtype for the treatment of BPH. However, although effective in reducing BPH there are still significant postural hypotension side effects associated with these drugs.

α_2-AR agonists were initially developed as centrally acting anti-hypertensive agents, but in recent years they have been increasingly used for a wide range of other conditions. Relatively non-selective agonists such as tizanidine are used as anti-spasmodics and others such as lofexidine to treat opiate withdrawal and dexmedetomidine primarily for their sedative and analgesic properties. α_{2A}-AR-selective agonists such as guanabenz are centrally acting anti-hypertensives, whereas guanfacine is effective in ADHD and brimonidine in glaucoma. There are no selective α_2-AR antagonists currently in use therapeutically, although a number of second-generation anti-psychotics such as risperidone and paliperidone include α_2-AR antagonism in a broad spectrum of pharmacological activity.

Of the three major subgroups of ARs, the β-AR have been the most widely studied and targeted therapeutically. However given all these years of development and clinical use, it is interesting that so few highly subtype-selective molecules have made it into regular clinical use – the highly selective β_2-AR agonists including salmeterol, formoterol and vilanterol for asthma and COPD and the β_3-AR agonists

mirabegron, solabegron and vibegron for overactive bladder are the exceptions. Potential future improvements in β_2-AR agonists for the treatment of asthma and COPD are discussed in detail (see chapter on "Asthma and COPD" in this volume). β_1-AR agonists such as denopamine and dobutamine had limited and very specific uses to maintain the function of the failing heart short term as long-term use (>few days) is associated with cardiac toxicity. β-AR antagonists, on the other hand, are used in a wide range of conditions including cardiac arrhythmias, heart failure, anxiety, ischaemic heart disease and hypertension. Some of the β_1-AR antagonists have some intrinsic sympathomimetic activity (partial agonism), and clinical studies have shown that agents without ISA are safer long term. Also, whilst some drugs are described as β_1-AR selective, the majority in clinical use display only modest selectivity and show significant blockade of β_2-AR that limits their use, particularly in patients with asthma. Whilst development of highly β_1-AR-selective antagonists for heart disease would reduce β_2-AR-mediated side effects and thus improve safety of these drugs in patients with concomitant asthma, they are not likely to improve the overall cardiac/clinical effectiveness of these compounds. Indeed, as catecholamine activation of cardiac β_2-AR would remain unopposed, they may be slightly less effective. However, they would be considerably safer in those with asthma, thus, for the first time, making the life-saving properties of this class of drugs an option for those with heart disease and asthma. Possibly one of the biggest changes in drugs acting at β-AR on the horizon is not the development of highly selective agents but the potential for improvement in the outcomes of cancer, with widespread use of well-known, well-tolerated and cheaply available β-AR antagonists.

Recent studies have made it clear that many of the AR subtypes couple to multiple G proteins, may have G protein independent actions and display complex signalling profiles (Littmann et al. 2015; da Silva Junior et al. 2017; Woo et al. 2019; Proudman and Baker 2021; De Pascali et al. 2022). The receptors are expressed in a very wide variety of cell types, and the level and pattern of expression determines the type of response observed following stimulation of the sympathetic nervous system. Although for β-AR clinical drug responses so far seem to be through typical catecholamine conformation Gs-cAMP-medicated pathways, the subtypes involved in certain α-AR physiological responses and potential signalling cascades involved are less certain. For α-AR, there are few highly selective drugs (α_{1A}-AR agonists and antagonists being the exception but these have not found specific clinical uses). There clearly is scope for the development of α_1- and α_2-AR subtype-selective agents, and with their development, it may be that clinical uses beyond those already established for non-selective agents may come to light.

References

Abiko LA, Dias Teixeira R, Engilberge S, Grahl A, Muhlethaler T, Sharpe T et al (2022) Filling of a water-free void explains the allosteric regulation of the beta1-adrenergic receptor by cholesterol. Nat Chem 14(10):1133–1141

Adkins JC, Balfour JA (1998) Brimonidine. A review of its pharmacological properties and clinical potential in the management of open-angle glaucoma and ocular hypertension. Drugs Aging 12(3):225–241

Ahlquist RP (1948) A study of the adrenotropic receptors. Am J Physiol 153(3):586–600

Ahn S, Kahsai AW, Pani B, Wang QT, Zhao S, Wall AL et al (2017) Allosteric "beta-blocker" isolated from a DNA-encoded small molecule library. Proc Natl Acad Sci U S A 114(7): 1708–1713

Ahn S, Pani B, Kahsai AW, Olsen EK, Husemoen G, Vestergaard M et al (2018) Small-molecule positive allosteric modulators of the beta2-adrenoceptor isolated from DNA-encoded libraries. Mol Pharmacol 94(2):850–861

Akinaga J, Garcia-Sainz JA, Pupo AS. (2019) Updates in the function and regulation of alpha1 -adrenoceptors. Br J Pharmacol 176(14):2343–2357

Albinana V, Recio-Poveda L, Gonzalez-Peramato P, Martinez-Pineiro L, Botella LM, Cuesta AM (2022) Blockade of beta2-adrenergic receptor reduces inflammation and oxidative stress in clear cell renal cell carcinoma. Int J Mol Sci 23(3)

ALLHAT Collaborative Research Group (2000) Major cardiovascular events in hypertensive patients randomized to doxazosin vs chlorthalidone: the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). JAMA 283(15):1967–1975

Altosaar KBP, Bond RA, Bylund DB, Cotecchia S, Devost D, Doze VA, Eikenburg DC, Gora S, Goupil E, Graham RM, Hébert T, Hieble JP, Hills R, Kan S, Machkalyan G, Michel MC, Minneman KP, Parra S, Perez D, Sleno R, Summers R, Zylbergold P (2019) Adrenoceptors (version 2019.3) in the IUPHAR/BPS guide to pharmacology database. IUPHAR. https://doi.org/10.2218/gtopdb/F4/2021.3

Aparici M, Gomez-Angelats M, Vilella D, Otal R, Carcasona C, Vinals M et al (2012) Pharmacological characterization of abediterol, a novel inhaled beta(2)-adrenoceptor agonist with long duration of action and a favorable safety profile in preclinical models. J Pharmacol Exp Ther 342(2):497–509

Arch JR (2004) Do low-affinity states of beta-adrenoceptors have roles in physiology and medicine? Br J Pharmacol 143(5):517–518

Arch JR (2011) Challenges in beta(3)-adrenoceptor agonist drug development. Ther Adv Endocrinol Metab 2(2):59–64

Arch JR, Kaumann AJ (1993) Beta 3 and atypical beta-adrenoceptors. Med Res Rev 13(6):663–729

Arch JR, Ainsworth AT, Cawthorne MA, Piercy V, Sennitt MV, Thody VE et al (1984) Atypical beta-adrenoceptor on brown adipocytes as target for anti-obesity drugs. Nature 309(5964): 163–165

Azzi M, Charest PG, Angers S, Rousseau G, Kohout T, Bouvier M et al (2003) Beta-arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G protein-coupled receptors. Proc Natl Acad Sci U S A 100(20):11406–11411

Bajor LA, Balsara C, Osser DN (2022) An evidence-based approach to psychopharmacology for posttraumatic stress disorder (PTSD) – 2022 update. Psychiatry Res 317:114840

Baker JG (2005a) The selectivity of beta-adrenoceptor antagonists at the human beta1, beta2 and beta3 adrenoceptors. Br J Pharmacol 144(3):317–322

Baker JG (2005b) Site of action of beta-ligands at the human beta1-adrenoceptor. J Pharmacol Exp Ther 313(3):1163–1171

Baker JG (2005c) Evidence for a secondary state of the human beta3-adrenoceptor. Mol Pharmacol 68(6):1645–1655

Baker JG (2008) Antagonist affinity measurements at the Gi-coupled human histamine H3 receptor expressed in CHO cells. BMC Pharmacol 8:9

Baker JG (2010a) The selectivity of beta-adrenoceptor agonists at human beta1-, beta2- and beta3-adrenoceptors. Br J Pharmacol 160(5):1048–1061

Baker JG (2010b) A full pharmacological analysis of the three turkey β-adrenoceptors and comparison with the human β-adrenoceptors. PLoS ONE 5(11):e15487. https://doi.org/10.1371/journal.pone.0015487

Baker JG, Wilcox RG (2017) Beta-blockers, heart disease and COPD: current controversies and uncertainties. Thorax 72(3):271–276

Baker JG, Hall IP, Hill SJ (2002) Pharmacological characterization of CGP 12177 at the human beta (2)-adrenoceptor. Br J Pharmacol 137(3):400–408

Baker JG, Hall IP, Hill SJ (2003a) Agonist actions of "beta-blockers" provide evidence for two agonist activation sites or conformations of the human beta1-adrenoceptor. Mol Pharmacol 63(6):1312–1321

Baker JG, Hall IP, Hill SJ (2003b) Agonist and inverse agonist actions of beta-blockers at the human beta 2-adrenoceptor provide evidence for agonist-directed signaling. Mol Pharmacol 64(6):1357–1369

Baker JG, Hall IP, Hill SJ (2003c) Pharmacology and direct visualisation of BODIPY-TMR-CGP: a long-acting fluorescent beta2-adrenoceptor agonist. Br J Pharmacol 139(2):232–242

Baker JG, Hall IP, Hill SJ (2003d) Influence of agonist efficacy and receptor phosphorylation on antagonist affinity measurements: differences between second messenger and reporter gene responses. Mol Pharmacol 64(3):679–688

Baker JG, Hall IP, Hill SJ (2004) Temporal characteristics of cAMP response element-mediated gene transcription: requirement for sustained cAMP production. Mol Pharmacol 65(4):986–998

Baker JG, Middleton R, Adams L, May LT, Briddon SJ, Kellam B et al (2010) Influence of fluorophore and linker composition on the pharmacology of fluorescent adenosine A1 receptor ligands. Br J Pharmacol 159(4):772–786

Baker JG, Proudman RG, Tate CG (2011a) The pharmacological effects of the thermostabilising (m23) mutations and intra and extracellular (beta36) deletions essential for crystallisation of the Turkey beta-adrenoceptor. Naunyn Schmiedebergs Arch Pharmacol 384(1):71–91

Baker JG, Kemp P, March J, Fretwell L, Hill SJ, Gardiner SM (2011b) Predicting in vivo cardiovascular properties of beta-blockers from cellular assays: a quantitative comparison of cellular and cardiovascular pharmacological responses. FASEB J 25(12):4486–4497

Baker JG, Hill SJ, Summers RJ (2011c) Evolution of beta-blockers: from anti-anginal drugs to ligand-directed signalling. Trends Pharmacol Sci 32(4):227–234

Baker JG, Proudman RG, Hill SJ (2013) Impact of polymorphic variants on the molecular pharmacology of the two-agonist conformations of the human beta1-adrenoceptor. PLoS One 8(11):e77582

Baker JG, Proudman RG, Hill SJ (2014) Identification of key residues in transmembrane 4 responsible for the secondary, low-affinity conformation of the human beta1-adrenoceptor. Mol Pharmacol 85(5):811–829

Baker JG, Proudman RG, Hill SJ (2015) Salmeterol's extreme beta2 selectivity is due to residues in both extracellular loops and transmembrane domains. Mol Pharmacol 87(1):103–120

Baker JG, Gardiner SM, Woolard J, Fromont C, Jadhav GP, Mistry SN et al (2017) Novel selective beta1-adrenoceptor antagonists for concomitant cardiovascular and respiratory disease. FASEB J 31(7):3150–3166

Baker JG, Fromont C, Bruder M, Thompson KSJ, Kellam B, Hill SJ et al (2020) Using esterase selectivity to determine the in vivo duration of systemic availability and abolish systemic side effects of topical beta-blockers. ACS Pharmacol Transl Sci 3(4):737–748

Barends CR, Absalom A, van Minnen B, Vissink A, Visser A (2017) Dexmedetomidine versus midazolam in procedural sedation. A systematic review of efficacy and safety. PloS One 12(1): e0169525

Barth E, Albuszies G, Baumgart K, Matejovic M, Wachter U, Vogt J et al (2007) Glucose metabolism and catecholamines. Crit Care Med 35(9 Suppl):S508–S518

Battram C, Charlton SJ, Cuenoud B, Dowling MR, Fairhurst RA, Farr D et al (2006) In vitro and in vivo pharmacological characterization of 5-[(R)-2-(5,6-diethyl-indan-2-ylamino)-1-hydroxy-ethyl]-8-hydroxy-1H-quinolin-2-o ne (indacaterol), a novel inhaled beta(2) adrenoceptor agonist with a 24-h duration of action. J Pharmacol Exp Ther 317(2):762–770

Beak J, Huang W, Parker JS, Hicks ST, Patterson C, Simpson PC et al (2017) An oral selective alpha-1A adrenergic receptor agonist prevents doxorubicin cardiotoxicity. JACC Basic Transl Sci 2(1):39–53

Beattie D, Bradley M, Brearley A, Charlton SJ, Cuenoud BM, Fairhurst RA et al (2010) A physical properties based approach for the exploration of a 4-hydroxybenzothiazolone series of beta2-adrenoceptor agonists as inhaled long-acting bronchodilators. Bioorg Med Chem Lett 20(17): 5302–5307

Benkel T, Zimmermann M, Zeiner J, Bravo S, Merten N, Lim VJY et al (2022) How carvedilol activates beta(2)-adrenoceptors. Nat Commun 13(1):7109

Bergman J, Persson H, Wetterlin K (1969) 2 new groups of selective stimulants of adrenergic beta-receptors. Experientia 25(9):899–901

Berridge MJ, Downes CP, Hanley MR (1982) Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. Biochem J 206(3):587–595

Berthelsen S, Pettinger WA (1977) A functional basis for classification of alpha-adrenergic receptors. Life Sci 21(5):595–606

Beta-2 Adrenergic Agonists (2012) LiverTox: clinical and research information on drug-induced liver injury, Bethesda. National Institute of Diabetes and Digestive and Kidney Diseases; last update September 2017

Billington CK, Penn RB, Hall IP (2017) beta2 Agonists. Handb Exp Pharmacol 237:23–40

Black JW, Crowther AF, Shanks RG, Smith LH, Dornhorst AC (1964) A new adrenergic betareceptor antagonist. Lancet 1(7342):1080–1081

Black JW, Duncan WA, Shanks RG (1965) Comparison of some properties of pronethalol and propranolol. Br J Pharmacol Chemother 25(3):577–591

Blaxall HS, Murphy TJ, Baker JC, Ray C, Bylund DB (1991) Characterization of the alpha-2C adrenergic receptor subtype in the opossum kidney and in the OK cell line. J Pharmacol Exp Ther 259(1):323–329

Blin N, Nahmias C, Drumare MF, Strosberg AD (1994) Mediation of most atypical effects by species homologues of the beta 3-adrenoceptor. Br J Pharmacol 112(3):911–919

Blue DR, Daniels DV, Gever JR, Jett MF, O'Yang C, Tang HM et al (2004) Pharmacological characteristics of Ro 115-1240, a selective alpha1A/1L-adrenoceptor partial agonist: a potential therapy for stress urinary incontinence. BJU Int 93(1):162–170

Bond RA, Clarke DE (1988) Agonist and antagonist characterization of a putative adrenoceptor with distinct pharmacological properties from the alpha- and beta-subtypes. Br J Pharmacol 95(3):723–734

Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R et al (1986) Beta 1- and beta 2-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective beta 1-receptor down-regulation in heart failure. Circ Res 59(3):297–309

Brittain RT, Farmer JB, Jack D, Martin LE, Simpson WT (1968) Alpha-[(t-Butylamino)methyl]-4-hydroxy-m-xylene-alpha 1,alpha 3-diol (AH.3365): a selective beta-adrenergic stimulant. Nature 219(5156):862–863

Brosda J, Jantschak F, Pertz HH (2014) alpha2-adrenoceptors are targets for antipsychotic drugs. Psychopharmacology (Berl) 231(5):801–812

Brown CM, MacKinnon AC, McGrath JC, Spedding M, Kilpatrick AT (1990) Alpha 2-adrenoceptor subtypes and imidazoline-like binding sites in the rat brain. Br J Pharmacol 99(4):803–809

Brucker BM, King J, Mudd PN Jr, McHale K (2022) Selectivity and maximum response of Vibegron and Mirabegron for beta3-Adrenergic receptors. Curr Ther Res Clin Exp 96:100674

Buxton BF, Jones CR, Molenaar P, Summers RJ (1987) Characterization and autoradiographic localization of beta-adrenoceptor subtypes in human cardiac tissues. Br J Pharmacol 92(2): 299–310

Bylund DB (1985) Heterogeneity of alpha-2 adrenergic receptors. Pharmacol Biochem Behav 22(5):835–843

Bylund DB (1988) Subtypes of alpha 2-adrenoceptors: pharmacological and molecular biological evidence converge. Trends Pharmacol Sci 9(10):356–361
Bylund DB (2005) Alpha-2 adrenoceptor subtypes: are more better? Br J Pharmacol 144(2): 159–160
Bylund DB, Ray-Prenger C, Murphy TJ (1988) Alpha-2A and alpha-2B adrenergic receptor subtypes: antagonist binding in tissues and cell lines containing only one subtype. J Pharmacol Exp Ther 245(2):600–607
Bylund DB, Eikenberg DC, Hieble JP, Langer SZ, Lefkowitz RJ, Minneman KP et al (1994) International Union of Pharmacology Nomenclature of adrenoceptors. Pharmacol Rev 46(2): 121–136
Campbell AP, Wakelin LP, Denny WA, Finch AM (2017) Homobivalent conjugation increases the allosteric effect of 9-aminoacridine at the alpha1-Adrenergic receptors. Mol Pharmacol 91(2): 135–144
Candelore MR, Deng L, Tota L, Guan XM, Amend A, Liu Y et al (1999) Potent and selective human beta(3)-adrenergic receptor antagonists. J Pharmacol Exp Ther 290(2):649–655
Cannon B, Nedergaard J (2004) Brown adipose tissue: function and physiological significance. Physiol Rev 84(1):277–359
Cannon WB, Rosenblueth A (1937) Autonomic neuroeffector systems. The Macmillan Company, New York
Catus SL, Gibbs ME, Sato M, Summers RJ, Hutchinson DS (2011) Role of beta-adrenoceptors in glucose uptake in astrocytes using beta-adrenoceptor knockout mice. Br J Pharmacol 162(8): 1700–1715
Cawthorne MA, Sennitt MV, Arch JR, Smith SA (1992) BRL 35135, a potent and selective atypical beta-adrenoceptor agonist. Am J Clin Nutr 55(1 Suppl):252S–257S
Chen X, Xu Y, Qu L, Wu L, Han GW, Guo Y et al (2019) Molecular mechanism for ligand recognition and subtype selectivity of alpha2C adrenergic receptor. Cell Rep 29(10):2936–43 e4
Chen J, Campbell AP, Wakelin LPG, Finch AM (2022) Characterisation of bis(4-aminoquinoline) s as alpha1A adrenoceptor allosteric modulators. Eur J Pharmacol 916:174659
Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS et al (2007) High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. Science 318(5854):1258–1265
Choy C, Raytis JL, Smith DD, Duenas M, Neman J, Jandial R et al (2016) Inhibition of beta2-adrenergic receptor reduces triple-negative breast cancer brain metastases: the potential benefit of perioperative beta-blockade. Oncol Rep 35(6):3135–3142
Christopoulos A (2002) Allosteric binding sites on cell-surface receptors: novel targets for drug discovery. Nat Rev Drug Discov 1(3):198–210
Chung FZ, Lentes KU, Gocayne J, Fitzgerald M, Robinson D, Kerlavage AR et al (1987) Cloning and sequence analysis of the human brain beta-adrenergic receptor. Evolutionary relationship to rodent and avian beta-receptors and porcine muscarinic receptors. FEBS Lett 211(2):200–206
Clarke WP, Bond RA (1998) The elusive nature of intrinsic efficacy. Trends Pharmacol Sci 19(7): 270–276
Clarke RW, Harris J (2002) RX 821002 as a tool for physiological investigation of alpha(2)-adrenoceptors. CNS Drug Rev 8(2):177–192
Clemente-Moragon A, Oliver E, Calle D, Cusso L, Tech MG, Pradillo JM et al (2022) Neutrophil beta1 adrenergic receptor blockade blunts stroke-associated neuroinflammation. Br J Pharmacol
Cohen ML, Bloomquist W, Kriauciunas A, Shuker A, Calligaro D (1999) Aryl propanolamines: comparison of activity at human beta3 receptors, rat beta3 receptors and rat atrial receptors mediating tachycardia. Br J Pharmacol 126(4):1018–1024
Cohen ML, Bloomquist W, Ito M, Lowell BB (2000) Beta3 receptors mediate relaxation in stomach fundus whereas a fourth beta receptor mediates tachycardia in atria from transgenic beta3 receptor knockout mice. Receptors Channels 7(1):17–23
Collins S (2011) Beta-adrenoceptor signaling networks in adipocytes for recruiting stored fat and energy expenditure. Front Endocrinol (Lausanne) 2:102

Cotecchia S, Schwinn DA, Randall RR, Lefkowitz RJ, Caron MG, Kobilka BK (1988) Molecular cloning and expression of the cDNA for the hamster alpha 1-adrenergic receptor. Proc Natl Acad Sci U S A 85(19):7159–7163

Cruickshank JM (1993) The xamoterol experience in the treatment of heart failure. Am J Cardiol 71(9):61C–64C

Cullum VA, Farmer JB, Jack D, Levy GP (1969) Salbutamol: a new, selective beta-adrenoceptive receptor stimulant. Br J Pharmacol 35(1):141–151

da Silva Junior ED, Sato M, Merlin J, Broxton N, Hutchinson DS, Ventura S et al (2017) Factors influencing biased agonism in recombinant cells expressing the human alpha1A -adrenoceptor. Br J Pharmacol 174(14):2318–2333

Dale HH (1906) On some physiological actions of ergot. J Physiol 34(3):163–206

Daly CJ, McGrath JC (2011) Previously unsuspected widespread cellular and tissue distribution of beta-adrenoceptors and its relevance to drug action. Trends Pharmacol Sci 32(4):219–226

Daniels DV, Gever JR, Jasper JR, Kava MS, Lesnick JD, Meloy TD et al (1999) Human cloned alpha1A-adrenoceptor isoforms display alpha1L-adrenoceptor pharmacology in functional studies. Eur J Pharmacol 370(3):337–343

Davis LE, Pogge EK, Garg R (2023) Are beta-blockers safe and effective after myocardial infarction in patients with COPD? JAAPA 36(3):13–15

De Pascali F, Ippolito M, Wolfe E, Komolov KE, Hopfinger N, Lemenze D et al (2022) beta2 -adrenoceptor agonist profiling reveals biased signalling phenotypes for the beta2 -adrenoceptor with possible implications for the treatment of asthma. Br J Pharmacol

Dehvari N, Sato M, Bokhari MH, Kalinovich A, Ham S, Gao J et al (2020) The metabolic effects of mirabegron are mediated primarily by beta3 -adrenoceptors. Pharmacol Res Perspect 8(5): e00643

Deluigi M, Morstein L, Schuster M, Klenk C, Merklinger L, Cridge RR et al (2022) Crystal structure of the alpha1B-adrenergic receptor reveals molecular determinants of selective ligand recognition. Nat Commun 13(1):382

Di Salvo J, Nagabukuro H, Wickham LA, Abbadie C, DeMartino JA, Fitzmaurice A et al (2017) Pharmacological characterization of a novel Beta 3 Adrenergic agonist, Vibegron: evaluation of Antimuscarinic receptor selectivity for combination therapy for overactive bladder. J Pharmacol Exp Ther 360(2):346–355

Diamanti E, Del Bello F, Carbonara G, Carrieri A, Fracchiolla G, Giannella M et al (2012) Might the observed alpha(2A)-adrenoreceptor agonism or antagonism of allyphenyline analogues be ascribed to different molecular conformations? Bioorg Med Chem 20(6):2082–2090

Dixon RA, Kobilka BK, Strader DJ, Benovic JL, Dohlman HG, Frielle T et al (1986) Cloning of the gene and cDNA for mammalian beta-adrenergic receptor and homology with rhodopsin. Nature 321(6065):75–79

do Vale GT, Ceron CS, Gonzaga NA, Simplicio JA, Padovan JC (2019) Three generations of beta-blockers: history, class differences and clinical applicability. Curr Hypertens Rev 15(1):22–31

Dooley DJ, Bittiger H, Reymann NC (1986) CGP 20712 A: a useful tool for quantitating beta 1- and beta 2-adrenoceptors. Eur J Pharmacol 130(1–2):137–139

Eason MG, Liggett SB (1993) Human alpha 2-adrenergic receptor subtype distribution: widespread and subtype-selective expression of alpha 2C10, alpha 2C4, and alpha 2C2 mRNA in multiple tissues. Mol Pharmacol 44(1):70–75

Eason MG, Liggett SB (1995) Identification of a Gs coupling domain in the amino terminus of the third intracellular loop of the alpha 2A-adrenergic receptor. Evidence for distinct structural determinants that confer Gs versus Gi coupling. J Biol Chem 270(42):24753–24760

Eason MG, Kurose H, Holt BD, Raymond JR, Liggett SB (1992) Simultaneous coupling of alpha 2-adrenergic receptors to two G-proteins with opposing effects. Subtype-selective coupling of alpha 2C10, alpha 2C4, and alpha 2C2 adrenergic receptors to Gi and Gs. J Biol Chem 267(22): 15795–15801

Edmondson SD, Zhu C, Kar NF, Di Salvo J, Nagabukuro H, Sacre-Salem B et al (2016) Discovery of Vibegron: a potent and selective beta3 Adrenergic receptor agonist for the treatment of overactive bladder. J Med Chem 59(2):609–623

Eglen RM, Bosse R, Reisine T (2007) Emerging concepts of guanine nucleotide-binding protein-coupled receptor (GPCR) function and implications for high throughput screening. Assay Drug Dev Technol 5(3):425–451

Eichel K, von Zastrow M (2018) Subcellular organization of GPCR signaling. Trends Pharmacol Sci 39(2):200–208

Elnatan J, Molenaar P, Rosenfeldt FL, Summers RJ (1994) Autoradiographic localization and quantitation of beta 1- and beta 2-adrenoceptors in the human atrioventricular conducting system: a comparison of patients with idiopathic dilated cardiomyopathy and ischemic heart disease. J Mol Cell Cardiol 26(3):313–323

Emorine LJ, Marullo S, Briend-Sutren MM, Patey G, Tate K, Delavier-Klutchko C et al (1989) Molecular characterization of the human beta 3-adrenergic receptor. Science 245(4922): 1118–1121

Erdozain AM, Brocos-Mosquera I, Gabilondo AM, Meana JJ, Callado LF (2019) Differential alpha2A- and alpha2C-adrenoceptor protein expression in presynaptic and postsynaptic density fractions of postmortem human prefrontal cortex. J Psychopharmacol 33(2):244–249

Evans BA, Papaioannou M, Hamilton S, Summers RJ (1999) Alternative splicing generates two isoforms of the beta3-adrenoceptor which are differentially expressed in mouse tissues. Br J Pharmacol 127(6):1525–1531

Evans BA, Sato M, Sarwar M, Hutchinson DS, Summers RJ (2010) Ligand-directed signalling at beta-adrenoceptors. Br J Pharmacol 159:1022–1038

Evans BA, Broxton N, Merlin J, Sato M, Hutchinson DS, Christopoulos A et al (2011) Quantification of functional selectivity at the human alpha(1A)-adrenoceptor. Mol Pharmacol 79(2): 298–307

Evans BA, Merlin J, Bengtsson T, Hutchinson DS (2019) Adrenoceptors in white, brown, and brite adipocytes. Br J Pharmacol 176(14):2416–2432

Fargin A, Raymond JR, Lohse MJ, Kobilka BK, Caron MG, Lefkowitz RJ (1988) The genomic clone G-21 which resembles a beta-adrenergic receptor sequence encodes the 5-HT1A receptor. Nature 335(6188):358–360

Flanagan CA (2016) GPCR-radioligand binding assays. Methods Cell Biol 132:191–215

Ford AP, Williams TJ, Blue DR, Clarke DE (1994) Alpha 1-adrenoceptor classification: sharpening Occam's razor. Trends Pharmacol Sci 15(6):167–170

Ford AP, Daniels DV, Chang DJ, Gever JR, Jasper JR, Lesnick JD et al (1997) Pharmacological pleiotropism of the human recombinant alpha1A-adrenoceptor: implications for alpha1-adrenoceptor classification. Br J Pharmacol 121(6):1127–1135

Frang H, Mukkala VM, Syysto R, Ollikka P, Hurskainen P, Scheinin M et al (2003) Nonradioactive GTP binding assay to monitor activation of g protein-coupled receptors. Assay Drug Dev Technol 1(2):275–280

Frazier EP, Michel-Reher MB, van Loenen P, Sand C, Schneider T, Peters SL et al (2011) Lack of evidence that nebivolol is a beta(3)-adrenoceptor agonist. Eur J Pharmacol 654(1):86–91

Frielle T, Collins S, Daniel KW, Caron MG, Lefkowitz RJ, Kobilka BK (1987) Cloning of the cDNA for the human beta 1-adrenergic receptor. Proc Natl Acad Sci U S A 84(22):7920–7924

Frielle T, Daniel KW, Caron MG, Lefkowitz RJ (1988) Structural basis of beta-adrenergic receptor subtype specificity studied with chimeric beta 1/beta 2-adrenergic receptors. Proc Natl Acad Sci U S A 85(24):9494–9498

Furchgott RF (1967) The pharmacological differentiation of adrenergic receptors. Ann N Y Acad Sci 139(3):553–570

Gaertner J, Fusi-Schmidhauser T (2022) Dexmedetomidine: a magic bullet on its way into palliative care-a narrative review and practice recommendations. Ann Palliat Med 11(4):1491–1504

Galaz-Montoya M, Wright SJ, Rodriguez GJ, Lichtarge O, Wensel TG (2017) beta2-Adrenergic receptor activation mobilizes intracellular calcium via a non-canonical cAMP-independent signaling pathway. J Biol Chem 292(24):9967–9974

Galitzky J, Langin D, Verwaerde P, Montastruc JL, Lafontan M, Berlan M (1997) Lipolytic effects of conventional beta 3-adrenoceptor agonists and of CGP 12,177 in rat and human fat cells: preliminary pharmacological evidence for a putative beta 4-adrenoceptor. Br J Pharmacol 122(6):1244–1250

Gbahou F, Rouleau A, Morisset S, Parmentier R, Crochet S, Lin JS et al (2003) Protean agonism at histamine H3 receptors in vitro and in vivo. Proc Natl Acad Sci U S A 100(19):11086–11091

Gerhardt CC, Gros J, Strosberg AD, Issad T (1999) Stimulation of the extracellular signal-regulated kinase 1/2 pathway by human beta-3 adrenergic receptor: new pharmacological profile and mechanism of activation. Mol Pharmacol 55(2):255–262

Gibbs ME, Summers RJ (2005) Contrasting roles for beta1, beta2 and beta3-adrenoceptors in memory formation in the chick. Neuroscience 131(1):31–42

Gillis RD, Botteri E, Chang A, Ziegler AI, Chung NC, Pon CK et al (2021) Carvedilol blocks neural regulation of breast cancer progression in vivo and is associated with reduced breast cancer mortality in patients. Eur J Cancer 147:106–116

Giovannitti JA Jr, Thoms SM, Crawford JJ (2015) Alpha-2 adrenergic receptor agonists: a review of current clinical applications. Anesth Prog 62(1):31–39

Golf S, Lovstad R, Hansson V (1985) Beta-adrenoceptor density and relative number of beta-adrenoceptor subtypes in biopsies from human right atrial, left ventricular, and right ventricular myocard. Cardiovasc Res 19(10):636–641

Graham RM, Perez DM, Hwa J, Piascik MT (1996) Alpha 1-adrenergic receptor subtypes. Molecular structure, function, and signaling. Circ Res 78(5):737–749

Granneman JG (2001) The putative beta4-adrenergic receptor is a novel state of the beta1-adrenergic receptor. Am J Physiol Endocrinol Metab 280(2):E199–E202

Gray K, Short J, Ventura S (2008) The alpha1A-adrenoceptor gene is required for the alpha1L-adrenoceptor-mediated response in isolated preparations of the mouse prostate. Br J Pharmacol 155(1):103–109

Grundmann M, Kostenis E (2015) Label free biosensors in GPCR screening. In: Prazeres DMF, Martins SAM (eds) G-protein receptor screening assays – methods and protocols. 1272. Springer, New York, pp 199–214

Haapalinna A, Viitamaa T, MacDonald E, Savola JM, Tuomisto L, Virtanen R et al (1997) Evaluation of the effects of a specific alpha 2-adrenoceptor antagonist, atipamezole, on alpha 1- and alpha 2-adrenoceptor subtype binding, brain neurochemistry and behaviour in comparison with yohimbine. Naunyn Schmiedebergs Arch Pharmacol 356(5):570–582

Halme M, Sjoholm B, Savola JM, Scheinin M (1995) Recombinant human alpha 2-adrenoceptor subtypes: comparison of [3H]rauwolscine, [3H]atipamezole and [3H]RX821002 as radioligands. Biochim Biophys Acta 1266(2):207–214

Han C, Abel PW, Minneman KP (1987) Heterogeneity of alpha 1-adrenergic receptors revealed by chlorethylclonidine. Mol Pharmacol 32(4):505–510

Harms HH (1976) Isoproterenol antagonism of cardioselective beta adrenergic receptor blocking agents: a comparative study of human and Guinea-pig cardiac and bronchial beta adrenergic receptors. J Pharmacol Exp Ther 199(2):329–335

Hatanaka T, Ukai M, Watanabe M, Someya A, Ohtake A, Suzuki M et al (2013) In vitro and in vivo pharmacological profile of the selective beta3-adrenoceptor agonist mirabegron in rats. Naunyn Schmiedebergs Arch Pharmacol 386(3):247–253

Hein P, Michel MC (2007) Signal transduction and regulation: are all alpha1-adrenergic receptor subtypes created equal? Biochem Pharmacol 73(8):1097–1106

Heitz A, Schwartz J, Velly J (1983) Beta-adrenoceptors of the human myocardium: determination of beta 1 and beta 2 subtypes by radioligand binding. Br J Pharmacol 80(4):711–717

Hicks PE, Cavero I, Manoury P, Lefevre-Borg F, Langer SZ (1987) Comparative analysis of beta-1 adrenoceptor agonist and antagonist potency and selectivity of cicloprolol, xamoterol and pindolol. J Pharmacol Exp Ther 242(3):1025–1034

Hicks A, McCafferty GP, Riedel E, Aiyar N, Pullen M, Evans C et al (2007) GW427353 (solabegron), a novel, selective beta3-adrenergic receptor agonist, evokes bladder relaxation and increases micturition reflex threshold in the dog. J Pharmacol Exp Ther 323(1):202–209

Hieble JP (2000) Adrenoceptor subclassification: an approach to improved cardiovascular therapeutics. Pharm Acta Helv 74(2–3):163–171

Hieble JP, Bylund DB, Clarke DE, Eikenburg DC, Langer SZ, Lefkowitz RJ et al (1995) International Union of Pharmacology. X. Recommendation for nomenclature of alpha 1-adrenoceptors: consensus update. Pharmacol Rev 47(2):267–270

Hill SJ, Baker JG, Rees S (2001) Reporter-gene systems for the study of G-protein-coupled receptors. Curr Opin Pharmacol 1(5):526–532

Hoffmann C, Leitz MR, Oberdorf-Maass S, Lohse MJ, Klotz KN (2004) Comparative pharmacology of human beta-adrenergic receptor subtypes – characterization of stably transfected receptors in CHO cells. Naunyn Schmiedebergs Arch Pharmacol 369(2):151–159

Horie K, Obika K, Foglar R, Tsujimoto G (1995) Selectivity of the imidazoline alpha-adrenoceptor agonists (oxymetazoline and cirazoline) for human cloned alpha 1-adrenoceptor subtypes. Br J Pharmacol 116(1):1611–1618

Hutchinson DS, Bengtsson T, Evans BA, Summers RJ (2002) Mouse beta 3a- and beta 3b-adrenoceptors expressed in Chinese hamster ovary cells display identical pharmacology but utilize distinct signalling pathways. Br J Pharmacol 135(8):1903–1914

Hutchinson DS, Sato M, Evans BA, Christopoulos A, Summers RJ (2005) Evidence for pleiotropic signaling at the mouse beta3-adrenoceptor revealed by SR59230A [3-(2-Ethylphenoxy)-1-[(1, S)-1,2,3,4-tetrahydronapth-1-ylamino]-2S-2-propa nol oxalate]. J Pharmacol Exp Ther 312(3): 1064–1074

Hutchinson DS, Chernogubova E, Sato M, Summers RJ, Bengtsson T (2006) Agonist effects of zinterol at the mouse and human beta(3)-adrenoceptor. Naunyn Schmiedebergs Arch Pharmacol 373(2):158–168

Igawa Y, Michel MC (2013) Pharmacological profile of beta3-adrenoceptor agonists in clinical development for the treatment of overactive bladder syndrome. Naunyn Schmiedebergs Arch Pharmacol 386(3):177–183

Insel PA, Sriram K, Gorr MW, Wiley SZ, Michkov A, Salmeron C et al (2019) GPCRomics: an approach to discover GPCR drug targets. Trends Pharmacol Sci 40(6):378–387

Isogaya M, Sugimoto Y, Tanimura R, Tanaka R, Kikkawa H, Nagao T et al (1999) Binding pockets of the beta(1)- and beta(2)-adrenergic receptors for subtype-selective agonists. Mol Pharmacol 56(5):875–885

Jarrott B, Louis WJ, Summers RJ (1979) The effect of a series of clonidine analogues on [3H] clonidine binding in rat cerebral cortex. Biochem Pharmacol 28(1):141–144

Jasper JR, Motulsky HJ, Insel PA (1988) Characterization of a bromoacetylated derivative of pindolol as a high affinity, irreversible beta adrenergic antagonist in cultured cells. J Pharmacol Exp Ther 244(3):820–824

Jasper JR, Lesnick JD, Chang LK, Yamanishi SS, Chang TK, Hsu SA et al (1998) Ligand efficacy and potency at recombinant alpha2 adrenergic receptors: agonist-mediated [35S]GTPgammaS binding. Biochem Pharmacol 55(7):1035–1043

Johnson RD, Minneman KP (1987) Differentiation of alpha 1-adrenergic receptors linked to phosphatidylinositol turnover and cyclic AMP accumulation in rat brain. Mol Pharmacol 31(3):239–246

Joseph SS, Lynham JA, Molenaar P, Grace AA, Colledge WH, Kaumann AJ (2003) Intrinsic sympathomimetic activity of (−)-pindolol mediated through a (−)-propranolol-resistant site of the beta1-adrenoceptor in human atrium and recombinant receptors. Naunyn Schmiedebergs Arch Pharmacol 368(6):496–503

Joseph SS, Lynham JA, Colledge WH, Kaumann AJ (2004a) Binding of (−)-[3H]-CGP12177 at two sites in recombinant human beta 1-adrenoceptors and interaction with beta-blockers. Naunyn Schmiedebergs Arch Pharmacol 369(5):525–532

Joseph SS, Lynham JA, Grace AA, Colledge WH, Kaumann AJ (2004b) Markedly reduced effects of (−)-isoprenaline but not of (−)-CGP12177 and unchanged affinity of beta-blockers at Gly389-beta1-adrenoceptors compared to Arg389-beta1-adrenoceptors. Br J Pharmacol 142(1):51–56

Jung JH, Kim J, MacDonald R, Reddy B, Kim MH, Dahm P (2017) Silodosin for the treatment of lower urinary tract symptoms in men with benign prostatic hyperplasia. Cochrane Database Syst Rev 11(11):CD012615

Kalinovich A, Dehvari N, Aslund A, van Beek S, Halleskog C, Olsen J et al (2020) Treatment with a beta-2-adrenoceptor agonist stimulates glucose uptake in skeletal muscle and improves glucose homeostasis, insulin resistance and hepatic steatosis in mice with diet-induced obesity. Diabetologia 63(8):1603–1615

Kaumann AJ (1996) (−)-CGP 12177-induced increase of human atrial contraction through a putative third beta-adrenoceptor. Br J Pharmacol 117(1):93–98

Kaumann AJ (1997) Four beta-adrenoceptor subtypes in the mammalian heart. Trends Pharmacol Sci 18(3):70–76

Kaumann AJ, Birnbaumer L (1973) Proceedings: adrenergic receptors in heart muscle: similarity of apparent affinities of beta-blockers for receptors mediating adenyl-cyclase activity, inotropic and chronotropic effects of catecholamines. Acta Physiol Lat Am 23(6):619–620

Kaumann AJ, Blinks JR (1980) Beta-adrenoceptor blocking agents as partial agonists in isolated heart muscle: dissociation of stimulation and blockade. Naunyn Schmiedebergs Arch Pharmacol 311(3):237–248

Kaumann AJ, Lobnig BM (1986) Mode of action of (−)-pindolol on feline and human myocardium. Br J Pharmacol 89(1):207–218

Kaumann AJ, Molenaar P (2008) The low-affinity site of the beta1-adrenoceptor and its relevance to cardiovascular pharmacology. Pharmacol Ther 118(3):303–336

Kaumann AJ, Preitner F, Sarsero D, Molenaar P, Revelli JP, Giacobino JP (1998) (−)-CGP 12177 causes cardiostimulation and binds to cardiac putative beta 4-adrenoceptors in both wild-type and beta 3-adrenoceptor knockout mice. Mol Pharmacol 53(4):670–675

Kaumann AJ, Engelhardt S, Hein L, Molenaar P, Lohse M (2001) Abolition of (−)-CGP 12177-evoked cardiostimulation in double beta1/beta2-adrenoceptor knockout mice. Obligatory role of beta1-adrenoceptors for putative beta4-adrenoceptor pharmacology. Naunyn Schmiedebergs Arch Pharmacol 363(1):87–93

Kenakin TP (1982) Theoretical and practical problems with the assessment of intrinsic efficacy of agonists: efficacy of reputed beta-1 selective adrenoceptor agonists for beta-2 adrenoceptors. J Pharmacol Exp Ther 223(2):416–423

Kenakin T (1999) Efficacy in drug receptor theory: outdated concept or under-valued tool? Trends Pharmacol Sci 20(10):400–405

Kenakin T (2001) Inverse, protean, and ligand-selective agonism: matters of receptor conformation. FASEB J 15(3):598–611

Kenakin T (2004) Efficacy as a vector: the relative prevalence and paucity of inverse agonism. Mol Pharmacol 65(1):2–11

Kenakin T (2007) Functional selectivity through protean and biased agonism: who steers the ship? Mol Pharmacol 72(6):1393–1401

Kenny BA, Chalmers DH, Philpott PC, Naylor AM (1995) Characterization of an alpha 1D-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. Br J Pharmacol 115(6):981–986

Kern C, Meyer T, Droux S, Schollmeyer D, Miculka C (2009) Synthesis and pharmacological characterization of beta2-adrenergic agonist enantiomers: zilpaterol. J Med Chem 52(6): 1773–1777

Kim YS, Sainz RD, Molenaar P, Summers RJ (1991) Characterization of beta 1- and beta 2-adrenoceptors in rat skeletal muscles. Biochem Pharmacol 42(9):1783–1789

Kim YS, Sainz RD, Summers RJ, Molenaar P (1992) Cimaterol reduces beta-adrenergic receptor density in rat skeletal muscles. J Anim Sci 70(1):115–122

Kim-Fuchs C, Le CP, Pimentel MA, Shackleford D, Ferrari D, Angst E et al (2014) Chronic stress accelerates pancreatic cancer growth and invasion: a critical role for beta-adrenergic signaling in the pancreatic microenvironment. Brain Behav Immun 40:40–47

Kobilka BK, Matsui H, Kobilka TS, Yang-Feng TL, Francke U, Caron MG et al (1987a) Cloning, sequencing, and expression of the gene coding for the human platelet alpha 2-adrenergic receptor. Science 238(4827):650–656

Kobilka BK, Dixon RA, Frielle T, Dohlman HG, Bolanowski MA, Sigal IS et al (1987b) cDNA for the human beta 2-adrenergic receptor: a protein with multiple membrane-spanning domains and encoded by a gene whose chromosomal location is shared with that of the receptor for platelet-derived growth factor. Proc Natl Acad Sci U S A 84(1):46–50

Kompa AR, Summers RJ (1999) Desensitization and resensitization of beta 1- and putative beta 4-adrenoceptor mediated responses occur in parallel in a rat model of cardiac failure. Br J Pharmacol 128(7):1399–1406

Konkar AA, Zhai Y, Granneman JG (2000a) beta1-adrenergic receptors mediate beta3-adrenergic-independent effects of CGP 12177 in brown adipose tissue. Mol Pharmacol 57(2):252–258

Konkar AA, Zhu Z, Granneman JG (2000b) Aryloxypropanolamine and catecholamine ligand interactions with the beta(1)-adrenergic receptor: evidence for interaction with distinct conformations of beta(1)-adrenergic receptors. J Pharmacol Exp Ther 294(3):923–932

Kozlowska H, Schlicker E, Kozlowski M, Baranowska M, Malinowska B (2006) Potential involvement of a propranolol-insensitive atypical beta-adrenoceptor the vasodilator effect of cyanopindolol in the human pulmonary artery. J Physiol Pharmacol 57(3):317–328

Kuenzel WJ, Kusiak JW, Augustine PC, Pitha J (1983) Effect of a beta-adrenergic antagonist on blood pressure, heart rate and beta-adrenoceptors in Turkey poults. Comp Biochem Physiol C Comp Pharmacol Toxicol 76(2):371–375

Lands AM, Luduena FP, Buzzo HJ (1967a) Differentiation of receptors responsive to isoproterenol. Life Sci 6(21):2241–2249

Lands AM, Arnold A, McAuliff JP, Luduena FP, Brown TG Jr (1967b) Differentiation of receptor systems activated by sympathomimetic amines. Nature 214(5088):597–598

Langer SZ (1974) Presynaptic regulation of catecholamine release. Biochem Pharmacol 23(13): 1793–1800

Langer SZ (2015) alpha2-adrenoceptors in the treatment of major neuropsychiatric disorders. Trends Pharmacol Sci 36(4):196–202

Laurila JM, Wissel G, Xhaard H, Ruuskanen JO, Johnson MS, Scheinin M (2011) Involvement of the first transmembrane segment of human alpha(2) -adrenoceptors in the subtype-selective binding of chlorpromazine, spiperone and spiroxatrine. Br J Pharmacol 164(5):1558–1572

Lee S (2019) Dexmedetomidine: present and future directions. Korean J Anesthesiol 72(4): 323–330

Leonardi A, Hieble JP, Guarneri L, Naselsky DP, Poggesi E, Sironi G et al (1997) Pharmacological characterization of the uroselective alpha-1 antagonist rec 15/2739 (SB 216469): role of the alpha-1L adrenoceptor in tissue selectivity, part I. J Pharmacol Exp Ther 281(3):1272–1283

Leppik RA, Lazareno S, Mynett A, Birdsall NJ (1998) Characterization of the allosteric interactions between antagonists and amiloride analogues at the human alpha2A-adrenergic receptor. Mol Pharmacol 53(5):916–925

Leppik RA, Mynett A, Lazareno S, Birdsall NJ (2000) Allosteric interactions between the antagonist prazosin and amiloride analogs at the human alpha(1A)-adrenergic receptor. Mol Pharmacol 57(3):436–445

Lewis CJ, Gong H, Brown MJ, Harding SE (2004) Overexpression of beta 1-adrenoceptors in adult rat ventricular myocytes enhances CGP 12177A cardiostimulation: implications for 'putative' beta 4-adrenoceptor pharmacology. Br J Pharmacol 141(5):813–824

Li F, De Godoy M, Rattan S (2004) Role of adenylate and guanylate cyclases in beta1-, beta2-, and beta3-adrenoceptor-mediated relaxation of internal anal sphincter smooth muscle. J Pharmacol Exp Ther 308(3):1111–1120

Li Z, Li J, Liu L, Deng W, Liu Q, Liu R et al (2020) Structural insight into the mechanism of 4-aminoquinolines selectivity for the alpha2A-adrenoceptor. Drug Des Devel Ther 14:2585–2594

Limbird LE (1988) Receptors linked to inhibition of adenylate cyclase: additional signaling mechanisms. FASEB J 2(11):2686–2695

Lipworth B, Wedzicha J, Devereux G, Vestbo J, Dransfield MT (2016) Beta-blockers in COPD: time for reappraisal. Eur Respir J 48(3):880–888

Littmann T, Gottle M, Reinartz MT, Kalble S, Wainer IW, Ozawa T et al (2015) Recruitment of beta-arrestin 1 and 2 to the beta2-adrenoceptor: analysis of 65 ligands. J Pharmacol Exp Ther 355(2):183–190

Liu YL, Nwosu UC, Rice PJ (1998) Relaxation of isolated human myometrial muscle by beta2-adrenergic receptors but not beta1-adrenergic receptors. Am J Obstet Gynecol 179(4):895–898

Liu X, Ahn S, Kahsai AW, Meng KC, Latorraca NR, Pani B et al (2017) Mechanism of intracellular allosteric beta2AR antagonist revealed by X-ray crystal structure. Nature 548(7668):480–484

Liu X, Masoudi A, Kahsai AW, Huang LY, Pani B, Staus DP et al (2019) Mechanism of beta2AR regulation by an intracellular positive allosteric modulator. Science 364(6447):1283–1287

Liu X, Kaindl J, Korczynska M, Stossel A, Dengler D, Stanek M et al (2020) An allosteric modulator binds to a conformational hub in the beta2 adrenergic receptor. Nat Chem Biol 16(7):749–755

Lofling LL, Stoer NC, Sloan EK, Chang A, Gandini S, Ursin G et al (2022) Beta-blockers and breast cancer survival by molecular subtypes: a population-based cohort study and meta-analysis. Br J Cancer 127(6):1086–1096

Lomasney JW, Cotecchia S, Lorenz W, Leung WY, Schwinn DA, Yang-Feng TL et al (1991) Molecular cloning and expression of the cDNA for the alpha 1A-adrenergic receptor. The gene for which is located on human chromosome 5. J Biol Chem 266(10):6365–6369

Louis SN, Nero TL, Iakovidis D, Jackman GP, Louis WJ (1999) LK 204-545, a highly selective beta1-adrenoceptor antagonist at human beta-adrenoceptors. Eur J Pharmacol 367(2–3):431–435

Lowe MD, Lynham JA, Grace AA, Kaumann AJ (2002) Comparison of the affinity of beta-blockers for two states of the beta 1-adrenoceptor in ferret ventricular myocardium. Br J Pharmacol 135(2):451–461

Lubawski I, Wale J (1969) Studies with LB 46, a new beta-receptor blocking drug. Eur J Pharmacol 6(3):345–348

Ma X, Hu Y, Batebi H, Heng J, Xu J, Liu X et al (2020) Analysis of beta2AR-Gs and beta2AR-Gi complex formation by NMR spectroscopy. Proc Natl Acad Sci U S A 117(37):23096–23105

MacDonald E, Kobilka BK, Scheinin M (1997) Gene targeting – homing in on alpha 2-adrenoceptor-subtype function. Trends Pharmacol Sci 18(6):211–219

MacLennan SJ, Luong LA, Jasper JR, To ZP, Eglen RM (1997) Characterization of alpha 2-adrenoceptors mediating contraction of dog saphenous vein: identity with the human alpha 2A subtype. Br J Pharmacol 121(8):1721–1729

Maiga A, Merlin J, Marcon E, Rouget C, Larregola M, Gilquin B et al (2013) Orthosteric binding of rho-Da1a, a natural peptide of snake venom interacting selectively with the alpha1A-adrenoceptor. PloS One 8(7):e68841

Malinowska B, Schlicker E (1996) Mediation of the positive chronotropic effect of CGP 12177 and cyanopindolol in the pithed rat by atypical beta-adrenoceptors, different from beta 3-adrenoceptors. Br J Pharmacol 117(5):943–949

Man In't Veld AJ, Schalekamp MA (1981) Pindolol acts as beta-adrenoceptor agonist in orthostatic hypotension. Br Med J (Clin Res Ed) 283(6290):561

Masureel M, Zou Y, Picard LP, van der Westhuizen E, Mahoney JP, Rodrigues J et al (2018) Structural insights into binding specificity, efficacy and bias of a beta(2)AR partial agonist. Nat Chem Biol 14(11):1059–1066

Merlin J, Sato M, Chia LY, Fahey R, Pakzad M, Nowell CJ et al (2018) Rosiglitazone and a beta3-adrenoceptor agonist are both required for functional Browning of White adipocytes in culture. Front Endocrinol (Lausanne) 9:249

Michel MC, Korstanje C (2016) beta3-adrenoceptor agonists for overactive bladder syndrome: role of translational pharmacology in a repositioning clinical drug development project. Pharmacol Ther 159:66–82

Michel AD, Loury DN, Whiting RL (1989a) Differences between the alpha 2-adrenoceptor in rat submaxillary gland and the alpha 2A-and alpha 2B-adrenoceptor subtypes. Br J Pharmacol 98(3):890–897

Michel MC, Brodde OE, Schnepel B, Behrendt J, Tschada R, Motulsky HJ et al (1989b) [3H] idazoxan and some other alpha 2-adrenergic drugs also bind with high affinity to a nonadrenergic site. Mol Pharmacol 35(3):324–330

Michel MC, Ochodnicky P, Summers RJ (2010) Tissue functions mediated by beta(3)-adrenoceptors-findings and challenges. Naunyn Schmiedebergs Arch Pharmacol 382:103–108

Michel MC, Michel-Reher MB, Hein P (2020) A systematic review of inverse Agonism at adrenoceptor subtypes. Cells 9(9)

Millan MJ, Maiofiss L, Cussac D, Audinot V, Boutin JA, Newman-Tancredi A (2002) Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. J Pharmacol Exp Ther 303(2):791–804

Milligan G (2003) Principles: extending the utility of [35S]GTP gamma S binding assays. Trends Pharmacol Sci 24(2):87–90

Minneman KP (1988) Alpha 1-adrenergic receptor subtypes, inositol phosphates, and sources of cell Ca2+. Pharmacol Rev 40(2):87–119

Minneman KP, Hedberg A, Molinoff PB (1979a) Comparison of beta adrenergic receptor subtypes in mammalian tissues. J Pharmacol Exp Ther 211(3):502–508

Minneman KP, Hegstrand LR, Molinoff PB (1979b) The pharmacological specificity of beta-1 and beta-2 adrenergic receptors in rat heart and lung in vitro. Mol Pharmacol 16(1):21–33

Minneman KP, Theroux TL, Hollinger S, Han C, Esbenshade TA (1994) Selectivity of agonists for cloned alpha 1-adrenergic receptor subtypes. Mol Pharmacol 46(5):929–936

Miralles A, Olmos G, Sastre M, Barturen F, Martin I, Garcia-Sevilla JA (1993) Discrimination and pharmacological characterization of I2-imidazoline sites with [3H]idazoxan and alpha-2-adrenoceptors with [3H]RX821002 (2-methoxy idazoxan) in the human and rat brains. J Pharmacol Exp Ther 264(3):1187–1197

Mitchell TH, Ellis RD, Smith SA, Robb G, Cawthorne MA (1989) Effects of BRL 35135, a beta-adrenoceptor agonist with novel selectivity, on glucose tolerance and insulin sensitivity in obese subjects. Int J Obes (Lond) 13(6):757–766

Molenaar P (2003) The 'state' of beta-adrenoceptors. Br J Pharmacol 140(1):1–2

Molenaar P, Russell F, Pitha J, Summers R (1988) Persistent beta-adrenoceptor blockade with alkylating pindolol (BIM) in Guinea-pig left atria and trachea. Biochem Pharmacol 37(19): 3601–3607

Molenaar P, Russell FD, Shimada T, Summers RJ (1990) Densitometric analysis of beta 1- and beta 2-adrenoceptors in Guinea-pig atrioventricular conducting system. J Mol Cell Cardiol 22(4): 483–495

Molenaar P, Sarsero D, Kaumann AJ (1997a) Proposal for the interaction of non-conventional partial agonists and catecholamines with the 'putative beta 4-adrenoceptor' in mammalian heart. Clin Exp Pharmacol Physiol 24(9–10):647–656

Molenaar P, Sarsero D, Arch JR, Kelly J, Henson SM, Kaumann AJ (1997b) Effects of (−)-RO363 at human atrial beta-adrenoceptor subtypes, the human cloned beta 3-adrenoceptor and rodent intestinal beta 3-adrenoceptors. Br J Pharmacol 120(2):165–176

Molenaar P, Bartel S, Cochrane A, Vetter D, Jalali H, Pohlner P et al (2000) Both beta(2)- and beta (1)-adrenergic receptors mediate hastened relaxation and phosphorylation of phospholamban and troponin I in ventricular myocardium of Fallot infants, consistent with selective coupling of beta(2)-adrenergic receptors to G(s)-protein. Circulation 102(15):1814–1821

Morales A (2001) Yohimbine in erectile dysfunction: would an orphan drug ever be properly assessed? World J Urol 19(4):251–255

Morrow AL, Creese I (1986) Characterization of alpha 1-adrenergic receptor subtypes in rat brain: a reevaluation of [3H]WB4104 and [3H]prazosin binding. Mol Pharmacol 29(4):321–330

Mukaida S, Sato M, Oberg AI, Dehvari N, Olsen JM, Kocan M et al (2019) BRL37344 stimulates GLUT4 translocation and glucose uptake in skeletal muscle via beta2-adrenoceptors without causing classical receptor desensitization. Am J Physiol Regul Integr Comp Physiol 316(5): R666–RR77

Murphy TJ, Bylund DB (1988) Characterization of alpha-2 adrenergic receptors in the OK cell, an opossum kidney cell line. J Pharmacol Exp Ther 244(2):571–578

Nagiri C, Kobayashi K, Tomita A, Kato M, Kobayashi K, Yamashita K et al (2021) Cryo-EM structure of the beta3-adrenergic receptor reveals the molecular basis of subtype selectivity. Mol Cell 81(15):3205–15 e5

Nedergaard J, Bengtsson T, Cannon B (2007) Unexpected evidence for active brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab 293(2):E444–E452

Nelson LE, Lu J, Guo T, Saper CB, Franks NP, Maze M (2003) The alpha2-adrenoceptor agonist dexmedetomidine converges on an endogenous sleep-promoting pathway to exert its sedative effects. Anesthesiology 98(2):428–436

Nevzorova J, Bengtsson T, Evans BA, Summers RJ (2002) Characterization of the beta-adrenoceptor subtype involved in mediation of glucose transport in L6 cells. Br J Pharmacol 137(1):9–18

Newcorn JH, Krone B, Dittmann RW (2022) Nonstimulant treatments for ADHD. Child Adolesc Psychiatr Clin N Am 31(3):417–435

Nikolaev VO, Bunemann M, Schmitteckert E, Lohse MJ, Engelhardt S (2006) Cyclic AMP imaging in adult cardiac myocytes reveals far-reaching beta1-adrenergic but locally confined beta2-adrenergic receptor-mediated signaling. Circ Res 99(10):1084–1091

Nobles KN, Xiao K, Ahn S, Shukla AK, Lam CM, Rajagopal S et al (2011) Distinct phosphorylation sites on the beta(2)-adrenergic receptor establish a barcode that encodes differential functions of beta-arrestin. Sci Signal 4(185):ra51

Nureki I, Kobayashi K, Tanaka T, Demura K, Inoue A, Shihoya W et al (2022) Cryo-EM structures of the beta3 adrenergic receptor bound to solabegron and isoproterenol. Biochem Biophys Res Commun 611:158–164

Obika K, Shibata K, Horie K, Foglar R, Kimura K, Tsujimoto G (1995) NS-49, a novel alpha 1a-adrenoceptor-selective agonist characterization using recombinant human alpha 1-adrenoceptors. Eur J Pharmacol 291(3):327–334

O'Connell TD, Jensen BC, Baker AJ, Simpson PC (2014) Cardiac alpha1-adrenergic receptors: novel aspects of expression, signaling mechanisms, physiologic function, and clinical importance. Pharmacol Rev 66(1):308–333

O'Donnell SR, Wanstall JC (1980) Evidence that ICI 118, 551 is a potent, highly Beta 2-selective adrenoceptor antagonist and can be used to characterize Beta-adrenoceptor populations in tissues. Life Sci 27(8):671–677

Oliver G, Schäfer EA (1894) On the physiological action of extracts of the suprarenal capsule. J Physiol 16:1–4

Oliver G, Schafer EA (1895) The physiological effects of extracts of the suprarenal capsules. J Physiol 18(3):230–276

Oshita M, Kigoshi S, Muramatsu I (1991) Three distinct binding sites for [3H]-prazosin in the rat cerebral cortex. Br J Pharmacol 104(4):961–965

Pak MD, Fishman PH (1996) Anomalous behavior of CGP 12177A on beta 1-adrenergic receptors. J Recept Signal Transduct Res 16(1–2):1–23

Pani B, Ahn S, Rambarat PK, Vege S, Kahsai AW, Liu A et al (2021) Unique positive cooperativity between the beta-Arrestin-biased beta-blocker carvedilol and a small molecule positive allosteric modulator of the beta2-Adrenergic receptor. Mol Pharmacol 100(5):513–525

Peltonen JM, Pihlavisto M, Scheinin M (1998) Subtype-specific stimulation of [35S]GTPgammaS binding by recombinant alpha2-adrenoceptors. Eur J Pharmacol 355(2–3):275–279

Perala M, Hirvonen H, Kalimo H, Ala-Uotila S, Regan JW, Akerman KE et al (1992) Differential expression of two alpha 2-adrenergic receptor subtype mRNAs in human tissues. Brain Res Mol Brain Res 16(1–2):57–63

Persson H, Olsson T (1970) Some pharmacological properties of terbutaline (INN), 1-(3,5-dihydroxyphenyl)-2-(T-butylamino)-ethanol. A new sympathomimetic beta-receptor-stimulating agent. Acta Med Scand Suppl:11–19

Piascik MT, Perez DM (2001) Alpha1-adrenergic receptors: new insights and directions. J Pharmacol Exp Ther 298(2):403–410

Piascik MT, Guarino RD, Smith MS, Soltis EE, Saussy DL Jr, Perez DM (1995) The specific contribution of the novel alpha-1D adrenoceptor to the contraction of vascular smooth muscle. J Pharmacol Exp Ther 275(3):1583–1589

Pietri-Rouxel F, Strosberg AD (1995) Pharmacological characteristics and species-related variations of beta 3-adrenergic receptors. Fundam Clin Pharmacol 9(3):211–218

Pihlavisto M, Sjoholm B, Scheinin M, Wurster S (1998) Modulation of agonist binding to recombinant human alpha2-adrenoceptors by sodium ions. Biochim Biophys Acta 1448(1): 135–146

Pitha J, Hughes BA, Kusiak JW, Dax EM, Baker SP (1982) Regeneration of beta-adrenergic receptors in senescent rats: a study using an irreversible binding antagonist. Proc Natl Acad Sci U S A 79(14):4424–4427

Piwnica D, Rosignoli C, de Menonville ST, Alvarez T, Schuppli Nollet M, Roye O et al (2014) Vasoconstriction and anti-inflammatory properties of the selective alpha-adrenergic receptor agonist brimonidine. J Dermatol Sci 75(1):49–54

Powell CE, Slater IH (1958) Blocking of inhibitory adrenergic receptors by a dichloro analog of isoproterenol. J Pharmacol Exp Ther 122(4):480–488

Preitner F, Muzzin P, Revelli JP, Seydoux J, Galitzky J, Berlan M et al (1998) Metabolic response to various beta-adrenoceptor agonists in beta3-adrenoceptor knockout mice: evidence for a new beta-adrenergic receptor in brown adipose tissue. Br J Pharmacol 124(8):1684–1688

Prichard BN (1988) Beta-blockage therapy and cardiovascular disease. Past, present, and future. Postgrad Med:8–18

Procopiou PA, Barrett VJ, Bevan NJ, Biggadike K, Box PC, Butchers PR et al (2010) Synthesis and structure-activity relationships of long-acting beta2 adrenergic receptor agonists incorporating metabolic inactivation: an antedrug approach. J Med Chem 53(11):4522–4530

Proudman RGW, Baker JG (2021) The selectivity of alpha-adrenoceptor agonists for the human alpha1A, alpha1B, and alpha1D-adrenoceptors. Pharmacol Res Perspect 9(4):e00799

Proudman RGW, Pupo AS, Baker JG (2020) The affinity and selectivity of alpha-adrenoceptor antagonists, antidepressants, and antipsychotics for the human alpha1A, alpha1B, and alpha1D-adrenoceptors. Pharmacol Res Perspect 8(4):e00602

Proudman RGW, Akinaga J, Baker JG (2022a) The signaling and selectivity of alpha-adrenoceptor agonists for the human alpha2A, alpha2B and alpha2C-adrenoceptors and comparison with human alpha1 and beta-adrenoceptors. Pharmacol Res Perspect 10(5):e01003

Proudman RGW, Akinaga J, Baker JG (2022b) The affinity and selectivity of alpha-adrenoceptor antagonists, antidepressants and antipsychotics for the human alpha2A, alpha2B, and alpha2C-adrenoceptors and comparison with human alpha1 and beta-adrenoceptors. Pharmacol Res Perspect 10(2):e00936

Qu L, Zhou Q, Xu Y, Guo Y, Chen X, Yao D et al (2019) Structural basis of the diversity of adrenergic receptors. Cell Rep 29(10):2929–35 e4

Quaresma B, Pimenta AR, Santos da Silva AC, Pupo AS, Romeiro LAS, Silva CLM et al (2019) Revisiting the pharmacodynamic uroselectivity of alpha (1)-adrenergic receptor antagonists. J Pharmacol Exp Ther 371(1):106–112

Quint JK, Herrett E, Bhaskaran K, Timmis A, Hemingway H, Wedzicha JA et al (2013) Effect of beta blockers on mortality after myocardial infarction in adults with COPD: population based cohort study of UK electronic healthcare records. BMJ 347:f6650

Quinton L, Girard E, Maiga A, Rekik M, Lluel P, Masuyer G et al (2010) Isolation and pharmacological characterization of AdTx1, a natural peptide displaying specific insurmountable antagonism of the alpha1A-adrenoceptor. Br J Pharmacol 159(2):316–325

Raskind MA, Dobie DJ, Kanter ED, Petrie EC, Thompson CE, Peskind ER (2000) The alpha1-adrenergic antagonist prazosin ameliorates combat trauma nightmares in veterans with posttraumatic stress disorder: a report of 4 cases. J Clin Psychiatry 61(2):129–133

Rasmussen SG, Choi HJ, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC et al (2007) Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. Nature 450(7168):383–387

Rasmussen DB, Bodtger U, Lamberts M, Torp-Pedersen C, Gislason G, Lange P et al (2020) Beta-blocker use and acute exacerbations of COPD following myocardial infarction: a Danish nationwide cohort study. Thorax 75(11):928–933

Regan JW, Kobilka TS, Yang-Feng TL, Caron MG, Lefkowitz RJ, Kobilka BK (1988) Cloning and expression of a human kidney cDNA for an alpha 2-adrenergic receptor subtype. Proc Natl Acad Sci U S A 85(17):6301–6305

Rizza RA, Cryer PE, Haymond MW, Gerich JE (1980) Adrenergic mechanisms for the effects of epinephrine on glucose production and clearance in man. J Clin Invest 65(3):682–689

Roberts SJ, Russell FD, Molenaar P, Summers RJ (1995) Characterization and localization of atypical beta-adrenoceptors in rat ileum. Br J Pharmacol 116(6):2549–2556

Roberts SJ, Papaioannou M, Evans BA, Summers RJ (1997) Functional and molecular evidence for beta 1-, beta 2- and beta 3-adrenoceptors in human colon. Br J Pharmacol 120(8):1527–1535

Ross EJ, Prichard BN, Kaufman L, Robertson AI, Harries BJ (1967) Preoperative and operative management of patients with phaeochromocytoma. Br Med J 1(5534):191–198

Ruuskanen JO, Xhaard H, Marjamaki A, Salaneck E, Salminen T, Yan YL et al (2004) Identification of duplicated fourth alpha2-adrenergic receptor subtype by cloning and mapping of five receptor genes in zebrafish. Mol Biol Evol 21(1):14–28

Sabio M, Jones K, Topiol S (2008) Use of the X-ray structure of the beta2-adrenergic receptor for drug discovery. Part 2: identification of active compounds. Bioorg Med Chem Lett 18(20): 5391–5395

Sarsero D, Molenaar P, Kaumann AJ (1998) Validity of (−)-[3H]-CGP 12177A as a radioligand for the 'putative beta4-adrenoceptor' in rat atrium. Br J Pharmacol 123(3):371–380

Sarsero D, Molenaar P, Kaumann AJ, Freestone NS (1999) Putative beta 4-adrenoceptors in rat ventricle mediate increases in contractile force and cell Ca2+: comparison with atrial receptors and relationship to (−)-[3H]-CGP 12177 binding. Br J Pharmacol 128(7):1445–1460

Sarsero D, Russell FD, Lynham JA, Rabnott G, Yang I, Fong KM et al (2003) (−)-CGP 12177 increases contractile force and hastens relaxation of human myocardial preparations through a propranolol-resistant state of the beta 1-adrenoceptor. Naunyn Schmiedebergs Arch Pharmacol 367(1):10–21

Sato M, Horinouchi T, Hutchinson DS, Evans BA, Summers RJ (2007) Ligand-directed signaling at the beta3-adrenoceptor produced by SR59230A relative to receptor agonists. Mol Pharmacol 72:1359–1368

Sato M, Hutchinson DS, Evans BA, Summers RJ (2008) The beta3-adrenoceptor agonist 4-[[(Hexylamino)carbonyl]amino]-N-[4-[2-[[(2S)-2-hydroxy-3-(4-hydroxyphenoxy)prop yl] amino]ethyl]-phenyl]-benzenesulfonamide (L755507) and antagonist (S)-N-[4-[2-[[3-[3-(acetamidomethyl)phenoxy]-2-hydroxypropyl]amino]-ethyl]phenyl] benzenesulfonamide (L748337) activate different signaling pathways in Chinese hamster ovary-K1 cells stably expressing the human beta3-adrenoceptor. Mol Pharmacol 74(5):1417–1428

Sato M, Dehvari N, Oberg AI, Dallner OS, Sandstrom AL, Olsen JM et al (2014) Improving type 2 diabetes through a distinct adrenergic signaling pathway involving mTORC2 that mediates glucose uptake in skeletal muscle. Diabetes 63(12):4115–4129

Sato T, Baker J, Warne T, Brown GA, Leslie AG, Congreve M et al (2015) Pharmacological analysis and structure determination of 7-methylcyanopindolol-bound beta1-adrenergic receptor. Mol Pharmacol 88(6):1024–1034

Sawangkoon S, Miyamoto M, Nakayama T, Hamlin RL (2000) Acute cardiovascular effects and pharmacokinetics of carvedilol in healthy dogs. Am J Vet Res 61(1):57–60

Schena G, Caplan MJ (2019) Everything you always wanted to know about beta3-AR * (*but were afraid to ask). Cells 8(4)

Schwinn DA, Lomasney JW, Lorenz W, Szklut PJ, Fremeau RT Jr, Yang-Feng TL et al (1990) Molecular cloning and expression of the cDNA for a novel alpha 1-adrenergic receptor subtype. J Biol Chem 265(14):8183–8189

Schwinn DA, Johnston GI, Page SO, Mosley MJ, Wilson KH, Worman NP et al (1995) Cloning and pharmacological characterization of human alpha-1 adrenergic receptors: sequence corrections and direct comparison with other species homologues. J Pharmacol Exp Ther 272(1):134–142

Sharma D, Farrar JD (2020) Adrenergic regulation of immune cell function and inflammation. Semin Immunopathol 42(6):709–717

Shibata K, Foglar R, Horie K, Obika K, Sakamoto A, Ogawa S et al (1995) KMD-3213, a novel, potent, alpha 1a-adrenoceptor-selective antagonist: characterization using recombinant human alpha 1-adrenoceptors and native tissues. Mol Pharmacol 48(2):250–258

Simonneaux V, Ebadi M, Bylund DB (1991) Identification and characterization of alpha 2D-adrenergic receptors in bovine pineal gland. Mol Pharmacol 40(2):235–241

Slack RJ, Barrett VJ, Morrison VS, Sturton RG, Emmons AJ, Ford AJ et al (2013) In vitro pharmacological characterization of vilanterol, a novel long-acting beta2-adrenoceptor agonist with 24-hour duration of action. J Pharmacol Exp Ther 344(1):218–230

Sloan EK, Priceman SJ, Cox BF, Yu S, Pimentel MA, Tangkanangnukul V et al (2010) The sympathetic nervous system induces a metastatic switch in primary breast cancer. Cancer Res 70(18):7042–7052

Soave M, Stoddart LA, Brown A, Woolard J, Hill SJ (2016) Use of a new proximity assay (NanoBRET) to investigate the ligand-binding characteristics of three fluorescent ligands to the human beta1-adrenoceptor expressed in HEK-293 cells. Pharmacol Res Perspect 4(5): e00250

Soave M, Briddon SJ, Hill SJ, Stoddart LA (2020) Fluorescent ligands: bringing light to emerging GPCR paradigms. Br J Pharmacol 177(5):978–991

Spear HC, Griswold D (1948) The use of dibenamine in pheochromocytoma; report of a case. N Engl J Med 239(20):736–739

Staehelin M, Simons P, Jaeggi K, Wigger N (1983) CGP-12177. A hydrophilic beta-adrenergic receptor radioligand reveals high affinity binding of agonists to intact cells. J Biol Chem 258(6): 3496–3502

Stahle H (2000) A historical perspective: development of clonidine. Best Pract Res Clin Anaesthiol 14:237–246

Starke K, Montel H, Gayk W, Merker R (1974) Comparison of the effects of clonidine on pre- and postsynaptic adrenoceptors in the rabbit pulmonary artery. Alpha-sympathomimetic inhibition of neurogenic vasoconstriction. Naunyn Schmiedebergs Arch Pharmacol 285(2):133–150

Stiles GL, Taylor S, Lefkowitz RJ (1983) Human cardiac beta-adrenergic receptors: subtype heterogeneity delineated by direct radioligand binding. Life Sci 33(5):467–473

Storch U, Straub J, Erdogmus S, Gudermann T, Mederos YSM (2017) Dynamic monitoring of Gi/o-protein-mediated decreases of intracellular cAMP by FRET-based Epac sensors. Pflugers Arch 469(5–6):725–737

Strange PG (2008) Agonist binding, agonist affinity and agonist efficacy at G protein-coupled receptors. Br J Pharmacol 153(7):1353–1363

Strosberg AD (1997) Structure and function of the beta 3-adrenergic receptor. Annu Rev Pharmacol Toxicol 37:421–450

Sugimoto Y, Fujisawa R, Tanimura R, Lattion AL, Cotecchia S, Tsujimoto G et al (2002) Beta(1)-selective agonist (−)-1-(3,4-dimethoxyphenetylamino)-3-(3,4-dihydroxy)-2-propanol [(−)-RO363] differentially interacts with key amino acids responsible for beta(1)-selective binding in resting and active states. J Pharmacol Exp Ther 301(1):51–58

Summers RJ, Molenaar P, Stephenson JA (1987) Autoradiographic localisation of receptors in the cardiovascular system. Trends Pharmacol Sci 8:272–276

Summers RJ, Molnaar P, Russell F, Elnatan J, Jones CR, Buxton BF et al (1989) Coexistence and localization of beta 1- and beta 2-adrenoceptors in the human heart. Eur Heart J 10(Suppl B):11–21

Sykes DA, Charlton SJ (2012) Slow receptor dissociation is not a key factor in the duration of action of inhaled long-acting beta2-adrenoceptor agonists. Br J Pharmacol 165(8):2672–2683

Takasu T, Ukai M, Sato S, Matsui T, Nagase I, Maruyama T et al (2007) Effect of (R)-2-(2-aminothiazol-4-yl)-4′-{2-[(2-hydroxy-2-phenylethyl)amino]ethyl} acetanilide (YM178), a novel selective beta3-adrenoceptor agonist, on bladder function. J Pharmacol Exp Ther 321(2):642–647

Tam SW, Worcel M, Wyllie M (2001) Yohimbine: a clinical review. Pharmacol Ther 91(3): 215–243

Taniguchi T, Inagaki R, Murata S, Akiba I, Muramatsu I (1999) Microphysiometric analysis of human alpha1a-adrenoceptor expressed in Chinese hamster ovary cells. Br J Pharmacol 127(4): 962–968

Tattersfield AE, Cragg DJ (1983) Effect of ICI 118551 on bronchial beta-adrenoceptor function and exercise heart rate in normal man. Br J Clin Pharmacol 16(6):587–590

The Xamoterol in Severe Heart Failure Study Group (1990) Xamoterol in severe heart failure. Lancet 336(8706):1–6

Theroux TL, Esbenshade TA, Peavy RD, Minneman KP (1996) Coupling efficiencies of human alpha 1-adrenergic receptor subtypes: titration of receptor density and responsiveness with inducible and repressible expression vectors. Mol Pharmacol 50(5):1376–1387

Thomsen W, Frazer J, Unett D (2005) Functional assays for screening GPCR targets. Curr Opin Biotechnol 16(6):655–665

Udumyan R, Montgomery S, Fang F, Almroth H, Valdimarsdottir U, Ekbom A et al (2017) Beta-blocker drug use and survival among patients with pancreatic adenocarcinoma. Cancer Res 77(13):3700–3707

Uhlen S, Porter AC, Neubig RR (1994) The novel alpha-2 adrenergic radioligand [3H]-MK912 is alpha-2C selective among human alpha-2A, alpha-2B and alpha-2C adrenoceptors. J Pharmacol Exp Ther 271(3):1558–1565

Uhlen S, Dambrova M, Nasman J, Schioth HB, Gu Y, Wikberg-Matsson A et al (1998) [3H] RS79948-197 binding to human, rat, Guinea pig and pig alpha2A-, alpha2B- and alpha2C-adrenoceptors. Comparison with MK912, RX821002, rauwolscine and yohimbine. Eur J Pharmacol 343(1):93–101

Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A et al (2015) Proteomics. Tissue-based map of the human proteome. Science 347(6220):1260419

U'Prichard DC, Greenberg DA, Snyder SH (1977) Binding characteristics of a radiolabeled agonist and antagonist at central nervous system alpha noradrenergic receptors. Mol Pharmacol 13(3): 454–473

Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka B, Weinstein H et al (2007) Functional selectivity and classical concepts of quantitative pharmacology. J Pharmacol Exp Ther 320(1): 1–13

Urits I, Patel A, Zusman R, Virgen CG, Mousa M, Berger AA et al (2020) A comprehensive update of Lofexidine for the management of opioid withdrawal symptoms. Psychopharmacol Bull 50(3):76–96

Vago T, Bevilacqua M, Dagani R, Meroni R, Frigeni G, Santoliss C et al (1984) Comparison of rat and human left ventricle beta-adrenergic receptors: subtype heterogeneity delineated by direct radioligand binding. Biochem Biophys Res Commun 121(1):346–354

van Wieringen JP, Michel-Reher MB, Hatanaka T, Ueshima K, Michel MC (2013) The new radioligand [(3)H]-L 748,337 differentially labels human and rat beta3-adrenoceptors. Eur J Pharmacol 720(1–3):124–130

Vrydag W, Alewijnse AE, Michel MC (2009) Do gene polymorphisms alone or in combination affect the function of human beta3-adrenoceptors? Br J Pharmacol 156(1):127–134

Wagner MJ, Cranmer LD, Loggers ET, Pollack SM (2018) Propranolol for the treatment of vascular sarcomas. J Exp Pharmacol 10:51–58

Walter M, Lemoine H, Kaumann AJ (1984) Stimulant and blocking effects of optical isomers of pindolol on the sinoatrial node and trachea of Guinea pig. Role of beta-adrenoceptor subtypes in the dissociation between blockade and stimulation. Naunyn Schmiedebergs Arch Pharmacol 327(2):159–175

Wang T, Li Z, Cvijic ME, Zhang L, Sum CS (2004) Measurement of cAMP for Gαs- and Gαi Protein-Coupled Receptors (GPCRs). In: Assay guidance manual [Internet]. Eli Lilly and Company and the National Center for Advancing Translational Sciences, Bethesda, MD. Available from: https://www.ncbi.nlm.nih.gov/books/NBK464633/?report=reader

Wang J, Hanada K, Staus DP, Makara MA, Dahal GR, Chen Q et al (2017) Galphai is required for carvedilol-induced beta1 adrenergic receptor beta-arrestin biased signaling. Nat Commun 8(1): 1706

Wang Y, Shi Q, Li M, Zhao M, Reddy Gopireddy R, Teoh JP et al (2021) Intracellular beta1-adrenergic receptors and organic cation transporter 3 mediate Phospholamban phosphorylation to enhance cardiac contractility. Circ Res 128(2):246–261

Warne T, Serrano-Vega MJ, Baker JG, Moukhametzianov R, Edwards PC, Henderson R et al (2008) Structure of a beta1-adrenergic G-protein-coupled receptor. Nature 454(7203):486–491

Weerink MAS, Struys M, Hannivoort LN, Barends CRM, Absalom AR, Colin P (2017) Clinical pharmacokinetics and pharmacodynamics of dexmedetomidine. Clin Pharmacokinet 56(8): 893–913

Wei W, Smrcka AV (2022) Subcellular beta-adrenergic receptor signaling in cardiac physiology and disease. J Cardiovasc Pharmacol 80(3):334–341

Weinshank RL, Zgombick JM, Macchi M, Adham N, Lichtblau H, Branchek TA et al (1990) Cloning, expression, and pharmacological characterization of a human alpha 2B-adrenergic receptor. Mol Pharmacol 38(5):681–688

Wetterlin KIL, Svensson LA (1968) Inventors

Wetzel JM, Miao SW, Forray C, Borden LA, Branchek TA, Gluchowski C (1995) Discovery of alpha 1a-adrenergic receptor antagonists based on the L-type Ca2+ channel antagonist niguldipine. J Med Chem 38(10):1579–1581

White CW, Choong YT, Short JL, Exintaris B, Malone DT, Allen AM et al (2013) Male contraception via simultaneous knockout of alpha1A-adrenoceptors and P2X1-purinoceptors in mice. Proc Natl Acad Sci U S A 110(51):20825–20830

Williams TJ, Blue DR, Daniels DV, Davis B, Elworthy T, Gever JR et al (1999) In vitro alpha1-adrenoceptor pharmacology of Ro 70-0004 and RS-100329, novel alpha1A-adrenoceptor selective antagonists. Br J Pharmacol 127(1):252–258

Wilson AL, Seibert K, Brandon S, Cragoe EJ Jr, Limbird LE (1991) Monovalent cation and amiloride analog modulation of adrenergic ligand binding to the unglycosylated alpha 2B-adrenergic receptor subtype. Mol Pharmacol 39(4):481–486

Wisler JW, DeWire SM, Whalen EJ, Violin JD, Drake MT, Ahn S et al (2007) A unique mechanism of beta-blocker action: carvedilol stimulates beta-arrestin signaling. Proc Natl Acad Sci U S A 104(42):16657–16662

Woo AY, Ge XY, Pan L, Xing G, Mo YM, Xing RJ et al (2019) Discovery of beta-arrestin-biased beta2-adrenoceptor agonists from 2-amino-2-phenylethanol derivatives. Acta Pharmacol Sin 40(8):1095–1105

Wu Y, Zeng L, Zhao S (2021) Ligands of adrenergic receptors: A structural point of view. Biomol Ther 11(7)

Xiang YK (2011) Compartmentalization of beta-adrenergic signals in cardiomyocytes. Circ Res 109(2):231–244

Yamashima T (2003) Jokichi Takamine (1854-1922), the samurai chemist, and his work on adrenalin. J Med Biogr 11(2):95–102

Yanagisawa T, Sato T, Yamada H, Sukegawa J, Nunoki K (2000) Selectivity and potency of agonists for the three subtypes of cloned human beta-adrenoceptors expressed in Chinese hamster ovary cells. Tohoku J Exp Med 192(3):181–193

Yuan D, Liu Z, Kaindl J, Maeda S, Zhao J, Sun X et al (2020) Activation of the alpha2B adrenoceptor by the sedative sympatholytic dexmedetomidine. Nat Chem Biol 16(5):507–512

Zhang M, Chen F, Sun X, Huang Y, Zeng Y, Chen J et al (2023) Sympathetic beta(2)-adrenergic receptor blockade overcomes docetaxel resistance in prostate cancer. Biochem Biophys Res Commun 657:69–79

Zhong H, Minneman KP (1999) Differential activation of mitogen-activated protein kinase pathways in PC12 cells by closely related alpha1-adrenergic receptor subtypes. J Neurochem 72(6):2388–2396

Signalling of Adrenoceptors: Canonical Pathways and New Paradigms

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Abstract

The concept of G protein-coupled receptors initially arose from studies of the β-adrenoceptor, adenylyl cyclase, and cAMP signalling pathway. Since then both canonical G protein-coupled receptor signalling pathways and emerging paradigms in receptor signalling have been defined by experiments focused on adrenoceptors. Here, we discuss the evidence for G protein coupling specificity of the nine adrenoceptor subtypes. We summarise the ability of each of the adrenoceptors to activate proximal signalling mediators including cAMP, calcium, mitogen-activated protein kinases, and protein kinase C pathways. Finally, we highlight the importance of precise spatial and temporal control of adrenoceptor signalling that is controlled by the localisation of receptors at intracellular membranes and in larger protein complexes.

Keywords

Adrenergic receptors · Adrenoceptors · Compartmentalised signalling · G proteins · Intracellular receptors · Signalling

1 Introduction

The classical view of the activation and signalling of G protein-coupled receptors (GPCRs) is defined by research on adrenoceptors (Beavo and Brunton 2002). Ligand binding to the receptor induces a conformational change, which facilitates the exchange of GDP for GTP in the Gα subunit of the heterotrimeric G protein complex. This nucleotide exchange leads to the dissociation of the Gα from the Gβγ subunits and activation of downstream second messenger signalling pathways such as cAMP, calcium, inositol trisphosphate ($InsP_3$), and mitogen-activated protein kinase (MAPK) cascades. These second messenger signalling pathways feedback to desensitise and internalise many (but not all) receptors via the recruitment of G protein receptor kinases (GRKs) and β-arrestins. However, the generation of the same limited second messengers by so many GPCRs (each cell expresses ~100 different GPCRs (Insel et al. 2012)) seemed insufficient to explain the complex signalling and vast range of observed physiological outcomes. The advent of a wide range of biosensors with the sensitivity to quantify signalling at a cellular and subcellular level has revealed a much more intricate control of GPCR signalling (reviewed in Halls 2019; Halls and Canals 2018). Using these technological advances, research on adrenoceptors has been primarily responsible for shifting our view of the activation and regulation of GPCR signalling. As a result, the importance of the texture of the initiated intracellular signal – or its spatiotemporal properties – is now recognised as key for GPCRs to initiate unique physiological outcomes (reviewed in Calebiro and Grimes 2020; DeFea 2011; Eichel and von

Zastrow 2018; Jong et al. 2018; Weinberg and Puthenveedu 2019). Here, we will provide an overview of the canonical signalling pathways activated by adrenoceptors. We then highlight research that reveals added depth to the repertoire of GPCR signalling: from adrenoceptors at intracellular membranes to the signalling of adrenoceptors in larger protein complexes.

2 G Protein Coupling of Adrenoceptors

In the last decade, X-ray crystal and cryogenic electron microscopy (cryo-EM) structures of adrenoceptors (ARs) bound to their cognate G proteins have emerged; these include structures for the α_{1A}-AR (Toyoda et al. 2023; Su et al. 2023), α_{2A}-AR (Xu et al. 2022; Fink et al. 2022), α_{2B}-AR (Yuan et al. 2020), β_1-AR (Su et al. 2020), β_2-AR (Rasmussen et al. 2011), and β_3-AR (Nagiri et al. 2021). These high-resolution structures confirmed the molecular interactions that were previously elucidated using biochemical methods, for example, the key interaction between the α5-helix of the Gα subunit and the intracellular side of the adrenoceptor transmembrane bundle (Milligan and Rees 1999).

Adrenoceptors are divided into the α_1-AR, α_2-AR, and β-AR subfamilies based on their ligand selectivity and G protein coupling (reviewed in Bylund 1988). The canonical signalling pathway utilised by the α_1-ARs involves coupling to the $G\alpha_{q/11}$ subfamily of heterotrimeric G proteins. The α_2-ARs couple to the inhibitory $G\alpha_{i/o}$ subfamily, and the β-ARs couple to the stimulatory $G\alpha_{s/olf}$ subfamily. It is important to remember that within these broad coupling classifications, each individual adrenoceptor subtype displays a more complex G protein coupling profile (Table 1). However, while adrenoceptors can couple to a range of G proteins in recombinant systems, it is not yet clear whether the coupling is relevant in vivo. For example, $G\alpha_{15}$ is promiscuous in its coupling to many GPCRs, including the β_2-AR (Wu et al. 1995), although it would rarely play a major role in adrenoceptor signalling as its expression is largely restricted to hematopoietic cell lineages (Amatruda et al. 1991).

2.1 G Protein Coupling of the α_1-ARs

The α_1-ARs couple to the $G\alpha_{q/11}$ subfamily. The activation of $G\alpha_{q/11}$ proteins leads to the stimulation of phospholipase C β (PLCβ) that hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol trisphosphate ($InsP_3$) and diacylglycerol (DAG). These in turn act as second messengers to activate downstream targets: $InsP_3$ can stimulate $InsP_3$ receptors (IP_3R) to release calcium from the endoplasmic or sarcoplasmic reticulum, and DAG directly activates protein kinase C (PKC) to phosphorylate several targets (see Sect. 3.4).

While it was known that α_1-ARs stimulated $InsP_3$ production, determination of the G protein subtype that mediated this signalling was more challenging. In fact, the G protein species that couples to the α_1-ARs was only confirmed *after*

Table 1 G protein coupling of adrenoceptors. Each adrenoceptor shows promiscuous coupling to multiple G proteins beyond the canonical classification of α_1-ARs as $G\alpha_{q/11}$-coupled receptors (Wu et al. 1992), α_2-ARs as $G\alpha_{i/o}$-coupled receptors (Cotecchia et al. 1990), and β-ARs as $G\alpha_{s/olf}$-coupled receptors (Gilman 1987). This table lists direct evidence for coupling of the different receptors to distinct Gα subtypes. For the G protein chimera assay, only G protein coupling that reached the high-confidence threshold is reported in the table (Inoue et al. 2019)

	Gα protein family			
Receptor	$G\alpha_{s/olf}$	$G\alpha_{i/o}$	$G\alpha_{q/11}$	$G\alpha_{12/13}$
α_{1A}-AR	$G\alpha_s$ (Inoue et al. 2019; Avet et al. 2022) $G\alpha_{olf}$ (Inoue et al. 2019)		$G\alpha_q$ (Wu et al. 1992; Inoue et al. 2019; Avet et al. 2022) $G\alpha_{11}$ (Wu et al. 1992; Avet et al. 2022) $G\alpha_{14}$ (Inoue et al. 2019; Avet et al. 2022) $G\alpha_{15}$ (Avet et al. 2022)	$G\alpha_{13}$ (Inoue et al. 2019)
α_{1B}-AR	$G\alpha_s$ (Inoue et al. 2019) $G\alpha_{olf}$ (Inoue et al. 2019)		$G\alpha_q$ (Wu et al. 1992; Inoue et al. 2019) $G\alpha_{11}$ (Wu et al. 1992)	$G\alpha_{14}$ (Inoue et al. 2019)
α_{1D}-AR	$G\alpha_s$ (Inoue et al. 2019) $G\alpha_{olf}$ (Inoue et al. 2019)		$G\alpha_q$ (Wu et al. 1992; Inoue et al. 2019) $G\alpha_{11}$ (Wu et al. 1992)	$G\alpha_{14}$ (Inoue et al. 2019)
α_{2A}-AR	$G\alpha_s$ (Avet et al. 2022)	$G\alpha_{i1}$ (Inoue et al. 2019; Avet et al. 2022) $G\alpha_{i2}$ (Avet et al. 2022) $G\alpha_{i3}$ (Inoue et al. 2019) $G\alpha_{oA}$ (Inoue et al. 2019; Avet et al. 2022) $G\alpha_{oB}$ (Avet et al. 2022) $G\alpha_z$ (Inoue et al. 2019; Avet et al. 2022)	$G\alpha_q$ (Avet et al. 2022) $G\alpha_{11}$ (Avet et al. 2022) $G\alpha_{14}$ (Avet et al. 2022) $G\alpha_{15}$ (Avet et al. 2022)	$G\alpha_{12}$ (Avet et al. 2022) $G\alpha_{13}$ (Avet et al. 2022)
α_{2B}-AR	$G\alpha_s$ (Nasman et al. 2001)	$G\alpha_{i1}$ (Nasman et al. 2001; Inoue et al. 2019; Avet et al. 2022) $G\alpha_{i2}$ (Avet et al. 2022) $G\alpha_{i3}$ (Inoue et al. 2019)	$G\alpha_{15}$ (Avet et al. 2022)	$G\alpha_{12}$ (Inoue et al. 2019)

		$G\alpha_{oA}$ (Inoue et al. 2019; Avet et al. 2022) $G\alpha_{oB}$ (Avet et al. 2022) $G\alpha_{z}$ (Inoue et al. 2019; Avet et al. 2022)		
α_{2C}-AR		$G\alpha_{i1}$ (Inoue et al. 2019; Avet et al. 2022) $G\alpha_{i2}$ (Avet et al. 2022) $G\alpha_{i3}$ (Inoue et al. 2019) $G\alpha_{oA}$ (Inoue et al. 2019; Avet et al. 2022) $G\alpha_{oB}$ (Avet et al. 2022) $G\alpha_{z}$ (Inoue et al. 2019; Avet et al. 2022)	$G\alpha_{15}$ (Avet et al. 2022)	$G\alpha_{13}$ (Avet et al. 2022)
β_1-AR	$G\alpha_{s}$ (Inoue et al. 2019; Lukasheva et al. 2020; Avet et al. 2022) $G\alpha_{olf}$ (Inoue et al. 2019)	$G\alpha_{i2}$ (Lukasheva et al. 2020) $G\alpha_{oA}$ (Avet et al. 2022) $G\alpha_{oB}$ (Avet et al. 2022) $G\alpha_{z}$ (Lukasheva et al. 2020; Avet et al. 2022)	$G\alpha_{q}$ (Avet et al. 2022) $G\alpha_{14}$ (Inoue et al. 2019; Avet et al. 2022) $G\alpha_{15}$ (Avet et al. 2022)	$G\alpha_{12}$ (Lukasheva et al. 2020; Avet et al. 2022) $G\alpha_{13}$ (Avet et al. 2022)
β_2-AR	$G\alpha_{s}$ (Masuho et al. 2015; Inoue et al. 2019; Olsen et al. 2020; Avet et al. 2022) $G\alpha_{olf}$ (Masuho et al. 2015; Inoue et al. 2019; Olsen et al. 2020)	$G\alpha_{i2}$ (Lukasheva et al. 2020; Olsen et al. 2020) $G\alpha_{oA}$ (Masuho et al. 2015; Olsen et al. 2020) $G\alpha_{oB}$ (Masuho et al. 2015; Olsen et al. 2020) $G\alpha_{z}$ (Masuho et al. 2015; Lukasheva et al. 2020; Olsen et al. 2020)	$G\alpha_{q}$ (Inoue et al. 2019) $G\alpha_{14}$ (Inoue et al. 2019) $G\alpha_{15}$ (Masuho et al. 2015; Olsen et al. 2020; Avet et al. 2022)	$G\alpha_{12}$ (Lukasheva et al. 2020)
β_3-AR	$G\alpha_{s}$ (Inoue et al. 2019; Avet et al. 2022) $G\alpha_{olf}$ (Inoue et al. 2019)			

characterisation of the G protein coupling of both α_2-ARs and β-ARs because the pharmacological tools to interrogate the $G\alpha_{q/11}$ subfamily were unavailable at the time. The α_1-ARs were shown to couple to $G\alpha_{q/11}$ by systematically measuring the production of inositol phosphates after transfection of individual $G\alpha_{q/11}$ subunits into COS-7 cells expressing the α_1-AR subtypes (Wu et al. 1992). This study suggested that all three α_1-AR subtypes couple robustly to $G\alpha_q$ and $G\alpha_{11}$ to stimulate inositol phosphate production. The different subtypes also stimulated $G\alpha_{14}$- and $G\alpha_{15}$-dependent inositol phosphate production to varying degrees. For example, the α_{1A}-AR is a poor activator of both $G\alpha_{14}$ and $G\alpha_{15}$ compared to the α_{1B}-AR and α_{1D}-AR (Wu et al. 1992). The α_1-ARs can also couple to the $G\alpha_{12/13}$ subfamily to activate Rho kinase that mediates the contractile response stimulated by α_{1D}-ARs in the rat aorta and α_{1A}-ARs in the rat caudal artery (Mueed et al. 2004) (see Sect. 4.3).

Recently, the coupling profiles of the three α_1-ARs were comprehensively assessed across all G protein subfamilies in vitro by two independent laboratories (Table 1). One approach used an assay that relies on $G\alpha_q$ protein chimeras, whereby the six residues at the C-terminal end of the α5-helix responsible for GPCR interaction in $G\alpha_q$ were systematically replaced with the six C-terminal residues of each Gα subunit of interest (Inoue et al. 2019). Activation of the $G\alpha_q$ chimera stimulates a plasma membrane-localised metalloprotease to shed an alkaline phosphatase-fused transforming growth factor α reporter. It should be noted that this assay cannot distinguish between $G\alpha_{i1}$ and $G\alpha_{i2}$ nor between $G\alpha_q$ and $G\alpha_{11}$ coupling as the six C-terminal residues are identical for these two Gα pairs. Nonetheless, the $G\alpha_q$ protein chimera assay allows a non-biased comparison of the activation of each different Gα subunit by measuring the same signalling response. The authors set a high-confidence threshold on whether they determined a particular receptor coupled to a particular G protein, which maximised the rate of true positives compared to false positives across all 148 GPCRs (Inoue et al. 2019). Another G protein screening study used a G protein effector membrane translocation assay, termed GEMTA (Avet et al. 2022). This assay measures the translocation of a particular Gα subunit to the plasma membrane after G protein activation using enhanced bystander bioluminescence resonance energy transfer.

Using the G protein chimera strategy, while it appeared that stimulation of the α_{1A}-AR by noradrenaline caused concentration-dependent activation of all G protein subtypes, only the activation of $G\alpha_s$, $G\alpha_{olf}$, $G\alpha_q$, $G\alpha_{13}$, and $G\alpha_{14}$ met the high-confidence threshold (Inoue et al. 2019). The robust activation of $G\alpha_{14}$ contrasted previous reports of relatively poor $G\alpha_{14}$ activation by the α_{1A}-AR (Wu et al. 1992). Using the GEMTA measurement, the α_{1A}-AR was shown to activate all four $G\alpha_{q/11}$ members as well as $G\alpha_s$ (Avet et al. 2022). This suggests that the previous study by Wu and colleagues may not have been sensitive enough to detect low levels of $G\alpha_{14}$ and $G\alpha_{15}$ activation (Wu et al. 1992). In the case of the α_{1B}-AR, the coupling appears to be surprisingly robust for $G\alpha_s$ $G\alpha_{olf}$, $G\alpha_q$, and $G\alpha_{14}$ (Inoue et al. 2019). Concentration-dependent coupling was also detected for $G\alpha_{i/o}$ members and $G\alpha_{13}$; however, this did not meet the high-confidence threshold. The α_{1D}-AR appears to be the most selective of the α_1-AR subtypes in its G protein coupling profile, having the ability to activate only $G\alpha_q$, $G\alpha_{14}$, $G\alpha_s$, and $G\alpha_{olf}$ (Inoue et al. 2019).

2.2 G Protein Coupling of the α_2-ARs

The α_2-ARs are classified as $G\alpha_{i/o}$-coupled receptors (Cotecchia et al. 1990). α_2-AR-mediated activation of the $G\alpha_{i/o}$ heterotrimer initiates the dissociation of $G\beta\gamma$ subunits. The $G\alpha_{i/o}$ subunit acts on adenylyl cyclase (AC) to inhibit the production of cAMP (Jakobs et al. 1978; Sabol and Nirenberg 1979).

Early studies demonstrated that the α_2-ARs can couple to more diverse $G\alpha$ subtypes beyond the $G\alpha_{i/o}$ subfamily (Table 1). For example, using radiolabelled GTPγS binding assays, the α_{2B}-AR was found to couple to both $G\alpha_{i1}$ and $G\alpha_s$ (Nasman et al. 2001). There are also temporal differences in G protein signalling activated by the different α_2-AR subtypes. G protein signalling downstream of the murine α_{2C}-AR receptor was deactivated at a slower rate than that of the α_{2A}-AR, potentially as a result of the slower rate of dissociation of noradrenaline from the α_{2C}-AR (Bünemann et al. 2001).

Recently, and as described for the α_1-ARs, large-scale α_2-AR-G protein profiling has been undertaken. Together these complementary techniques showed that the α_{2A}-AR has the broadest G protein coupling profile of the three α_2-AR subtypes (Avet et al. 2022; Inoue et al. 2019). As expected, the α_{2A}-AR is a strong activator of $G\alpha_{i/o}$ chimera subunits, with secondary activation of $G\alpha_{s/olf}$, $G\alpha_{q/11}$, and $G\alpha_{12/13}$ also detected (Avet et al. 2022). Interestingly, using the G protein chimera strategy, the concentration-dependent activation of $G\alpha_{s/olf}$, $G\alpha_{q/11}$, and $G\alpha_{12/13}$ families by the α_{2A}-AR did not meet the high-confidence threshold (Inoue et al. 2019). The α_{2B}-AR displayed a similar promiscuity profile in the G protein chimera assay, causing concentration-dependent activation of all G proteins in the $G\alpha_{s/olf}$, $G\alpha_{i/o}$, $G\alpha_{q/11}$, and $G\alpha_{12/13}$ families (Inoue et al. 2019). However, only activation of the $G\alpha_{i/o}$ family and $G\alpha_{12}$ reached the high-confidence threshold. The GEMTA measurement only detected activation of $G\alpha_{15}$ in addition to the canonical $G\alpha_{i/o}$ subunits (Avet et al. 2022). The α_{2C}-AR displayed the most selectivity, principally activating $G\alpha_{i/o}$ subunits with only minor activation of $G\alpha_{15}$ and $G\alpha_{13}$ (Avet et al. 2022; Inoue et al. 2019). There also appeared to be a small, concentration-dependent activation of $G\alpha_q$ and $G\alpha_{14}$ using the G protein chimera assay that did not meet the high-confidence threshold (Inoue et al. 2019). Together these studies suggest that the specificity of $G\alpha_{i/o}$ subfamily signalling varies depending on the particular α_2-AR subtype in question.

2.3 G Protein Coupling of the β-ARs

Each of the three β-AR subtypes is well characterised as $G\alpha_{s/olf}$-coupled receptors. Their activation of $G\alpha_s$ induces the stimulation of ACs, converting ATP to the second messenger cAMP. Within the $G\alpha_s$ subfamily, there are two members: $G\alpha_s$ and $G\alpha_{olf}$. The β-ARs can couple to both $G\alpha_s$ and $G\alpha_{olf}$ in cells transiently transfected with receptor cDNAs (Inoue et al. 2019; Masuho et al. 2015) (Table 1). However, the expression of $G\alpha_{olf}$ is restricted to regions such as the

olfactory system and striatum; therefore, most of the physiological effects of β-ARs are mediated through $G\alpha_s$ (Drinnan et al. 1991; Jones and Reed 1989).

β_1-AR G protein coupling has been assessed by multiple groups. In general, the β_1-AR is the least selective of the β-ARs for coupling to the canonical G protein subtype, $G\alpha_s$. In addition to $G\alpha_s$, the β_1-AR activates members of each of the $G\alpha_{i/o}$, $G\alpha_{q/11}$, and $G\alpha_{12/13}$ subfamilies (Avet et al. 2022; Inoue et al. 2019; Lukasheva et al. 2020). Despite this promiscuity (Table 1), different studies report the activation of distinct Gα subunits within the subfamilies, likely due to differences in assay technologies.

The β_2-AR is predominantly a $G\alpha_s$-coupled receptor (Limbird et al. 1980). However, it is well documented that the β_2-AR can also signal through $G\alpha_{i/o}$. For example, aortic contraction in mice in response to isoprenaline can be reversed by pre-treatment with pertussis toxin (Davel et al. 2014). This is mediated by the β_2-AR as the effect is lost in β_2-AR knockout mice. Lefkowitz and colleagues investigated the G protein coupling of the β_2-AR further and proposed a switch from $G\alpha_s$ to $G\alpha_{i/o}$ coupling controlled by protein kinase A (PKA) phosphorylation of the receptor (Daaka et al. 1997) (see Sect. 3.3). Consistent with these observations, in vitro coupling studies of the β_2-AR suggest that in addition to coupling to $G\alpha_{s/olf}$, the receptor can weakly couple to $G\alpha_{i/o}$ subunits and also to $G\alpha_{14}$ and $G\alpha_{15}$ (Avet et al. 2022; Inoue et al. 2019; Masuho et al. 2015; Olsen et al. 2020).

Analogous to the β_1-AR and β_2-AR, the β_3-AR also displays secondary coupling to G proteins other than $G\alpha_s$. The β_3-AR can couple to $G\alpha_{i/o}$ subunits to limit the amount of cAMP produced through its own $G\alpha_s$ signalling in adipocytes (Chaudhry et al. 1994). This is a key feedback mechanism that controls β_3-AR-driven lipolysis in adipocytes, downstream of the $G\alpha_s$-cAMP-PKA pathway (Murphy et al. 1993). While stimulation of the β_3-AR did appear to cause some concentration-dependent activation of $G\alpha_{i/o}$, $G\alpha_q$, and $G\alpha_{14}$ using the G protein chimera assay in HEK293 cells, this did not meet the high-confidence threshold (Inoue et al. 2019).

3 Adrenoceptor Effects on Proximal Signalling Mediators

In the following sections, we summarise how stimulation of adrenoceptors can impact common signal transduction pathways, such as cAMP, calcium, mitogen-activated protein kinases (MAPKs), and PKC. While we have considered each principal signalling pathway in isolation, it is important to remember that these pathways are inherently linked in the cell. For example, cAMP can both activate and inhibit calcium mobilisation and MAPK signalling (reviewed in Dumaz and Marais 2005; Halls and Cooper 2011; Stork and Schmitt 2002). Calcium can both activate and inhibit cAMP signalling (Halls and Cooper 2011), and PLC activation of PKC activity can occur downstream of either $G\alpha_{q/11}$ or $G\alpha_s$-cAMP signalling (Oude Weernink et al. 2007). In addition to the regulation of GPCR internalisation, both GRKs and β-arrestins have a scaffolding and signalling role (reviewed in DeFea 2011; Penela et al. 2019). Moreover, adrenoceptor ligands are capable of biased signalling – the preferential activation of one signalling pathway over another (see

Chapter "Drugs Interacting with Adrenoceptors: Agonists, Antagonists, Inverse Agonists, Biased Agonists, Allosteric Ligands" for more detail) – and most adrenoceptors can impact signalling processes beyond the canonical pathways described here. More detail on additional signalling activated by adrenoceptors can be found in the IUPHAR/BPS Guide to Pharmacology database (Altosaar et al. 2019).

3.1 cAMP Signalling

Upon agonist binding, β-ARs activate $G\alpha_s$ proteins to stimulate AC to produce cAMP (Fig. 1a). The duration of the cAMP response is controlled by the activity of phosphodiesterases (PDEs), responsible for the hydrolysis of cAMP to 5'-AMP. Increases in cAMP promote the activation of PKA, and PKA can phosphorylate many substrates including cAMP response element binding protein (CREB) (Gonzalez and Montminy 1989). CREB acts as a transcription factor, switching on the expression of an array of different genes, including *FOS*, *GEM*, *PCK1*, and *PDE4D* (Tsvetanova and von Zastrow 2014; Zhang et al. 2005). PKA activity also results in a negative feedback loop whereby PKA can phosphorylate the intracellular loops of the β_2-AR to cause rapid desensitisation (Hausdorff et al. 1989). Interestingly, while this pattern of PKA-mediated desensitisation holds true at the β_1-AR, it does not occur at the β_3-AR (Rapacciuolo et al. 2003). In addition to PKA, cAMP can also activate other effectors, including nucleotide-gated ion channels, the exchange protein directly activated by cAMP (Epac)1 and Epac2, and the Popeye domain-containing proteins (POPDC).

cAMP signalling by adrenoceptors is highly spatially regulated in order to elicit distinct cellular effects (see also Sects. 4 and 5). The most comprehensive example of this regulation is β-AR-induced cAMP signalling in cardiomyocytes (reviewed in Agarwal et al. 2022; Zaccolo and Pozzan 2002). Even though cAMP is a small and highly soluble molecule, the wide diffusion of cAMP throughout the cell is limited by the activity of PDEs. Localised PDEs can generate "sinks" with low concentrations of cAMP, to spatially restrict cAMP to certain subcellular compartments (Mongillo et al. 2004). This then confines the activation of PKA to a particular subcellular region, controlling which protein effectors are phosphorylated. In addition to spatial control of cAMP itself, in rat cardiomyocytes, the location of the β_1-AR and β_2-ARs is also spatially regulated, with the β_2-ARs restricted to the T-tubules of the cells (Nikolaev et al. 2010). The importance of this spatial regulation of β-AR-cAMP to normal physiology is demonstrated by the loss of receptor subtype organisation in a rat model of chronic heart failure (Nikolaev et al. 2010). Of note, the β_1-AR and β_2-ARs do not show the same spatial organisation in human cardiomyocytes, suggesting that the organisation of intracellular signalling is likely to vary depending on the species. In the healthy human heart, there is a high proportion of β_1-AR expression compared to the other two β-AR subtypes. Of the β-AR in the human heart, approximately 77% are β_1-AR and 23% are β_2-AR, with a low level of β_3-AR expression (Bristow et al. 1986; Moniotte

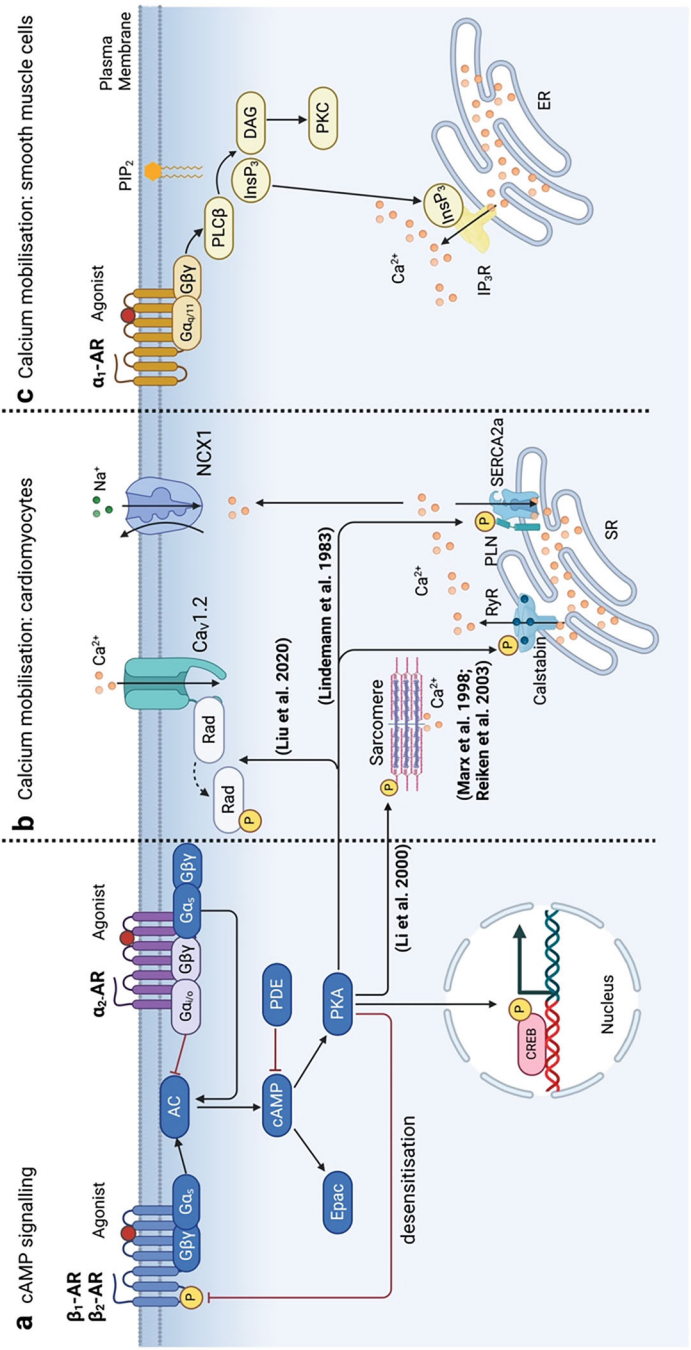

Fig. 1 Schematic representations of adrenoceptor-mediated cAMP signalling and calcium mobilisation. (**a**) The stimulation of $G\alpha_s$-coupled adrenoceptors (e.g. β-ARs or α_2-ARs) activates adenylyl cyclase (AC), which increases the production of the second messenger cAMP. Increased cAMP levels promote the activation of effectors such as Epac (exchange protein activated by cAMP) and PKA. PKA can phosphorylate several targets, including the cAMP response element binding protein (CREB), which acts as a transcription factor of gene expression. AC activity can be inhibited following stimulation of $G\alpha_{i/o}$-coupled adrenoceptors (e.g. α_1-ARs). Phosphodiesterases (PDEs) hydrolyse cAMP to limit the duration of cAMP signalling. (**b**) Calcium (Ca^{2+}) mobilisation in cardiomyocytes is regulated by the activity of PKA following the stimulation of $G\alpha_s$-coupled β_1-AR or β_2-ARs (reviewed in Papa et al. 2022). PKA

Fig. 1 (continued) phosphorylates the RGK GTPase, Rad, an inhibitor of the L-type calcium channel Ca_V1, resulting in Ca_V1 opening and calcium influx from the extracellular space (Liu et al. 2020). PKA also phosphorylates calstabin (now known as FKBP1A), a subunit of the ryanodine receptor (RyR) complex to allow the release of calcium from the sarcoplasmic reticulum (SR) (Marx et al. 1998; Reiken et al. 2003). Cytosolic calcium then binds to sarcomeres to initiate muscle contraction (Li et al. 2000). Contraction stops when calcium is removed from the cytoplasm by the sarcoplasmic/endoplasmic reticulum calcium ATPase type 2A (SERCA2a) and the sodium/calcium exchanger 1 (NCX1). PKA can also phosphorylate phospholamban (PLN), to prevent its inhibitory interaction with SERCA2a, thereby enhancing the removal of cytosolic calcium (Lindemann et al. 1983). (**c**) In smooth muscle cells, calcium mobilisation is primarily regulated by the $G\alpha_{q/11}$-coupled α_1-ARs. When activated, $G\alpha_q$ stimulates PLCβ, which converts the membrane-associated PIP_2 into inositol trisphosphate ($InsP_3$) and diacylglycerol (DAG). $InsP_3$ binds to the $InsP_3$ receptor (IP_3R) on the endoplasmic reticulum (ER), causing calcium to be released from the ER. This elevation in cytosolic calcium causes smooth muscle contraction. Black lines indicate activation of signalling pathways, and red lines indicate the inhibition of signalling pathways

et al. 2001). While the β_2-ARs are present at lower levels of expression than the β_1-ARs, much of the cAMP produced in human cardiomyocytes in response to β-AR stimulation occurs via the β_2-AR subtype (reviewed in Brodde 1991).

α_2-ARs broadly act to inhibit cAMP production (Fig. 1a). However, due to their G protein coupling promiscuity (see Sect. 2.2), they can also have a stimulatory effect on cAMP. Depending on the availability of different Gα subtypes, α_2-ARs can produce a "bell-shaped" cAMP concentration-response curve – inhibiting the production of cAMP at lower agonist concentrations (through $G\alpha_{i/o}$) while stimulating cAMP (through $G\alpha_s$) at higher agonist concentrations (Eason et al. 1992; Nasman et al. 2001; Proudman et al. 2022).

3.2 Calcium Mobilisation

Calcium mobilisation (both influx from the extracellular space and release from intracellular stores) is crucial for the regulation of numerous essential cellular functions, including cardiac and skeletal muscle contraction, motility, secretion, and synaptic transmission (reviewed in Wehrens et al. 2005). The α_1-ARs are defined by their coupling to the $G\alpha_{q/11}$ family of G proteins (see Sect. 2.1). Activation of the three α_1-AR subtypes leads to PLCβ-mediated hydrolysis of the membrane lipid PIP_2 into $InsP_3$ and DAG (Fig. 1c). $InsP_3$ then travels to intracellular calcium stores at the endoplasmic reticulum (ER) or sarcoplasmic reticulum (in muscle cells). $InsP_3$ binding to the IP_3R opens this calcium channel to elevate cytosolic calcium. The α_1-ARs can couple to additional G proteins and activate multiple signalling pathways beyond PLCβ, including MAPK cascades (see also Sect. 3.3). This signalling has been extensively reviewed elsewhere (Cotecchia 2010; Endoh 2016; O'Connell et al. 2014; Perez 2020).

The α_2-ARs can increase calcium mobilisation, with the pathways underlying the increase being highly dependent on cell type; responses can be $G\alpha_{i/o}$-dependent or independent (as determined using pertussis toxin), and can involve either modulation of the plasma membrane-localised calcium channel or release of calcium from intracellular stores (via a $G\alpha_{i/o}$-Gβγ-PLC pathway) (Dorn et al. 1997; Holmberg et al. 1998; Michel et al. 1989; Salm and McCarthy 1990). Activation of endogenous and exogenous α_2-ARs can also inhibit calcium mobilisation (Gollasch et al. 1991; Soini et al. 1997). In differentiated PC-12 cells transfected with either the α_{2A}-AR or α_{2B}-AR, stimulation of the receptors caused both a $G\alpha_{i/o}$-mediated inhibition of the $Ca_V2.2$ calcium current and a smaller increase in the Ca_V1 calcium current that was unaffected by pertussis toxin (Soini et al. 1997).

One of the most well-studied physiological roles of β-ARs is the regulation of cardiac function, where cAMP-dependent calcium signalling pathways regulate muscle contraction (Fig. 1b). In general, heartbeat is induced by excitation-contraction coupling, a process that requires highly coordinated calcium release and reuptake (reviewed in Bers 2002). The action potential in cardiomyocytes (usually defined as atrial or ventricular) activates L-type calcium channels (Ca_V1) to allow an additional, small inward calcium current that activates ryanodine

receptors (RyRs) and calcium influx from the sarcoplasmic reticulum to cause a transient elevation of cytosolic calcium (Bers 2002). The binding of cytosolic calcium to sarcomeric proteins initiates contraction. Muscle contraction ensues as calcium is removed from the cytosol. Elevated cytosolic calcium also activates calcium/calmodulin-dependent protein kinase (CaMKII), which phosphorylates and inactivates phospholamban, a sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA2a) inhibitor, to allow calcium to be pumped back to the sarcoplasmic reticulum via SERCA2a (Bers 2002). Any remaining cytosolic calcium is extruded out of the cell by the sodium/calcium exchanger, NCX1 (Bers 2002).

In response to activation of the sympathetic nervous system, β-AR signalling can augment cardiac muscle contraction via a $G\alpha_s$-AC-cAMP-PKA pathway (reviewed in Papa et al. 2022). PKA acts as a nodal point and phosphorylates a series of effector molecules that potentiate calcium mobilisation, including Ca_V1, RyRs, phospholamban, and sarcomeric proteins (Fig. 1b). PKA phosphorylation of the L-type calcium channel inhibitor, Rad (a RGK GTPase), inactivates it to prevent inhibition of calcium channels and therefore facilitates a further increase in calcium influx (Liu et al. 2020). PKA phosphorylation of RyRs at Ser2808 (RyR1) and Ser2843 (RyR2) facilitates the dissociation of calstabin (now known as FKBP1A); this primes the channel for opening and increases calcium release from the sarcoplasmic reticulum (Marx et al. 1998; Reiken et al. 2003). PKA phosphorylation of phospholamban at Ser1 leads to dissociation of phospholamban from SERCA2a, allowing calcium to be pumped back into the sarcoplasmic reticulum (Lindemann et al. 1983). PKA also phosphorylates sarcomeric proteins such as troponin I and myosin binding protein C, which reduce the binding affinity between the myofilaments and calcium to promote muscle relaxation (Li et al. 2000; Rosas et al. 2015).

3.3 MAPK Signalling Pathway

The extracellular signal-regulated kinase (ERK1/2) MAPK cascade plays an important role in gene transcription, cell proliferation, and differentiation; it is highly regulated and involves the sequential activation of the protein kinases Ras-Raf-MEK-ERK (reviewed in Eblen 2018). Activation of the ERK MAPK signalling pathway by adrenoceptors has been reported to variously depend on the Gα subunit, the released Gβγ subunits, or β-arrestins (Fig. 2). It is likely that the precise mechanism varies with cell type, receptor expression (endogenous vs exogenous), the subcellular site of signalling, and the sensitivity of the various detection assays (see following sections). Nevertheless, the consensus is that activation of ERK signalling by adrenoceptors is dependent on the upstream effectors Src, Ras, and Rap (see below).

3.3.1 Src

The β_2-AR is the most studied adrenoceptor subtype that regulates MAPK activity. Daaka et al. (1997) were the first to report that the Gβγ subunits of $G\alpha_i$ mediated

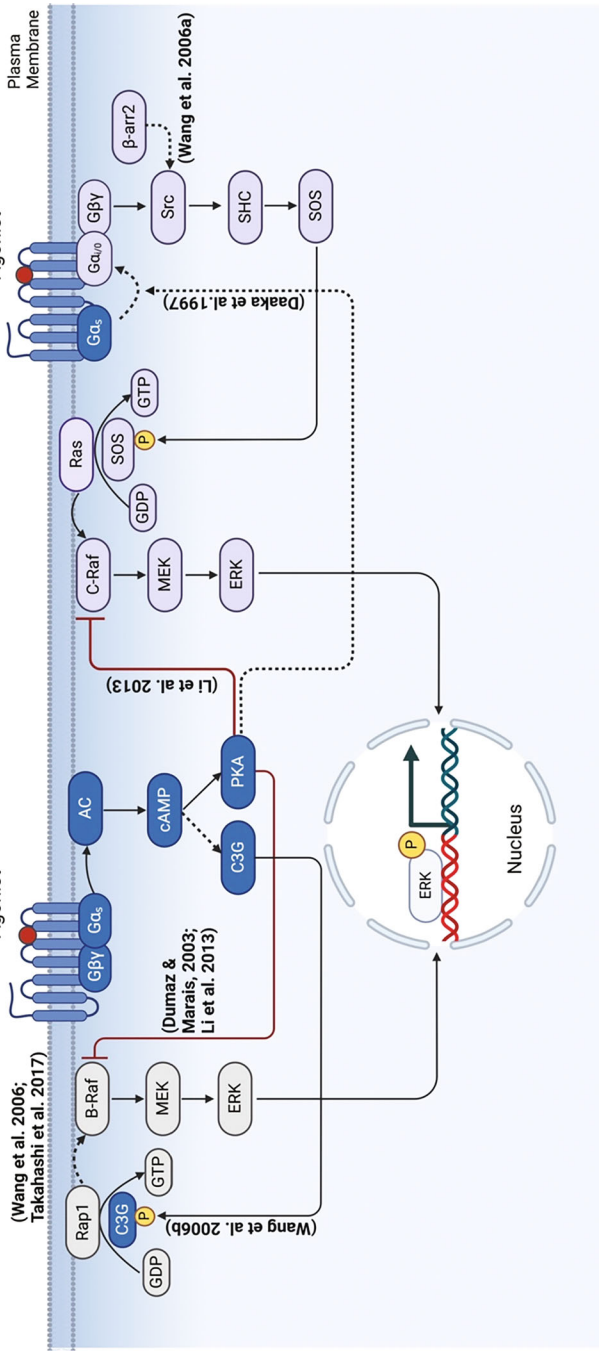

Fig. 2 Schematic representations of adrenoceptor-activated ERK1/2 MAPK signalling. Activation of $G\alpha_s$-coupled adrenoceptors causes MAPK/ERK signalling via several proposed mechanisms. Direct cAMP stimulation leads to phosphorylation of C3G, the guanine nucleotide exchange factor for Rap1 (Wang et al. 2006b). C3G promotes Rap1 activation by catalysing the exchange of Rap1-bound GDP for GTP. Rap1 can couple to ERK signalling via its interaction with B-Raf (Takahashi et al. 2017; Wang et al. 2006b). This Rap1-B-Raf-ERK signalling, however, can be terminated by PKA phosphorylation of B-Raf. Similarly, PKA can also phosphorylate C-Raf, inhibiting the Ras-C-Raf-ERK signalling pathway (Li et al. 2013). PKA may also mediate the G protein coupling switch of adrenoceptors from $G\alpha_s$ to $G\alpha_i$ (Daaka et al. 1997). The released $G\beta\gamma$ subunit of $G\alpha_i$ can activate Src-dependent ERK signalling. In addition, β-arrestin 2 is proposed to recruit Src to activate ERK signalling downstream of $G\alpha_i$-coupled adrenoceptors (Wang et al. 2006a). Black lines indicate activation of signalling pathways, and red lines indicate the inhibition of signalling pathways. Solid lines show experimentally determined pathways, whereas dashed lines indicate hypothetical pathways for which there is currently little or no evidence

activation of ERK signalling by the β_2-AR. They proposed that after PKA phosphorylation and desensitisation of the β_2-AR, the G protein coupling of the receptor switched from $G\alpha_s$ to $G\alpha_i$. The $G\beta\gamma$ subunits released from the $G\alpha_i$ protein then activated a Src-SHC-SOS-Ras pathway that ultimately led to ERK signalling. Although unable to replicate the G protein coupling switch using the same cell system, a later study confirmed a dominant role of Src in the activation of ERK signalling even in response to stimulation of a mutant β_2-AR that lacked PKA phosphorylation sites (β_2-AR (PKA-))(Friedman et al. 2002). Other studies have also supported the idea that Src has a primary role in stimulating β_2-AR ERK activation (Ma et al. 2000; O'Hayre et al. 2017).

ERK MAPK activity can also be induced by the α_1-AR, and this is likely dependent on synergistic activation of the β_2-AR (Sabri et al. 2000). The β-AR agonist isoprenaline and the selective α_1-AR agonist A-61603 both induce biphasic ERK activation in α_1-AR-expressing HEK293/EBNA cells (Copik et al. 2015). The high potency phase of ERK activation was blocked by a selective β_2-AR antagonist, whereas the low potency response was sensitive to an α_1-AR-selective antagonist. While there is a consensus that activation of ERK MAPK signalling by the α_1-AR is at least partially dependent on β_2-AR signalling (Copik et al. 2015; Sabri et al. 2000), the precise mechanism remains unclear (Copik et al. 2015; Huang et al. 2007; Lee et al. 2003; Liu et al. 2011; Perez-Aso et al. 2013). It is possible that this synergistic ERK MAPK activity depends on β_2-AR activation of AC-PKA (see following sections), or the direct activation of Src by the β_2-AR. In addition, several reports suggest that receptor internalisation is required for α_1-AR-mediated ERK activity (Liu et al. 2011; Perez-Aso et al. 2013).

3.3.2 Rap1

The β_2-AR activates ERK signalling via a $G\alpha_s$-cAMP-PKA pathway in a number of cell types (Schmitt and Stork 2000; Vossler et al. 1997; Wan and Huang 1998). Activation of ERK was independent of $G\alpha_{i/o}$ and $G\beta\gamma$ subunits, and involved PKA-mediated activation of a Rap1-B-Raf-MEK-ERK cascade (Schmitt and Stork 2000; Wan and Huang 1998). It is proposed that PKA phosphorylates Rap1 to create binding sites for the scaffold protein, 14-3-3. 14-3-3 would then facilitate an interaction between Rap1 and the scaffolding protein, KSR (Kinase Suppressor of Ras), which usually exists in a heterodimer with B-Raf (Takahashi et al. 2017). To the best of our knowledge, this model (specifically the scaffolding proteins 14-3-3 and KSR) has not been experimentally validated in response to activation of the β_2-AR. Below, we focus on the evidence in the literature for generalised cAMP modulation of ERK activity. However, it is yet to be determined whether these are the same pathways that control ERK signalling downstream of the β-AR-cAMP pathway.

Rap1 belongs to the Ras family of GTPases. Despite a high sequence homology, Rap1 and Ras bind to and activate different effectors and pathways (reviewed in Bos 1998; Shah et al. 2019). Rap1 was initially thought to functionally antagonise Ras signalling by sequestering away Ras effectors, such as Raf (Bos 1998). As such, the presence of a β_2-AR-cAMP-Rap1-B-Raf-MEK-ERK pathway has been questioned

(Armaiz-Pena et al. 2013; Obara et al. 2004; Schmitt and Stork 2002; Wang et al. 2006b). For example, application of an Epac (a RapGEF)-activating cAMP analogue caused an increase in a perinuclear pool of Rap1, but failed to activate ERK (Enserink et al. 2002). In contrast, another RapGEF, C3G, stimulated Rap1-ERK activation by relocalising perinuclear Rap1 to the plasma membrane, where Rap1 was able to signal through B-Raf (Wang et al. 2006b). In addition to emphasising the specificity of the RapGEF family, this also highlights the importance of subcellular location in the cAMP-mediated activation of Rap1-ERK signalling. Consistent with location-dependent activation of Rap1-ERK pathways, a study using a Rap1 biosensor and live-cell TIRF imaging observed Rap1 activation of plasma membrane ERK, but not cytosolic or nuclear ERK (Keyes et al. 2020). This is strong evidence that apparently contradictory observations can be explained by the variable sensitivity of traditional methods, which typically lack the resolution to capture the spatiotemporal texture of a particular signal.

3.3.3 Ras

In addition to activation of ERK signalling, cAMP can inhibit this kinase cascade via Ras (Obara et al. 2004; Schmitt and Stork 2002; Wang et al. 2006b). Activation of a cAMP-PKA-Src-C3G-Rap1 pathway allows Rap1 to effectively "trap" C-Raf and sequester Ras-ERK signalling (Schmitt and Stork 2002). In addition, PKA can also terminate ERK signalling via phosphorylation of C-Raf and B-Raf, which disrupts the active Raf-Ras complex (Dumaz and Marais 2003; Li et al. 2013; Takahashi et al. 2013). However, application of techniques with greater spatial and temporal resolution has revealed a more complex regulation of ERK by Ras and Rap1 downstream of cAMP signalling. It was shown that isoprenaline-mediated activation of Ras leads to an early and transient ERK signal (Li et al. 2016; Takahashi et al. 2013). This Ras-mediated transient ERK response was rapidly terminated by PKA phosphorylation of Raf, whereas Rap1-induced ERK signalling has a longer duration (Takahashi et al. 2013). Thus, the combined spatiotemporal activation of Ras and Rap1 in response to cAMP or PKA stimulation potentially plays a crucial role in the fine-tuning of adrenoceptor-mediated ERK signalling pathways.

3.3.4 β-Arrestins

Activation of the β_1-AR and β_2-AR leads to the recruitment of β-arrestins, named for their ability to promote the homologous desensitisation of β-ARs in concert with GRK (Lohse et al. 1990). GRKs phosphorylate intracellular serine/threonine residues of agonist-activated receptors, principally within the third intracellular loop and C-terminal tail, resulting in the recruitment of β-arrestins and receptor internalisation (reviewed in Ferguson 2001). This paradigm seems to hold for the α_{1B}-ARs. The α_{1B}-AR is phosphorylated by GRKs, and multiple phosphorylation sites have been identified in the third intracellular loop and C-terminal tail of the α_{1A}-AR and α_{1D}-AR, with in silico analysis implicating GRKs (reviewed in Akinaga et al. 2019; García-Sáinz et al. 2000). All α_1-ARs can internalise in response to agonist (reviewed in Akinaga et al. 2019; García-Sáinz et al. 2000). However, not all adrenoceptors are phosphorylated and internalised. For example, the β_3-AR does not

undergo phosphorylation by GRKs or internalisation following activation (reviewed in Okeke et al. 2019). Moreover, while the α_{2A}-AR and α_{2B}-AR are phosphorylated by GRKs and undergo internalisation, the α_{2C}-AR appears to undergo β-arrestin-dependent internalisation in the absence of receptor phosphorylation (DeGraff et al. 1999; Jewell-Motz and Liggett 1996).

In addition to their role in receptor internalisation, β-arrestins were later suggested to also serve as independent signal transducers, with one of the most well-studied examples being β-arrestin-mediated activation of ERK signalling (reviewed in Luttrell and Lefkowitz 2002; Shenoy and Lefkowitz 2011). Similar to the reports of G protein-cAMP-mediated modulation of ERK signalling, the studies deciphering β-arrestin-dependent ERK signalling are conflicting. Extensive research has shown that β-arrestins are critical in initiating G protein-independent ERK signalling (as reviewed in Luttrell and Lefkowitz 2002; Shenoy and Lefkowitz 2011); however, this arrestin-dependent signal transduction has been challenged in recent years (Eichel et al. 2018; Grundmann et al. 2018; Hamed et al. 2021; Luttrell et al. 2018; O'Hayre et al. 2017). Using the same cellular system (HEK293 cells), genetic ablation of β-arrestins enhanced β_2-AR- but diminished β_1-AR-induced ERK signalling (Eichel et al. 2018; O'Hayre et al. 2017). In line with this, knockout of β-arrestins had a variable effect on the β_2-AR-induced phosphorylation of ERK in different CRISPR/Cas9 and siRNA HEK293 cell clones (Luttrell et al. 2018). Despite the variable effects of β-arrestin knockout in different studies, ERK signalling in response to adrenoceptor stimulation was never fully abolished (see "Src" and "Rap1" subheadings, above). This suggests a dual role for G proteins and β-arrestins in mediating activation of ERK signalling in response to adrenoceptor stimulation. Consistent with this idea, Src-dependent activation of ERK in response to stimulation of the α_2-AR requires β-arrestin 2 as a scaffold protein (Wang et al. 2006a). Thus, these data suggest that β-arrestins have a more important role in assembling components of the G protein-mediated ERK signalling cascade, rather than directly activating ERK signalling itself (Gutkind and Kostenis 2018; Luttrell et al. 2018). This is perhaps also consistent with the additional roles of GRKs as signalling hubs (that can be independent of their kinase activity); these GRK signalling hubs can also coordinate and influence non-GPCR signalling (reviewed in Penela et al. 2019).

3.4 PKC Activity

Stimulation of the $G\alpha_{q/11}$-coupled α_1-ARs leads to activation of PLC, to produce $InsP_3$ and DAG. $InsP_3$ has a key role in the regulation of calcium mobilisation (see Sect. 3.2). The primary role attributed to DAG is the recruitment of PKC that translocates from the cytoplasm to the plasma membrane and phosphorylates a myriad of downstream targets. In addition to $G\alpha_{q/11}$-coupled receptors, the $G\alpha_s$-coupled β-ARs can also activate PKC. This occurs via the cAMP effector Epac, which activates and recruits PLCε. The activation of PKC by a β-AR-cAMP-Epac pathway occurs in HEK293 cells (Schmidt et al. 2001) and rat cardiac myocytes (Li et al. 2015; Oestreich et al. 2007). Activation of this signalling pathway has also

been reported for other receptors such as nociceptors in rat dorsal root ganglion (Hucho et al. 2005) and prostaglandin E1 receptors in N1E-115 neuroblastoma cells (Schmidt et al. 2001).

One of the most well-studied roles for PKC in adrenoceptor signalling is receptor desensitisation. The activation of conventional PKCs (cPKC: PKCα, PKCβ_I, PKCβ_{II} and PKCγ) induced rapid α_{1B}-AR homologous desensitisation in HEK293 cells (Kienitz et al. 2016; Niemeyer et al. 2019; Renkhold et al. 2022). α_{1B}-AR mutants with deleted PKC (or GRK2) phosphorylation sites had a significantly reduced level of acute receptor desensitisation, and receptor desensitisation was independent of arrestin activity (Renkhold et al. 2022). Similarly, β_1-AR-mediated cAMP activity was differentially desensitised by distinct PKC isoforms, with a rank order of PKCβ_{II}>PKCα>PKCε>PKCζ (Guimond et al. 2005). Some studies have also demonstrated that the stimulation of PKC following α_1-AR activation can augment the desensitisation of β-ARs. α_1-AR activation inhibited the activity of β-AR-modulated cardiac chloride and calcium channels, which was mediated in part by PKC (Belevych et al. 2004; Chen et al. 1996; Oleksa et al. 1996). In transgenic mice with overexpressed α_{1B}-ARs, GRK2 activity and subsequent β-AR desensitisation were enhanced independently of GRK2 protein levels (Akhter et al. 1997), and were later correlated with the increased expression of PKCδ, PKCε, and PKCβ_{II} (Lemire et al. 1998).

In addition to receptor desensitisation, activation of specific PKC isoforms by the α_1-AR can modulate the expression and function of membrane-localised ion channels. Activation of the α_{1B}-AR resulted in the recruitment of PKCα, which transiently inhibited the potassium channels $K_{Ca}3.1$ (Kienitz et al. 2016) and $K_{ir}3.1$ (GIRK) (Niemeyer et al. 2019). In contrast, the recruitment of novel PKC (nPKC: PKCδ, PKCε, PKCη, and PKCθ), specifically PKCδ, facilitated $K_{Ca}3.1$ activity in response to α_{1B}-AR stimulation (Kienitz et al. 2016; Renkhold et al. 2022). Activation of the α_{1A}-AR by phenylephrine increases the membrane expression and current of $K_{Ca}3.1$, and this is negatively regulated by a constitutively active PKCβ_{II} (Braun et al. 2020). Overall, the specific effect of PKC isoforms on membrane ion channel activity in response to adrenoceptor stimulation is, like other adrenoceptor signalling pathways, highly dependent on various factors including receptor subtype, the agonist exposure time, and cell type.

4 Adrenoceptor Signalling from Intracellular Membranes

The presence of β_2-ARs within endosomal membranes following receptor endocytosis was established nearly 30 years ago (Moore et al. 1995). Traditionally however, internalisation events were considered a consequence of continual agonist activation at the cell surface. Physical disruption of GPCR-G protein coupling at the plasma membrane was understood to functionally desensitise the receptor and initiate G protein-independent signalling (reviewed in Ellisdon and Halls 2016). The emergence of novel Förster resonance energy transfer (FRET)-based and conformation-specific biosensors has since challenged these perceptions by revealing active, G

protein-bound β_2-ARs within early endosomal membranes (Bowman et al. 2016; Irannejad et al. 2013). The adrenoceptor family has since become an exemplar of signalling from intracellular membranes and has helped to redefine where canonical GPCR signalling can occur within the cell.

4.1 β_2-AR in Endosomes

Following ligand-induced internalisation from the plasma membrane, endosomal pools of the β_2-AR adopt an active conformation that persists for several hours (Kim et al. 2021). These active endosomal receptors recruit $G\alpha_s$ to generate a functionally significant second wave of cAMP (Irannejad et al. 2013; Kim et al. 2021) and to initiate ERK signalling (Kwon et al. 2022). Propagation of these endosomal cAMP and ERK signals to the nucleus controls gene transcription (Tsvetanova and von Zastrow 2014; Kwon et al. 2022; Willette et al. 2023). For example, endosomal cAMP production is sufficient to activate the CREB transcription factor to drive downstream transcription of a subset of genes including *PCK1* (Bowman et al. 2016; Tsvetanova and von Zastrow 2014). Comparable, or even greater, cAMP accumulation at the plasma membrane does not activate CREB nor induce *PCK1* expression (Tsvetanova and von Zastrow 2014). These differences in location-specific cAMP production also have functional consequences at the protein level: endosomal – but not cell surface – cAMP favours the dephosphorylation of a subset of protein phosphatase 2A (PP2A) target proteins (Tsvetanova et al. 2021). The specificity of both transcriptional responses and protein dephosphorylation for endosomal cAMP highlights a functional role for endosome-initiated signals. Additionally, it provides a point of differentiation between plasma membrane and endosomal receptor pools and reveals the ability of the β_2-AR to generate unique location-specific outcomes.

4.2 β_1-AR in the Golgi

The Golgi routinely traffics GPCRs during their synthetic journey. Accordingly, Golgi-localised receptors may either be awaiting transit to another location or may reflect a pool of spare receptors (reviewed in Crilly and Puthenveedu 2021). A third possibility arises, exemplified by the β_1-AR, in which Golgi-resident receptors represent an active in situ pool that does not originate from another cellular compartment. Activation of Golgi-residing β_1-ARs occurs following ligand access to this organelle via either passive diffusion (e.g. dobutamine) or facilitated transport (e.g. adrenaline) (Irannejad et al. 2017). Subsequent $G\alpha_s$ protein recruitment and activation promote cAMP production that is sufficient to activate local PKA pools (Irannejad et al. 2017). Compartmentalisation of β_1-AR and AC at the Golgi likely occurs in a cell type-specific manner, as there was no effect of adrenaline on AC activity in thymocytes (Buchwalow et al. 1981). While direct visualisation of ACs at the Golgi has not been reported, functional studies in numerous cell types suggest the presence of active AC in the Golgi (Buchwalow et al. 1981; Cheng and Farquhar

1976a, b). The inability to visually confirm AC localisation in the Golgi to date is likely due to the poor specificity of AC-directed antibodies and the relatively low expression levels of the endogenous protein (reviewed in Hanoune and Defer 2001).

In cardiomyocytes, β_1-ARs are endogenously distributed both at the cell surface and at the Golgi. Stimulation of Golgi-resident β_1-ARs leads to activation of $G\alpha_s$ and an increase in cAMP production (Irannejad et al. 2017). Local accumulation of cAMP at the Golgi, which is spatially distinct from the cell surface cAMP pool, may be associated with both physiological and pathophysiological consequences. Activation of cAMP-mediated pathways in the Golgi has been linked to the stimulation of calcium release from this organelle (Yang et al. 2015). β_1-ARs expressed at the Golgi may therefore serve as a local source of cAMP production, mediating Golgi-dependent calcium release. Given that the release of calcium from the Golgi is crucial for many of the functions of this organelle, this suggests a potential physiological role for Golgi-localised β_1-ARs in protein sorting and/or trafficking. Activation of β_1-AR-$G\alpha_s$-cAMP signalling at the Golgi in cardiomyocytes can also stimulate phosphatidylinositol-4-phosphate (PI4P) hydrolysis via an intermediary Epac/A kinase anchoring protein 6 (AKAP6, also known as mAKAPβ)/PLCε complex (Nash et al. 2019). Signals downstream of this intracellular receptor pool may also have functional and clinically relevant consequences. β_1-AR-mediated increases in cardiomyocyte cell area are more effectively reduced by permeable receptor antagonists that are able to access receptors inside the cell (Nash et al. 2019). These observations have crucial clinical significance given that β-blockers are used therapeutically for the treatment of cardiovascular disease (Nash et al. 2019). The use of a β_1-AR-selective β-blocker that only antagonises the Golgi-localised receptor pool would likely have much greater anti-hypertrophic efficacy. As has been demonstrated for other GPCRs (Jensen et al. 2017; Mai et al. 2021), a greater understanding of the location of pathophysiological signalling will likely result in β-blockers with greater efficacy.

In addition to β_1-ARs at the Golgi, cardiomyocytes also express β_1-ARs at the cell surface (Bathe-Peters et al. 2021). The generation of cAMP strictly at the cell surface is maintained by the activity of local PDEs. Stimulation of cell surface β_1-ARs activates a key calcium regulator, phospholamban, which is embedded within the membrane of the sarcoplasmic reticulum (Myagmar et al. 2017). Phospholamban activation relieves its inhibitory effect on calcium uptake into the sarcoplasmic reticulum stores, to promote the calcium release crucial for muscle contraction (reviewed in MacLennan and Kranias 2003). Activation of the β_1-ARs at the plasma membrane, via phospholamban activation and presumably calcium release, therefore has a predominant role in driving cardiomyocyte contractility (Myagmar et al. 2017). The proximity of cell surface β_1-ARs to the extracellular environment facilitates rapid responses such as contraction. Conversely, activation of intracellular receptors may contribute to longer-term effects such as modulation of protein expression. As such, the relative location of each receptor pool enables the differential access of second messenger signals to the appropriate signalling machinery in order to confer the required specificity and speed of responses.

4.3 α_{1A}-AR and α_{1B}-AR in the Nucleus

The α_{1A}-AR and α_{1B}-AR localise to, and signal from, the nucleus in cardiomyocytes (Wright et al. 2008) (Fig. 3). In contrast to intracellular β-ARs, cardiomyocyte α_1-ARs have no detectable expression at the plasma membrane; instead, they are distributed exclusively at the nuclear membrane (Wright et al. 2012). The cognate signalling partners of the α_{1A}-AR and α_{1B}-AR, $G\alpha_{q/11}$ and PLC, also co-distribute with these receptors at the nucleus under basal conditions (Wright et al. 2008). However, it remains for future studies to show $G\alpha_q$ recruitment or PLC activation in response to α_1-AR stimulation to definitively confirm the ability of these receptors to signal via canonical G protein-mediated pathways at the nucleus.

Activation of nuclear-localised α_{1A}-AR promotes the accumulation of phosphorylated ERK adjacent to the plasma membrane (Wright et al. 2008). While stimulation of nuclear α_{1B}-ARs was also suggested to increase the phosphorylation of ERK, this was not statistically verified (Wright et al. 2008). Another study found no change in the level of agonist-induced phosphorylation of ERK following reconstitution of the α_{1B}-AR into α_{1A}-AR/α_{1B}-AR double knockout cardiomyocytes (Huang et al. 2007). Instead of directly increasing ERK phosphorylation itself, it was suggested that the α_{1B}-AR may form heterodimers with the α_{1A}-AR at the nucleus to modulate downstream signalling (Wright et al. 2012). Analysis of α_1-AR mRNA in cultured cardiomyocytes reveals that 100% of myocytes express α_{1B}-AR mRNA, while only 50% express α_{1A}-AR mRNA (Myagmar et al. 2017). Given that approximately half of the myocyte population expresses the α_{1B}-AR alone, a unique – but currently unknown – functional role for this receptor subtype is likely to exist. In support of this, the activation of α_{1B}-AR in α_{1A}-AR knockout cardiomyocytes was reported to induce contraction (Myagmar et al. 2017). However, it is possible that the α_{1B}-AR in this knockout cell line adopts a contractile phenotype to compensate for the absence of α_{1A}-AR expression.

The α_{1A}-AR has a number of well-established roles in cardiomyocytes. α_{1A}-AR-mediated ERK phosphorylation at the nucleus is protective against cell death induced by cytotoxic stimuli (Huang et al. 2007). The receptor also activates PKCδ to phosphorylate cardiac troponin I; this results in contraction via changes in sarcomere length (Wu et al. 2014). Cardiomyocyte contraction was not observed following stimulation of mutant α_{1A}-ARs that are unable to localise to the nucleus (Wu et al. 2014). This suggests that the location of α_{1A}-ARs at the nucleus is essential for function. Consistent with this idea, activation of α_{1A}-ARs in isolated nuclei is sufficient to induce PKC activity (Wu et al. 2014). These findings collectively highlight the importance of location for α_{1A}-AR signalling.

The proximity of α_1-AR-mediated signals to the transcriptional machinery in the nucleus could suggest an additional role for these receptors in transcriptional regulation. This is the case for many other GPCRs that are localised at the nucleus, such as the metabotropic glutamate $mGlu_5$ receptor and the proteinase-activated receptor, PAR2 (Jong et al. 2009; Joyal et al. 2014). Non-specific α-AR activation does modulate gene expression in cardiomyocytes (Appert-Collin et al. 2007), and ERK phosphorylation in response to dabuzalgron, a partial and selective α_{1A}-AR agonist,

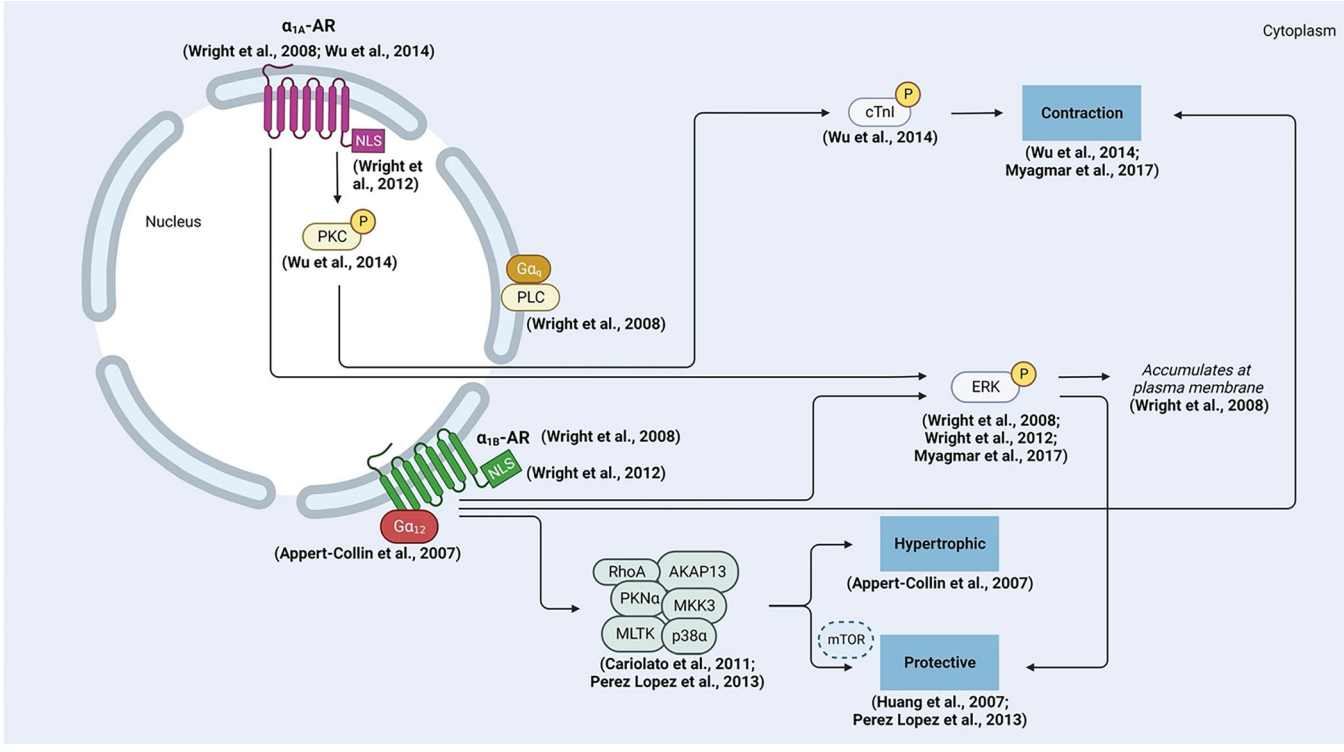

Fig. 3 Schematic representation of nuclear-localised α_{1A}-AR and α_{1B}-AR signalling in cardiomyocytes. The α_{1A}-AR localises to the inner nuclear membrane (INM) where it mediates cardiomyocyte contraction via PKCδ activation and phosphorylation of cardiac troponin (cTnI) at a PKC target site (Wu et al. 2014). α_{1A}-AR activation also promotes an increase in phosphorylated ERK (pERK) that accumulates near the plasma membrane (PM) (Myagmar et al. 2017; Wright et al. 2008). pERK signals downstream of activated α_{1A}-AR are protective against cytotoxic stimuli (Huang et al. 2007). α_{1B}-AR activation promotes formation of a macromolecular protein complex involving the protein scaffold AKAP13 (also known as AKAP-Lbc), the GTPase RhoA, protein kinase N α (PKNα), and members of the MAPK pathway (Cariolato et al. 2011; Pérez López et al. 2013). Complex formation promotes p38α-MAPK activation and leads to hypertrophic

Fig. 3 (continued) responses such as increases in cell area and transcription of hypertrophic genes (Appert-Collin et al. 2007). p38α-MAPK activation mediated by this protein complex also contributes to cardioprotective effects likely via mammalian target of rapamycin (mTOR)-driven protein modulation (Pérez López et al. 2013). While this pathway requires upstream activation of RhoA by $G\alpha_{12}$ (Appert-Collin et al. 2007), direct coupling of the receptor and $G\alpha_{12}$ has not been shown (see Table 1, Sect. 2.1). Activation of the α_{1B}-AR may also promote ERK phosphorylation, and induce contraction in cardiomyocytes (Myagmar et al. 2017). Signalling effectors, $G\alpha_{q/11}$ and PLC, have been identified in cardiomyocte nuclear fractions under basal conditions (Wright et al. 2008); however, direct coupling of $G\alpha_{q/11}$ to either α_1-AR in cardiomyocyte nuclei has not been demonstrated. While here we show the α_{1B}-AR within the outer nuclear membrane (ONM) due to the fact that the receptor signals into the cytosol, this has not been experimentally determined. Both the α_{1A}-AR and α_{1B}-AR are localised to the nucleus via C-terminal nuclear localisation sequences (NLS) (Wright et al. 2012). Dotted lines indicate proposed connections, and solid lines show experimentally determined pathways

preserved the expression of genes linked to mitochondrial function and ATP synthesis in an in vivo model of cardiotoxicity (Baek et al. 2021). However, the location of the α_{1A}-AR in these two studies was not determined (Appert-Collin et al. 2007). Nonetheless, it is likely that gene transcription can be attributed to nuclear-localised α-ARs given that these receptors are only found in the nucleus in cardiomyocytes (Wright et al. 2012). Ligand access to the nucleus likely occurs via the same carrier-mediated transport mechanisms identified for noradrenaline access to Golgi-localised β_1-ARs (Nash et al. 2019).

5 Adrenoceptor Signalling in Protein Complexes

In addition, and complementary, to distinct intracellular locations, the organisation of signalling effectors in close proximity to GPCRs can confer unique signalling responses. This has been well demonstrated in rat cardiomyocytes where the differential distribution of β_1-AR and β_2-AR in distinct membrane domains, and in proximity to different protein complexes, allows the β_2-AR to control excitation-contraction coupling (reviewed in Halls 2019; Schleicher and Zaccolo 2018). In these cells, the assembly of a β_2-AR-protein complex at the T-tubules (including the scaffold protein AKAP5 (also known as AKAP79 in humans or AKAP150 in rodents), AC, and calcium channels) restricts cAMP to a very localised region. Within these T-tubules, the activation of the β_2-AR causes a 26-fold increase in cAMP concentration compared to that detected in the bulk cytosol (Bastug-Ozel et al. 2019). In heart failure models, the compartmentation of β_2-AR signalling is lost, with only a 2.4-fold increase in cAMP concentration in response to receptor activation within T-tubules versus the bulk cytosol (Bastug-Ozel et al. 2019). In the following sections, we will summarise some additional examples of the unique signalling of adrenoceptors that occurs due to the assembly of a GPCR-protein complex.

5.1 Activation of the β_2-AR by Ultra-Low Ligand Concentrations

In HEK293 cells and human cardiac fibroblasts, the β_2-AR is pre-assembled with a large protein complex at the plasma membrane that confers sensitivity of the receptor to ultra-low concentrations of ligand (Civciristov et al. 2018; Civciristov and Halls 2019). Femtomolar concentrations of isoprenaline cause a sustained increase in cAMP and nuclear ERK, leading to the transcription of a unique set of genes compared to higher ligand concentrations (Civciristov et al. 2018). This high-sensitivity signalling of the β_2-AR is dependent on the co-assembly of the receptor with AKAP5, AKAP12 (also known as AKAP250), AC2, PDE4D5, PKA, G proteins ($G\alpha_{i/o}$, $G\alpha_s$, $G\beta\gamma$), and β-arrestins (Civciristov et al. 2018). Similar sensitivity was identified in the non-tumour breast cell line, MCF-10A, where femtomolar concentrations of isoprenaline promoted cell adhesion (Bruzzone et al. 2014). This high-sensitivity signalling was mediated by a sub-population of β_2-ARs found in

lipid-rich plasma membrane domains with cell adhesion induced via activation of a $G\alpha_s$-AC-cAMP-Epac pathway (Bruzzone et al. 2014). High-sensitivity signalling of the β_2-AR is not limited to cell lines. Activation of the β_2-AR by clenbuterol in mouse soleus muscle and C2C12 myoblasts, at concentrations 250-fold lower than the EC_{50} (reviewed in Arch and Kaumann 1993), results in increased glucose uptake and palmitate oxidation (Ngala et al. 2008, 2009). These studies illustrate the importance of the local receptor environment (including surrounding proteins) in exerting an allosteric influence on GPCR signalling; it is likely these responses occur following activation of only one or two receptors per cell (Calabrese and Giordano 2021; Civciristov et al. 2018; Civciristov and Halls 2019).

5.2 The β_2-AR-$Ca_V1.2$-AMPA Receptor Complex Contributes to Synaptic Plasticity

In the brain, the β_2-AR assembles within large protein complexes to control synaptic transmission and plasticity. The composition of these protein complexes is very well defined and includes all effector and regulatory proteins of the classical cAMP signalling cascade (reviewed in Man et al. 2020). At post-synaptic sites, the β_2-AR is localised in a complex with $Ca_V1.2$ (Davare et al. 2001) and the AMPA ionotropic glutamate receptor GluA1 subunit (Joiner et al. 2010). The β_2-AR-$Ca_V1.2$ complex is involved in long-term potentiation induced by prolonged theta tetanus at 5–10 Hz for 90–180 s, a rhythm that is implicated in spatial learning (Qian et al. 2012, 2017; White et al. 2008). The scaffold protein, AKAP5, links the proteins together by directly binding $Ca_V1.2$, AC, PKA, and PP2B; $Ca_V1.2$ directly binds PP2A and PP2B and interacts with the C-terminus of the β_2-AR (it is not yet known whether this is a direct interaction) (reviewed in Man et al. 2020). The β_2-AR-GluA1 complex can increase synaptic transmission via increased retention of GluA1 at the cell surface (Joiner et al. 2010). The scaffold protein, post-synaptic density 95 (PSD-95), links the proteins together by directly binding the β_2-AR and the auxiliary subunits of AMPA receptors, the transmembrane AMPA receptor regulatory proteins (TARPs); in addition, the PSD-95 homolog synapse-associated protein 97 (SAP97) directly binds GluA1 (reviewed in Man et al. 2020). Both PSD-95 and SAP97 can interact with AKAP5, which in turn recruits PKA, PP2B, and ACs to the complex (reviewed in Man et al. 2020). Given the location of both complexes at post-synaptic sites, the overlap of protein components (e.g. AKAP5), and that AMPA-induced depolarisation activates $Ca_V1.2$, it is tempting to speculate that the β_2-AR forms a large signalling hub involving both $Ca_V1.2$ and the AMPA receptor.

The assembly of the complex limits the cAMP produced by β_2-AR stimulation to the immediate vicinity of the receptor. Cell-attached recording experiments (where the electrode physically isolates a section of the membrane) revealed that activation of the β_2-AR only increased $Ca_V1.2$ open probability when the agonist albuterol was applied inside the patch electrode itself (Davare et al. 2001). This is consistent with the observation that only GluA1 subunits that co-immunoprecipitated with the

β_2-AR were phosphorylated by PKA in response to β_2-AR activation (Joiner et al. 2010). Together, these observations suggest that the cAMP produced by activation of the β_2-AR in this complex is limited to travel only 200 nm from the receptor (Man et al. 2020).

5.3 An α_1-AR Complex with AKAP13 for Activation of p38 MAPK

α_1-AR signalling is also driven by the assembly of a protein complex (Fig. 3). In HEK293 cells, stimulation of either the α_{1A}-AR or α_{1B}-AR activates $G\alpha_{12/13}$ and the GEF activity of AKAP13 (also known as AKAP-Lbc) (Appert-Collin et al. 2007). AKAP13-mediated replacement of GDP for GTP activates RhoA, which recruits its effector, protein kinase N α (PKNα), to the AKAP13 scaffold. PKNα subsequently serves a dual function, by acting as an additional scaffold and also activating members of the MAPK family. This PKNα-induced signal transduction cascade ultimately activates p38 MAPK. It remains unknown whether there are any points of differentiation between the α_{1A}-AR- and α_{1B}-AR-induced AKAP13 signalling complex. The phenylephrine-induced increase in p38 MAPK phosphorylation in cardiomyocytes is also dependent on AKAP13, which assembles PKNα, MAPK, and p38 MAPK (Pérez López et al. 2013). Disabling the scaffolding ability of AKAP13 in intact hearts prevents stress-induced compensatory hypertrophy, which highlights a role of this protein complex in cardioprotection (Pérez López et al. 2013). Consistent with this idea, non-specific activation of cardiomyocyte α-ARs promotes cellular hypertrophy and transcription of hypertrophic genes via a pathway involving $G\alpha_{12}$, AKAP13, and RhoA (Appert-Collin et al. 2007). The formation and activation of this macromolecular complex is specific for p38 MAPK and does not affect the activity of other MAPK members, such as ERK and JNK (Cariolato et al. 2011; Pérez López et al. 2013). This observation is consistent with the idea that cells use protein complexes to control specificity in signalling.

6 Conclusion

The concept of GPCRs initially arose from the realisation that cAMP formation was dependent on three proteins with separate functions: ligand recognition (receptor), information transduction (G protein), and cAMP synthesis (AC) (reviewed in Beavo and Brunton 2002; Gilman 1987; Hill 2006). This insight was followed by the cloning of the β_2-AR (Dixon et al. 1986), and since then, adrenoceptors (particularly the β_2-AR) have defined many paradigms for GPCRs. It is now clear that adrenoceptors activate multiple G proteins (in addition to their cognate $G\alpha_s$, $G\alpha_{i/o,}$ or $G\alpha_{q/11}$ classification), couple to diverse signalling pathways, signal differently depending on their location within the cell, and modify their signalling by co-location with other scaffolds and effectors. The development of high-resolution approaches to observe and quantify GPCR behaviour, particularly in an endogenous

setting, continues at pace (reviewed in Calebiro and Grimes 2020; Soave et al. 2021). The application of these high-resolution approaches to study GPCRs in their local environment will no doubt further illuminate the variety of cellular responses that can be controlled by this fascinating receptor family.

References

Agarwal SR, Sherpa RT, Moshal KS, Harvey RD (2022) Compartmentalized cAMP signaling in cardiac ventricular myocytes. Cell Signal 89:110172. https://doi.org/10.1016/j.cellsig.2021.110172

Akhter SA, Milano CA, Shotwell KF, Cho M-C, Rockman HA, Lefkowitz RJ, Koch WJ (1997) Transgenic mice with cardiac overexpression of alpha1B-adrenergic receptors: in vivo alpha1-adrenergic receptor-mediated regulation of beta-adrenergic signaling. J Biol Chem 272:21253–21259. https://doi.org/10.1074/jbc.272.34.21253

Akinaga J, García-Sáinz JA, Pupo AS (2019) Updates in the function and regulation of α1-adrenoceptors. Br J Pharmacol 176:2343–2357. https://doi.org/10.1111/bph.14617

Altosaar K, Balaji P, Bond RA, Bylund DB, Cotecchia S, Devost D, Doze VA, Eikenburg DC, Gora S, Goupil E, Graham RM, Hébert T, Hieble JP, Hills R, Kan S, Machkalyan G, Michel MC, Minneman KP, Parra S, Perez D, Sleno R, Summers RJ, Zylbergold P (2019) Adrenoceptors (version 2019.3) in the IUPHAR/BPS Guide to Pharmacology Database. IUPHAR/BPS Guide Pharmacol CITE. https://doi.org/10.2218/gtopdb/F4/2021.3

Amatruda TT, Steele DA, Slepak VZ, Simon MI (1991) G alpha 16, a G protein alpha subunit specifically expressed in hematopoietic cells. Proc Natl Acad Sci U S A 88:5587–5591. https://doi.org/10.1073/pnas.88.13.5587

Appert-Collin A, Cotecchia S, Nenniger-Tosato M, Pedrazzini T, Diviani D (2007) The A-kinase anchoring protein (AKAP)-Lbc-signaling complex mediates alpha1 adrenergic receptor-induced cardiomyocyte hypertrophy. Proc Natl Acad Sci U S A 104:10140–10145. https://doi.org/10.1073/pnas.0701099104

Arch JR, Kaumann AJ (1993) Beta 3 and atypical beta-adrenoceptors. Med Res Rev 13:663–729. https://doi.org/10.1002/med.2610130604

Armaiz-Pena GN, Allen JK, Cruz A, Stone RL, Nick AM, Lin YG, Han LY, Mangala LS, Villares GJ, Vivas-Mejia P, Rodriguez-Aguayo C, Nagaraja AS, Gharpure KM, Wu Z, English RD, Soman KV, Shahzad MMK, Zigler M, Deavers MT, Zien A, Soldatos TG, Jackson DB, Wiktorowicz JE, Torres-Lugo M, Young T, De Geest K, Gallick GE, Bar-Eli M, Lopez-Berestein G, Cole SW, Lopez GE, Lutgendorf SK, Sood AK (2013) Src activation by β-adrenoreceptors is a key switch for tumour metastasis. Nat Commun 4:1403. https://doi.org/10.1038/ncomms2413

Avet C, Mancini A, Breton B, Le Gouill C, Hauser AS, Normand C, Kobayashi H, Gross F, Hogue M, Lukasheva V, St-Onge S, Carrier M, Héroux M, Morissette S, Fauman EB, Fortin JP, Schann S, Leroy X, Gloriam DE, Bouvier M (2022) Effector membrane translocation biosensors reveal G protein and βarrestin coupling profiles of 100 therapeutically relevant GPCRs. Elife 11:e74101. https://doi.org/10.7554/eLife.74101

Baek M, DiMaio F, Anishchenko I, Dauparas J, Ovchinnikov S, Lee GR, Wang J, Cong Q, Kinch LN, Schaeffer RD, Millán C, Park H, Adams C, Glassman CR, DeGiovanni A, Pereira JH, Rodrigues AV, van Dijk AA, Ebrecht AC, Opperman DJ, Sagmeister T, Buhlheller C, Pavkov-Keller T, Rathinaswamy MK, Dalwadi U, Yip CK, Burke JE, Garcia KC, Grishin NV, Adams PD, Read RJ, Baker D (2021) Accurate prediction of protein structures and interactions using a three-track neural network. Science 373:871–876. https://doi.org/10.1126/science.abj8754

Bastug-Ozel Z, Wright PT, Kraft AE, Pavlovic D, Howie J, Froese A, Fuller W, Gorelik J, Shattock MJ, Nikolaev VO (2019) Heart failure leads to altered beta2-adrenoceptor/cyclic adenosine

monophosphate dynamics in the sarcolemmal phospholemman/Na,K ATPase microdomain. Cardiovasc Res 115:546–555. https://doi.org/10.1093/cvr/cvy221

Bathe-Peters M, Gmach P, Boltz H-H, Einsiedel J, Gotthardt M, Hübner H, Gmeiner P, Lohse MJ, Annibale P (2021) Visualization of beta-adrenergic receptor dynamics and differential localization in cardiomyocytes. Proc Natl Acad Sci U S A 118:e2101119118. https://doi.org/10.1073/pnas.2101119118

Beavo JA, Brunton LL (2002) Cyclic nucleotide research -- still expanding after half a century. Nat Rev Mol Cell Biol 3:710–718. https://doi.org/10.1038/nrm911

Belevych AE, Juranek I, Harvey RD (2004) Protein kinase C regulates functional coupling of β1-adrenergic receptors to Gi/o-mediated responses in cardiac myocytes. FASEB J 18:1–19. https://doi.org/10.1096/fj.03-0647fje

Bers DM (2002) Cardiac excitation-contraction coupling. Nature 415:198–205. https://doi.org/10.1038/415198a

Bos JL (1998) All in the family? New insights and questions regarding interconnectivity of Ras, Rap1 and Ral. EMBO J 17:6776–6782. https://doi.org/10.1093/emboj/17.23.6776

Bowman SL, Shiwarski DJ, Puthenveedu MA (2016) Distinct G protein-coupled receptor recycling pathways allow spatial control of downstream G protein signaling. J Cell Biol 214:797–806. https://doi.org/10.1083/jcb.201512068

Braun C, Parks XX, Qudsi H, Lopes CM (2020) PKCbeta-II specifically regulates KCNQ1/KCNE1 channel membrane localization. J Mol Cell Cardiol 138:283–290. https://doi.org/10.1016/j.yjmcc.2019.10.010

Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R, Zera P, Menlove R, Shah P, Jamieson S (1986) Beta 1- and beta 2-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective beta 1-receptor down-regulation in heart failure. Circ Res 59:297–309. https://doi.org/10.1161/01.RES.59.3.297

Brodde OE (1991) Beta 1- and beta 2-adrenoceptors in the human heart: properties, function, and alterations in chronic heart failure. Pharmacol Rev 43:203–242

Bruzzone A, Saulière A, Finana F, Sénard J-M, Lüthy I, Galés C (2014) Dosage-dependent regulation of cell proliferation and adhesion through dual β2-adrenergic receptor/cAMP signals. FASEB J 28:1342–1354. https://doi.org/10.1096/fj.13-239285

Buchwalow IB, Kopiov OV, Schulze W (1981) Ultracytochemical localization of adenylate cyclase activity in rat thymocytes. Histochemistry 72:625–634. https://doi.org/10.1007/bf00493279

Bünemann M, Bücheler MM, Philipp M, Lohse MJ, Hein L (2001) Activation and deactivation kinetics of alpha 2A- and alpha 2C-adrenergic receptor-activated G protein-activated inwardly rectifying K+ channel currents. J Biol Chem 276:47512–47517. https://doi.org/10.1074/jbc.M108652200

Bylund DB (1988) Subtypes of alpha 2-adrenoceptors: pharmacological and molecular biological evidence converge. Trends Pharmacol Sci 9:356–361. https://doi.org/10.1016/0165-6147(88)90254-4

Calabrese EJ, Giordano J (2021) Ultra low doses and biological amplification: approaching Avogadro's number. Pharmacol Res 170:105738. https://doi.org/10.1016/j.phrs.2021.105738

Calebiro D, Grimes J (2020) G protein-coupled receptor pharmacology at the single-molecule level. Annu Rev Pharmacol Toxicol 60:73–87. https://doi.org/10.1146/annurev-pharmtox-010919-023348

Cariolato L, Cavin S, Diviani D (2011) A-kinase anchoring protein (AKAP)-Lbc anchors a PKN-based signaling complex involved in α1-adrenergic receptor-induced p38 activation. J Biol Chem 286:7925–7937. https://doi.org/10.1074/jbc.M110.185645

Chaudhry A, MacKenzie RG, Georgic LM, Granneman JG (1994) Differential interaction of beta 1- and beta 3-adrenergic receptors with Gi in rat adipocytes. Cell Signal 6:457–465. https://doi.org/10.1016/0898-6568(94)90093-0

Chen L, El-Sherif N, Boutjdir M (1996) Alpha1-adrenergic activation inhibits beta-adrenergic stimulated unitary Ca2+ currents in cardiac ventricular myocytes. Circ Res 79:184–193. https://doi.org/10.1161/01.RES.79.2.184

Cheng H, Farquhar MG (1976a) Presence of adenylate cyclase activity in Golgi and other fractions from rat liver. I Biochemical determination. J Cell Biol 70:660–670. https://doi.org/10.1083/jcb.70.3.660

Cheng H, Farquhar MG (1976b) Presence of adenylate cyclase activity in Golgi and other fractions from rat liver. II. Cytochemical localization within Golgi and ER membranes. J Cell Biol 70:671–684. https://doi.org/10.1083/jcb.70.3.671

Civciristov S, Ellisdon AM, Suderman R, Pon CK, Evans BA, Kleifeld O, Charlton SJ, Hlavacek WS, Canals M, Halls ML (2018) Preassembled GPCR signaling complexes mediate distinct cellular responses to ultralow ligand concentrations. Sci Signal 11:eaan1188. https://doi.org/10.1126/scisignal.aan1188

Civciristov S, Halls ML (2019) Signalling in response to sub-picomolar concentrations of active compounds: pushing the boundaries of GPCR sensitivity. Br J Pharmacol 176:2382–2401. https://doi.org/10.1111/bph.14636

Copik AJ, Baldys A, Nguyen K, Sahdeo S, Ho H, Kosaka A, Dietrich PJ, Fitch B, Raymond JR, Ford APDW, Button D, Milla ME (2015) Isoproterenol acts as a biased agonist of the alpha-1A-adrenoceptor that selectively activates the MAPK/ERK pathway. PloS One 10:e0115701. https://doi.org/10.1371/journal.pone.0115701

Cotecchia S (2010) The α1-adrenergic receptors: diversity of signaling networks and regulation. J Recept Signal Transduct Res 30:410–419. https://doi.org/10.3109/10799893.2010.518152

Cotecchia S, Kobilka BK, Daniel KW, Nolan RD, Lapetina EY, Caron MG, Lefkowitz RJ, Regan JW (1990) Multiple second messenger pathways of alpha-adrenergic receptor subtypes expressed in eukaryotic cells. J Biol Chem 265:63–69

Crilly SE, Puthenveedu MA (2021) Compartmentalized GPCR signaling from intracellular membranes. J Membr Biol 254:259–271. https://doi.org/10.1007/s00232-020-00158-7

Daaka Y, Luttrell LM, Lefkowitz RJ (1997) Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. Nature 390:88–91. https://doi.org/10.1038/36362

Davare MA, Avdonin V, Hall DD, Peden EM, Burette A, Weinberg RJ, Horne MC, Hoshi T, Hell JW (2001) A beta2 adrenergic receptor signaling complex assembled with the Ca2+ channel Cav1.2. Science 293:98–101. https://doi.org/10.1126/science.293.5527.98

Davel AP, Brum PC, Rossoni LV (2014) Isoproterenol induces vascular oxidative stress and endothelial dysfunction via a Giα-coupled β2-adrenoceptor signaling pathway. PloS One 9:e91877. https://doi.org/10.1371/journal.pone.0091877

DeFea KA (2011) Beta-arrestins as regulators of signal termination and transduction: how do they determine what to scaffold? Cell Signal 23:621–629. https://doi.org/10.1016/j.cellsig.2010.10.004

DeGraff JL, Gagnon AW, Benovic JL, Orsini MJ (1999) Role of arrestins in endocytosis and signaling of alpha2-adrenergic receptor subtypes. J Biol Chem 274:11253–11259. https://doi.org/10.1074/jbc.274.16.11253

Dixon RA, Kobilka BK, Strader DJ, Benovic JL, Dohlman HG, Frielle T, Bolanowski MA, Bennett CD, Rands E, Diehl RE, Mumford RA, Slater EE, Sigal IS, Caron MG, Lefkowitz RJ, Strader CD (1986) Cloning of the gene and cDNA for mammalian beta-adrenergic receptor and homology with rhodopsin. Nature 321:75–79. https://doi.org/10.1038/321075a0

Dorn GW 2nd, Oswald KJ, McCluskey TS, Kuhel DG, Liggett SB (1997) Alpha 2A-adrenergic receptor stimulated calcium release is transduced by Gi-associated G(beta gamma)-mediated activation of phospholipase C. Biochemistry 36:6415–6423. https://doi.org/10.1021/bi970080s

Drinnan SL, Hope BT, Snutch TP, Vincent SR (1991) G(olf) in the basal ganglia. Mol Cell Neurosci 2:66–70. https://doi.org/10.1016/1044-7431(91)90040-u

Dumaz N, Marais R (2003) Protein kinase A blocks Raf-1 activity by stimulating 14-3-3 binding and blocking Raf-1 interaction with Ras. J Biol Chem 278:29819–29823. https://doi.org/10.1074/jbc.C300182200

Dumaz N, Marais R (2005) Integrating signals between cAMP and the RAS/RAF/MEK/ERK signalling pathways. Based on the anniversary prize of the Gesellschaft fur Biochemie und Molekularbiologie lecture delivered on 5 July 2003 at the special FEBS meeting in Brussels. FEBS J 272:3491–3504. https://doi.org/10.1111/j.1742-4658.2005.04763.x

Eason MG, Kurose H, Holt BD, Raymond JR, Liggett SB (1992) Simultaneous coupling of alpha 2-adrenergic receptors to two G-proteins with opposing effects. Subtype-selective coupling of alpha 2C10, alpha 2C4, and alpha 2C2 adrenergic receptors to Gi and Gs. J Biol Chem 267:15795–15801

Eblen ST (2018) Chapter four - extracellular-regulated kinases: signaling from Ras to ERK substrates to control biological outcomes. In: Tew KD, Fisher PB (eds) Advances in cancer research, vol 138. Academic Press, pp 99–142

Eichel K, Jullié D, Barsi-Rhyne B, Latorraca NR, Masureel M, Sibarita J-B, Dror RO, von Zastrow M (2018) Catalytic activation of β-arrestin by GPCRs. Nature 557:381–386. https://doi.org/10.1038/s41586-018-0079-1

Eichel K, von Zastrow M (2018) Subcellular organization of GPCR signaling. Trends Pharmacol Sci 39:200–208. https://doi.org/10.1016/j.tips.2017.11.009

Ellisdon AM, Halls ML (2016) Compartmentalization of GPCR signalling controls unique cellular responses. Biochem Soc Trans 44:562–567. https://doi.org/10.1042/BST20150236

Endoh M (2016) Cardiac α1-adrenoceptors and inotropy: myofilament Ca2+ sensitivity, intracellular Ca2+ mobilization, signaling pathway, and pathophysiological relevance. Circ Res 119:587–590. https://doi.org/10.1161/circresaha.116.309502

Enserink JM, Christensen AE, de Rooij J, van Triest M, Schwede F, Genieser HG, Døskeland SO, Blank JL, Bos JL (2002) A novel Epac-specific cAMP analogue demonstrates independent regulation of Rap1 and ERK. Nat Cell Biol 4:901–906. https://doi.org/10.1038/ncb874

Ferguson SS (2001) Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. Pharmacol Rev 53:1–24

Fink EA et al (2022) Structure-based discovery of nonopioid analgesics acting through the α2A-adrenergic receptor. Science 377:eabn7065. https://doi.org/10.1126/science.abn7065

Friedman J, Babu B, Clark RB (2002) β2-adrenergic receptor lacking the cyclic AMP-dependent protein kinase consensus sites fully activates extracellular signal-regulated kinase 1/2 in human embryonic kidney 293 cells: lack of evidence for Gs/Gi switching. Mol Pharmacol 62:1094–1102. https://doi.org/10.1124/mol.62.5.1094

García-Sáinz JA, Vázquez-Prado J, del Carmen ML (2000) Alpha 1-adrenoceptors: function and phosphorylation. Eur J Pharmacol 389:1–12. https://doi.org/10.1016/s0014-2999(99)00896-1

Gilman AG (1987) G proteins: transducers of receptor-generated signals. Annu Rev Biochem 56:615–649. https://doi.org/10.1146/annurev.bi.56.070187.003151

Gollasch M, Hescheler J, Spicher K, Klinz FJ, Schultz G, Rosenthal W (1991) Inhibition of Ca2+ channels via alpha 2-adrenergic and muscarinic receptors in pheochromocytoma (PC-12) cells. Am J Physiol 260:C1282–C1289. https://doi.org/10.1152/ajpcell.1991.260.6.C1282

Gonzalez GA, Montminy MR (1989) Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. Cell 59:675–680. https://doi.org/10.1016/0092-8674(89)90013-5

Grundmann M, Merten N, Malfacini D, Inoue A, Preis P, Simon K, Rüttiger N, Ziegler N, Benkel T, Schmitt NK, Ishida S, Müller I, Reher R, Kawakami K, Inoue A, Rick U, Kühl T, Imhof D, Aoki J, König GM, Hoffmann C, Gomeza J, Wess J, Kostenis E (2018) Lack of beta-arrestin signaling in the absence of active G proteins. Nat Commun 9:341. https://doi.org/10.1038/s41467-017-02661-3

Guimond J, Mamarbachi AM, Allen BG, Rindt H, Hébert TE (2005) Role of specific protein kinase C isoforms in modulation of β1- and β2-adrenergic receptors. Cell Signal 17:49–58. https://doi.org/10.1016/j.cellsig.2004.05.012

Gutkind JS, Kostenis E (2018) Arrestins as rheostats of GPCR signalling. Nat Rev Mol Cell Biol 19:615–616. https://doi.org/10.1038/s41580-018-0041-y

Halls ML (2019) Localised GPCR signalling as revealed by FRET biosensors. Curr Opin Cell Biol 57:48–56. https://doi.org/10.1016/j.ceb.2018.11.001

Halls ML, Canals M (2018) Genetically encoded FRET biosensors to illuminate compartmentalised GPCR signalling. Trends Pharmacol Sci 39:148–157. https://doi.org/10.1016/j.tips.2017.09.005

Halls ML, Cooper DM (2011) Regulation by Ca2+-signaling pathways of adenylyl cyclases. Cold Spring Harb Perspect Biol 3:a004143. https://doi.org/10.1101/cshperspect.a004143

Hamed O, Joshi R, Michi AN, Kooi C, Giembycz MA (2021) β2-adrenoceptor agonists promote extracellular signal-regulated kinase 1/2 dephosphorylation in human airway epithelial cells by canonical, cAMP-driven signaling independently of β-arrestin 2. Mol Pharmacol 100:388–405. https://doi.org/10.1124/molpharm.121.000294

Hanoune J, Defer N (2001) Regulation and role of adenylyl cyclase isoforms. Annu Rev Pharmacol Toxicol 41:145–174. https://doi.org/10.1146/annurev.pharmtox.41.1.145

Hausdorff WP, Bouvier M, O'Dowd BF, Irons GP, Caron MG, Lefkowitz RJ (1989) Phosphorylation sites on two domains of the beta 2-adrenergic receptor are involved in distinct pathways of receptor desensitization. J Biol Chem 264:12657–12665

Hill SJ (2006) G-protein-coupled receptors: past, present and future. Br J Pharmacol 147(Suppl 1): S27–S37. https://doi.org/10.1038/sj.bjp.0706455

Holmberg CI, Kukkonen JP, Bischoff A, Näsman J, Courtney MJ, Michel MC, Akerman KE (1998) Alpha2B-adrenoceptors couple to Ca2+ increase in both endogenous and recombinant expression systems. Eur J Pharmacol 363:65–74. https://doi.org/10.1016/s0014-2999(98)00780-8

Huang Y, Wright CD, Merkwan CL, Baye NL, Liang Q, Simpson PC, O'Connell TD (2007) An alpha1A-adrenergic-extracellular signal-regulated kinase survival signaling pathway in cardiac myocytes. Circulation 115:763–772. https://doi.org/10.1161/circulationaha.106.664862

Hucho TB, Dina OA, Levine JD (2005) Epac mediates a cAMP-to-PKC signaling in inflammatory pain: an isolectin B4(+) neuron-specific mechanism. J Neurosci 25:6119–6126. https://doi.org/10.1523/jneurosci.0285-05.2005

Inoue A, Raimondi F, Kadji FMN, Singh G, Kishi T, Uwamizu A, Ono Y, Shinjo Y, Ishida S, Arang N, Kawakami K, Gutkind JS, Aoki J, Russell RB (2019) Illuminating G-protein-coupling selectivity of GPCRs. Cell 177:1933–1947.e1925. https://doi.org/10.1016/j.cell.2019.04.044

Insel PA, Murray F, Yokoyama U, Romano S, Yun H, Brown L, Snead A, Lu D, Aroonsakool N (2012) cAMP and Epac in the regulation of tissue fibrosis. Br J Pharmacol 166:447–456. https://doi.org/10.1111/j.1476-5381.2012.01847.x

Irannejad R, Pessino V, Mika D, Huang B, Wedegaertner PB, Conti M, von Zastrow M (2017) Functional selectivity of GPCR-directed drug action through location bias. Nat Chem Biol 13: 799–806. https://doi.org/10.1038/nchembio.2389

Irannejad R, Tomshine JC, Tomshine JR, Chevalier M, Mahoney JP, Steyaert J, Rasmussen SG, Sunahara RK, El-Samad H, Huang B, von Zastrow M (2013) Conformational biosensors reveal GPCR signalling from endosomes. Nature 495:534–538. https://doi.org/10.1038/nature12000

Jakobs KH, Saur W, Schultz G (1978) Inhibition of platelet adenylate cyclase by epinephrine requires GTP. FEBS Lett 85:167–170. https://doi.org/10.1016/0014-5793(78)81272-1

Jensen DD, Lieu T, Halls ML, Veldhuis NA, Imlach WL, Mai QN, Poole DP, Quach T, Aurelio L, Conner J, Herenbrink CK, Barlow N, Simpson JS, Scanlon MJ, Graham B, McCluskey A, Robinson PJ, Escriou V, Nassini R, Materazzi S, Geppetti P, Hicks GA, Christie MJ, Porter CJH, Canals M, Bunnett NW (2017) Neurokinin 1 receptor signaling in endosomes mediates sustained nociception and is a viable therapeutic target for prolonged pain relief. Sci Transl Med 9:eaal3447. https://doi.org/10.1126/scitranslmed.aal3447

Jewell-Motz EA, Liggett SB (1996) G protein-coupled receptor kinase specificity for phosphorylation and desensitization of alpha2-adrenergic receptor subtypes. J Biol Chem 271:18082–18087. https://doi.org/10.1074/jbc.271.30.18082

Joiner ML, Lise MF, Yuen EY, Kam AY, Zhang M, Hall DD, Malik ZA, Qian H, Chen Y, Ulrich JD, Burette AC, Weinberg RJ, Law PY, El-Husseini A, Yan Z, Hell JW (2010) Assembly of a beta2-adrenergic receptor--GluR1 signalling complex for localized cAMP signalling. EMBO J 29:482–495. https://doi.org/10.1038/emboj.2009.344

Jones DT, Reed RR (1989) Golf: an olfactory neuron specific-G protein involved in odorant signal transduction. Science 244:790–795. https://doi.org/10.1126/science.2499043

Jong YI, Harmon SK, O'Malley KL (2018) GPCR signalling from within the cell. Br J Pharmacol 175:4026–4035. https://doi.org/10.1111/bph.14023

Jong YJ, Kumar V, O'Malley KL (2009) Intracellular metabotropic glutamate receptor 5 (mGluR5) activates signaling cascades distinct from cell surface counterparts. J Biol Chem 284:35827–35838. https://doi.org/10.1074/jbc.M109.046276

Joyal JS, Nim S, Zhu T, Sitaras N, Rivera JC, Shao Z, Sapieha P, Hamel D, Sanchez M, Zaniolo K, St-Louis M, Ouellette J, Montoya-Zavala M, Zabeida A, Picard E, Hardy P, Bhosle V, Varma DR, Gobeil F Jr, Beauséjour C, Boileau C, Klein W, Hollenberg M, Ribeiro-da-Silva A, Andelfinger G, Chemtob S (2014) Subcellular localization of coagulation factor II receptor-like 1 in neurons governs angiogenesis. Nat Med 20:1165–1173. https://doi.org/10.1038/nm.3669

Keyes J, Ganesan A, Molinar-Inglis O, Hamidzadeh A, Zhang J, Ling M, Trejo J, Levchenko A, Zhang J (2020) Signaling diversity enabled by Rap1-regulated plasma membrane ERK with distinct temporal dynamics. Elife 9:e57410. https://doi.org/10.7554/eLife.57410

Kienitz M-C, Vladimirova D, Müller C, Pott L, Rinne A (2016) Receptor species-dependent desensitization controls KCNQ1/KCNE1 K+ channels as downstream effectors of Gq protein-coupled receptors. J Biol Chem 291:26410–26426. https://doi.org/10.1074/jbc.M116.746974

Kim H, Lee HN, Choi J, Seong J (2021) Spatiotemporal characterization of GPCR activity and function during endosomal trafficking pathway. Anal Chem 93:2010–2017. https://doi.org/10.1021/acs.analchem.0c03323

Kwon Y, Mehta S, Clark M, Walters G, Zhong Y, Lee HN, Sunahara RK, Zhang J (2022) Non-canonical β-adrenergic activation of ERK at endosomes. Nature 611(7934):173–179. https://doi.org/10.1038/s41586-022-05343-3. Epub 2022 Oct 26. PMID: 36289326; PMCID: PMC10031817

Lee D, Robeva A, Chen Z, Minneman KP (2003) Mutational uncoupling of α1A-adrenergic receptors from G proteins also uncouples mitogenic and transcriptional responses in PC12 cells. J Pharmacol Exp Ther 306:471–477. https://doi.org/10.1124/jpet.103.050500

Lemire I, Allen BG, Rindt H, Hebert TE (1998) Cardiac-specific overexpression of alpha1B-AR regulates beta-AR activity via molecular crosstalk. J Mol Cell Cardiol 30:1827–1839. https://doi.org/10.1006/jmcc.1998.0746

Li L, Cai H, Liu H, Guo T (2015) β-Adrenergic stimulation activates protein kinase Cε and induces extracellular signal-regulated kinase phosphorylation and cardiomyocyte hypertrophy. Mol Med Rep 11:4373–4380. https://doi.org/10.3892/mmr.2015.3316

Li L, Desantiago J, Chu G, Kranias EG, Bers DM (2000) Phosphorylation of phospholamban and troponin I in β-adrenergic-induced acceleration of cardiac relaxation. Am J Physiol Heart Circ Physiol 278:H769–H779. https://doi.org/10.1152/ajpheart.2000.278.3.H769

Li Y, Dillon TJ, Takahashi M, Earley KT, Stork PJS (2016) Protein kinase A-independent Ras protein activation cooperates with Rap1 protein to mediate activation of the extracellular signal-regulated kinases (ERK) by cAMP. J Biol Chem 291:21584–21595. https://doi.org/10.1074/jbc.M116.730978

Li Y, Takahashi M, Stork PJS (2013) Ras-mutant cancer cells display B-Raf binding to Ras that activates extracellular signal-regulated kinase and is inhibited by protein kinase A phosphorylation. J Biol Chem 288:27646–27657. https://doi.org/10.1074/jbc.M113.463067

Limbird LE, Gill DM, Lefkowitz RJ (1980) Agonist-promoted coupling of the beta-adrenergic receptor with the guanine nucleotide regulatory protein of the adenylate cyclase system. Proc Natl Acad Sci U S A 77:775–779. https://doi.org/10.1073/pnas.77.2.775

Lindemann JP, Jones LR, Hathaway DR, Henry BG, Watanabe AM (1983) Beta-adrenergic stimulation of phospholamban phosphorylation and Ca2+-ATPase activity in Guinea pig ventricles. J Biol Chem 258:464–471

Liu F, He K, Yang X, Xu N, Liang Z, Xu M, Zhao X, Han Q, Zhang Y (2011) α1A-adrenergic receptor induces activation of extracellular signal-regulated kinase 1/2 through endocytic pathway. PloS One 6:e21520. https://doi.org/10.1371/journal.pone.0021520

Liu G, Papa A, Katchman AN, Zakharov SI, Roybal D, Hennessey JA, Kushner J, Yang L, Chen B-X, Kushnir A, Dangas K, Gygi SP, Pitt GS, Colecraft HM, Ben-Johny M, Kalocsay M, Marx SO (2020) Mechanism of adrenergic CaV1.2 stimulation revealed by proximity proteomics. Nature 577:695–700. https://doi.org/10.1038/s41586-020-1947-z

Lohse MJ, Benovic JL, Codina J, Caron MG, Lefkowitz RJ (1990) β-Arrestin: a protein that regulates β-adrenergic receptor function. Science 248(4962):1547–1550. https://doi.org/10.1126/science.2163110. PMID: 2163110

Lukasheva V, Devost D, Le Gouill C, Namkung Y, Martin RD, Longpré JM, Amraei M, Shinjo Y, Hogue M, Lagacé M, Breton B, Aoki J, Tanny JC, Laporte SA, Pineyro G, Inoue A, Bouvier M, Hébert TE (2020) Signal profiling of the β(1)AR reveals coupling to novel signalling pathways and distinct phenotypic responses mediated by β(1)AR and β(2)AR. Sci Rep 10:8779. https://doi.org/10.1038/s41598-020-65636-3

Luttrell LM, Lefkowitz RJ (2002) The role of β-arrestins in the termination and transduction of G-protein-coupled receptor signals. J Cell Sci 115:455–465. https://doi.org/10.1242/jcs.115.3.455

Luttrell LM, Wang J, Plouffe B, Smith JS, Yamani L, Kaur S, Jean-Charles P-Y, Gauthier C, Lee M-H, Pani B, Kim J, Ahn S, Rajagopal S, Reiter E, Bouvier M, Shenoy SK, Laporte SA, Rockman HA, Lefkowitz RJ (2018) Manifold roles of beta-arrestins in GPCR signaling elucidated with siRNA and CRISPR/Cas9. Sci Signal 11:eaat7650. https://doi.org/10.1126/scisignal.aat7650

Ma YC, Huang J, Ali S, Lowry W, Huang XY (2000) Src tyrosine kinase is a novel direct effector of G proteins. Cell 102:635–646. https://doi.org/10.1016/s0092-8674(00)00086-6

MacLennan DH, Kranias EG (2003) Phospholamban: a crucial regulator of cardiac contractility. Nat Rev Mol Cell Biol 4:566–577. https://doi.org/10.1038/nrm1151

Mai QN, Shenoy P, Quach T, Retamal JS, Gondin AB, Yeatman HR, Aurelio L, Conner JW, Poole DP, Canals M, Nowell CJ, Graham B, Davis TP, Briddon SJ, Hill SJ, Porter CJH, Bunnett NW, Halls ML, Veldhuis NA (2021) A lipid-anchored neurokinin 1 receptor antagonist prolongs pain relief by a three-pronged mechanism of action targeting the receptor at the plasma membrane and in endosomes. J Biol Chem 296:100345. https://doi.org/10.1016/j.jbc.2021.100345

Man KNM, Navedo MF, Horne MC, Hell JW (2020) β(2) adrenergic receptor complexes with the L-type Ca(2+) channel Ca(V)1.2 and AMPA-type glutamate receptors: paradigms for pharmacological targeting of protein interactions. Annu Rev Pharmacol Toxicol 60:155–174. https://doi.org/10.1146/annurev-pharmtox-010919-023404

Marx SO, Ondrias K, Marks AR (1998) Coupled gating between individual skeletal muscle Ca2+ release channels (ryanodine receptors). Science 281:818–821. https://doi.org/10.1126/science.281.5378.818

Masuho I, Ostrovskaya O, Kramer GM, Jones CD, Xie K, Martemyanov KA (2015) Distinct profiles of functional discrimination among G proteins determine the actions of G protein-coupled receptors. Sci Signal 8:ra123. https://doi.org/10.1126/scisignal.aab4068

Michel MC, Brass LF, Williams A, Bokoch GM, LaMorte VJ, Motulsky HJ (1989) Alpha 2-adrenergic receptor stimulation mobilizes intracellular Ca2+ in human erythroleukemia cells. J Biol Chem 264:4986–4991

Milligan G, Rees S (1999) Chimaeric Gα proteins: their potential use in drug discovery. Trends Pharmacol Sci 20:118–124. https://doi.org/10.1016/S0165-6147(99)01320-6

Mongillo M, McSorley T, Evellin S, Sood A, Lissandron V, Terrin A, Huston E, Hannawacker A, Lohse MJ, Pozzan T, Houslay MD, Zaccolo M (2004) Fluorescence resonance energy transfer-based analysis of cAMP dynamics in live neonatal rat cardiac myocytes reveals distinct functions of compartmentalized phosphodiesterases. Circ Res 95:67–75. https://doi.org/10.1161/01.Res.0000134629.84732.11

Moniotte S, Kobzik L, Feron O, Trochu J-N, Gauthier C, Balligand J-L (2001) Upregulation of beta3-adrenoceptors and altered contractile response to inotropic amines in human failing myocardium. Circulation 103:1649–1655. https://doi.org/10.1161/01.CIR.103.12.1649

Moore RH, Sadovnikoff N, Hoffenberg S, Liu S, Woodford P, Angelides K, Trial JA, Carsrud ND, Dickey BF, Knoll BJ (1995) Ligand-stimulated beta 2-adrenergic receptor internalization via the constitutive endocytic pathway into rab5-containing endosomes. J Cell Sci 108(Pt 9): 2983–2991. https://doi.org/10.1242/jcs.108.9.2983

Mueed I, Bains P, Zhang L, Macleod KM (2004) Differential participation of protein kinase C and Rho kinase in alpha 1-adrenoceptor mediated contraction in rat arteries. Can J Physiol Pharmacol 82:895–902. https://doi.org/10.1139/y04-086

Murphy GJ, Kirkham DM, Cawthorne MA, Young P (1993) Correlation of β3-adrenoceptor-induced activation of cyclic amp-dependent protein kinase with activation of lipolysis in rat white adipocytes. Biochem Pharmacol 46:575–581. https://doi.org/10.1016/0006-2952(93)90540-D

Myagmar B-E, Flynn JM, Cowley PM, Swigart PM, Montgomery MD, Thai K, Nair D, Gupta R, Deng DX, Hosoda C, Melov S, Baker AJ, Simpson PC (2017) Adrenergic receptors in individual ventricular myocytes. Circ Res 120:1103–1115. https://doi.org/10.1161/CIRCRESAHA.117.310520

Nagiri C, Kobayashi K, Tomita A, Kato M, Kobayashi K, Yamashita K, Nishizawa T, Inoue A, Shihoya W, Nureki O (2021) Cryo-EM structure of the β3-adrenergic receptor reveals the molecular basis of subtype selectivity. Mol Cell 81:3205–3215.e3205. https://doi.org/10.1016/j.molcel.2021.06.024

Nash CA, Wei W, Irannejad R, Smrcka AV (2019) Golgi localized beta1-adrenergic receptors stimulate Golgi PI4P hydrolysis by PLCepsilon to regulate cardiac hypertrophy. Elife 8:e48167. https://doi.org/10.7554/eLife.48167

Nasman J, Kukkonen JP, Ammoun S, Akerman KE (2001) Role of G-protein availability in differential signaling by alpha 2-adrenoceptors. Biochem Pharmacol 62:913–922. https://doi.org/10.1016/s0006-2952(01)00730-4

Ngala RA, O'Dowd J, Wang SJ, Agarwal A, Stocker C, Cawthorne MA, Arch JR (2008) Metabolic responses to BRL37344 and clenbuterol in soleus muscle and C2C12 cells via different atypical pharmacologies and beta2-adrenoceptor mechanisms. Br J Pharmacol 155:395–406. https://doi.org/10.1038/bjp.2008.244

Ngala RA, O'Dowd J, Wang SJ, Stocker C, Cawthorne MA, Arch JR (2009) Beta2-adrenoceptors and non-beta-adrenoceptors mediate effects of BRL37344 and clenbuterol on glucose uptake in soleus muscle: studies using knockout mice. Br J Pharmacol 158:1676–1682. https://doi.org/10.1111/j.1476-5381.2009.00472.x

Niemeyer A, Rinne A, Kienitz M-C (2019) Receptor-specific regulation of atrial GIRK channel activity by different Ca2+-dependent PKC isoforms. Cell Signal 64:109418. https://doi.org/10.1016/j.cellsig.2019.109418

Nikolaev VO, Moshkov A, Lyon AR, Miragoli M, Novak P, Paur H, Lohse MJ, Korchev YE, Harding SE, Gorelik J (2010) Beta2-adrenergic receptor redistribution in heart failure changes cAMP compartmentation. Science 327:1653–1657. https://doi.org/10.1126/science.1185988

O'Connell TD, Jensen BC, Baker AJ, Simpson PC (2014) Cardiac alpha1-adrenergic receptors: novel aspects of expression, signaling mechanisms, physiologic function, and clinical importance. Pharmacol Rev 66:308–333. https://doi.org/10.1124/pr.112.007203

O'Hayre M, Eichel K, Avino S, Zhao X, Steffen DJ, Feng X, Kawakami K, Aoki J, Messer K, Sunahara R, Inoue A, von Zastrow M, Gutkind JS (2017) Genetic evidence that β-arrestins are dispensable for the initiation of β(2)-adrenergic receptor signaling to ERK. Sci Signal 10: eaal3395. https://doi.org/10.1126/scisignal.aal3395

Obara Y, Labudda K, Dillon TJ, Stork PJS (2004) PKA phosphorylation of Src mediates Rap1 activation in NGF and cAMP signaling in PC12 cells. J Cell Sci 117:6085–6094. https://doi.org/10.1242/jcs.01527

Oestreich EA, Wang H, Malik S, Kaproth-Joslin KA, Blaxall BC, Kelley GG, Dirksen RT, Smrcka AV (2007) Epac-mediated activation of phospholipase C epsilon plays a critical role in beta-adrenergic receptor-dependent enhancement of Ca2+ mobilization in cardiac myocytes. J Biol Chem 282:5488–5495. https://doi.org/10.1074/jbc.M608495200

Okeke K, Angers S, Bouvier M, Michel MC (2019) Agonist-induced desensitisation of β(3) -adrenoceptors: where, when, and how? Br J Pharmacol 176:2539–2558. https://doi.org/10.1111/bph.14633

Oleksa LM, Hool LC, Harvey RD (1996) Alpha1-adrenergic inhibition of the beta-adrenergically activated Cl- current in Guinea pig ventricular myocytes. Circ Res 78:1090–1099. https://doi.org/10.1161/01.RES.78.6.1090

Olsen RHJ, DiBerto JF, English JG, Glaudin AM, Krumm BE, Slocum ST, Che T, Gavin AC, McCorvy JD, Roth BL, Strachan RT (2020) TRUPATH, an open-source biosensor platform for interrogating the GPCR transducerome. Nat Chem Biol 16:841–849. https://doi.org/10.1038/s41589-020-0535-8

Oude Weernink PA, Han L, Jakobs KH, Schmidt M (2007) Dynamic phospholipid signaling by G protein-coupled receptors. Biochim Biophys Acta Biomembr 1768:888–900. https://doi.org/10.1016/j.bbamem.2006.09.012

Papa A, Kushner J, Marx SO (2022) Adrenergic regulation of calcium channels in the heart. Annu Rev Physiol 84:285–306. https://doi.org/10.1146/annurev-physiol-060121-041653

Penela P, Ribas C, Sánchez-Madrid F, Mayor F (2019) G protein-coupled receptor kinase 2 (GRK2) as a multifunctional signaling hub. Cell Mol Life Sci 76:4423–4446. https://doi.org/10.1007/s00018-019-03274-3

Perez DM (2020) α1-adrenergic receptors in neurotransmission, synaptic plasticity, and cognition. Front Pharmacol 11:581098. https://doi.org/10.3389/fphar.2020.581098

Pérez López I, Cariolato L, Maric D, Gillet L, Abriel H, Diviani D (2013) A-kinase anchoring protein Lbc coordinates a p38 activating signaling complex controlling compensatory cardiac hypertrophy. Mol Cell Biol 33:2903–2917. https://doi.org/10.1128/mcb.00031-13

Perez-Aso M, Segura V, Montó F, Barettino D, Noguera MA, Milligan G, D'Ocon P (2013) The three α1-adrenoceptor subtypes show different spatio-temporal mechanisms of internalization and ERK1/2 phosphorylation. Biochim Biophys Acta 1833:2322–2333. https://doi.org/10.1016/j.bbamcr.2013.06.013

Proudman RGW, Akinaga J, Baker JG (2022) The signaling and selectivity of α-adrenoceptor agonists for the human α2A, α2B and α2C-adrenoceptors and comparison with human α1 and β-adrenoceptors. Pharmacol Res Perspect 10:e01003. https://doi.org/10.1002/prp2.1003

Qian H, Matt L, Zhang M, Nguyen M, Patriarchi T, Koval OM, Anderson ME, He K, Lee HK, Hell JW (2012) β2-adrenergic receptor supports prolonged theta tetanus-induced LTP. J Neurophysiol 107:2703–2712. https://doi.org/10.1152/jn.00374.2011

Qian H, Patriarchi T, Price JL, Matt L, Lee B, Nieves-Cintrón M, Buonarati OR, Chowdhury D, Nanou E, Nystoriak MA, Catterall WA, Poomvanicha M, Hofmann F, Navedo MF, Hell JW (2017) Phosphorylation of Ser1928 mediates the enhanced activity of the L-type Ca2+ channel Cav1.2 by the β2-adrenergic receptor in neurons. Sci Signal 10:eaaf9659. https://doi.org/10.1126/scisignal.aaf9659

Rapacciuolo A, Suvarna S, Barki-Harrington L, Luttrell LM, Cong M, Lefkowitz RJ, Rockman HA (2003) Protein kinase A and G protein-coupled receptor kinase phosphorylation mediates beta-1 adrenergic receptor endocytosis through different pathways. J Biol Chem 278:35403–35411. https://doi.org/10.1074/jbc.M305675200

Rasmussen SG, DeVree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS, Thian FS, Chae PS, Pardon E, Calinski D, Mathiesen JM, Shah ST, Lyons JA, Caffrey M, Gellman SH, Steyaert J, Skiniotis G, Weis WI, Sunahara RK, Kobilka BK (2011) Crystal structure of the β2 adrenergic receptor-Gs protein complex. Nature 477:549–555. https://doi.org/10.1038/nature10361

Reiken S, Lacampagne A, Zhou H, Kherani A, Lehnart SE, Ward C, Huang F, Gaburjakova M, Gaburjakova J, Rosemblit N, Warren MS, He K-l, Yi G-h, Wang J, Burkhoff D, Vassort G, Marks AR (2003) PKA phosphorylation activates the calcium release channel (ryanodine

receptor) in skeletal muscle : defective regulation in heart failure. J Cell Biol 160:919–928. https://doi.org/10.1083/jcb.200211012

Renkhold L, Kollmann R, Inderwiedenstraße L, Kienitz M-C (2022) PKC-isoform specific regulation of receptor desensitization and KCNQ1/KCNE1 K+ channel activity by mutant α1B-adrenergic receptors. Cell Signal 91:110228. https://doi.org/10.1016/j.cellsig.2021.110228

Rosas PC, Liu Y, Abdalla MI, Thomas CM, Kidwell DT, Dusio GF, Mukhopadhyay D, Kumar R, Baker KM, Mitchell BM, Powers PA, Fitzsimons DP, Patel BG, Warren CM, Solaro RJ, Moss RL, Tong CW (2015) Phosphorylation of cardiac myosin-binding protein-C is a critical mediator of diastolic function. Circ Heart Fail 8:582–594. https://doi.org/10.1161/CIRCHEARTFAILURE.114.001550

Sabol SL, Nirenberg M (1979) Regulation of adenylate cyclase of neuroblastoma x glioma hybrid cells by alpha-adrenergic receptors. I. Inhibition of adenylate cyclase mediated by alpha receptors. J Biol Chem 254:1913–1920

Sabri A, Pak E, Alcott SA, Wilson BA, Steinberg SF (2000) Coupling function of endogenous alpha1- and beta-adrenergic receptors in mouse cardiomyocytes. Circ Res 86:1047–1053. https://doi.org/10.1161/01.RES.86.10.1047

Salm AK, McCarthy KD (1990) Norepinephrine-evoked calcium transients in cultured cerebral type 1 astroglia. Glia 3:529–538. https://doi.org/10.1002/glia.440030612

Schleicher K, Zaccolo M (2018) Using cAMP sensors to study cardiac nanodomains. J Cardiovasc Dev Dis 5:17. https://doi.org/10.3390/jcdd5010017

Schmidt M, Evellin S, Weernink PAO, Fv D, Rehmann H, Lomasney JW, Jakobs KH (2001) A new phospholipase-C–calcium signalling pathway mediated by cyclic AMP and a Rap GTPase. Nat Cell Biol 3:1020–1024. https://doi.org/10.1038/ncb1101-1020

Schmitt JM, Stork PJ (2002) PKA phosphorylation of Src mediates cAMP's inhibition of cell growth via Rap1. Mol Cell 9:85–94. https://doi.org/10.1016/s1097-2765(01)00432-4

Schmitt JM, Stork PJS (2000) Beta2-adrenergic receptor activates extracellular signal-regulated kinases (ERKs) via the small G protein Rap1 and the serine/threonine kinase B-Raf. J Biol Chem 275:25342–25350. https://doi.org/10.1074/jbc.M003213200

Shah S, Brock EJ, Ji K, Mattingly RR (2019) Ras and Rap1: a tale of two GTPases. Semin Cancer Biol 54:29–39. https://doi.org/10.1016/j.semcancer.2018.03.005

Shenoy SK, Lefkowitz RJ (2011) β-Arrestin-mediated receptor trafficking and signal transduction. Trends Pharmacol Sci 32:521–533. https://doi.org/10.1016/j.tips.2011.05.002

Soave M, Stoddart LA, White CW, Kilpatrick LE, Goulding J, Briddon SJ, Hill SJ (2021) Detection of genome-edited and endogenously expressed G protein-coupled receptors. FEBS J 288:2585–2601. https://doi.org/10.1111/febs.15729

Soini SL, Duzic E, Lanier SM, Åkerman KEO (1997) Dual modulation of calcium channel current via recombinant α2-adrenoceptors in pheochromocytoma (PC-12) cells. Pflugers Arch 435:280–285. https://doi.org/10.1007/s004240050513

Stork PJ, Schmitt JM (2002) Crosstalk between cAMP and MAP kinase signaling in the regulation of cell proliferation. Trends Cell Biol 12:258–266. https://doi.org/10.1016/s0962-8924(02)02294-8

Su M, Zhu L, Zhang Y, Paknejad N, Dey R, Huang J, Lee M-Y, Williams D, Jordan KD, Eng ET, Ernst OP, Meyerson JR, Hite RK, Walz T, Liu W, Huang X-Y (2020) Structural basis of the activation of heterotrimeric Gs-protein by isoproterenol-bound β1-adrenergic receptor. Mol Cell 80:59–71.e54. https://doi.org/10.1016/j.molcel.2020.08.001

Su M, Wang J, Xiang G et al (2023) Structural basis of agonist specificity of α1A-adrenergic receptor. Nat Commun 14:4819. https://doi.org/10.1038/s41467-023-40524-2

Takahashi M, Dillon TJ, Liu C, Kariya Y, Wang Z, Stork PJS (2013) Protein kinase A-dependent phosphorylation of Rap1 regulates its membrane localization and cell migration. J Biol Chem 288:27712–27723. https://doi.org/10.1074/jbc.M113.466904

Takahashi M, Li Y, Dillon TJ, Stork PJS (2017) Phosphorylation of Rap1 by cAMP-dependent protein kinase (PKA) creates a binding site for KSR to sustain ERK activation by cAMP. J Biol Chem 292:1449–1461. https://doi.org/10.1074/jbc.M116.768986

Toyoda Y, Zhu A, Kong F et al (2023) Structural basis of α1A-adrenergic receptor activation and recognition by an extracellular nanobody. Nat Commun 14:3655. https://doi.org/10.1038/s41467-023-39310-x

Tsvetanova NG, Trester-Zedlitz M, Newton BW, Peng GE, Johnson JR, Jimenez-Morales D, Kurland AP, Krogan NJ, von Zastrow M (2021) Endosomal cAMP production broadly impacts the cellular phosphoproteome. J Biol Chem 297:100907. https://doi.org/10.1016/j.jbc.2021.100907

Tsvetanova NG, von Zastrow M (2014) Spatial encoding of cyclic AMP signaling specificity by GPCR endocytosis. Nat Chem Biol 10:1061–1065. https://doi.org/10.1038/nchembio.1665

Vossler MR, Yao H, York RD, Pan M-G, Rim CS, Stork PJS (1997) cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. Cell 89:73–82. https://doi.org/10.1016/S0092-8674(00)80184-1

Wan Y, Huang X-Y (1998) Analysis of the Gs/mitogen-activated protein kinase pathway in mutant S49 cells. J Biol Chem 273:14533–14537. https://doi.org/10.1074/jbc.273.23.14533

Wang Q, Lu R, Zhao J, Limbird LE (2006a) Arrestin serves as a molecular switch, linking endogenous alpha2-adrenergic receptor to Src-dependent, but not Src-independent, ERK activation. J Biol Chem 281:25948–25955. https://doi.org/10.1074/jbc.M605415200

Wang Z, Dillon TJ, Pokala V, Mishra S, Labudda K, Hunter B, Stork PJS (2006b) Rap1-mediated activation of extracellular signal-regulated kinases by cyclic AMP is dependent on the mode of Rap1 activation. Mol Cell Biol 26:2130–2145. https://doi.org/10.1128/MCB.26.6.2130-2145.2006

Wehrens XHT, Lehnart SE, Marks AR (2005) Intracellular calcium release and cardiac disease. Annu Rev Physiol 67:69–98. https://doi.org/10.1146/annurev.physiol.67.040403.114521

Weinberg ZY, Puthenveedu MA (2019) Regulation of G protein-coupled receptor signaling by plasma membrane organization and endocytosis. Traffic 20:121–129. https://doi.org/10.1111/tra.12628

White JA, McKinney BC, John MC, Powers PA, Kamp TJ, Murphy GG (2008) Conditional forebrain deletion of the L-type calcium channel Ca V 1.2 disrupts remote spatial memories in mice. Learn Mem 15:1–5. https://doi.org/10.1101/lm.773208

Willette BKA, Zhang JF, Zhang J, Tsvetanova NG (2023) Endosome positioning coordinates spatially selective GPCR signaling. Nat Chem Biol. https://doi.org/10.1038/s41589-023-01390-7. Epub ahead of print. PMID: 37500769

Wright CD, Chen Q, Baye NL, Huang Y, Healy CL, Kasinathan S, O'Connell TD (2008) Nuclear alpha1-adrenergic receptors signal activated ERK localization to caveolae in adult cardiac myocytes. Circ Res 103:992–1000. https://doi.org/10.1161/circresaha.108.176024

Wright CD, Wu SC, Dahl EF, Sazama AJ, O'Connell TD (2012) Nuclear localization drives α1-adrenergic receptor oligomerization and signaling in cardiac myocytes. Cell Signal 24:794–802. https://doi.org/10.1016/j.cellsig.2011.11.014

Wu D, Katz A, Lee CH, Simon MI (1992) Activation of phospholipase C by alpha 1-adrenergic receptors is mediated by the alpha subunits of Gq family. J Biol Chem 267:25798–25802

Wu D, Kuang Y, Wu Y, Jiang H (1995) Selective coupling of beta 2-adrenergic receptor to hematopoietic-specific G proteins. J Biol Chem 270:16008–16010. https://doi.org/10.1074/jbc.270.27.16008

Wu SC, Dahl EF, Wright CD, Cypher AL, Healy CL, O'Connell TD (2014) Nuclear localization of alpha1A-adrenergic receptors is required for signaling in cardiac myocytes: an "inside-out" alpha1-AR signaling pathway. J Am Heart Assoc 3:e000145. https://doi.org/10.1161/JAHA.113.000145

Xu J et al (2022) Structural insights into ligand recognition, activation, and signaling of the α2A adrenergic receptor. Sci Adv 8:eabj5347. https://doi.org/10.1126/sciadv.abj5347

Yang Z, Kirton HM, MacDougall DA, Boyle JP, Deuchars J, Frater B, Ponnambalam S, Hardy ME, White E, Calaghan SC, Peers C, Steele DS (2015) The Golgi apparatus is a functionally distinct Ca2+ store regulated by the PKA and Epac branches of the β1-adrenergic signaling pathway. Sci Signal 8:ra101. https://doi.org/10.1126/scisignal.aaa7677

Yuan D, Liu Z, Kaindl J, Maeda S, Zhao J, Sun X, Xu J, Gmeiner P, Wang HW, Kobilka BK (2020) Activation of the α(2B) adrenoceptor by the sedative sympatholytic dexmedetomidine. Nat Chem Biol 16:507–512. https://doi.org/10.1038/s41589-020-0492-2

Zaccolo M, Pozzan T (2002) Discrete microdomains with high concentration of cAMP in stimulated rat neonatal cardiac myocytes. Science 295:1711–1715. https://doi.org/10.1126/science.1069982

Zhang X, Odom DT, Koo S-H, Conkright MD, Canettieri G, Best J, Chen H, Jenner R, Herbolsheimer E, Jacobsen E, Kadam S, Ecker JR, Emerson B, Hogenesch JB, Unterman T, Young RA, Montminy M (2005) Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. Proc Natl Acad Sci U S A 102:4459–4464. https://doi.org/10.1073/pnas.0501076102

Presynaptic Adrenoceptors

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Contents

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Abstract

Presynaptic α_2-adrenoceptors are localized on axon terminals of many noradrenergic and non-noradrenergic neurons in the peripheral and central nervous systems. Their activation by exogenous agonists leads to inhibition of the exocytotic release of noradrenaline and other transmitters from the neurons. Most often, the α_{2A}-receptor subtype is involved in this inhibition. The chain of molecular events between receptor occupation and inhibition of the exocytotic release of transmitters has been determined. Physiologically released endogenous noradrenaline elicits retrograde autoinhibition of its own release. Some clonidine-like α_2-receptor agonists have been used to treat hypertension. Dexmedetomidine is used for prolonged sedation in the intensive care; It also has a strong analgesic effect. The α_2-receptor antagonist mirtazapine increases the noradrenaline concentration in the synaptic cleft by interrupting physiological autoinhibion of release. It belongs to the most effective antidepressive drugs. β_2-Adrenoceptors are also localized on axon terminals in the peripheral and central nervous systems. Their activation leads to enhanced transmitter release, however, they are not activated by endogenous adrenaline.

Keywords

Adrenoceptor · Alpha-adrenoceptor · Axon terminal · Beta-adrenoceptor · Clonidine · Dexmedetomidine · Exocytosis · Mirtazapine · Noradrenaline · Presynaptic receptor · Synaptic transmission · Transmitter release · Voltage-gated calcium channel

1 Introduction, Historical Overview

Brown and Gillespie (1957) have shown that dibenamine and phenoxybenzamine, two adrenoceptor blocking drugs, enhanced the release of noradrenaline in the spleen evoked by electrical stimulation of the splenic nerves in anesthetized cats. In the following years, it was repeatedly observed that several antagonists selective for α-adrenoceptors increase the release of noradrenaline in sympathetically innervated tissues. For example, Blakeley and Summers (1978) showed that

piperoxan (that, unlike dibenamine and phenoxybenzamine, does not inhibit the neuronal and extraneuronal uptake of noradrenaline) also enhances the release of noradrenaline.

In the 1970s, many new observations were made. It was observed that the α-adrenoceptor antagonists were not uniform in their effects. Some of them preferentially increased the release of noradrenaline from the presynaptic axon terminals (yohimbine, rauwolscine); others blocked preferentially the postsynaptic vascular responses to noradrenaline. Clonidine was also identified as an agonist with selectivity for the presynaptic release-inhibiting α-receptor (vs. the postsynaptic receptor involved in vasoconstriction). These observations led to the conclusion that the presynaptic α-receptors must be different from the postsynaptic α-receptors.

Already in 1971–1972 Salomon Langer and Klaus Starke put up the hypothesis that sympathetic noradrenergic axon terminals possess presynaptic α-receptors: Activation of these receptors by noradrenaline released from the same axon terminals leads to feedback inhibition of the release of noradrenaline. This hypothesis was verified during the second half of the 1970s. Salomon Langer (1974) suggested that "the postsynaptic alpha-receptor that mediates the response of the effector organ should be referred to as α_1, while the presynaptic alpha-receptor that regulates transmitter release should be called α_2."

This early phase of research on presynaptic adrenoceptors was reviewed among others by Salomon Langer (1974, 1997) and Klaus Starke (1977, 1981, 1987).

In the 1980s, presynaptic α-adrenoceptors were classified as α_2-receptors. Postsynaptic α-receptors, mediating vasoconstriction, are mostly α_1-receptors, but in some blood vessels activation of α_2-receptors can also lead to vasoconstriction (Flavahan et al. 1987). The function of presynaptic α_2-receptors was shown in many peripheral tissues and in the central nervous system. The α_2-receptors were localized mostly as autoreceptors on the axon terminals of noradrenergic neurons, but also as heteroreceptors on axon terminals of other neurons, for example, cholinergic and dopaminergic neurons.

Between 1986 and 1991, the primary amino acid sequences of nine adrenoceptors were determined by cloning, and these adrenoceptors are named today: α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, and β_3 (Hein and Kobilka 1995; Alexander et al. 2021). Soon, transgenic mouse lines were generated in which one or more adrenoceptor subtypes were deleted. With the help of these transgenic animals, but also conventionally using a large series of α_2-receptor antagonists, the presynaptic adrenoceptors were definitively identified (Hein et al. 1999a, b). In most cases, the presynaptic α-adrenoceptor appeared to be the α_{2A}-receptor. A more recent review on presynaptic adrenoceptors was published by Gilsbach and Hein (2008).

2 Anatomical Localization of Presynaptic Adrenoceptors

Adrenoceptors were identified by detection of their mRNA, by detection of their protein with immunohistochemistry, or by detection of their protein with a Western blot. Importantly, many antibodies against adrenoceptors that were used in

immunohistochemical and Western blot studies were not stringently tested for their specificity for the studied target receptor. Therefore, the results obtained with such antibodies must be interpreted with caution.

The localization of the adrenoceptors to the axon terminals was performed with light and electron microscopy, with in situ hybridization, or by using purified presynaptic membranes or synaptosomes. In in situ hybridization studies, mRNA was detected in the somatic regions of the neurons. However, it can be expected that after translation the receptor proteins are transported from the somatic regions to the axon terminals. Studies showing presynaptic localization of adrenoceptors are listed in Table 1.

Combining immunohistochemistry with confocal microscopy, Wang et al. (2013) demonstrated the presence of α_{2A}-adrenoceptors on sympathetic axon terminals of the heart of the mouse. Using in situ hybridization, mRNA for α_{2A}-, α_{2B}-, α_{2C}-, and α_{1C}-adrenoceptors in the somata of rat superior cervical ganglion (SCG) neurons was shown (Vidovic and Hill 1997) – the dominant receptor was the α_{2A}-receptor. Notably, the adrenoceptor denoted as "α_{1C}" by Vidovic and Hill (1997) is named today as "α_{1A}" (Alexander et al. 2021). After synthesis in the somatic regions of SCG sympathetic neurons, the adrenoceptors are transported to the axon terminals of postganglionic sympathetic neurons in the tissues. Their activation will affect noradrenaline release from the axon terminals.

The presynaptic localization of adrenoceptors was shown in neurons involved in nociception. The perikarya of primary nociceptive neurons lie in the dorsal root ganglia, and the axons of these pseudounipolar neurons project to the periphery and to the dorsal horn of the spinal cord. In situ hybridization experiments performed by Nicholson et al. (2005) indicated that cells in dorsal root ganglia of the rat express α_{2C}-, α_{1A}-, and α_{1B}-adrenoceptor mRNA. Expression of mRNA for α_{2A}-, α_{2B}-, α_{1D}-, β_1-, or β_2-receptor was not seen in these studies.

Riedl et al. (2009) used well-characterized antibodies and confocal microscopy to study the localization of receptors in the peripheral and central axon terminals of primary nociceptive neurons in rats. Axon terminals in both regions possess α_{2A}- and α_{2C}-receptors. The α_{2A}-receptors are co-localized with substance P (a known co-transmitter of primary nociceptive neurons) and with delta opioid receptors in the peripheral as well as in the central axon terminals of the primary nociceptive neurons. Appropriately, clonidine inhibits the release of calcitonin gene-related peptide (CGRP; another co-transmitter of primary nociceptive neurons) in spinal cord synaptosomes. Notably, therapeutically used α_2-receptor agonists, like dexmedetomidine, have sedative and analgesic effects. The basis of the analgesic effect could be the α_2-receptor-mediated presynaptic inhibition of the release of glutamate, substance P, and CGRP from the central axon terminals of the primary nociceptive neurons.

The lateral spinal nucleus (LSN) of the spinal cord is involved in sensory perception. Using antibodies against α_{2C}-receptors and electron microscopy, Olave and Maxwell (2004) demonstrated the presence of α_{2C}-receptors on axon terminals targeting LSN neurons in rats.

Table 1 Presynaptic localization of adrenoceptors

Species	Organ/tissue	Technique	Localisation	Authors
Mouse				
Mouse	Heart	Confocal microscopy Immunohistochemistry	Presynaptic terminals possess α_{2A} receptors, α_{2B} and α_{2C} Receptors are localized in the blood vessel wall	Wang et al. (2013)
Rat				
Rat	Superior cervical ganglion (SCG)	In situ hybridization	Expressed: α_{2A} (= dominant), α_{2B} and α_{2C} Expressed: α_{1C} (named today α_{1A})	Vidovic and Hill (1997)
Rat	Dorsal root ganglion	In situ hybridization	Expressed: α_{2C}, α_{1A}, and α_{1B} mRNA Not expressed: α_{2A}, α_{2B}, α_{1D}, β_1 or β_2 mRNA	Nicholson et al. (2005)
Rat	Superficial dorsal spinal cord Skin	Confocal microscopy Immunohistochemistry Slices, Synaptosomes	α_{2A}- and α_{2C}-receptors are expressed in axon terminals in the spinal cord α_{2A}-receptors, delta opioid receptors (DORs), and substance P are co-expressed in axon terminals in the spinal cord and in the skin α_{2A}-receptors, DORs, and substance P are co-expressed in spinal cord synaptosomes Clonidine and a DOR agonist suppress calcitonin gene-related peptide (CGRP) release from the synaptosomes	Riedl et al. (2009)
Rat	Spinal cord Lateral spinal nucleus (LSN)	Electron microscopy Immunohistochemistry	Presynaptic axon terminals to LSN neurons possess α_{2C}-receptors	Olave and Maxwell (2004)
Rat	Locus coeruleus	Light microscopy Electron microscopy Immunohistochemistry	Axon terminals express α_{2A}-receptors. The axon terminals are mostly non-catecholaminergic and form symmetric and asymmetric synapses	Aoki et al. (1994)
Rat	Hippocampus	Light microscopy Electron microscopy Immunohistochemistry	Presynaptic α_{2A}-receptors in non-catecholaminergic and catecholaminergic terminals	Milner et al. (1998)
Rat	Cortexhippocampus	Receptor autoradiography	Cholinergic immunotoxin injected into the basal forebrain The densities of α_2-, β- and 5-HT_{2A}-receptors in the projection regions decrease	Heider et al. (1997)
Monkey				
Monkey	Prefrontal cortex	Electron microscopy Immunohistochemistry	α_{2A}-receptors in axon terminals; mostly preterminal regions; axo-axonic interaction	Aoki et al. (1998)
Human				
Human	Postmortem brain	Western blot Pre- and postsynaptic densities purified	Prefrontal cortex: α_{2A} 5% presyn., 95% postsyn α_{2C} 60% presyn., 40% postsyn	Erdozain et al. (2019)

Presynaptic adrenoceptors were detected in three brain regions. In the locus coeruleus, α_{2A}-receptors were observed with electron microscopy on non-catecholaminergic and catecholaminergic axon terminals (Aoki et al. 1994). Similarly, Milner et al. (1998) saw α_{2A}-receptors on non-catecholaminergic and catecholaminergic terminals in the hippocampus. Heider et al. (1997) destroyed cholinergic neurons by injecting a cholinergic neurotoxin into the basal forebrain of rats. Using receptor autoradiography, they observed after a latency a decrease in the number of α_2-, β-, and 5-HT_{2A}-receptors in the projection regions of the destroyed cholinergic neurons. The conclusion is justified that these receptors were originally located on the axon terminals of cholinergic neurons.

Adrenoceptors in the prefrontal cortex of the monkey were analyzed by combining immunohistochemistry with electron microscopy (Aoki et al. 1998): Axonal α_{2A}-receptors occurred mostly at pre-terminal regions, suggesting axo-axonic interactions.

Erdozain et al. (2019) wanted to determine the position of α_{2A}- and α_{2C}-receptors in the prefrontal cortex of the human brain. Brain material was obtained post mortem, and pre- and postsynaptic densities were purified using biochemical techniques. The concentration of adrenoceptor proteins was determined by Western blotting. The α_{2A}-receptors were predominantly postsynaptically localized (5% in the presynaptic densities, 95% in the postsynaptic densities). The α_{2C}-receptors were similarly distributed in the two synaptic domains (60% in the presynaptic densities, 40% in the postsynaptic densities).

3 Methods Used for Studying the Function of Presynaptic Adrenoceptors

Presynaptic adrenoceptors were most often studied in vitro in isolated organs or tissues that were superfused or perfused with physiological salt solutions.

Neurotransmitter release was mostly elicited by electrical stimulation of axons in the tissues or, seldom, by superfusion of a salt solution with high K^+ concentration. Physiologically, an action potential traveling along the axon depolarizes the axon terminal, which activates voltage-gated Ca^{2+} channels, and the Ca^{2+} influx triggers the transmitter vesicle release machinery (Szabo and Starke 2021). Several kinds of experiments were performed to verify that the transmitter release elicited by electrical stimulation of the superfused tissues is similar to the physiological exocytotic release mechanism. For example, it was shown that inhibition of voltage-gated Na^+ channels by tetrodotoxin abolishes physiological transmitter release and also transmitter release in response to electrical stimulation. Similarly, Ca^{2+} removal or inhibition of voltage-gated Ca^{2+} channels prevents transmitter release in these in vitro preparations.

It is difficult to measure transmitter release directly from the axon terminals into the synaptic cleft. In most experiments, the overflow or spillover of the released transmitter from the synaptic cleft (or junctional space) into the superfusion fluid or blood plasma was determined. These “surrogate” parameters correlate reliably with

the real transmitter release from the axon terminals. In the following text, the expression "transmitter release" will be regularly used, although actually overflow or spillover was determined.

Most often, transmitter release was quantified by using radioactive tracers of the neurotransmitters. Noradrenergic, dopaminergic, and serotoninergic axon terminals were labeled by preincubation with [^{3}H]-noradrenaline, [^{3}H]-dopamine, and [^{3}H]-serotonin, respectively. Cholinergic axon terminals were labeled by preincubation with the acetylcholine precursor [^{3}H]-choline. More reliable measures of transmitter release can be obtained, when the neuronal reuptake of released [^{3}H]-noradrenaline, [^{3}H]-dopamine, [^{3}H]-serotonin, and [^{3}H]-acetylcholine (or of its metabolite [^{3}H]-choline) is inhibited (e.g., by desipramine, nomifensine, fluoxetine, or hemicholine, respectively). In most of these radiotracer experiments, the total [^{3}H] overflow was measured, which includes the radiolabeled neurotransmitter and its radiolabeled metabolites.

In a minority of the studies, the endogenous neurotransmitters noradrenaline or dopamine in the perfusion / superfusion fluid were directly determined with chromatographic methods, including high-pressure liquid chromatography (HPLC). In some early studies, the amount of released vasoactive substances was detected with bioassays (e.g., Blakeley and Summers 1978). Dopamine release in brain slices can also be determined with the electrochemical method "fast cyclic voltammetry."

An indirect measure of transmitter release is the postsynaptic response to electrical stimulation of presynaptic axons. For example, electrical stimulation of the sympathetic axons of a blood vessel elicits α_1-adrenoceptor-mediated vasoconstriction; electrical stimulation of the sympathetic axons of the heart elicits a β_1-adrenoceptor-mediated increase in the heart rate.

4 Function of Presynaptic α-Adrenoceptors

The field has been summarized in several major reviews: Starke 1977, 1981, 1987, 2001; Starke et al. 1989; Langer 1997; Boehm and Kubista 2002; Kubista and Boehm 2006; Gilsbach and Hein 2008; Schlicker and Feuerstein 2017.

4.1 Presynaptic α-Adrenoceptors: In Vitro Studies

The function of presynaptic α-adrenoceptors has been very often studied in vitro, and Table 2 shows the most important and representative findings. Presynaptic release-inhibiting α-receptors were identified among others in mice, rats, rabbits, guinea pigs, and monkeys.

4.1.1 Presynaptic α-Adrenoceptors in Mice

In mice, activation of presynaptic α_2-receptors led to an inhibition of noradrenaline release from the sympathetic neurons of the heart, kidney, and vas deferens (see Table 2). Activation of presynaptic α_2-receptors also inhibited noradrenaline release

Table 2 Function of presynaptic α-adrenoceptors in vitro

Species	Organ/tissue	Effect of activation/blockade of α-receptors	Receptor involved	Authors
Mouse				
Mouse also α_{2A}-, α_{2B}-, α_{2C}-, $\alpha_{2A/C}$-receptor KO	Heart	Noradrenaline and UK-14304 inhibit el. stim. evoked [^{3}H]-NA release Autoinhibition operates	α_{2A}, α_{2C}	Hein et al. (1999a)
Mouse	Vas deferens Cerebral cortex	In both tissues: El. stim. evoked [^{3}H]-NA release is enhanced by rauwolscine Autoinhibition operates	α_{2A}, α_{2C} (α_{2B})	Scheibner et al. (2001), Trendelenburg et al. (2003)
Mouse also $\alpha_{2A\,=\,2D}$-receptor KO	Atria, vas deferens, hippocampus, occipito-parietal cortex	UK-14304 inhibits and rauwolscine enhances el. stim. evoked [^{3}H]-NA release Autoinhibition operates The autoreceptor already functions at postnatal day P1	$\alpha_{2A\,=\,2D}$	Schelb et al. (2001)
Mouse also α_{2A}-receptor KO	Myenteric plexus longitudinal muscle	Medetomidine inhibits el. stim. evoked [^{3}H]-NA release el. stim. evoked [^{3}H]-ACh release	α_{2A}	Scheibner et al. (2002)
Mouse also α_{2A}-receptor KO	Perfused kidney	El. stim. evoked NA release is enhanced by phentolamine and in α_{2A}-receptor KO animals Autoinhibition operates	α_{2A}	Hoch et al. (2011)
Mouse	Vas deferens Axon terminals	Clonidine inhibits el. stim. evoked increase in $[Ca^{2+}]_{intracellular}$ The α_2-receptors are activated by endogenous noradrenaline	α_2	O'Connor et al. (1999)
Mouse	Bed nucleus of the stria terminalis (BNST)	UK-14304 suppresses excitatory synaptic transmission The effect of UK-14304 is diminished in α_{2A}-knockout mice	α_{2A}	Egli et al. (2005)

Rat				
Rat	Heart	NA, oxymetazoline, and xylometazoline inhibit el. stim. evoked [^{3}H]-NA release Phenoxybenzamine prevents the inhibition	α_2	Fuder et al. (1986)
Rat	Heart atrium submandibular gland	UK-14304 and methoxamine decrease and α_2-antagonists increase el. stim. evoked [^{3}H]-NA release Imidazoline or α_1-receptors are not involved	$\alpha_{2A\,=\,2D}$	Limberger et al. (1992)
Rat	Heart ventricle slices	UK-14304 suppresses el. stim. evoked [^{3}H]-NA release	α_2	Akers and Cassis (2000)
Rat	Perfused heart	UK-14304 inhibits el. stim. evoked NA release: ischemia attenuates the effect of UK- 14304 Yohimbine enhances el. stim. evoked NA release: Ischemia attenuates the effect of yohimbine Presynaptic recurrent autoinhibition operates	α_2	Grimm et al. (2001)
Rat	Perfused mesenteric artery	Yohimbine enhances el. stim. evoked NA release	α_2	Tsuda et al. (1992)
Rat	Portal vein	Yohimbine enhances el. stim. evoked [^{3}H]-NA release	α_2	Ortiz de Urbina et al. (1992)
Rat	Inferior vena cava	Clonidine and B-HT920 inhibit [^{3}H]-NA release Idazoxan enhances [^{3}H]-NA release	α_2	Molderings and Göthert (1990)
Rat	Urinary bladder	Clonidine inhibits and yohimbine enhances el. stim. evoked [^{3}H]-NA release	α_2	Somogyi and de Groat (1990)
Rat	Occipital cortex	Noradrenaline inhibits el. stim. evoked [^{3}H]-NA release Phentolamine and yohimbine enhance the release. Autoinhibition operates		Taube et al. (1977)
Rat	Spinal cord		α_{2A}	Umeda et al. (1997)

(continued)

Table 2 (continued)

Species	Organ/tissue	Effect of activation/blockade of α-receptors	Receptor involved	Authors
		Clonidine and dexmedetomidine inhibit el. stim. evoked evoked ^{3}[H]-NA release α_2-Antagonists enhance [^{3}H]-NA release Autoinhibition operates		
Rat	Pineal gland	Clonidine and oxymetazoline inhibit K^+-evoked release of [^{3}H]-NA Yohimbine enhances K^+-evoked release of [^{3}H]-NA	α_2	Pelayo et al. (1977)
Rat	Septal region	UK-14304 inhibits el. stim. evoked [^{3}H]-5-HT release Idazoxan enhances the evoked release: the α_2-receptors are tonically activated	α_2	Rutz et al. (2007)
Rat	Cultured hippocampal neurons	Noradrenaline and clonidine inhibit: – action potential evoked release of excitatory transmitter – action potential evoked release of inhibitory transmitter	$\alpha_{2A\,=\,2D}$	Boehm (1999)
Rat	Neonatal spinal cord slices	Noradrenaline and clonidine presynaptically inhibit excitatory synaptic input to sympathetic preganglionic neurons.	α_{2A}	Miyazaki et al. (1998)
Rat	Tuberomammillary nucleus	Noradrenaline presynaptically inhibits GABAergic synaptic transmission (IPSCs)	α_{2A}	Nakamura et al. (2013)
Rat	Ventrolateral preoptic nucleus Dissociated neurons	Noradrenaline presynaptically inhibits GABAergic neurotransmission	α_2	Matsuo et al. (2003)
Rat	Hypothalamic paraventricular nucleus	Noradrenaline presynaptically inhibits GABAergic neurotransmission	α_2	Han et al. (2002)

Rat	Primary nociceptive neurons	UK-14304 presynaptically inhibits excitatory neurotransmission in the spinal cord Yohimbine antagonizes the effect of UK-14304	α_2	Chen et al. (2011)
Rat	Cultured superior cervical ganglion neurons	Noradrenaline and clonidine inhibit cholinergic excitatory synaptic transmission	α_2	Stephens and Mochida (2005)
Rabbit				
Rabbit	Perfused heart	Oxymetazoline and naphazoline inhibit sympathetic stim. evoked release of endogenous noradrenaline Phentolamine enhances the evoked release of noradrenaline. Autoinhibition operates Oxymetazoline and naphazoline inhibit vagal stim. evoked parasympathetic transmission	Not determined	Starke (1972a)
Rabbit	Perfused heart	Exogenous noradrenaline inhibits el. stim. evoked [^{14}C]-NA release by activating α-receptors		Starke (1972b)
Rabbit	Pulmonary artery	α-Agonists inhibit el. stim. evoked [^{3}H]-NA release	"Presynaptic α-adrenoceptor" later named α_2	Starke et al. (1975a, b)
Rabbit	Caudate nucleus slices	UK-14304 inhibits el. stim. evoked release of endogenous DA (measured by voltammetry) Rauwolscine and oxymetazoline antagonize the inhibition by UK-14304	α_{2A}	Trendelenburg et al. (1994)
Other species				
Guinea pig	Ileal myenteric plexus	α_2-agonists inhibit and antagonists enhance el. stim. evoked ^{3}[H]-NA release	α_{2B}	Blandizzi et al. (1993)
		Autoinhibition operates		
		α_2-agonists inhibit el. stim. evoked [^{3}H]-ACh release	α_{2A}	

(continued)

Table 2 (continued)

Species	Organ/tissue	Effect of activation/blockade of α-receptors	Receptor involved	Authors
Pig	Brain cortex	UK-14304 inhibits el. stim. evoked [^{3}H]-NA release	α_{2A}	Trendelenburg et al. (1996)
African green monkey	Cerebral cortex	α_2-antagonists enhance el. stim. evoked [^{3}H]-NA release Autoinhibition operates	$\alpha_{2A=}\alpha_{2D}$	Trendelenburg et al. (1997a)

Abbreviations: ACh, acetylcholine; el. stim., electrical stimulation; B-HT920, α_2-receptor agonist; GABA, γ-aminobutyric acid; IPSC, inhibitory postsynaptic current; KO, receptor knockout (the function of the receptor was blocked by modification of its gene); NA, noradrenaline; UK-14304, α_2-agonist named today brimonidine; 5-HT, 5-hydroxytryptamine, serotonin

in several brain regions, like the occipito-parietal cortex and hippocampus. The release of acetylcholine from parasympathetic axon terminals in the gut was also suppressed after activation of their presynaptic α_2-receptors.

In the majority of cases, the α_2-receptors of noradrenergic axon terminals were also activated by noradrenaline released from the same axon terminals, i.e., presynaptic autoinhibition of transmitter release operated.

Using transgenic mice in which one or more of the α_2-receptor subtypes were deleted, the presynaptic release-inhibiting receptors were unequivocally identified. On principle, all three α_2-receptor subtypes (α_{2A}, α_{2B}, and α_{2C}) can mediate presynaptic inhibition (Trendelenburg et al. 2003), but in most organs the α_{2A}-subtype is the dominant presynaptic α_2-receptor. In the heart and the cerebral cortex, axon terminals possess both α_{2A}- and α_{2C}-receptors (Hein et al. 1999a; Scheibner et al. 2001; Trendelenburg et al. 2003). Under certain experimental conditions, a role even of the third subtype, the α_{2B}-receptor, could be established (Trendelenburg et al. 2003). It should be noted that the α_{2A}-receptors of mice were frequently denoted as α_{2D}-receptors, because they differ in ligand recognition profile from the α_{2A}-receptors of rabbits and humans. The reason for this difference is a point mutation in the fifth transmembrane domain of the mouse (and rat) receptor (Starke 2001).

4.1.2 Presynaptic α-Adrenoceptors in Rats

In rats, activation of presynaptic α_2-receptors led to a decrease in noradrenaline release in several sympathetically innervated organs or tissues: heart, mesenteric artery, portal vein, inferior vena cava, submandibular gland, and urinary bladder (see Table 2). For demonstrating physiological autoinhibition of noradrenaline release, the presynaptic axons must be stimulated at the appropriate frequency and the duration of stimulating trains must be within appropriate ranges: only under such conditions will be adrenoceptors in the axon terminals appropriately activated. Indeed, under appropriate stimulating conditions α_2-antagonists enhanced the release of noradrenaline in most cases, suggesting that physiological autoinhibition of noradrenaline release via presynaptic α_2-receptors is a widespread phenomenon.

The subtype of the presynaptic α_2-receptor in sympathetically innervated tissues of the rat was not frequently identified. The results of experiments performed by Limberger et al. (1992) suggest, however, that the most prominent presynaptic α_2-receptor in the rat is of the α_{2A}-subtype (which is similar to the α_{2A}-receptor of mice and slightly different from the α_2-receptor of humans and rabbits; see Sect. 4.1.1).

The function of presynaptic α_2-receptors in the central nervous system has also been intensively studied. Early studies of Taube et al. (1977) have shown that noradrenaline inhibits and the antagonists phentolamine and yohimbine enhance the electrically evoked release of [^{3}H]-noradrenaline in the occipital cortex. These observations indicate that presynaptic α_2-autoreceptors mediate recurrent inhibition of transmitter release by noradrenaline. [^{3}H]-noradrenaline release in the spinal cord was suppressed by clonidine and dexmedetomidine (Umeda et al. 1997). In the same experiments, [^{3}H] noradrenaline release was enhanced by the antagonists yohimbine, CH-38083, and the α_{2A}-selective compound BRL44408. However, the α_{2B}-selective antagonist ARC239 did not affect transmitter release. The authors

concluded that presynaptic autoinhibition of noradrenaline release was mediated by α_{2A}-receptors. [^{3}H]-noradrenaline release in the pineal gland was also inhibited by clonidine and oxymetazoline and enhanced by yohimbine. Finally, activation and blockade of α_2-heteroreceptors on axon terminals of serotoninergic neurons led to inhibition and enhancement, respectively, of the release of [^{3}H]-serotonin.

Modulation of synaptic transmission by presynaptic receptors was also studied in electrophysiological experiments. Thus, activation of presynaptic α_2-receptors led to inhibition of synaptic transmission in the hippocampus (inhibitory and excitatory transmission), ventrolateral preoptic nucleus (GABAergic transmission), hypothalamic paraventricular nucleus (GABAergic transmission), spinal cord (excitatory transmission), superior cervical ganglia (cholinergic excitatory transmission), and primary nociceptive neurons (excitatory transmission). The subtype of the α_2-receptor was not systemically studied in these experiments. When it was determined, the receptor appeared to be the rat orthologue of the α_{2A}-receptor (Nakamura et al. 2013; Boehm 1999).

4.1.3 Presynaptic α-Adrenoceptors in Rabbits

The seminal experiments of Klaus Starke in the 1970s were performed on rabbit pulmonary arteries and rabbit hearts (see Table 2).

In the rabbit pulmonary artery, several α-receptor agonists inhibited the electrical stimulation-evoked [^{3}H]-noradrenaline overflow, indicating presynaptic inhibition of transmitter release (Starke et al. 1975a, b). The α-receptor agonists also elicited direct vasoconstriction. The experiments identified α-receptor agonists with higher affinity for the presynaptic α-receptor (oxymetazoline, α-methyl-noradrenaline, tramazoline, clonidine) than for the postsynaptic α-receptor. Preferential agonists for the postsynaptic α-receptor were also found (phenylephrine, methoxamine).

In isolated perfused rabbit hearts, the imidazoline derivatives oxymetazoline and naphazoline inhibited the electrical stimulation-evoked release of endogenous noradrenaline from sympathetic axon terminals (noradrenaline was determined fluorimetrically following alumina adsorption and elution) (Starke 1972a). The electrical stimulation-evoked increase in heart rate was also inhibited by the imidazolines. The effect of oxymetazoline on noradrenaline release was antagonized by phentolamine. Oxymetazoline and naphazoline also interfered with the bradycardic effect observed during stimulation of vagal axons, suggesting that presynaptic release-inhibiting α-receptors are also localized on the axon terminals of cholinergic neurons.

In similar experiments on isolated perfused rabbit hearts, Starke (1972b) showed that exogeneous noradrenaline inhibited the electrical stimulation-evoked [^{14}C]-noradrenaline release by activating α-receptors. Klaus Starke pointed to the possibility that endogenous noradrenaline released from the sympathetic axon terminals may elicit autoreceptor-mediated feedback inhibition of transmitter release.

Trendelenburg et al. (1994) electrically stimulated caudate nucleus slices obtained from rabbits and determined the release of endogenous dopamine with fast cycling voltammetry. The α_2-agonist UK-14304 inhibited the release of dopamine. Based on the potencies of a series of antagonists against UK-14304, the

authors suggested that the presynaptic α_2-receptor on dopaminergic axon terminals was of the α_{2A}-subtype.

4.1.4 Presynaptic α-Adrenoceptors in Other Species

Presynaptic α-receptors were also observed in tissues of guinea pigs, pigs, and monkeys (see Table 2).

In superfused guinea pig ileum, several α_2-receptor agonists inhibited the electrical stimulation-evoked release of [^{3}H]-noradrenaline and [^{3}H]-acetylcholine. Based on the potencies of the agonists and their interactions with α_2-antagonists, the authors concluded that the inhibition of noradrenaline release was mediated by α_{2B}-receptors, whereas the inhibition of the release of acetylcholine was mediated by α_{2A}-receptors. The α_2-antagonists enhanced the release of noradrenaline, and this indicates that the release of the sympathetic neurotransmitter is controlled by autoinhibition.

Pig brain cortex slices were electrically stimulated to release [^{3}H]-noradrenaline. The α_2-receptor agonist UK-14304 suppressed the release of [^{3}H]-noradrenaline (international nonproprietary name [INN] of UK-14304 = brimonidine). Based on the rightward shifts of the concentration–response curve of UK-14304 by a series of α-receptor antagonists, it was concluded that the presynaptic release-inhibiting α_2-receptor was of the α_{2A}-subtype.

Cortical slices were prepared from brains of African green monkeys and electrically stimulated to release [^{3}H]-noradrenaline. A series of α-antagonists enhanced the release of [^{3}H]-noradrenaline indicating that presynaptic autoinhibition occurs. Based on the potencies of the α-antagonists, the authors arrived at the conclusion that the genetic α_{2A}-receptor (which pharmacologically behaves as an α_{2D}-receptor in this species) was involved in autoinhibition.

4.2 Presynaptic α-Adrenoceptors: In Vivo Studies

The function of presynaptic α-adrenoceptors was studied in two kinds of in vivo experiments (see Table 3). Modulation of transmitter release in the brain was studied in microdialysis experiments. The involvement of α-adrenoceptors in the release of noradrenaline in peripheral organs was analyzed by observing cardiovascular responses and changes in blood plasma catecholamines in living animals.

4.2.1 Microdialysis Experiments

For microdialysis, a catheter is inserted into a stereotactically identified small brain region. Artificial cerebrospinal fluid is then pumped into the region, and the effluate, which reflects the composition of the extracellular space, is analyzed with HPLC coupled with electrochemical detection. The technique is well suited for studying transmitter release in the target brain regions of conscious or anesthetized animals (see Table 3).

The most robust data on the function of presynaptic α-receptors was obtained in mice: dexmedetomidine applied via the microdialysis catheter directly to the axon

Table 3 Function of presynaptic α-adrenoceptors in vivo

Species	Determined parameter/technique	Effect of activation/blockade of α-receptors	Receptor involved	Authors
Mouse				
Mouseconscious	Medial prefrontal cortex Microdialysis	Dexmedetomidine applied via the dialysis catheter suppresses NA release The effect of dexmedetomidine is diminished in α_{2A}-adrenoceptor KO mice	α_{2A}	Ihalainen and Tanila (2002)
Mouseanesthetized	Dentate gyrus Voltammetry	El. stim. in the locus coeruleus elicits NA release in the gyrus dentatus Idazoxan (applied i.p.) potentiates el. stim. evoked NA release	Probably α_2	Yavich et al. (2005)
Rat				
Ratconscious	Cerebral cortex Microdialysis	Idazoxan applied via the dialysis catheter enhances NA release	α_2	Dennis et al. (1987)
Ratconscious	Prefrontal cortex Microdialysis	Clonidine applied s.c. suppresses NA and DA release Mianserin and yohimbine applied s.c. enhance NA and DA release Endogenous NA activates presynaptic α_2-receptors on noradrenergic and dopaminergic axon terminals	α_2	Tanda et al. (1996)
Ratconscious	Frontal cortex Hippocampus Microdialysis	Frontal cortex: Mirtazapine (s.c. applied) increases NA and DA release but does not affect 5-HT release Hippocampus: Mirtazapine enhances NA release but does not affect 5-HT release	α_2	Millan et al. (2000)
Ratconscious	Microdialysis Dorsal hippocampus Prefrontal cortex	Mirtazapine applied i.p. enhances NA and 5-HT release Mirtazapine applied i.p. enhances NA and DA release Endogenous NA activates presynaptic α_2-receptors on noradrenergic, serotonergic, and dopaminergic axon terminals	α_2	Yamauchi et al. (2012)
Ratconscious	Prefrontal cortex Microdialysis	α_2-agonist BRL44408 applied via the dialysis catheter enhances NA release	α_{2A}	Pudovkina et al. (2001)

Rat conscious	Striatum microdialysis	α_2-agonist S18616 applied s.c. suppresses NA release Several antagonists applied s.c. enhance NA release S18616 applied s.c. suppresses DA release	α_{2A} and α_{2C} α_2	Gobert et al. (2004)
Rat	Pithed rat	Clonidine and BHT-920 suppress the el. stim. evoked increase in plasma NA concentration	α2	Szemeredi et al. (1989)
Ratconscious	Portal vein NA release	Oxymetazoline decreases and yohimbine enhances el. stim. evoked NA release α2-receptors are dysfunctional in spontaneously hypertensive rats	α2	Remie and Zaagsma (1986), Remie et al. (1992)
Ratanesthetized	Stomach Acid secretion	α_2-agonists presynaptically inhibit vagal stim. evoked acid secretion	α_{2A}	Blandizzi et al. (1995)
Ratconscious	Blood plasma NA conc.	Yohimbine enhances stress-induced increase in plasma NA concentration	α_2	Johansson and Ehrenström (1988)
Ratanesthe-tized	Blood plasma NA determined. Prefrontal cortex (microdialysis)	Clonidine, rilmenidine, and moxonidine (imidazoline-like) and guanabenz lower blood pressure, heart rate, and the plasma NA concentration At the same doses they also lower the NA concentration in the prefrontal cortex	α_2	Szabo et al. (2001)
Dog				
Doganesthetized	Sympathetic nerve to the heart stimulated. NA in coronary sinus determined	Clonidine decreased and phenoxybenzamine increased the el. stim. evoked NA release in the heart	αprobably α_2	Yamaguchi et al. (1977)
Dog anesthetized	Sympathetic nerve to the liver stimulated. NA in hepatic venous blood determined	Clonidine inhibits and yohimbine enhances the el. stim. evoked NA release in the liver Negative feedback of NA release functions	αprobably α_2	Yamaguchi (1982)
Rabbit				
Rabbitpithed	Sympathetic outflow el. stimulated NA spillover into the blood is determined	α-Methyl-noradrenaline and clonidine lower NA spillover (effect antagonized by yohimbine) Yohimbine and rauwolscine increase NA spillover	α_2	Majewski et al. (1983a)

(continued)

Table 3 (continued)

Species	Determined parameter/technique	Effect of activation/blockade of α-receptors	Receptor involved	Authors
Rabbitconscious	NA spillover into the blood is determined	α-Methyl-noradrenaline lowers NA spillover and the adrenaline plasma concentration Yohimbine and rauwolscine increase NA spillover	α_2	Majewski et al. (1983b)
Rabbitanesthetized	Renal sympathetic nerve activity (RSNA) measured Plasma NA determined	Clonidine lowered the plasma NA conc. and decreased the ratio "plasma NA/RSNA" Yohimbine and rauwolscine increased the plasma NA conc. and increased the ratio "plasma NA/RSNA" Endogenous presynaptic autoinhibition operates physiologically	α_2	Szabo et al. (1989)
Rabbitanesthetized	Renal sympathetic nerve activity (RSNA) measured Renal NA spillover and total body NA spillover determined	Yohimbine enhances the ratio "renal NA spillover/RSNA" and the ratio "total body NA spillover/RSNA" Presynaptic autoinhibition of NA release from renal sympathetic axon terminals operates during physiological impulse traffic in the sympathetic neurons	α_2	Szabo et al. (1992)
Rabbitpithed	Sympathetic outflow el. stimulated	Moxinidine, rilmenidine, and UK-14304 (three imidazoline α_2-agonists) lowered the plasma NA concentration		Urban et al. (1995a, b)

Abbreviations: el. stim., electrical stimulation; B-HT920, α_2-receptor agonist; DA, dopamine; i.p., intraperitoneally applied; KO, receptor knockout (the function of the receptor was blocked by modification of its gene); NA, noradrenaline; RSNA, renal sympathetic nerve activity; s.c., subcutaneously applied; UK-14304, α_2-agonist named today brimonidine; 5-HT, 5-hydroxytryptamine, serotonin

terminals in the prefrontal cortex lowered the noradrenaline concentration in the vicinity of the axon terminals (Ihalainen and Tanila 2002). The effect of dexmedetomidine was diminished in α_{2A}-adrenoceptor knockout mice, pointing to the involvement of α_{2A}-receptors. Electrical stimulation of the locus coeruleus in mice elicited noradrenaline release in the dentate gyrus of the hippocampus, a projection region of locus coeruleus neurons (noradrenaline was determined in these experiments with voltammetry). Intraperitoneal injection of the α_2-antagonist idazoxan enhanced the electrical stimulation-evoked noradrenaline release in the dentate gyrus (Yavich et al. 2005). This result is compatible with the function of presynaptic negative feedback inhibition at noradrenergic axon terminals in the dentate gyrus.

In rats, idazoxan applied via the microdialysis catheter directly to the axon terminals in the cerebral cortex increased the extracellular noradrenaline concentration in the vicinity (Dennis et al. 1987): It is very likely that noradrenaline released from the axon terminals tonically activated presynaptic α_2-receptors. In similar experiments, the locally applied α-antagonist BRL44408 increased the extracellular noradrenaline concentration in the prefrontal cortex – suggesting the operation of α_{2A}-receptor-mediated presynaptic autoinhibition (Pudovkina et al. 2001).

In the microdialysis studies cited above, the site of action of the α_2-receptor ligands was restricted to the adrenergic axon terminals: The effects of the ligands accurately reflected presynaptic modulation of transmitter release. In several other studies described below, the drugs were applied intraperitoneally or subcutaneously, and effects on neurotransmitter concentrations in brain regions were determined with microdialysis. The observed effects are probably presynaptic effects on the axon terminals of projection neurons; however, effects on the somatodendritic regions of the neurons cannot be excluded. A subcutaneously injected α_2-agonist lowered and an α_2-antagonist increased the noradrenaline concentration in the striatum – the findings are compatible with presynaptic release-modulating α_2-receptors on noradrenergic axon terminals (Gobert et al. 2004). The striatal dopamine concentration was also lowered by the α_2-agonist (Gobert et al. 2004). In two microdialysis studies performed on rats, the subcutaneously administered antidepressive drugs mianserin and mirtazapine increased the noradrenaline concentration in the frontal cortex and the hippocampus (Tanda et al. 1996; Millan et al. 2000) – probably by interrupting presynaptic α_2-receptor-mediated autoinhibition.

4.2.2 Function of Presynaptic α-Adrenoceptors in the Peripheral Nervous System

The typical presynaptic α-adrenoceptor is the α_2-receptor. α_2-Receptors are localized at many sites in the sympathetic and parasympathetic systems (see Fig. 1). It is difficult to study the function of peripheral presynaptic α_2-receptors in living animals because the injected pharmacological tools, α_2-agonists and α_2-antagonists, can simultaneously affect the sympathetic and parasympathetic systems at many sites. The verification of a pure presynaptic effect is hardly possible. In some experiments described below, it was attempted to restrict the site of action of the pharmacological tools to the presynaptic axon terminals.

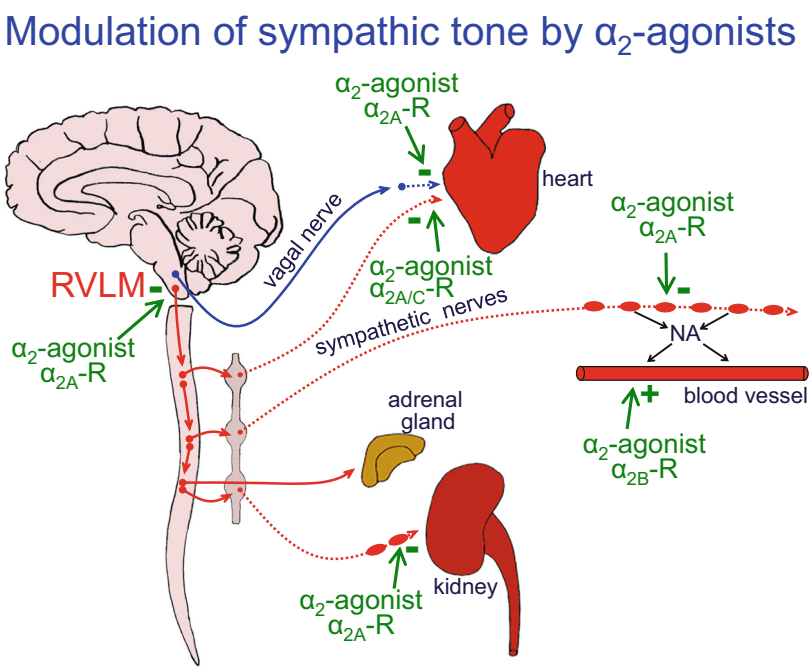

Fig. 1 Modulation of the sympathetic tone by α_2-agonists. α_2-receptor agonists can affect the sympathetic tone and the cardiovascular system at many sites. (1) α_2-agonists can lower sympathetic tone by inhibiting presympathetic neurons in the rostral ventrolateral medulla oblongata (RVLM). Probably α_{2A}-receptors are involved in this central sympathoinhibition. (2) α_2-agonists can activate presynaptic α_{2A}-receptors in blood vessels and in the kidney and thereby inhibit noradrenaline release. (3) Sympathetic axon terminals in the heart possess α_{2A}- and α_{2C}-receptors: Activation of these receptors also leads to inhibition of noradrenaline release. (4) α_2-agonists can presynaptically inhibit acetylcholine release from cardiac vagal axon terminals - putatively α_{2A}-receptors are involved. (5) By activating α_{2B}-receptors in vascular smooth muscle cells, α_2-agonists can increase vascular tone and blood pressure

Szemeredi et al. (1989) electrically stimulated the entire sympathetic outflow in pithed rats (see Table 3). The evoked increase in the plasma noradrenaline concentration was depressed by α_2-agonists, indicating that noradrenaline release was suppressed in the majority of sympathetically innervated organs. In the experiments of Remie and Zaagsma (1986) and Remie et al. (1992), the sympathetic nerves of the portal vein were electrically stimulated in rats, and blood was sampled from the portal vein downstream from the site of stimulation for the determination of the plasma noradrenaline concentration. The α_2-agonist oxymetazoline inhibited and the α_2-antagonist yohimbine enhanced, respectively, the stimulation-evoked noradrenaline release from the sympathetic neurons. The results point to presynaptic α_2-receptors in axon terminals of postganglionic sympathetic neurons in the portal vein, which can be activated by exogenous α_2-agonists and by endogenous noradrenaline. Presynaptic α_2-receptor activation by several α_2-agonists inhibited vagal stimulation-evoked acid secretion in the stomach, and the authors suggested the

involvement of α_{2A}-receptors (Blandizzi et al. 1995). These above-mentioned in vivo experiments allow localization of the release-modulating α_2-receptors to axon terminals (Remie and Zaagsma 1986; Remie et al. 1992; Blandizzi et al. 1995). They have, however, the disadvantage that transmitter release was elicited by an artificial electrical stimulation pattern, instead of ongoing physiological impulse traffic.

In some experiments in rats, systemic injection of α_2-agonists lowered the systemic plasma noradrenaline concentration, whereas injection of α_2-antagonists increased it. Although the involvement of α_2-receptors on sympathetic axon terminals in these effects is likely, effects of the applied drugs on α_2-receptors in the central nervous system cannot be excluded (Johansson and Ehrenström 1988; Szabo et al. 2001).

Yamaguchi et al. (1977, 1982) studied the function of α-receptors in anesthetized dogs. In the first series of experiments, the sympathetic nerves to the heart were electrically stimulated and noradrenaline in the coronary sinus was determined by a radiometric enzymatic assay. Clonidine decreased and phenoxybenzamine increased the stimulation-evoked noradrenaline release. In the second series of experiments, the sympathetic nerves to the liver were stimulated and noradrenaline in the hepatic veins was determined. Again, clonidine inhibited and yohimbine increased stimulation-evoked noradrenaline release. The experiments of Yamaguchi et al. shed light on the function of α-receptors on axon terminals of sympathetic nerves in the heart and the liver: These inhibitory receptors can be activated by an exogenous agonist and also by endogenous noradrenaline released from the axon terminals themselves. The effects in the experiments of Yamaguchi et al. were elicited by clonidine and yohimbine; therefore, α_2-receptors were surely involved – in the "old times"; however, Yamaguchi et al. did not yet name the receptors as α_2-receptors.

The function of presynaptic α_2-receptors in the sympathetic nervous system of the rabbit was studied in a series of experiments in the laboratory of Klaus Starke (Majewski et al. 1983a, b; Szabo et al. 1989, 1992, 2001; Urban et al. 1995a, b). In pithed rabbits, an artificial sympathetic tone was established by electrical stimulation of preganglionic sympathetic axons in the spinal canal. α-Methyl-noradrenaline and clonidine lowered, whereas yohimbine and rauwolscine increased, the noradrenaline spillover into the blood – this points to presynaptic inhibitory α_2-receptors in axon terminals of postganglionic sympathetic neurons in the majority of organs (Majewski et al. 1983a). The α_2-receptors also mediate recurrent presynaptic autoinhibition by endogenous noradrenaline. Similarly, in pithed rabbits with electrically stimulated sympathetic outflow, the three imidazoline α_2-agonists moxonidine, rilmenidine, and UK-14304 lowered the blood plasma noradrenaline concentration (Urban et al. 1995a, b). In conscious rabbits, α-methyl-noradrenaline decreased the spillover of noradrenaline into the blood, and the antagonists yohimbine and rauwolscine markedly increased it (Majewski et al. 1983b). The latter observations in conscious rabbits support the hypothesis of presynaptic autoinhibition at sympathetic axon terminals; however, an involvement of central nervous α_2-receptors in the effects of the antagonists could not be excluded.

In complex experiments on anesthetized rabbits, we analyzed presynaptic autoinhibition under physiological conditions (Fig. 2) (Szabo et al. 1992). Renal

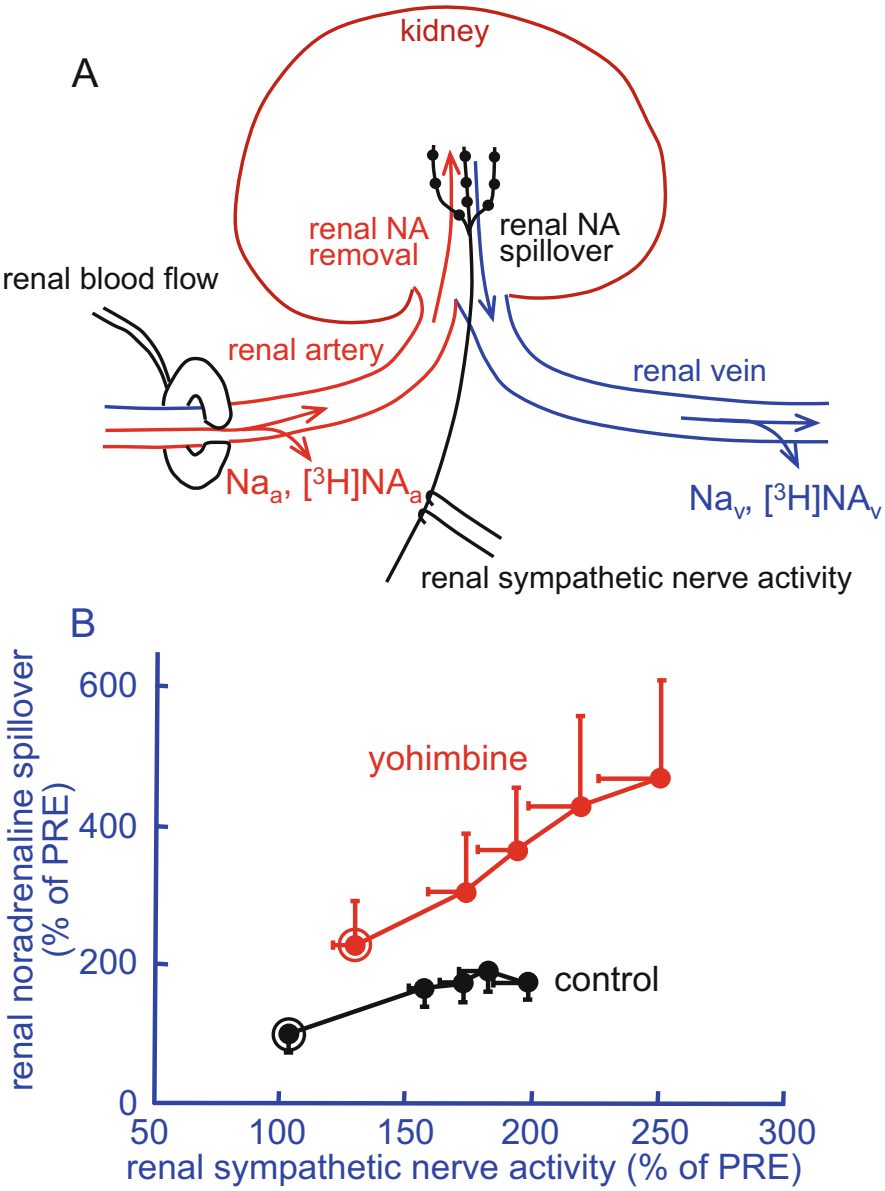

Fig. 2 α_2-Adrenoceptor-mediated autoinhibition of noradrenaline release operates under physiological conditions. (**a**) In anesthetized rabbits renal noradrenaline (NA) spillover was determined and the activity of a renal sympathetic nerve was electrically recorded. The nerve was not interrupted and was not electrically stimulated. Noradrenaline release in the kidney was solely due to ongoing physiological sympathetic nerve activity. In order to obtain a wide range of sympathetic nerve activities, the baroreceptor reflex was activated by controlled hypotension. (**b**) At any given renal sympathetic nerve activity, the sympathetic axon terminals released more noradrenaline in the presence of the α_2-antagonist yohimbine (YOH; injected i.v.) than in the control group (CON). This is a proof of the operation of the α_2-receptor-mediated autoinhibition of noradrenaline release from renal sympathetic nerves. Modified from Szabo et al. (1992)

postganglionic sympathetic nerve activity was quantified by counting action potentials in the nerve. Renal noradrenaline spillover from the synaptic cleft into the blood was determined simultaneously. The α_2-antagonist yohimbine increased the quotient "renal noradrenaline spillover / renal sympathetic nerve activity," indicating that axon terminals release more noradrenaline, when their α_2-receptors are inactivated. These results show that α_2-receptor-mediated presynaptic autoinhibition of noradrenaline release operates under truly physiological conditions, i.e., with ongoing sympathetic nerve impulse traffic (the sympathetic nerve was not interrupted and not stimulated).

Summary

Many in vitro experiments were performed, and they showed that presynaptic α_2-receptors are ubiquitous. Their activation leads to presynaptic inhibition of transmitter release in many peripheral organs and in many regions of the central nervous system. In most cases, the presynaptic α_2-receptors mediate autoinhibition of noradrenaline release by released endogenous noradrenaline.

The number of in vivo experiments, in which the function of presynaptic α_2-receptors was studied, is much lower than the number of in vitro experiments. And even less is the number of in vivo experiments in which the function of presynaptic α_2-receptors was unequivocally verified under truly physiological conditions (physiological impulse traffic in the sympathetic axons).

5 Function of Presynaptic β-Adrenoceptors

The function of presynaptic β-adrenoceptors was studied in mice, rats, rabbits, guinea pigs, and monkeys. The most important studies are summarized in Table 4.

5.1 Presynaptic β-Adrenoceptors: In Vitro Studies

In mice, activation of presynaptic β_2-receptors led to an enhancement of noradrenaline release from the sympathetic neurons of the heart atrium, spleen, and vas deferens and also from noradrenergic axon terminals in the cortex (Trendelenburg et al. 2000). Excitatory synaptic transmission in the bed nucleus of the stria terminalis was also enhanced after activation of presynaptic β_2-receptors (Egli et al. 2005).

In several studies, activation of presynaptic β_2-receptors in the rat heart led to an increase of noradrenaline release from sympathetic axon terminals. Presynaptic recurrent autofacilitation via β_2-receptors seemed to operate in some cases. The experiments of Apparsundaram and Eikenburg (1995) point to several features of presynaptic facilitation by β_2-receptors. These authors measured the electrical stimulation-evoked release of endogenous noradrenaline in the perfused rat heart. Phentolamine enhanced noradrenaline release, indicating presynaptic autoinhibition via α_2-receptors. The β_2-agonist salbutamol facilitated noradrenaline release at low

Table 4 Observations on the function of presynaptic β-adrenoceptors in vitro and in vivo

Species	Organ/tissue	Effect of activation/blockade of α-receptors	Receptor involved	Authors
In vitro				
Mouse				
Mouse	Heart atrium Spleen Vas deferens Occipito-parietal cortex	Isoprenaline and salbutamol enhance el. stim. evoked [^{3}H]-NA release No effect in the vas deferens No effect in the cortex	β_2	Trendelenburg et al. (2000)
Mouse	Bed nucleus of the stria terminalis (dBNST)	Isoprenaline and noradrenaline enhance excitatory synaptic transmission Timolol and ICI-118.551 prevent the effects of noradrenaline	β_2	Egli et al. (2005)
Rat				
Rat	Atrium	Isoprenaline enhances [^{3}H]-NA release (the effect is prevented by propranolol) The enhancement functions only in the presence of α_2-blockade	β_2?	Kazanietz and Enero (1989)
Rat	Heart ventricle slices	Salbutamol enhances el. stim. evoked ^{3}[H]-NA release	β_2	Akers and Cassis (2000)
Rat	Perfused heart	Salbutamol enhances el. stim. evoked NA release: stronger effect at low stim. frequency and in the presence of α_2-blockade Presynaptic recurrent autofacilitation does not operate	β_2	Apparsundaram and Eikenburg (1995)
Rat	Perfused heart	Terbutaline slightly enhances el. stim. evoked NA release: ischemia potentiates the effect of terbutaline ICI-118.551 inhibits el. stim. evoked NA release: ischemia potentiates the effect of ICI-118.551 Presynaptic recurrent autofacilitation operates	β_2	Grimm et al. (2001)
Rat	Portal vein	Yohimbine enhances el. stim. evoked [^{3}H]-NA release The effect of yohimbine is diminished by several β-antagonists Presynaptic recurrent autofacilitation may be operating	β_1?	Ortiz de Urbina et al. (1992)

Rat	Urinary bladder Detrusor muscle	Mirabegron and isoprenaline inhibit el. stim. evoked [^{3}H]-ACh release **Indirect effect:** β_3-receptors are postsynaptically localized in smooth muscle cells; adenosine is produced in smooth muscle cells; as retrograde messenger adenosine activates inhibitory A_1-adenosine receptors in cholinergic axon terminals	β_3	Silva et al. (2017, 2020)
Rat	Cerebral cortex Synaptosomes	Isoprenaline and Sp-cAMP enhance 4-aminopyridine-evoked glutamate release	β	Wang (2002)
Rat	Cerebral cortex Synaptosomes	Isoprenaline and 8-Br-cAMP facilitate 4-aminopyridine-evoked glutamate release Propranolol blocks the effect of isoprenaline	β	Wang et al. (2002)
Rat	Amygdala	Isoprenaline enhances the amplitude of EPSCs Paired-pulse ratio is lowered → presyn. action Isoprenaline facilitates Ca^{2+} influx through P/Q Ca^{2+} channels	β	Huang et al. (1996)
Rat	Ventromedial hypothalamic nucleus	Noradrenaline and formoterol presynaptically facilitate glutamatergic transmission β_2-antagonist ICI-118.551 prevents facilitation β_1-antagonist atenolol does not affect facilitation	β_2	Lee et al. (2007)
Rat	Medial prefrontal cortex	Isoprenaline enhances mEPSC frequency mEPSC amplitude is not affected Isoprenaline increases the amplitude of glutamatergic eEPSCs (paired pulse ratio is lowered) Presynaptic mode of action; cAMP/PKA signaling	β	Ji et al. (2008)
Rat	Insular cortex	Isoprenaline enhances GABAergic transmission between FS-pyramidal cells	β	Koyanagi et al. (2010)
Rat	Cortex, layer V pyramidal neurons	Isoproterenol enhances presynaptically glutamatergic transmission	β	Kobayashi et al. (2009)
Rat	Visual cortex		β	Terakado (2014)

(continued)

Table 4 (continued)

Species	Organ/tissue	Effect of activation/blockade of α-receptors	Receptor involved	Authors
		Isoprenaline presynaptically facilitates GABAergic synaptic transmission: mIPSC-frequency ↑		
Rat	Cerebellum, interneuron (basket cell) → Purkinje cell transmission	Noradrenaline → eIPSC in Purkinje cells ↑, PPR ↓ (presynaptic effect)Isoproterenol: same effect β_2-antagonist ICI-118.551 prevents facilitation β_1-antagonist CGP20712A has no effect on facilitation β_2-adrenoceptor on the soma of basket cells: frequency ↑	β_2	Saitow et al. (2000)
Rat	Hippocampus Resistance vessels	The β_3-agonists CL 316243, BRL 37344, and ZD 2079 **do not affect** el. stim. evoked ^{3}[H]-NA, ^{3}[H]-5-HT, and ^{3}[H]-ACh release in the hippocampus CL 316243 **does not affect** spinal stimulation-evoked blood pressure increase in pithed rats	β_3: no role	Zelaszczyk et al. (2005)
Guinea pig				
Guinea pig	Vas deferens	Isoprenaline and clenbuterol facilitate presynaptically el. stim. evoked release of endogenous noradrenaline and ATP	β_2	Todorov et al. (2001)
Guinea pig	Vas deferens	Isoprenaline increases the amplitude of el. stim. evoked excitatory junction potentials (EJPs; purinergic transmission)	β	Hardy and Brock (2001)
In vivo				
Ratconscious	Portal vein NA release	Fenoterol strongly enhances the release of NA evoked by local el. stim The effect of fenoterol is antagonized by ICI- 118.551, but not by an β_1-selective antagonist In the presence of endogenous activation of α_2-receptors, the stimulatory effect of the β_2-agonist is greatly attenuated	β_2	Remie et al. (1988a, b)

Doganesthetized	Sympathetic nerve to the heart stimulated NA in coronary sinus determined	Isoprenaline increased and sotalol decreased the el. stim. evoked NA release in the heart Endogenous autofacilitation of NA release via presynaptic β-receptors is possible	β	Yamaguchi et al. (1977)
Rabbit anesthetized	NA spillover into the blood is determined	Adrenaline increased the NA spillover Propranolol prevented the effect Adrenaline was first taken up by the noradrenaline transporter into sympathetic axon terminals, and activated β-receptors after release from the terminals	β	Majewski et al. (1982)

Abbreviations: ACh, acetylcholine; el. stim., electrical stimulation; GABA, γ-aminobutyric acid; ICI-118.551, β_2-selective antagonist; eIPSC, evoked inhibitory postsynaptic current; EPSC, excitatory postsynaptic current; FS, fast-spiking neuron; mIPSC, miniature inhibitory postsynaptic current; NA, noradrenaline; PKA, protein kinase A; PPR, paired pulse ratio

stimulation frequency. At an intermediate stimulation frequency, salbutamol facilitated release only in the presence of phentolamine: Obviously, presynaptic β_2- and α_2-receptors interact with each other, probably at the second messenger level. At a high stimulation frequency, salbutamol did not affect noradrenaline release even in the presence of phentolamine: The transmitter release mechanism was probably already saturated by high Ca^{2+} concentrations in the axon terminal. Propranolol did not influence the noradrenaline release in the experiments of Apparsundaram and Eikenburg (1995), indicating that endogenous noradrenaline-induced recurrent autofacilitation via β_2-receptors does not operate.

In the short study of Ortiz de Urbina et al. (1992), yohimbine enhanced the electrical stimulation-evoked [^{3}H]-noradrenaline release in the rat portal vein – indicating presynaptic α_2-receptor-mediated autoinhibition. When added in the presence of yohimbine, propranolol lowered the release of [^{3}H]-noradrenaline, and the authors suggest that this indicates noradrenaline-induced presynaptic autofacilitation via β_1-receptors.

The modulation of synaptic transmission via presynaptic β-receptors was also studied in electrophysiological experiments on slices prepared from rat brains (Table 4). In several cortical regions, in the amygdala and in the hypothalamus isoprenaline, formoterol or noradrenaline enhanced excitatory or inhibitory synaptic transmission with a presynaptic action. While the involvement of β-receptors was very likely in most experiments, the involvement of β_2-receptors was verified only by Lee et al. (2007) and Saitow et al. (2000) using a selective β_2-receptor agonist (formoterol), a selective β_2-antagonist (IC-I118.551), and selective β_1-antagonists (atenolol and CGP20712A). Physiological activation of β-receptors on glutamatergic or GABAergic axon terminals has not been seen. But it is conceivable that noradrenaline or adrenaline released from adrenergic axon terminals activates presynaptic β-receptors on adjacent glutamatergic or GABAergic axon terminals.

In the guinea pig vas deferens, isoprenaline and the β_2-adrenoceptor selective agonist clenbuterol enhanced the electrically evoked release of noradrenaline and ATP and the excitatory junction potentials recorded in postsynaptic smooth muscle cells. Presynaptic β_2-receptors were involved in these effects (Todorov et al. 2001).

The β_3-receptor agonists CL 316243, BRL 37344, and ZD 2079 did not affect the release of [^{3}H]-noradrenaline, [^{3}H]-serotonin, and [^{3}H]-acetylcholine evoked by electrical stimulation in the rat hippocampus (Zelaszczyk et al. 2005).

Several more recent studies were aimed to clarify whether activation of β_3-receptors affects transmitter release from peripheral axon terminals. The logical hypothesis is that activation of a $G\alpha_s$-protein-coupled presynaptic receptor enhances transmitter release from axon terminals. The question of the role of presynaptic β_3-receptors arose while analyzing the mode of action of the β_3-selective drug mirabegron. Mirabegron is the first-in-class β_3-selective drug used clinically to treat the overactive bladder syndrome (Michel and Korstanje 2016; Igawa et al. 2019).

Acetylcholine released from postganglionic parasympathetic fibers activates M_3 muscarinic acetylcholine receptors of the detrusor muscle of the urinary bladder and elicits its contraction. The localization of β_3-receptors in the detrusor muscle and in

the urothelium was shown by immunohistochemical techniques (Silva et al. 2017, 2020). One of the mechanisms of bladder relaxation by mirabegron is probably via the β_3-receptor–Gα_s-protein–adenylate cyclase–cAMP–direct detrusor relaxation pathway (e.g., Maki et al. 2019; but see Frazier et al. 2005; Igawa et al. 2019). Immunohistochemical studies do not show presynaptic β_3-receptors on cholinergic axon terminals (e.g., Silva et al. 2017; but see Coelho et al. 2017). Despite the lack of presence of β_3-receptors in cholinergic axons, mirabegron inhibits acetylcholine release from cholinergic axon terminals in the rat and human detrusor muscle (Silva et al. 2017, 2020). The authors suggest the following sequence of actions: β_3-receptor activation in the detrusor muscle cell – Gα_s-protein activation – adenylate cyclase activation – cAMP production – adenosine monophosphate production by phosphodiesterases – adenosine production by a nucleotidase – transport of adenosine from the detrusor muscle cell to the extracellular space by the equilibrative nucleoside transporter 1 (ENT1) – activation of the G$\alpha_{i/o}$-protein-coupled A_1-adenosine receptor in cholinergic axon terminals – inhibition of acetylcholine release. Thus, the second mode of detrusor muscle relaxation is due to inhibition of acetylcholine release with the above-described complex mechanism.

Unexpectedly, activation of the Gα_s-protein-coupled β_3-receptors leads to presynaptic inhibition of acetylcholine release: the β_3-receptor is postsynaptically localized, and the postsynaptically generated retrograde messenger adenosine activates presynaptic inhibitory A_1-adenosine receptors in cholinergic axon terminals.

5.2 Presynaptic β-Adrenoceptors: In Vivo Studies

Remie et al. (1988a, b) performed experiments on conscious rats: The sympathetic nerves of the portal vein were electrically stimulated, and blood was sampled from the portal vein downstream from the site of stimulation for the determination of the plasma noradrenaline concentration. Fenoterol directly injected into the portal vein strongly enhanced the noradrenaline release elicited by electrical stimulation of the sympathetic nerves of the portal vein. The effect of fenoterol was antagonized by the β_2-antagonist ICI-118.551 but not by the β_1-antagonist CGP20712A, pointing to the involvement of presynaptic β_2-receptors. Physiological activation of presynaptic α_2-receptors by endogenous noradrenaline attenuated the β_2-receptor-mediated facilitation of transmitter release.

It must be noted that in in vivo experiments like those of Remie et al. (1988a, b) interpretation problems due to in vivo complexity arise. For example, the β_2-agonist fenoterol could affect the noradrenaline release from the sympathetic axons of the portal vein not only by a direct presynaptic action on axon terminals. Fenoterol is a strong vasodilator in the systemic circulation, and this leads to sympathetic activation via the baroreflex – the noradrenaline release from sympathetic neurons increases, also in the portal vein. Indeed, in the experiments of Remie et al. (1988a, b) the pre-stimulation basal noradrenaline determined in the portal vein increased up to threefold. However, in the discussed experiments fenoterol also

increased the noradrenaline release evoked by local electrical stimulation of the portal vein sympathetic axons – a clear indication of an effect of fenoterol on the axon terminals in the portal vein.

Yamaguchi et al. (1977) stimulated in anesthetized dogs the sympathetic nerves to the heart and determined the noradrenaline release into the coronary sinus. Systemic administration (i.v.) of isoprenaline or sotalol enhanced or lowered, respectively, the electrical stimulation-evoked noradrenaline release into the coronary sinus. Isoprenaline activates β_1- and β_2-receptors and sotalol is an antagonist of both β-receptors (and even blocks repolarizing HERG/KvLQT1 K^+ channels). Therefore, the interpretation of the observations of Yamaguchi et al. (1977) is that axon terminals of postsynaptic synaptic neurons in the heart of dogs possess presynaptic facilitatory β-receptors, which can also be activated by endogenous catecholamines.

Majewski et al. (1982) determined the noradrenaline spillover rate in anesthetized rabbits. Intravenously administered adrenaline increased the noradrenaline spillover, and this effect was prevented by propranolol. It is therefore likely that sympathetic axon terminals in the rabbit possess facilitatory β-receptors. Interestingly, the facilitatory effect of adrenaline was abolished by the noradrenaline uptake inhibitor desipramine: The authors suggest that the injected adrenaline was taken up into the axon terminals of the noradrenaline transporter, then released, and then activated presynaptic facilitatory β-receptors.

6 Function of Presynaptic Adrenoceptors in Man

6.1 Presynaptic α-Adrenoceptors

6.1.1 Presynaptic α-Adrenoceptors: In Vitro Studies

The function of presynaptic α-adrenoceptors in human tissues has been often studied in vitro – Table 5 shows the important and representative findings.

In most sympathetically innervated tissues, activation of α_2-receptors by agonists like clonidine, UK-14304 (later named brimonidine) or oxymetazoline, suppressed noradrenaline release. This phenomenon was observed in the right atrium and in papillary muscles of the heart (Matkó et al. 1994; Rump et al. 1995a; Münch et al. 1996). Release inhibiting α_2-receptors were also detected in digital arteries, renal arteries, gastric arteries, and ileocolic arteries (e.g., Moulds and Stevens 1983; Guimarães et al. 1998). The saphenous vein and the corpus cavernosum are also endowed with α_2-receptors, the activation of which led to a decrease in noradrenaline release in these tissues (Molderings et al. 1989; Molderings and Göthert 1995). Activation of α_2-receptors of noradrenergic sympathetic axon terminals in the iris-ciliary body, dental pulp, and kidney suppressed the transmitter release as well.

Cholinergic axon terminals in the stomach and *Taenia coli* also possess α_2-receptors, and their activation lowers acetylcholine release (Del Tacca et al. 1970; Leclere and Lefebvre 2002).

Several research groups have shown that noradrenaline release in the human (neo)cortex can be lowered by activating presynaptic α_2-receptors (Feuerstein et al.

Table 5 Observations on the function of presynaptic α- and β-adrenoceptors in humans

Species	Organ/tissue	Effect of activation/blockade of α-receptors	Receptor involved	Authors
In vitro				
Man	Right atrial appendage	Isoprenaline enhances el. stim. evoked [^{3}H]-NA release The effect is antagonized by ICI-118.551 but not by atenolol	β_2	Rump et al. (1994)
Man	Right atrial appendage	UK-14304 inhibits el. stim. evoked [^{3}H]-NA release (autoinhibition-free condition) Series of α-ligands shift the conc.-response curve of UK-14304 to the right	α_{2C}	Rump et al. (1995a)
Man	Right atrial appendage	UK-14304 inhibits and yohimbine enhances el. stim. evoked NA release Presynaptic α_2-receptors may be physiologically activated Terbutaline increases el. stim. evoked NA overflow Pindolol lowers el. stim. evoked NA overflow Presynaptic β_2-receptors may be physiologically activated.	α_2 β_2	Münch et al. (1996)
Man	Right atrial appendage	Oxymetazoline inhibits and idazoxan enhances el. stim. evoked [^{3}H]-NA release Fenoterol enhances el. stim. evoked [^{3}H]-NA release: ICI-118.551 antagonizes the effect	α_2 β_2	Abadie et al. (1996)
Man	Pulmonary artery strips	Adrenaline, isoprenaline, and procaterol facilitate el. stim. evoked [^{3}H]-NA release ICI-118.551 and propranolol (but not atenolol) antagonize the effect of isoprenaline	β_2	Göthert and Hentrich (1985)
Man	Papillary muscle	Xylazine lowers el. stim. evoked [^{3}H]-NA release CH-38083 (α_2-antagonist) enhances el. stim. evoked [^{3}H]-NA release Presynaptic autoinhibition functions	α_2	Matkó et al. (1994)
Man	Digital arteries, metatarsal veins	Isoprenaline and salbutamol facilitate el. stim. evoked [^{3}H]-NA release: Propranolol antagonizes the effects The β_2-receptors are not tonically activated (by endogenous adrenaline released from axon terminals)	β_2	Moulds and Stevens (1983)
Man	Gastric and ileocolic arteries	α_2-antagonists facilitate el. stim. evoked [^{3}H]-NA release The potencies (EC_{30} values) of eight antagonists at facilitating [^{3}H]-NA release determined	α_{2A}	Guimarães et al. (1998)

(continued)

Table 5 (continued)

Species	Organ/tissue	Effect of activation/blockade of α-receptors	Receptor involved	Authors
Man	Corpus cavernosum strips	Rauwolscine enhances el. stim. evoked [^{3}H]-NA release	α_2	de Tejada et al. (1989)
Man	Corpus cavernosum	B-HT 920 (α_2-agonist) inhibits el. stim. evoked [^{3}H]-NA release Rauwolscine facilitates [^{3}H]-NA release	α_2	Molderings et al. (1989)
Man	Saphenous vein strips	El. stim. evoked [^{3}H]-NA release measured Rauwolscine potently antagonizes the inhibitory effect of oxymetazoline The potencies of a series of antagonists at facilitating [^{3}H]-NA release determined	α_{2A}	Molderings and Göthert (1995)
Man	Saphenous vein strips	Adrenaline, isoprenaline, and procaterol facilitate el. stim. evoked [^{3}H]-NA release Propranolol and ICI-118.551 antagonize the effect of isoprenaline Adrenaline released from axon terminals does not facilitate [^{3}H]-NA release	β_2	Molderings et al. (1988)
Man	Renal artery strips	UK-14303 inhibits el. stim. evoked [^{3}H]-NA release: The effect is antagonized by rauwolscine Rauwolscine alone enhances el. stim. evoked [^{3}H]-NA release: Feedback inhibition operates	α_2	Rump et al. (1991)
Man	Kidney cortical slices	UK-14304 inhibits el. stim. evoked [^{3}H]-NA release (autoinhibition-free condition) Series of α-ligands shift the conc.-response curve of UK-14304 to the right	α_{2A}	Trendelenburg et al. (1997b)
Man	Kidney cortical slices	Isoprenaline enhances el. stim. evoked [^{3}H]-NA release The effect is antagonized by ICI-118.551 but not by atenolol	β_2	Rump et al. (1995b)
Man	Urinary bladder detrusor muscle	Mirabegron and isoprenaline inhibit el. stim. evoked [^{3}H]-ACh release **Indirect effect:** β_3-receptors are postsynaptically localized in smooth muscle cells; adenosine is produced in smooth muscle cells; as retrograde messenger adenosine activates inhibitory A_1-adenosine receptors in cholinergic axon terminals	β_3	Silva et al. (2017, 2020)

Man	Dental pulp	NA and UK-14304 (but not clonidine) inhibit el. stim. evoked [^{3}H]-NA release Rauwolscine enhances [^{3}H]-NA release Presynaptic autoinhibition operates	α_2	Parker et al. (1994)
Man	Dental pulp	Isoprenaline and salbutamol enhance el. stim. evoked [^{3}H]-NA release The effect is prevented by ICI-188.551 Autofacilitation is not operating	β_2	Parker et al. (1998)
Man	Stomach (proximal) muscle strips	UK-14304 inhibits the el. stim. evoked [^{3}H]-ACh release Rauwolscine antagonizes the effect	α_2	Leclere and Lefebvre (2002)
Man	Taenia coli	Noradrenaline inhibits the el. stim. evoked ACh release	α	Del Tacca et al. (1970)
Man	Iris-ciliary body	Clonidine inhibits el. stim. evoked [^{3}H]-NA release: Yominbine antagonizes the effect Yominbine enhances evoked [^{3}H]-NA release: Presynaptic autoinhibition operates	α_2	Jumblatt et al. (1993)
Man	Neocortical slices	UK 14304 inhibits the el. stim. evoked [^{3}H]-NA release Rauwolscine enhances the el. stim. evoked [^{3}H]-NA release: Autoinhibition operates β_1- and β_2-antagonists are ineffective	α_2	Feuerstein et al. (1990)
Man	Neocortical slices	NA inhibits el. stim. evoked [^{3}H]-NA release Under autoinhibition conditions: 9 antagonists enhance el. stim. evoked [^{3}H]-NA release	α_{2A}	Feuerstein et al. (2000)
Man	Cortical slices	Clonidine and oxymetazoline inhibit el. stim. evoked [^{3}H]-NA release: Yohimbine antagonizes the effect of clonidine Presynaptic autoinhibition operates The pattern of antagonism of the effect of clonidine by prazosin, AR-C 239, mianserin, and ORG 20350 suggests the involvement of $\alpha_{2A/D}$-receptors	$\alpha_{2A/D}$	Raiteri et al. (1992)
Man	Neocortical slices	(+)-Oxaprotiline (NAT inhibitor) lowers el. stim. evoked [^{3}H]-5-HT release Phentolamine, rauwolscine, and idazoxan enhance el. stim. evoked [^{3}H]-5-HT release NA released from neighboring axon terminals activates α_2-heteroceptors on serotoninergic axon terminals	α_2	Feuerstein et al. (1993)

(continued)

Table 5 (continued)

Species	Organ/tissue	Effect of activation/blockade of α-receptors	Receptor involved	Authors
Man	Neocortical slices	NA inhibits the el. stim. evoked [^{3}H]-ACh release Idazoxan antagonizes the effect	α_2	Beani et al. (1992)
In vivo				
Man	NA kinetics in the forearm determined	Intraarterial infusion of adrenaline enhances the NA spillover in the forearm Intraarterial infusion of phentolamine: inconclusive observations	β	Chang et al. (1994)
Man	Control vs. heart failure	Clonidine (infused into the brachial artery) lowered forearm NA spillover in controls No clonidine effect in patients with heart failure	α_2	Aggarwal et al. (2001)
Man	Heart failure	Patients with genetically defect α_{2C}-receptors develop more serious heart failure (NYHA class ↑; LV ejection fraction ↓; dp/dtmax ↓)	α_{2C}	Brede et al. (2002)
Man	Plasma melatonin determined	Clonidine decreases the plasma melatonin concentration Clonidine probably activates presynaptic α_2-receptors on sympathetic axon terminals.	α_2	Lewy et al. (1986)
Man	Plasma melatonin determined	The α_2-antagonist Org 3770 increases the plasma melatonin concentration Org 3770 probably blocks presynaptic α_2-receptors on sympathetic axon terminals	α_2	Palazidou et al. (1989)
Man	Melatonin determined in urine	Clonidine decreases the urine melatonin concentration in depressed patients	α_2	Paparrigopoulos et al. (2001)

Abbreviations: ACh, acetylcholine; el. stim., electrical stimulation; ICI-118.551, β_2-selective antagonist; NA, noradrenaline; NAT, noradrenaline transporter; UK-14304, α_2-agonist named today brimonidine; 5-HT, 5-hydroxytryptamine, serotonin

1990, 2000; Raiteri et al. 1992). Similarly, serotonin and acetylcholine release from the respective axon terminals can be controlled by inhibitory α_2-receptors.

The subtype of the α_2-receptors involved in presynaptic inhibition in humans was determined only in few experiments). With one exception (α_{2C}-receptor; Rump et al. 1995a), the receptor was identified as an α_{2A}-receptor (e.g., Molderings and Göthert 1995; Guimarães et al. 1998).

In most experiments on most tissues, α_2-antagonists, given alone, enhanced noradrenaline release in the tissues. These observations indicate that released noradrenaline elicits an α_2-autoreceptor-mediated feedback inhibition of noradrenaline release.

6.1.2 Presynaptic α-Adrenoceptors: In Vivo Studies

There are only a few publications on the function of presynaptic α_2-receptors in living humans (Table 5 shows the important findings).

Aggarwal et al. (2001) employed radiotracer techniques for studying noradrenaline kinetics: Clonidine infused into the brachial artery lowered the forearm spillover of noradrenaline in healthy individuals – an effect very likely mediated by α_2-receptors on sympathetic axon terminals. Clonidine had no effect in patients with heart failure. The sympathetic system is strongly activated in patients with chronic heart failure; accordingly the plasma noradrenaline concentration is strongly elevated. It is conceivable that clonidine did not inhibit noradrenaline release in patients with heart failure, because the presynaptic α_2-receptors were already maximally activated by endogenous noradrenaline.

Schäfers et al. (1999) studied the cardiovascular effects of α-methyl-noradrenaline in healthy conscious humans. α-Methyl-noradrenaline is the active metabolite of α-methyl-dopa, which is mostly used to treat hypertension during pregnancy. α-Methyl-noradrenaline is an agonist at α_2- and β-adrenoceptors, and it does not cross the blood–brain barrier. Intravenous infusion of α-methyl-noradrenaline elicited a series of β-adrenoceptor-mediated effects: increases in heart rate, systolic blood pressure, cardiac output, blood glucose, and free fatty acids. Diastolic blood pressure and total peripheral resistance were lowered. Importantly, the plasma noradrenaline concentration was also decreased, and this effect was prevented by the α_2-antagonist yohimbine. The parsimonious interpretation of this latter observation is that α-methyl-noradrenaline lowered the plasma noradrenaline concentration by activating α_2-receptors on axon terminals of postganglionic sympathetic neurons.

The study of Brede et al. (2002) points to the role played by certain α_2-receptor subtypes during progression of heart failure. In α_{2A}- or α_{2C}-receptor knockout mice, the progression of heart failure was enhanced, probably because noradrenaline release from sympathetic axons was not sufficiently inhibited via presynaptic α_{2A}- or α_{2C}-autoreceptors. There is an obvious parallelism with a condition in humans: In patients carrying a deletion variant of the α_{2C}-receptor, chronic heart failure is more serious, including worse clinical status and reduced left ventricular function (Brede et al. 2002).

Melatonin secretion from the pineal gland is under sympathetic control. Intravenously administered clonidine lowers human plasma melatonin levels, probably by

activating α_2-receptors on noradrenergic axon terminals in the pineal gland. In contrast, oral administration of an α_2-antagonist increased the plasma melatonin concentration, suggesting α_2-autoreceptor-mediated feedback inhibition of noradrenaline release functions in the pineal gland (Palazidou et al. 1989).

6.2 Presynaptic β-Adrenoceptors

6.2.1 Presynaptic β-Adrenoceptors: In Vitro Studies

There are a limited number of studies on the function of presynaptic β-adrenoceptors in human tissues in vitro – Table 5 shows these studies.

The non-selective β-agonist isoprenaline and the β_2-selective agonists fenoterol, salbutamol, or terbutaline were used in these studies to activate β-adrenoceptors. To verify involvement of β_2-adrenoceptors, the β_2-selective antagonist ICI-118.551 was used in several studies.

In several sympathetically innervated tissues, activation of β_2-receptors facilitated noradrenaline release from sympathetic axon terminals: right atrium (Rump et al. 1994; Abadie et al. 1996), pulmonary artery (Göthert and Hentrich 1985), digital arteries (Moulds and Stevens 1983), metatarsal veins, saphenous vein (Molderings et al. 1988), kidney (Rump et al. 1995b), and dental pulp.

In the study of Münch et al. (1996), the β_2-agonist terbutaline increased, whereas the non-selective β-receptor antagonist pindolol lowered electrical stimulation-evoked NA overflow in the right atrium. Based on the inhibitory effect of the antagonist pindolol, it was suggested that presynaptic β_2-receptors are autoactivated by endogenous noradrenaline released from sympathetic axon terminals. Such retrograde synaptic signaling via β_2-receptors was not seen in other studies.

In the detrusor muscle of the urinary bladder of humans, activation of β_3-receptors leads to presynaptic inhibition of acetylcholine release from postganglionic parasympathetic axons with the following mechanism (Silva et al. 2017, 2020): β_3-receptors are postsynaptically localized in the detrusor muscle, and their activation triggers the production of adenosine. Adenosine, as a retrograde messenger, activates presynaptic inhibitory A_1-adenosine receptors in cholinergic axon terminals. The inhibition of acetylcholine release with this mechanism lowers the tonus of the detrusor muscle, and this is an important component of the effects of the β_3-selective agonist mirabegron, when it is clinically used to treat the overactive bladder syndrome.

6.2.2 Presynaptic β-Adrenoceptors: In Vivo Studies

The function of presynaptic β-receptors in living humans was studied by Chang et al. (1994). In complex kinetic experiments, the noradrenaline spillover in the forearm was determined. Infusion of a low dose of adrenaline into the brachial artery markedly increased the forearm noradrenaline spillover. This observation is compatible with the presence of facilitatory presynaptic β-receptors, probably β_2-receptors, on sympathetic axon terminals in the forearm vasculature.

7 Molecular Mechanisms Involved in Presynaptic Modulation

The molecular mechanisms involved in presynaptic modulation of transmitter release by adrenoceptors have not been extensively studied. Therefore, results obtained in experiments on the somatodentritic regions of neurons will be often discussed below. Results of experiments with other $G\alpha_{i/o}$- and $G\alpha_s$-coupled receptors will also be described in the following paragraphs.

Presynaptic signaling by G-protein-coupled receptors was comprehensively reviewed by Brown and Sihra (2008). Three mechanisms are frequently quoted for modulation of transmitter release from axon terminals (see Fig. 3): (1) Changes of transmembrane K^+ currents may affect the depolarization of the axon terminal. (2) Modulation of voltage-gated Ca^{2+} channels affects the Ca^{2+}-elicited exocytotic release of transmitters. (3) The third possibility for presynaptic modulation is direct interference with the synaptic vesicle exocytotic release machinery.

7.1 Presynaptic α_2-Adrenoceptors

Mechanisms of presynaptic inhibition of transmitter release after activation of α_2-receptors are illustrated in Fig. 3.

If present on axon terminals, activation of α_2-receptors leads to suppression of neurotransmitter release, and inhibitory $G\alpha_{o/i}$-proteins are nearly always involved in this suppression. Pertussis toxin, which inactivates $G\alpha_{o/i}$-proteins, prevents the presynaptic inhibition via α_2-receptors in most experiments. For example, pertussis toxin attenuates the noradrenergic inhibition of substance P release from cultured chick dorsal root ganglia sensory neurons (Holz et al. 1989).

7.1.1 Inhibition of Voltage-Gated Ca^{2+} Channels

Inhibition of voltage-gated Ca^{2+} channels is the most important mechanism by which $G\alpha_{o/i}$-coupled receptors suppress transmitter release. Transmitter release from axon terminals of neurons in the central nervous system is frequently mediated by P/Q-type voltage-gated Ca^{2+} channels ($Ca_v2.1$) (Catterall and Few 2008). On the other hand, transmitter release from axon terminals of peripheral autonomic neurons is mostly mediated by N-type voltage-gated Ca^{2+} channels ($Ca_v2.2$). For example, noradrenaline release from the rat tail artery and the human heart atrium is triggered by activation of N-type Ca^{2+} channels ($Ca_v2.2$) (Clasbrummel et al. 1989; Molderings et al. 2000).

Presynaptic calcium channels are kept in close vicinity to the vesicle release mechanism by an interaction between the "synprint" site of the α_1-subunit of the calcium channels and the proteins involved in vesicle release (syntaxin-1, SNAP25 and synaptotagmin-1) (Spafford and Zamponi 2003; Catterall and Few 2008; Gandini and Zamponi 2022). Activation of $G\alpha_{o/i}$-coupled receptors leads to a pertussis toxin-sensitive, fast (latency <100 ms), and voltage-dependent inhibition of voltage-gated Ca^{2+} channels in the axon terminals (see Fig. 3). After dissociation

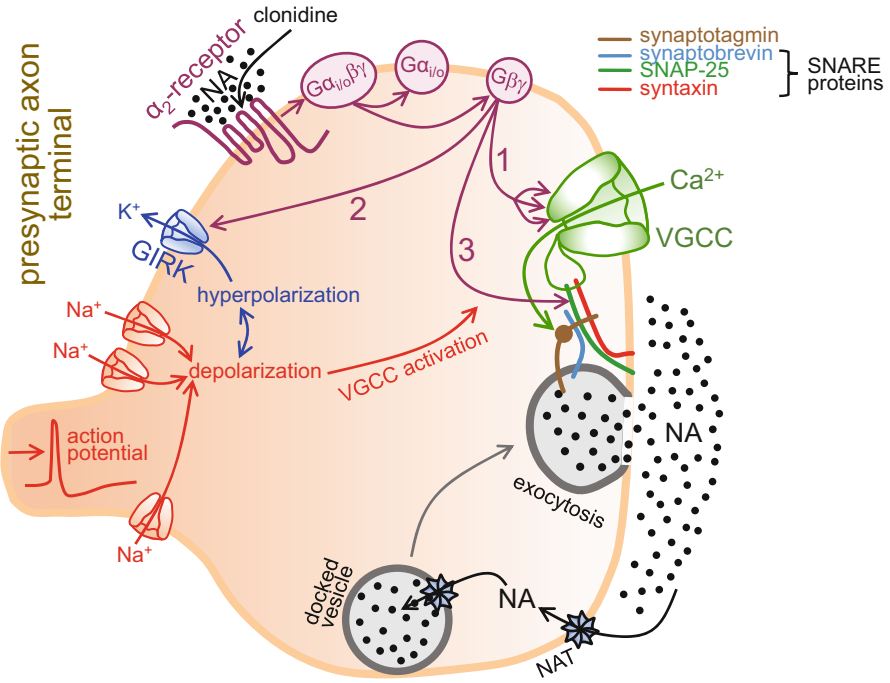

Fig. 3 Mechanism of presynaptic inhibition by α_2-receptors. An action potential reaches the axon terminal and activates there voltage-gated sodium channels. The ensuing depolarization activates voltage-gated calcium channels (VGCCs) of P/Q- or N-type. Ca^{2+} flows into the axoplasma, interacts with the Ca^{2+} sensor synaptotagmin, synaptotagmin activates the SNARE complex, and the transmitter noradrenaline (NA) is released via fusion of the synaptic vesicle with the membrane of the axon terminal (exocytotic release). Three probable mechanisms of inhibition of NA release after activation of α_2-receptors are shown (1,2, and 3). Endogenous noradrenaline or an exogenous drug like clonidine activates presynaptic α_2-receptors in the axon terminal, leading to activation of the heterotrimeric $G\alpha_{i/o}\beta\gamma$ complex. The $G\alpha_{i/o}$ and $G\beta\gamma$ components are released from each other. (1) The $G\beta\gamma$ dimer diffuses to the VGCC, interacts with it at three sites, resulting in inhibition of the VGCC. (2) The $G\beta\gamma$ dimer can also diffuse to K^+-channels (for example to G protein-activated inwardly rectifying K^+-channels; GIRK-channels) and activate them. An outward K^+-current develops, leading to hyperpolarization of the axon terminal. This means less activation of VGCCs and less transmitter release. (3) The $G\beta\gamma$ dimer can also diffuse to the SNARE complex and bind to SNAP-25. Interaction with this essential protein of the SNARE complex will lead to a "post-Ca^{2+} entry" kind of inhibition of the vesicle exocytosis

of the $\alpha_{o/i}\beta\gamma$ trimer, the $G\beta\gamma$-subunits associate with three regions of the α_1-subunit of Ca^{2+} channels (N terminus, linker between I and II domains, and the C terminus), eliciting an inhibition of the channels (Catterall and Few 2008). A strong depolarizing pre-pulse diminishes the inhibition of Ca^{2+} channels by $G\alpha_{o/i}$ receptors: This voltage dependency develops, because the depolarization leads to the dissociation of the $G\beta\gamma$-subunits from the Ca^{2+} channel.

In early experiments, Schofield (1990) showed that noradrenaline and clonidine inhibited, via activation of α_2-receptors, somatodendritic voltage-gated Ca^{2+}

channels in cultured rat superior cervical ganglion (SCG) neurons. It is likely that such inhibition also occurs in the axon terminals of these neurons and is the basis of the inhibition of noradrenaline release after activation of α_2-receptors. In similar experiments on SCG neurons, Ikeda (1996) showed that overexpression of $G\beta_1\gamma_2$, $G\beta_1\gamma_3$ and $G\beta_1\gamma_7$ dimers inhibits N-type voltage-gated Ca^{2+} channels and occludes their inhibition by noradrenaline. Nearly identical observations were made by Herlitze et al. (1996).

Delmas et al. (1999) also studied the role of G-proteins in the inhibition of voltage-gated Ca^{2+} channels by noradrenaline in cultured rat SCG neurons. The inhibition by noradrenaline was sensitive to pertussis toxin and was voltage-dependent. The inhibition by noradrenaline was attenuated by intracellular application of antibodies against several $G\alpha_o$- and $G\alpha_i$-protein subtypes and by antisense depletion of these G-protein subtypes. The noradrenergic inhibition was also attenuated by sequestering $G\beta\gamma$-subunits within the neurons. Again, it is likely that the inhibitory mechanism observed in the soma of the SCG neurons is identical with the mechanism operating within the axon terminals of the same neurons.

Electrically evoked [^{3}H]-noradrenaline release from cultured chick sympathetic neurons was inhibited by the α_2-agonist UK-14304 (today called brimonidine) via activation of $\alpha_{2A/D}$-receptors. UK-14304 simultaneously inhibited voltage-gated Ca^{2+} channels in the somatodendritic regions of these neurons (Trendelenburg et al. 2001).

With elegant experiments, Stephens and Mochida (2005) clarified the role of $G\beta\gamma$-subunits and N-type calcium ($Ca_v2.2$) channels in presynaptic inhibition elicited by activation of α_2-receptors. Between cultured rat sympathetic SCG neurons, fast synaptic transmission mediated by nicotinic acetylcholine receptors develops after several weeks in culture. This synaptic transmission was inhibited by noradrenaline and clonidine, and the inhibition was prevented by the α_2-antagonist yohimbine. It was also prevented by pertussis toxin, indicating involvement of $G\alpha_{o/i}$-proteins. Injection of purified $G\beta\gamma$-subunits into the presynaptic neuron inhibited the synaptic transmission and occluded the inhibition elicited by noradrenaline. Noradrenaline and intracellularly injected $G\beta\gamma$-subunits inhibited $Ca_v2.2$ (N-type) channel-mediated currents in SCG neurons, and this inhibition was attenuated by application of a strong depolarizing pre-pulse before activation of the Ca^{2+} currents. Moreover, action potential-independent transmitter release was not affected by noradrenaline, ruling out a direct inhibition of the vesicular release mechanism by noradrenaline.

7.1.2 Inhibition of the Vesicle Release Machinery

Schwartz (1997) observed that the electrically evoked [^{3}H]-noradrenaline release from cultured rat SCG neurons was inhibited by the α_2-agonists UK-14304 and oxymetazoline. However, these α_2-agonists did not affect the electrically evoked increase of the intracellular Ca^{2+} concentration. Later it was observed in several kinds of experiments that activation of $G\alpha_{o/i}$-coupled receptors can affect neurotransmitter release independently of changes in the intracellular Ca^{2+} concentration (see, e.g., Than and Szabo 2002; Szabo et al. 2004). It became obvious that in these

cases the transmitter release was inhibited at a site downward of Ca^{2+} entry into the axon terminal. These "post-Ca^{2+} entry" effects were most often observed after activation of presynaptic $GABA_B$-receptors and CB_1-cannabinoid receptors.

Blackmer et al. (2001) studied the effect of serotonin on synaptic transmission between reticulospinal axons and ventral horn neurons of the lamprey. Serotonin inhibited the neurotransmission. Microinjection of G-protein βγ subunits into the presynaptic axon terminal caused similar inhibition, and microinjection of a Gβγ subunit scavenger blocked the serotonin effect. The injection of the Gβγ subunits into the axon terminal did not affect, however, the influx of Ca^{2+} that triggered synaptic transmission. These results suggest that Gβγ subunits may affect transmitter release mechanisms directly. In biochemical experiments on PC12 cells, Blackmer et al. (2005) identified the type of interaction between Gβγ subunits and the synaptic vesicle release machinery. Gβγ subunits bind primarily to the C terminus of SNAP25 (Gerachshenko et al. 2005), which is a component of the vesicle release complex SNARE (see Fig. 3). Gβγ subunits compete with synaptotagmin for Ca^{2+}-dependent binding to the SNARE complex – this may lead to uncoupling of the vesicle fusion from the Ca^{2+} signal.

The SNARE complex (composed of SNAP25, synaptobrevin, and syntaxin) and the Ca^{2+} sensor synaptotagmin are the key actors in the vesicular release of neurotransmitters. The function of these key actors is modulated by a series of accessory proteins within the axon terminal. SNAP25 and several of the accessory proteins possess phosphorylation sites which can be targeted by protein kinase A (PKA) (Brown and Sihra 2008). $G\alpha_{o/i}$-coupled receptors inhibit adenylate cyclase and therefore indirectly inhibit protein kinase A. In an early study of Schoffelmeer et al. (1986) on rat brain slices, clonidine inhibited electrically evoked [^{3}H]-noradrenaline release, and part of this inhibition was due to a "post-Ca^{2+} entry" effect. Operation of adenylyl cyclase was necessary for this "post-Ca^{2+} entry" inhibition of the vesicle release machinery. Therefore, the possible chain of events was activation of α_2-receptors – $G\alpha_{o/i}$-mediated inhibition of adenylyl cyclase – decrease in cAMP level – less activation of protein kinase A – and less phosphorylation and activation of the vesicle release machinery.

7.1.3 Modulation of K^+ Channels in Axon Terminals

Activation of $G\alpha_{i/o}$-coupled receptors can lead to modulation of transmembrane K^+ currents (see Fig. 3). The α_{2A}- and α_{2C}-receptors can couple with G-protein-regulated inward rectifier K^+ channels (GIRK channels) and activate them (Bünemann et al. 2001). Activation of M_2 muscarinic acetylcholine receptors also leads to a $G\alpha_i$- and Gβγ-mediated activation of GIRK channels in superior cervical ganglion (SCG) neurons (Fernandez-Fernandez et al. 1999, 2001). Expectedly, an outward K^+ current would hyperpolarize the axon terminal and counteract the depolarization by an incoming action potential. Less activation of voltage-gated Ca^{2+} channels and less transmitter release would follow. However, strong evidence for the involvement of GIRK channels in the presynaptic inhibition by α_2-receptor agonists is lacking.

A role for K^+ channels in the presynaptic inhibition of neurotransmission elicited by activation of CB_1 cannabinoid receptors was repeatedly seen at cerebellar synapses. The CB_1-receptors are coupled to $G\alpha_{i/o}$-proteins, like the α_2-receptors. Daniel and Crepel (2001) observed that activation of CB_1-receptors inhibited glutamatergic synaptic transmission in the cerebellar cortex. Ca^{2+}-influx into the axon terminals was simultaneously inhibited. Both effects were absent, when an unidentified K^+ channel was blocked by the non-specific K^+ channel inhibitor 4-aminopyridine. The authors suggest that activation of CB_1-receptors primarily enhances outward K^+ currents, hyperpolarization occurs, and this leads to diminished activation of voltage-gated Ca^{2+} channels. Diana and Marty (2003) also observed an involvement of K^+ channels in presynaptic inhibition: An endogenous cannabinoid inhibited GABAergic neurotransmission presynaptically in the cerebellar cortex; one component of the presynaptic inhibition disappeared when K^+ channels in the presynaptic axon terminals were inactivated.

One may extrapolate the observations on the role of K^+ channels in presynaptic inhibition to presynaptic α_2-receptors. However, convincing direct evidence for the involvement of K^+-channels in the presynaptic inhibition by activated α_2-receptors is rare.

7.2 Presynaptic β-Adrenoceptors

Activation of β-adrenoceptors often increases neurotransmitter release from presynaptic axon terminals. $G\alpha_s$-proteins, adenylyl cyclase, and protein kinase A are frequently involved in this presynaptic enhancement.

Gereau and Conn (1994) observed that isoproterenol enhances glutamatergic neurotransmission in hippocampal brain slices with a presynaptic action. The enhancement was potentiated by a phosphodiesterase inhibitor and prevented by an inhibitor of protein kinase A.

Results of experiments on synaptosomes by Herrero and Sánchez-Prieto (1996) also point to the involvement adenylyl cyclase and cAMP-dependent protein kinase A in the enhancement of transmitter release after activation of β-receptors: Isoproterenol and forskolin enhanced intracellular cAMP and Ca^{2+} levels and Ca^{2+}-dependent glutamate release from the synaptosomes. Protein kinase A inhibitors diminished the enhancement of glutamate release. Wang (2002) and Wang et al. (2002) obtained very similar results in synaptosomes: The β-receptor agonist isoproterenol increased the intracellular Ca^{2+} concentration and the Ca^{2+}-evoked release of glutamate. A membrane-permeable cAMP analogue also increased the release of glutamate. The effects of isoproterenol on glutamate release were prevented by a protein kinase A inhibitor.

In the experiments of Saitow et al. (2005), short application (5 min) of the β-receptor agonist isoproterenol led to a long-lasting (>40 min) presynaptic potentiation (LTP) of GABAergic synaptic transmission between interneurons and Purkinje cells in the cerebellar cortex. Protein kinase A was involved in the LTP, and the Ca^{2+} sensitivity of the vesicle release machinery was enhanced.

Huang et al. (1996, 1998) studied excitatory synaptic transmission in the amygdala in slice preparations. Short application of isoproterenol elicited long-lasting presynaptic potentiation (LTP) of synaptic transmission, and the functions of adenylyl cyclase and protein kinase A were essential for the development of the LTP. The long-lasting enhancement of transmitter release was very likely the consequence of the long-lasting potentiation of Ca^{2+} currents via P/Q-type voltage-gated Ca^{2+} channels.

SNAP25 and its accessory proteins possess phosphorylation sites which can be targeted by protein kinase A (Nagy et al. 2004; Brown and Sihra 2008). However, it is not known whether phosphorylation of these proteins of the vesicle release machinery plays a role in the facilitation of transmitter release along the β-receptor-adenylyl cyclase-protein kinase A pathway.

As already mentioned above in Sect. 5.1, activation of β_3-receptors in the urinary bladder leads indirectly to inhibition of acetylcholine release from parasympathetic axon terminals (Silva et al. 2017, 2020). The β_3-receptors are localized in the detrusor smooth muscle cells. As expected from a $G\alpha_s$-protein-coupled receptor, its activation elicits cAMP production, adenosine monophosphate production, and adenosine production. Adenosine reaches the cholinergic axon terminals innervating the detrusor muscle as a retrograde messenger and activates there A_1-adenosine receptors, and this results in inhibition of acetylcholine release.

A_1-adenosine receptors are coupled with $G\alpha_{i/o}$-proteins and may lead to inhibition of acetylcholine release with mechanisms similar to those used by α_2-receptors to inhibit transmitter release (see Fig. 3): (1) modulation of voltage-gated Ca^{2+} channels in the axon terminals; (2) modulation of transmembrane K^+ currents; and (3) direct interference with the synaptic vesicle exocytotic release machinery.

8 Role of Presynaptic Adrenoceptors in the Effects of Therapeutically Used Drugs

8.1 α_2-Adrenoceptor Agonists

Clonidine was the first selective α_2-adrenoceptor agonist, and it played a decisive role in discriminating presynaptic α_2-adrenoceptors from postsynaptic α_1-adrenoceptors in peripheral organs with sympathetic innervation.

In 1962, Boehringer Ingelheim in Germany wanted to develop a new nasal decongestant resembling naphazoline chemically, which was already used for this indication. The chemist Helmut Stähle synthesized compound St155 (later named clonidine), and it had the expected nasal decongestant effect in animals. The effect of clonidine on α_2-receptors was discovered by serendipity, and it is amusing to read the personal recount by Helmut Stähle (2000): "... Dr Wolf, a physician and a member of the trial group, allowed his secretary, Mrs Schwandt, ... to administer herself a few drops of a 0.3% solution into her nostrils since she had a cold. There was, however, some surprise and embarrassment when the lady fell asleep for 24 h. She also developed a rather low blood pressure, a marked bradycardia and dryness of

the mouth. The dose amounted, as determined later, to the equivalent of approximately 20 tablets of Catapres." Catapres® is the proprietary name of clonidine. Further clinical studies verified that clonidine lowers blood pressure and heart rate and inhibits saliva secretion (Hoefke and Kobinger 1966). Clonidine has been long used to treat essential hypertension, but its use became rare because of its side effects. Today, clonidine has a role in treating hypertensive emergencies as well as in pain control and opioid withdrawal schemes.

8.1.1 Cardiovascular Applications of α_2-Adrenoceptor Agonists

Figure 1 gives an overview of the sites and mechanisms of action of α_2-agonists/clonidine-like drugs.

The antihypertensive effect of α-methyl-dopa was described by Oates (1960). It is still used to treat hypertension during pregnancy. α-Methyl-dopa is a prodrug; its antihypertensive effect is elicited by its active metabolite α-methyl-noradrenaline that activates α_2-receptors.

Clonidine and α-methyl-dopa are categorized in textbooks as "centrally acting antihypertensive drugs." It is thought that these compounds activate α_2-receptors in cardiovascular centers in the medulla oblongata, and this leads to a decrease in the activity of presympathetic neurons projecting from the rostral ventrolateral nucleus of the medulla oblongata (RVLM) to the intermediolateral column (IML) of the spinal cord (see Fig. 1). Notably, part of the inhibition of the presympathetic neurons in the RVLM is due to an α_2-receptor-mediated presynaptic inhibition of the glutamatergic input to the presympathetic neurons (Hayar and Guyenet 2000).

I believe that the expression "centrally acting antihypertensive drug" does not accurately describe the mechanism of action of clonidine-like drugs. These drugs lower the sympathetic tone of peripheral organs by simultaneously depressing the sympathetic outflow from the medulla oblongata but also the release of noradrenaline from postganglionic sympathetic neurons by activating presynaptic α_2-receptors on their axon terminals (Urban et al. 1995b; Szabo et al. 2001; for a review, see Szabo 2002) (see Fig. 1).

About 20 years after the discovery of clonidine, rilmenidine and moxonidine were introduced into antihypertensive therapy. Simultaneously, a new hypothesis arose: The new drugs and clonidine could elicit their antihypertensive effects by activating a novel receptor, the I_1 imidazoline receptor. The actual stand is that there are only imidazoline binding sites (Dardonville and Rozas 2004) but no receptors: "The Concise Guide to Pharmacology 2021/22, G-protein-coupled receptors" does not list "imidazoline receptors," only three non-GPCR binding sites for imidazolines are mentioned (I_1, I_2, and I_3) (Alexander et al. 2021). No corresponding genes emerged despite the sequencing of the human genome. For sure, rilmenidine and moxonidine are good α_2-receptor agonists: For example, they lower the firing rate of locus coeruleus neurons in rat brain slices by activating $\alpha_{2A/D}$-receptors in their somatodendritic regions (Szabo et al. 1996; Nörenberg et al. 1997). The many arguments against the imidazoline hypothesis outweigh the observations that support it. The sympathoinhibitory effects of clonidine-like drugs are best explained by activation of α_2-adrenoceptors (Urban et al. 1994, 1995a; for a review, see Szabo

2002). Clonidine is still on the market in several countries for chronically lowering blood pressure, but it has lost popularity for this indication, mostly because of its unwanted effects which are sedation and inhibition of saliva production. The chemically related α_2-agonist moxonidine is used as a second-line drug for lowering blood pressure.

8.1.2 Non-cardiovascular Applications of α_2-Adrenoceptor Agonists

Clonidine and two other α_2-adrenoceptor agonists, tizanidine and dexmedetomidine, are also used clinically to affect preferentially the central nervous system (CNS). We should recall the localization and the function of α_2-adrenoceptors as shown in Tables 1, 2, and 3. The most prominent noradrenergic nucleus in the CNS is the locus coeruleus located in the pons. Noradrenergic axons from the locus coeruleus project to most regions of the brain and the spinal cord. α_2-Receptors are synthesized in the somatodendritic regions of the neurons in the pons and transported to the axon terminals in the projection regions, for example the cortex. An α_2-agonist simultaneously elicits two effects: (1) It lowers the firing rate of locus coeruleus neurons by activating somatodendritic α_2-receptors (Szabo et al. 1996; Nörenberg et al. 1997), and (2) it lowers the release of noradrenaline from the axon terminals by activating presynaptic receptors on them. To make the system more complex, α_2-receptors are also localized on many serotoninergic, dopaminergic, cholinergic, GABAergic, and glutamatergic axon terminals: Activation of these receptors leads to presynaptic inhibition of neurotransmission by all these transmitters. Summarizing: α_2-Agonists cause ubiquitous somatodendritic and presynaptic neuronal inhibition in the CNS.

The only indication for tizanidine is the treatment of spasticity due to multiple sclerosis or spinal cord injury. Very likely, tizanidine acts on presynaptic α_2-receptors in the spinal cord and inhibits thereby the release of excitatory amino acids.

Dexmedetomidine and to a lesser degree clonidine are used for prolonged sedation of critically ill and mechanically ventilated patients (Giovannitti et al. 2015). They can also be used for procedural sedation and general anesthesia. The sedative effect is due to activation of somatodendritic α_2-receptors in the noradrenergic locus coeruleus neurons and activation of presynaptic α_2-receptors on the axon terminals of the same neurons. Dexmedetomidine and clonidine suppress sympathetic tone in peripheral tissues and lower systemic vascular resistance, blood pressure, heart rate, and cardiac output. Due to these effects, intra-operative fluctuations of cardiovascular parameters are damped by these α_2-agonists.

A recent meta-analysis suggests that intra-operative administration of dexmedetomidine elicits similar analgesia as intra-operative administration of the potent opioid remifentanil (Grape et al. 2019)! While eliciting analgesia, one possible site of action of an α_2-receptor agonist is the primary sensory neuron with its perikaryon located in the dorsal root ganglion: The α_2-agonist inhibits the release of glutamate and substance P (and of other neurotransmitters) from the axon terminals of this neuron (Holz et al. 1989).

Dexmedetomidine and clonidine are also used to mitigate delirium, including delirium elicited by alcohol withdrawal. It was recently demonstrated that clonidine combined with gabapentin can prevent alcohol withdrawal syndrome in hospitalized

trauma patients (McCullough et al. 2023). Clonidine and the more recently introduced α_2-receptor agonist lofeximide are established treatments of the opioid withdrawal syndrome (Albertson et al. 2014; Gorodetzky et al. 2017). Suppression of the overactive sympathetic nervous system during opioid withdrawal is an essential component of the effects of these drugs.

8.2 α_2-Adrenoceptor Antagonists

The tetracyclic antidepressant drugs mianserin and mirtazapine were developed by the pharmaceutical company AkzoNobel (Organon) and received patent protection in 1967 and 1976, respectively. The only difference between mirtazapine and mianserin is a nitrogen/carbon substitution in one of the four cycles. Pharmacologically and clinically the two drugs behave very similarly, and only mirtazapine will be discussed here. Due to its dual action (see below), mirtazapine is a strong antidepressant drug, in the rank order of efficacy of antidepressant drugs it occupies the second place after amitriptyline (Cipriani et al. 2018). In the rank order of the numbers of antidepressive drug prescriptions (defined daily doses [DDD] per year), mirtazapine occupies in Germany the fourth position.

At therapeutic plasma concentrations, mirtazapine blocks α_2-adrenoceptors. A PET study on healthy volunteers showed that administration of low therapeutic doses of mirtazapine already leads to an α_2-receptor occupancy in the brain in the range of 74–96% (Smith et al. 2007). With high potency mirtazapine also antagonizes H_1 histamine receptors and 5-HT_{2A} and 5-HT_3 serotonin receptors. Because it blocks H_1 histamine receptors, mirtazapine is also used, at low doses, to treat some sleep disorders (Sato et al. 2013).

The antidepressant action of mirtazapine is due to enhancement of monoaminergic synaptic transmission in several brain regions (De Boer et al. 1996; Bengtsson et al. 2000; Kaminska et al. 2014; for a review, see Invernizzi and Garattini 2004). Mirtazapine increases the noradrenaline concentration in the synaptic cleft primarily by antagonizing presynaptic α_2-autoreceptors on noradrenergic axon terminals (Yamauchi et al. 2012; Millan et al. 2000). Synaptic dopamine concentrations are also increased in some brain regions (Yamauchi et al. 2012; Millan et al. 2000). In these regions, dopamine is probably removed from the synaptic cleft not only by the dopamine transporter (DAT) but also by the noradrenaline transporter (NAT). It is the blockade of α_2-receptors in the noradrenergic axon terminals by mirtazapine which leads to enhanced synaptic dopamine concentrations (Devoto et al. 2004). Blockade of presynaptic α_2-heteroreceptors on serotoninergic axon terminals leads to enhanced synaptic serotonin concentrations in some brain regions (Yamauchi et al. 2012). An additional mechanism contributes to the enhancement of the synaptic concentrations of the monoamines: Mirtazapine blocks α_2-receptors also in the somatodendritic regions of the monoaminergic neurons and therefore increases their firing rate (Fukuyama et al. 2013).

8.3 Possible Developments of Drugs Interfering with Presynaptic α-Adrenoceptors

α_2-receptors are ubiquitously expressed in the human body (see Fig. 1). They are localized in the central nervous system on presynaptic axon terminals and in the somatodendritic regions of neurons. In most cases, they are also physiologically activated by endogenous catecholamines. In the periphery, α_2-receptors are mostly localized as presynaptic receptors on axon terminals of postganglionic sympathetic and parasympathetic neurons, but also on axon terminals of primary pain sensory neurons. The peripheral presynaptic α_2-receptors are also targets of endogenous catecholamines. In several tissues, α_2-receptors are also found in postsynaptic structures, for example in vascular smooth muscle cells.

Simple pharmacological logic indicates that ubiquitously expressed receptors are suboptimal drug targets. Agonists or antagonists of α_2-receptors will not only elicit the one desired therapeutic effect. Simultaneously they will elicit several or many unwanted effects. This explains that at present α_2-agonists are mostly used in small "therapeutic niches." The very effective and frequently prescribed antidepressive drug mirtazapine, an α_2-antagonist, has also remarkable unwanted effects.

The problems originating in the ubiquitousness of α_2-receptors could be solved either by restricting the drug action to certain α_2-receptor subtypes or by restricting the drug effect to a certain body compartment.

8.3.1 Possible Developments in the Field of α_2-Adrenoceptor Agonists

α_2-Agonists as Antihypertensive Drugs

α_2-Agonists like clonidine lost their position as first-line antihypertensive drugs, mostly because sedation and inhibition of saliva production were disturbing side effects. That the sedative effect is strong can be deduced from the discovery story ("sleeping secretary"; see above Sect. 8.1) and from the clinical practice, where α_2-agonists are used as sedatives.

First-line drugs for the treatment of arterial hypertension are angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers, calcium channel blockers, thiazide and loop diuretics, β-blockers, and mineralocorticoid receptor antagonists (Mancia et al. 2023). Clonidine, moxonidine, or rilmenidine are no longer recommended for the routine treatment of hypertension, but clonidine can be used in hypertensive emergencies (Mancia et al. 2023). In resistant hypertension, clonidine, as a fourth drug, is similarly effective as the mineralocorticoid receptor antagonist spironolactone (Krieger et al. 2018).

Peripheral presynaptic inhibition of noradrenaline release from sympathetic axon terminals by α_2-agonists is a very logical mechanism for the reduction of blood pressure. Both factors determining blood pressure (cardiac minute volume and total peripheral resistance) are lowered simultaneously. Compared with the first-line antihypertensive drugs, only the α_2-agonists have this dual mode of action! It is conceivable that a peripherally selective and $\alpha_{2A/C}$-receptor-selective α_2-agonist would be an effective and well-tolerable antihypertensive agent. Due to peripheral

selectivity, the most disturbing side effect, sedation, would be eliminated. The selectivity for the $\alpha_{2A/C}$-receptors would lead to preferential presynaptic inhibition; vasoconstriction via α_{2B}-receptors would be minimized.

Peripheral selectivity can be achieved by generating electrically charged drug molecules with low octanol/water distribution coefficient, which do not pass the blood–brain barrier (e,g., COPD drugs like tiotropium). However, electrically charged hydrophilic molecules are not absorbed from the gut; therefore, they are not suitable for long-term oral application.

A better option to generate peripherally selective α_2-agonists is to synthetize α_2-agonists which are substrates of transporters at the blood–brain barrier. The best-known drug transporter at the blood–brain barrier is P-glycoprotein (ABCB1 transporter), which is localized on the luminal side of blood vessel endothelial cells. It transports, for example, the μ-opioid receptor agonist loperamide at the blood–brain barrier back to the blood circulation. One technique to make drugs substrates of P-glycoprotein is to couple them chemically with polyethylene glycol. Coupling naloxone with polyethylene glycol yields naloxegol, and naloxegol is a substrate of P-glycoprotein and does not pass the blood–brain barrier. Summarizing: For cardiovascular applications α_2-receptor agonists should be synthesized, which are substrates of transporters at the blood–brain barrier, do not diffuse into the central nervous system, and therefore do not elicit central side effects like sedation.

α_2-Agonists for the Treatment of Chronic Heart Failure

According to the European Society of Cardiology, the following groups of drugs improve the prognosis of patients with chronic heart failure with reduced ejection fraction (McDonagh et al. 2021): angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers, valsartan combined with the neprilysin inhibitor sacubitril, β-blockers, mineralocorticoid receptor antagonists, and sodium-glucose cotransporter-2 inhibitors (SGLT2i).

Among several transmitter/hormonal systems the sympathetic nervous system is strongly activated during chronic heart failure. More specifically, the sympathetic axons in the heart release more noradrenaline in patients with heart failure than in control persons (Meredith et al. 1993). Patients with high rates of cardiac noradrenaline release have a worse prognosis than patients with low noradrenaline release rates (Kaye et al. 1995). When cardiac myocytes are chronically exposed to high noradrenaline concentrations, they are slowly damaged (Mann et al. 1992). Accordingly, it was suggested that the chronic deleterious effects of cardiac noradrenaline on myocardial function can be slowed by administration of β-blockers (Swedberg et al. 1979). As mentioned above, β-blockers are used today routinely to improve the prognosis of patients with heart failure.

When intense noradrenaline release from sympathetic axons of the heart causes harm to the heart, then it seems logical to suppress noradrenaline release as a therapeutic measure. Very logical drug targets to this aim are presynaptic α_2-receptors in cardiac sympathetic axon terminals. As shown abundantly in previous chapters, activation of $\alpha_{2A/C}$-receptors by α_2-agonists lowers noradrenaline release in the heart of animals and humans.

Clinical studies with the hypothesis that activation of α_2-receptors improves the prognosis of patients with chronic heart failure were planned. In initial experiments, it was shown that the α_2-agonist moxonidine dose dependently lowers the plasma noradrenaline concentration in patients with chronic heart failure (MOXSE trial; Swedberg et al. 2002). A randomized, double-blind, placebo-controlled trial including 4,533 patients with heart failure was planned and started (MOXCON trial; Cohn et al. 2003). However, after inclusion of 1,934 patients the study had to be prematurely terminated because of unexpected deaths (54 deaths in the moxonidine group vs. 32 deaths in the placebo group). This dramatic negative result was fully surprising, and there is no plausible explanation for the deaths. Understandably, the drug development program "moxonidine for heart failure" was stopped.

After this dramatic negative outcome of the MOXCON trial, it is probably wise not to pursue the development of α_2-agonists as treatments for chronic heart failure. Although the scientific rationale is very plausible, some limited preclinical studies could be performed with peripherally selective and $\alpha_{2A/C}$-receptor selective α_2-agonists in vitro and in vivo, also in mammals. α_{2A}- and α_{2C}-receptors play a protective role in heart failure (Brede et al. 2002); then it could be advantageous to selectively activate them in heart failure.

α_2-Agonists for Analgesia

The strong analgesic effects of α_2-adrenoceptors agonists (e.g., Grape et al. 2019) warrant further studies. Identification of the site of action of the α_2-agonists and of the subtypes of the involved α_2-receptors may lead to more effective analgesic drugs with more favorable side profiles.

8.3.2 Possible Developments in the Field of α_2-Adrenoceptor Antagonists

α_2-Antagonists as Antidepressive Drugs

As mentioned above, mirtazapine is the second most effective antidepressive drug. It primarily blocks α_2-receptors, thereby interrupting physiological α_2-receptor-mediated autoinhibition of noradrenaline release in the brain, and the noradrenaline concentration in the synaptic space increases (Yamauchi et al. 2012; Holm and Markham 1999; Millan et al. 2000). The most frequent side effects of mirtazapine are increased appetite, weight gain, sedation, and dry mouth. Mirtazapine increases body weight, body fat mass, and the blood concentration of leptin, but does not affect glucose homeostasis (Laimer et al. 2006). Unexpectedly, mirtazapine is practically devoid of cardiovascular side effects.

Mirtazapine has the following affinities for the human α_2-receptor subtypes (Proudman et al. 2022):

α_{2A}-receptor: K_i = 158 nM	α_{2B}-receptor: K_i = 810 nM	α_{2C}-receptor: K_i = 110 nM

Thus, mirtazapine has moderate affinity for the α_2-receptors, and it does not discriminate between the receptor subtypes.

A collateral observation: The antipsychotic drug risperidone and its active metabolite paliperidone possess slightly higher affinities for the α_2-receptor subtypes than mirtazapine (Proudman et al. 2022).

Mirtazapine has the following affinities for the human serotonin (5-HT) receptor subtypes (Millan et al. 2000):

5-HT_{1A}-recep.: K_i = 1.3 μM	5-HT_{1B}-recep.: $K_i > 6$ μM	5-HT_{1D}-recep.: K_i = 0.8 μM
5-HT_{2A}-recep.: K_i = 13 nM	5-HT_{2B}-recep.: K_i = 20 nM	5-HT_{2C}-recep.: K_i = 13 nM
5-HT_3-recep.: K_i = 20 nM		

Thus, mirtazapine has high affinity for the 5-HT_2- and 5-HT_3-receptor subtypes, but only low affinity for the 5-HT_1-receptor subtypes. Remarkably, the affinity of mirtazapine for 5-HT_2- and 5-HT_3-receptor subtypes is higher than its affinity for the α_2-receptor subtypes.

Mirtazapine has very high affinity for human H_1 histamine receptors: $K_i = 1.3$ nM (Appl et al. 2012). Affinities for the H_2-, H_3-, and H_4 histamine receptors are about three orders of magnitude lower. Remarkably, the affinity of mirtazapine for the H_1 histamine receptor is about 100-fold higher than its affinity for the α_2-receptor subtypes.

Sedation is a very frequent side effect (occurs in ≥10% of patients) of mirtazapine, and this can be explained by the high affinity of mirtazapine for the H_1 histamine receptor. Indeed, H_1 histamine receptor antagonists, for example diphenhydramine and doxylamine, are used in sleep disorders.

Increased appetite and substantial weight gain are also very frequent side effects of mirtazapine (occur in ≥10% of patients). Blockades of the H_1 histamine receptor and the 5-HT_{2C} serotonin receptor are robustly associated with weight gain (Rege 2008). As shown above, mirtazapine has high affinities for these two receptors.

How could be α_2-antagonists improved for the therapy of depression? Some ideas:

It is obvious that the blockade of H_1 histamine receptors and the 5-HT_{2C} serotonin receptors is the basis of the side effects weight gain and sedation. Novel selective α_2-antagonists should be synthesized, which have no affinities for these receptors, and generally, block only α_2-receptors.

Mirtazapine simultaneously blocks all three α_2-receptor subtypes. It would be interesting to synthesize and test a pure α_{2A}-selective antagonist. The presynaptic α_2-receptor in the monkey brain is of the α_{2A}-subtype (Trendelenburg et al. 1997a) – very probably also in the human brain.

Mirtazapine has no meaningful cardiovascular side effects. One would expect that it interferes in the central nervous system with the regulation of the cardiovascular system and, more importantly, with the α_2-receptor-mediated autoinhibition of noradrenaline release from sympathetic axons in the periphery. Intuitively, one would expect an increase of the sympathetic tone with vasoconstriction and increased cardiac output. Analysis of the effects of mirtazapine on the sympathetic system and on the cardiovascular system in humans could generate interesting knowledge.

References

Abadie C, Foucart S, Pagé P, Nadeau R (1996) Modulation of noradrenaline release from isolated human atrial appendages. J Auton Nerv Syst 61:269–276. https://doi.org/10.1016/s0165-1838(96)00093-8

Aggarwal A, Esler MD, Socratous F, Kaye DM (2001) Evidence for functional presynaptic alpha-2 adrenoceptors and their down-regulation in human heart failure. J Am Coll Cardiol 37:1246–1251. https://doi.org/10.1016/s0735-1097(01)01121-4

Akers WS, Cassis LA (2000) Presynaptic modulation of sympathetic neurotransmission in rat left ventricle slices: effect of pressure overload. J Neural Transm 107:885–902. https://doi.org/10.1007/s007020070040

Albertson TE, Chenoweth J, Ford J, Owen K, Sutter ME (2014) Is it prime time for alpha2-adrenocepter agonists in the treatment of withdrawal syndromes? J Med Toxicol 10:369–381. https://doi.org/10.1007/s13181-014-0430-3

Alexander SP, Christopoulos A, Davenport AP, Kelly E, Mathie A, Peters JA (2021) The concise guide to pharmacology 2021/22: G protein-coupled receptors. Br J Pharmacol 178(Suppl 1): S27–S156. https://doi.org/10.1111/bph.15538

Aoki C, Go CG, Venkatesan C, Kurose H (1994) Perikaryal and synaptic localization of α_{2A}-adrenergic receptor-like immunoreactivity. Brain Res 650:181–204. https://doi.org/10.1016/0006-8993(94)91782-5

Aoki C, Venkatesan C, Go CG, Forman R, Kurose H (1998) Cellular and subcellular sites for noradrenergic action in the monkey dorsolateral prefrontal cortex as revealed by the immunocytochemical localization of noradrenergic receptors and axons. Cereb Cortex 8:269–277. https://doi.org/10.1093/cercor/8.3.269

Apparsundaram S, Eikenburg DC (1995) Role of prejunctional beta adrenoceptors in rat cardiac sympathetic neurotransmission. J Pharmacol Exp Ther 272:519–526

Appl H, Holzammer T, Dove S, Haen E, Strasser A, Seifert R (2012) Interactions of recombinant human histamine H_1R, H_2R, H_3R, and H_4R receptors with 34 antidepressants and antipsychotics. Naunyn Schmiedebergs Arch Pharmacol 385:145–170. https://doi.org/10.1007/s00210-011-0704-0

Beani L, Bianchi C, Antonelli T, Caló G, Morari M, Ferioli V, Gaist G (1992) Comparison of [^{3}H]choline and D-[^{3}H]aspartate efflux from Guinea pig and human neocortex. J Neurochem 58:1454–1459. https://doi.org/10.1111/j.1471-4159.1992.tb11363.x

Bengtsson HJ, Kele J, Johansson J, Hjorth S (2000) Interaction of the antidepressant mirtazapine with alpha2-adrenoceptors modulating the release of 5-HT in different rat brain regions in vivo. Naunyn Schmiedebergs Arch Pharmacol 362:406–412. https://doi.org/10.1007/s002100000294

Blackmer T, Larsen EC, Takahashi M, Martin TF, Alford S, Hamm HE (2001) G protein βγ subunit-mediated presynaptic inhibition: regulation of exocytotic fusion downstream of Ca^{2+} entry. Science 292:293–297. https://doi.org/10.1126/science.1058803

Blackmer T, Larsen EC, Bartleson C, Kowalchyk JA, Yoon EJ, Preininger AM, Alford S, Hamm HE, Martin TF (2005) G protein βγ directly regulates SNARE protein fusion machinery for secretory granule exocytosis. Nat Neurosci 8:421–425. https://doi.org/10.1038/nn1423

Blakeley AG, Summers RJ (1978) The effects of piperoxan on uptake of noradrenaline and overflow of transmitter in the isolated blood perfused spleen of the cat. Br J Pharmacol 63:683–687. https://doi.org/10.1111/j.1476-5381.1978.tb17283.x

Blandizzi C, Tarkovacs G, Natale G, Del Tacca M, Vizi ES (1993) Functional evidence that [^{3}H]acetylcholine and [^{3}H]noradrenaline release from Guinea pig ileal myenteric plexus and noradrenergic terminals is modulated by different presynaptic alpha-2 adrenoceptor subtypes. J Pharmacol Exp Ther 267:1054–1060

Blandizzi C, Natale G, Colucci R, Carignani D, Lazzeri G, Del Tacca M (1995) Characterization of alpha α_2-adrenoceptor subtypes involved in the modulation of gastric acid secretion. Eur J Pharmacol 278:179–182. https://doi.org/10.1016/0014-2999(95)00170-p

Boehm S (1999) Presynaptic α_2-adrenoceptors control excitatory, but not inhibitory, transmission at rat hippocampal synapses. J Physiol 519:439–449. https://doi.org/10.1111/j.1469-7793.1999.0439m.x

Boehm S, Kubista H (2002) Fine tuning of sympathetic transmitter release via ionotropic and metabotropic presynaptic receptors. Pharmacol Rev 54:43–99. https://doi.org/10.1124/pr.54.1.43

Brede M, Wiesmann F, Jahns R, Hadamek K, Arnolt C, Neubauer S, Lohse MJ, Hein L (2002) Feedback inhibition of catecholamine release by two different α_2-adrenoceptor subtypes prevents progression of heart failure. Circulation 106:2491–2496. https://doi.org/10.1161/01.cir.0000036600.39600.66

Brown GL, Gillespie JS (1957) The output of sympathetic transmitter from the spleen of the cat. J Physiol 138:81–102. https://doi.org/10.1113/jphysiol.1957.sp005839

Brown DA, Sihra TS (2008) Presynaptic signaling by heterotrimeric G-proteins. Handb Exp Pharmacol 184:207–260. https://doi.org/10.1007/978-3-540-74805-2_8

Bünemann M, Bücheler MM, Philipp M, Lohse MJ, Hein L (2001) Activation and deactivation kinetics of α_{2A}- and α_{2C}-adrenergic receptor-activated G protein-activated inwardly rectifying K^+ channel currents. J Biol Chem 276:47512–47517. https://doi.org/10.1074/jbc.M108652200

Catterall WA, Few AP (2008) Calcium channel regulation and presynaptic plasticity. Neuron 59:882–901. https://doi.org/10.1016/j.neuron.2008.09.005

Chang PC, Kriek E, van Brummelen P (1994) Sympathetic activity and presynaptic adrenoceptor function in patients with longstanding essential hypertension. J Hypertens 12:179–190

Chen SR, Chen H, Yuan WX, Pan HL (2011) Increased presynaptic and postsynaptic α_2-adrenoceptor activity in the spinal dorsal horn in painful diabetic neuropathy. J Pharmacol Exp Ther 337:285–292. https://doi.org/10.1124/jpet.110.176586

Cipriani A, Furukawa TA, Salanti G, Chaimani A, Lauren Z Atkinson LZ et al. (2018) Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis. Lancet 391:1357–1366. https://doi.org/10.1016/S0140-6736(17)32802-7

Clasbrummel B, Osswald H, Illes P (1989) Inhibition of noradrenaline release by ω-conotoxin GVIA in the rat tail artery. Br J Pharmacol 96:101–110. https://doi.org/10.1111/j.1476-5381.1989.tb11789.x

Coelho A, Antunes-Lopes T, Gillespie J, Cruz F (2017) Beta-3 adrenergic receptor is expressed in acetylcholine-containing nerve fibers of the human urinary bladder: an immunohistochemical study. NeurourolUrodyn 36:1972–1980. https://doi.org/10.1002/nau.23224

Cohn JN, Pfeffer MA, Rouleau J, Sharpe N, Swedberg K, Straub M et al (2003) Adverse mortality effect of central sympathetic inhibition with sustained-release moxonidine in patients with heart failure (MOXCON). Eur J Heart Fail 5:659–667. https://doi.org/10.1016/s1388-9842(03)00163-6

Daniel H, Crepel F (2001) Control of Ca^{2+} influx by cannabinoid and metabotropic glutamate receptors in rat cerebellar cortex requires K^+ channels. J Physiol 537:793–800. https://doi.org/10.1111/j.1469-7793.2001.00793.x

Dardonville C, Rozas I (2004) Imidazoline binding sites and their ligands: an overview of the different chemical structures. Med Res Rev 24:639–661. https://doi.org/10.1002/med.20007

de Boer TH, Nefkens F, van Helvoirt A, van Delft AM (1996) Differences in modulation of noradrenergic and serotonergic transmission by the alpha-2 adrenoceptor antagonists, mirtazapine, mianserin and idazoxan. J Pharmacol Exp Ther 277:852–860

de Tejada SI, Kim N, Lagan I, Krane RJ, Goldstein I (1989) Regulation of adrenergic activity in penile corpus cavernosum. J Urol 142:1117–1121. https://doi.org/10.1016/s0022-5347(17)39009-2

Del Tacca M, Soldani G, Selli M, Crema A (1970) Action of catecholamines on release of acetylcholine from human taenia coli. Eur J Pharmacol 9:80–84. https://doi.org/10.1016/0014-2999(70)90323-7

Delmas P, Abogadie FC, Milligan G, Buckley NJ, Brown DA (1999) βγ dimers derived from G_o and G_i proteins contribute different components of adrenergic inhibition of Ca^{2+} channels in rat sympathetic neurones. J Physiol 518:23–36. https://doi.org/10.1111/j.1469-7793.1999.0023r.x

Dennis T, L'Heureux R, Carter C, Scatton B (1987) Presynaptic alpha-2 adrenoceptors play a major role in the effects of idazoxan on cortical noradrenaline release (as measured by in vivo dialysis) in the rat. J Pharmacol Exp Ther 241:642–649

Devoto P, Flore G, Pira L, Longu G, Gessa GL (2004) Mirtazapine-induced corelease of dopamine and noradrenaline from noradrenergic neurons in the medial prefrontal and occipital cortex. Eur J Pharmacol 487:105–111. https://doi.org/10.1016/j.ejphar.2004.01.018

Diana MA, Marty A (2003) Characterization of depolarization-induced suppression of inhibition using paired interneuron-Purkinje cell recordings. J Neurosci 23:5906–5918. https://doi.org/10.1523/JNEUROSCI.23-13-05906.2003

Egli RE, Kash TL, Choo K, Savchenko V, Matthews RT, Blakely RD, Winder DG (2005) Norepinephrine modulates glutamatergic transmission in the bed nucleus of the stria terminalis. Neuropsychopharmacology 30:657–668. https://doi.org/10.1038/sj.npp.1300639

Erdozain AM, Brocos-Mosquera I, Gabilondo AM, Meana JJ, Callado LF (2019) Differential α_{2A}- and α_{2C}-adrenoceptor protein expression in presynaptic and postsynaptic density fractions of postmortem human prefrontal cortex. J Psychopharmacol 33:244–249. https://doi.org/10.1177/0269881118798612

Fernandez-Fernandez JM, Wanaverbecq N, Halley P, Caulfield MP, Brown DA (1999) Selective activation of heterologously expressed G protein-gated K^+ channels by M_2 muscarinic receptors in rat sympathetic neurones. J Physiol 515:631–637. https://doi.org/10.1111/j.1469-7793.1999.631ab.x

Fernández-Fernández JM, Abogadie FC, Milligan G, Delmas P, Brown DA (2001) Multiple pertussis toxin-sensitive G-proteins can couple receptors to GIRK channels in rat sympathetic neurons when expressed heterologously, but only native G_i-proteins do so in situ. Eur J Neurosci 14:283–292. https://doi.org/10.1046/j.0953-816x.2001.01642.x

Feuerstein TJ, Dooley DJ, Seeger W (1990) Inhibition of norepinephrine and acetylcholine release from human neocortex by ω-conotoxin GVIA. J Pharmacol Exp Ther 252:778–785

Feuerstein TJ, Mutschler A, Lupp A, Van Velthoven V, Schlicker E, Göthert M (1993) Endogenous noradrenaline activates α_2-adrenoceptors on serotonergic nerve endings in human and rat neocortex. J Neurochem 61:474–480. https://doi.org/10.1111/j.1471-4159.1993.tb02148.x

Feuerstein TJ, Huber B, Vetter J, Aranda H, Van Velthoven V, Limberger N (2000) Characterization of the α_2-adrenoceptor subtype, which functions as α_2-autoreceptor in human neocortex. J Pharmacol Exp Ther 294:356–362

Flavahan NA, Cooke JP, Shepherd JT, Vanhoutte PM (1987) Human postjunctional alpha-1 and alpha-2 adrenoceptors: differential distribution in arteries of the limbs. J Pharmacol Exp Ther 241:361–365

Frazier EP, Mathy MJ, Peters SL, Michel MC (2005) Does cyclic AMP mediate rat urinary bladder relaxation by isoproterenol? J Pharmacol Exp Ther 313:260–267. https://doi.org/10.1124/jpet.104.077768

Fuder H, Braun HJ, Schimkus R (1986) Presynaptic alpha-2 adrenoceptor activation and coupling of the receptor-presynaptic effector system in the perfused rat heart: affinity and efficacy of phenethylamines and imidazoline derivatives. J Pharmacol Exp Ther 237:237–245

Fukuyama K, Tanahashi S, Hamaguchi T, Nakagawa M, Shiroyama T, Motomura E, Okada M (2013) Differential mechanisms underlie the regulation of serotonergic transmission in the dorsal and median raphe nuclei by mirtazapine: a dual probe microdialysis study. Psychopharmacology (Berl) 229:617–626. https://doi.org/10.1007/s00213-013-3122-9

Gandini MA, Zamponi GW (2022) Voltage-gated calcium channel nanodomains: molecular composition and function. FEBS J 289:614–633. https://doi.org/10.1111/febs.15759

Gerachshenko T, Blackmer T, Yoon EJ, Bartleson C, Hamm HE, Alford S (2005) Gβγ acts at the C terminus of SNAP-25 to mediate presynaptic inhibition. Nat Neurosci 8:597–605. https://doi.org/10.1038/nn1439

Gereau RW 4th, Conn PJ (1994) Presynaptic enhancement of excitatory synaptic transmission by β-adrenergic receptor activation. J Neurophysiol 72:1438–1442. https://doi.org/10.1152/jn.1994.72.3.1438

Gilsbach R, Hein L (2008) Presynaptic metabotropic receptors for acetylcholine and adrenaline/noradrenaline. Handb Exp Pharmacol 184:261–288. https://doi.org/10.1007/978-3-540-74805-2_9

Giovannitti JA Jr, Thoms SM, Crawford JJ (2015) Alpha-2 adrenergic receptor agonists: a review of current clinical applications. Anesth Prog 62:31–39. https://doi.org/10.2344/0003-3006-62.1.31

Gobert A, Billiras R, Cistarelli L, Millan MJ (2004) Quantification and pharmacological characterization of dialysate levels of noradrenaline in the striatum of freely-moving rats: release from adrenergic terminals and modulation by alpha2-autoreceptors. J Neurosci Methods 140:141–152. https://doi.org/10.1016/j.jneumeth.2004.04.040

Gorodetzky CW, Walsh SL, Martin PR, Saxon AJ, Gullo KL, Biswas K (2017) A phase III, randomized, multi-center, double blind, placebo controlled study of safety and efficacy of lofexidine for relief of symptoms in individuals undergoing inpatient opioid withdrawal. Drug Alcohol Depend 176:79–88. https://doi.org/10.1016/j.drugalcdep.2017.02.020

Göthert M, Hentrich F (1985) Identification of presynaptic β_2-adrenoceptors on the sympathetic nerve fibres of the human pulmonary artery. Br J Pharmacol 85:933–941. https://doi.org/10.1111/j.1476-5381.1985.tb11094.x

Grape S, Kirkham KR, Frauenknecht J, Albrecht E (2019) Intra-operative analgesia with remifentanil vs. dexmedetomidine: a systematic review and meta-analysis with trial sequential analysis. Anaesthesia 74:793–800. https://doi.org/10.1111/anae.14657

Grimm M, Kurz T, Schwarz M, Richardt D, Schäfer U, Katus HA, Richardt G (2001) Presynaptic regulation of cardiac norepinephrine release in ischemia. J Cardiovasc Pharmacol 38:58–68. https://doi.org/10.1097/00005344-200107000-00007

Guimarães S, Figueiredo IV, Caramona MM, Moura D, Paiva MQ (1998) Prejunctional α_{2A}-autoreceptors in the human gastric and ileocolic arteries. Naunyn Schmiedebergs Arch Pharmacol 358:207–211. https://doi.org/10.1007/pl00005244

Han SK, Chong W, Li LH, Lee IS, Murase K, Ryu PD (2002) Noradrenaline excites and inhibits GABAergic transmission in parvocellular neurons of rat hypothalamic paraventricular nucleus. J Neurophysiol 87:2287–2296. https://doi.org/10.1152/jn.2002.87.5.2287

Hardy TA, Brock JA (2001) Effects of modulating Ca^{2+} entry and activating prejunctional receptors on facilitation of excitatory junction potentials in the Guinea-pig vas deferens in vitro. Naunyn Schmiedebergs Arch Pharmacol 363:515–525. https://doi.org/10.1007/s002100000394

Hayar A, Guyenet PG (2000) Prototypical imidazoline-1 receptor ligand moxonidine activates alpha2-adrenoceptors in bulbospinal neurons of the RVL. J Neurophysiol 83:766–776. https://doi.org/10.1152/jn.2000.83.2.766

Heider M, Schliebs R, Rossner S, Bigl V (1997) Basal forebrain cholinergic immunolesion by 192IgG-saporin: evidence for a presynaptic location of subpopulations of α_2- and β-adrenergic as well as 5-HT_{2A} receptors on cortical cholinergic terminals. Neurochem Res 22:957–966. https://doi.org/10.1023/a:1022418708293

Hein L, Kobilka BK (1995) Adrenergic receptor signal transduction and regulation. Neuropharmacology 34:357–366. https://doi.org/10.1016/0028-3908(95)00018-2

Hein L, Altman JD, Kobilka BK (1999a) Two functionally distinct α_2-adrenergic receptors regulate sympathetic neurotransmission. Nature 402(6758):181–184. https://doi.org/10.1038/46040

Hein L, Limbird LE, Eglen RM, Kobilka BK (1999b) Gene substitution/knockout to delineate the role of α_2-adrenoceptor subtypes in mediating central effects of catecholamines and imidazolines. Ann N Y Acad Sci 881:265–271. https://doi.org/10.1111/j.1749-6632.1999.tb09368.x

Herlitze S, Garcia DE, Mackie K, Hille B, Scheuer T, Catterall WA (1996) Modulation of Ca^{2+} channels by G-protein βγ subunits. Nature 380:258–262. https://doi.org/10.1038/380258a0

Herrero I, Sánchez-Prieto J (1996) cAMP-dependent facilitation of glutamate release by β-adrenergic receptors in cerebrocortical nerve terminals. J Biol Chem 271:30554–30560. https://doi.org/10.1074/jbc.271.48.30554

Hoch H, Stegbauer J, Potthoff SA, Hein L, Quack I, Rump LC, Vonend O (2011) Regulation of renal sympathetic neurotransmission by renal α_{2A}-adrenoceptors is impaired in chronic renal failure. Br J Pharmacol 163:438–446. https://doi.org/10.1111/j.1476-5381.2011.01223.x

Hoefke W, Kobinger W (1966) Pharmakologische Wirkungen des 2-(2,6-Dichlorphenylamino)-2-imidazolin-hydrochlorids, einer neuen, antihypertensiven Substanz [pharmacological effects of 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride, a new antihypertensive substance]. Arzneimittelforschung 16:1038–1050

Holm KJ, Markham A (1999) Mirtazapine: a review of its use in major depression. Drugs 57:607–631. https://doi.org/10.2165/00003495-199957040-00010

Holz GG 4th, Kream RM, Spiegel A, Dunlap K (1989) G proteins couple α-adrenergic and $GABA_b$ receptors to inhibition of peptide secretion from peripheral sensory neurons. J Neurosci 9:657–666. https://doi.org/10.1523/JNEUROSCI.09-02-00657.1989

Huang CC, Hsu KS, Gean PW (1996) Isoproterenol potentiates synaptic transmission primarily by enhancing presynaptic calcium influx via P- and/or Q-type calcium channels in the rat amygdala. J Neurosci 16:1026–1033. https://doi.org/10.1523/JNEUROSCI.16-03-01026.1996

Huang CC, Wang SJ, Gean PW (1998) Selective enhancement of P-type calcium currents by isoproterenol in the rat amygdala. J Neurosci 18(6):2276–2282. https://doi.org/10.1523/JNEUROSCI.18-06-02276.1998

Igawa Y, Aizawa N, Michel MC (2019) β3 -adrenoceptors in the normal and diseased urinary bladder-what are the open questions? Br J Pharmacol 176:2525–2538. https://doi.org/10.1111/bph.14658

Ihalainen JA, Tanila H (2002) In vivo regulation of dopamine and noradrenaline release by alpha2A-adrenoceptors in the mouse prefrontal cortex. Eur J Neurosci 15:1789–1794. https://doi.org/10.1046/j.1460-9568.2002.02014.x

Ikeda SR (1996) Voltage-dependent modulation of N-type calcium channels by G-protein βγ subunits. Nature 380:255–258. https://doi.org/10.1038/380255a0

Invernizzi RW, Garattini S (2004) Role of presynaptic $alpha_2$-adrenoceptors in antidepressant action: recent findings from microdialysis studies. Prog Neuropsychopharmacol Biol Psychiatry 28:819–827. https://doi.org/10.1016/j.pnpbp.2004.05.026

Ji XH, Cao XH, Zhang CL, Feng ZJ, Zhang XH, Ma L, Li BM (2008) Pre- and postsynaptic β-adrenergic activation enhances excitatory synaptic transmission in layer V/VI pyramidal neurons of the medial prefrontal cortex of rats. Cereb Cortex 18:1506–1520. https://doi.org/10.1093/cercor/bhm177

Johansson P, Ehrenström F (1988) Presynaptic α-adrenoceptor regulation of transmitter release in the conscious rat. Comp Biochem Physiol C Comp Pharmacol Toxicol 89:65–69. https://doi.org/10.1016/0742-8413(88)90146-6

Jumblatt JE, Ohia SE, Hackmiller RC (1993) Prejunctional modulation of norepinephrine release in the human iris-ciliary body. Invest Ophthalmol Vis Sci 34:2790–2793

Kaminska K, Gołembiowska K, Rogóz Z (2014) The effect of risperidone on the mirtazapine-induced changes in extracellular monoamines in the rat frontal cortex. Pharmacol Rep 66:984–990. https://doi.org/10.1016/j.pharep.2014.06.009

Kaye DM, Lefkovits J, Jennings GL, Bergin P, Broughton A, Esler MD (1995) Adverse consequences of high sympathetic nervous activity in the failing human heart. J Am Coll Cardiol 26:1257–1263. https://doi.org/10.1016/0735-1097(95)00332-0

Kazanietz MG, Enero MA (1989) Modulation of noradrenaline release by presynaptic alpha-2 and beta adrenoceptors in rat atria. Effect of pretreatment with clenbuterol. Naunyn Schmiedebergs Arch Pharmacol 340:274–278. https://doi.org/10.1007/BF00168510

Kobayashi M, Kojima M, Koyanagi Y, Adachi K, Imamura K, Koshikawa N (2009) Presynaptic and postsynaptic modulation of glutamatergic synaptic transmission by activation of α_1-and

β-adrenoceptors in layer V pyramidal neurons of rat cerebral cortex. Synapse 63:269–281. https://doi.org/10.1002/syn.20604

Koyanagi Y, Yamamoto K, Oi Y, Koshikawa N, Kobayashi M (2010) Presynaptic interneuron subtype- and age-dependent modulation of GABAergic synaptic transmission by β-adrenoceptors in rat insular cortex. J Neurophysiol 103:2876–2888. https://doi.org/10.1152/jn.00972.2009

Krieger EM, Drager LF, Giorgi DMA, Pereira AC, Barreto-Filho JAS (2018) Spironolactone versus clonidine as a fourth-drug therapy for resistant hypertension: the ReHOT randomized study (resistant hypertension optimal treatment). Hypertension 71:681–690. https://doi.org/10.1161/HYPERTENSIONAHA.117.10662

Kubista H, Boehm S (2006) Molecular mechanisms underlying the modulation of exocytotic noradrenaline release via presynaptic receptors. Pharmacol Ther 112:213–242. https://doi.org/10.1016/j.pharmthera.2006.04.005

Laimer M, Kramer-Reinstadler K, Rauchenzauner M, Lechner-Schoner T, Strauss R et al (2006) Effect of mirtazapine treatment on body composition and metabolism. J Clin Psychiatry 67:421–424. https://doi.org/10.4088/jcp.v67n0313

Langer SZ (1974) Presynaptic regulation of catecholamine release. Biochem Pharmacol 23:1793–1800. https://doi.org/10.1016/0006-2952(74)90187-7

Langer SZ (1997) 25 years since the discovery of presynaptic receptors: present knowledge and future perspectives. Trends Pharmacol Sci 18:95–99. https://doi.org/10.1016/s0165-6147(96)01034-6

Leclere PG, Lefebvre RA (2002) Presynaptic modulation of cholinergic neurotransmission in the human proximal stomach. Br J Pharmacol 135:135–142. https://doi.org/10.1038/sj.bjp.0704471

Lee JG, Choi IS, Park EJ, Cho JH, Lee MG, Choi BJ, Jang IS (2007) β_2-adrenoceptor-mediated facilitation of glutamatergic transmission in rat ventromedial hypothalamic neurons. Neuroscience 144:1255–1265. https://doi.org/10.1016/j.neuroscience.2006.10.049

Lewy AJ, Siever LJ, Uhde TW, Markey SP (1986) Clonidine reduces plasma melatonin levels. J Pharm Pharmacol 38:555–556. https://doi.org/10.1111/j.2042-7158.1986.tb04639.x

Limberger N, Trendelenburg AU, Starke K (1992) Pharmacological characterization of presynaptic α_2-autoreceptors in rat submaxillary gland and heart atrium. Br J Pharmacol 107:246–255. https://doi.org/10.1111/j.1476-5381.1992.tb14494.x

Majewski H, Hedler L, Starke K (1982) The noradrenaline rate in the anaesthetized rabbit: facilitation by adrenaline. Naunyn Schmiedebergs Arch Pharmacol 321:20–27. https://doi.org/10.1007/BF00586343

Majewski H, Hedler L, Starke K (1983a) Evidence for a physiological role of presynaptic α_2-adrenoceptors: modulation of noradrenaline release in the pithed rabbit. Naunyn Schmiedebergs Arch Pharmacol 324:256–263

Majewski H, Hedler L, Starke K (1983b) Modulation of noradrenaline release in the conscious rabbit through α-adrenoceptors. Eur J Pharmacol 193:255–264

Maki T, Kajioka S, Itsumi M, Kareman E, Lee K, Shiota M, Eto M (2019) Mirabegron induces relaxant effects via cAMP signaling-dependent and -independent pathways in detrusor smooth muscle. Low Urin Tract Symptoms 11:O209–O217. https://doi.org/10.1111/luts.12247

Mancia G, Kreutz R, Brunström M, Burnier M, Grassi G, Januszewicz A (2023) 2023 ESH guidelines for the management of arterial hypertension. J Hypertens 41:1874–2071. https://doi.org/10.1097/HJH.0000000000003480

Mann DL, Kent RL, Parsons B, Cooper G IV (1992) Adrenergic effects on the biology of the adult mammalian cardiocyte. Circulation 85:790–804. https://doi.org/10.1161/01.cir.85.2.790

Matkó I, Fehér E, Vizi ES (1994) Receptor mediated presynaptic modulation of the release of noradrenaline in human papillary muscle. Cardiovasc Res 28:700–704. https://doi.org/10.1093/cvr/28.5.700

Matsuo S, Jang IS, Nabekura J, Akaike N (2003) α_2-adrenoceptor-mediated presynaptic modulation of GABAergic transmission in mechanically dissociated rat ventrolateral preoptic neurons. J Neurophysiol 89:1640–1648. https://doi.org/10.1152/jn.00491.2002

McCullough MA, Miller PR 3rd, Martin T, Rebo KA, Stettler GR, Martin RS et al (2023) Eliminating the benzos: a benzodiazepine-sparing approach to preventing and treating alcohol withdrawal syndrome. J Trauma Acute Care Surg. https://doi.org/10.1097/TA.0000000000004188

McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M et al (2021) 2021 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J 42: 3599–3726. https://doi.org/10.1093/eurheartj/ehab368

Meredith IT, Eisenhofer G, Lambert GW, Dewar EM, Jennings GL, Esler MD (1993) Cardiac sympathetic nervous activity in congestive heart failure. Evidence for increased neuronal norepinephrine release and preserved neuronal uptake. Circulation 88:136–145. https://doi.org/10.1161/01.cir.88.1.136

Michel MC, Korstanje C (2016) β3-adrenoceptor agonists for overactive bladder syndrome: role of translational pharmacology in a repositioning clinical drug development project. Pharmacol Ther 159:66–82. https://doi.org/10.1016/j.pharmthera.2016.01.007

Millan MJ, Gobert A, Rivet JM, Adhumeau-Auclair A, Cussac D, Newman-Tancredi A et al (2000) Mirtazapine enhances frontocortical dopaminergic and corticolimbic adrenergic, but not serotonergic, transmission by blockade of α_2-adrenergic and serotonin$_{2C}$ receptors: a comparison with citalopram. Eur J Neurosci 12:1079–1095. https://doi.org/10.1046/j.1460-9568.2000.00982.x

Milner TA, Lee A, Aicher SA, Rosin DL (1998) Hippocampal α_{2A}-adrenergic receptors are located predominantly presynaptically but are also found postsynaptically and in selective astrocytes. J Comp Neurol 395:310–327

Miyazaki T, Kobayashi H, Tosaka T (1998) Presynaptic inhibition by noradrenaline of the EPSC evoked in neonatal rat sympathetic preganglionic neurons. Brain Res 790:170–177. https://doi.org/10.1016/s0006-8993(97)01549-7

Molderings GJ, Göthert M (1990) Mutual interaction between presynaptic α_2-adrenoceptors and 5-HT1B receptors on the sympathetic nerve terminals of the rat inferior vena cava. Naunyn Schmiedebergs Arch Pharmacol 341:391–397. https://doi.org/10.1007/BF00176329

Molderings GJ, Göthert M (1995) Subtype determination of presynaptic α_2-autoreceptors in the rabbit pulmonary artery and human saphenous vein. Naunyn Schmiedebergs Arch Pharmacol 352:483–490. https://doi.org/10.1007/BF00169381

Molderings G, Likungu J, Zerkowski HR, Göthert M (1988) Presynaptic β_2-adrenoceptors on the sympathetic nerve fibres of the human saphenous vein: no evidence for involvement in adrenaline-mediated positive feedback loop regulating noradrenergic transmission. Naunyn Schmiedebergs Arch Pharmacol 337:408–414. https://doi.org/10.1007/BF00169532

Molderings GJ, Göthert M, Van Ahlen H, Porst H (1989) Noradrenaline release in human corpus cavernosum and its modulation via presynaptic α_2-adrenoceptors. Fundam Clin Pharmacol 3: 497–504. https://doi.org/10.1111/j.1472-8206.1989.tb00684.x

Molderings GJ, Likungu J, Göthert M (2000) N-type calcium channels control sympathetic neurotransmission in human heart atrium. Circulation 101:403–407. https://doi.org/10.1161/01.cir.101.4.403

Moulds RF, Stevens MJ (1983) Facilitatory prejunctional β-adrenoceptors in human arteries and veins. Gen Pharmacol 14:81–83. https://doi.org/10.1016/0306-3623(83)90069-1

Münch G, Kurz T, Urlbauer T, Seyfarth M, Richardt G (1996) Differential presynaptic modulation of noradrenaline release in human atrial tissue in normoxia and anoxia. Br J Pharmacol 118: 1855–1861. https://doi.org/10.1111/j.1476-5381.1996.tb15614.x

Nagy G, Reim K, Matti U, Brose N, Binz T, Rettig J, Neher E, Sørensen JB (2004) Regulation of releasable vesicle pool sizes by protein kinase A-dependent phosphorylation of SNAP-25. Neuron 41:417–429. https://doi.org/10.1016/s0896-6273(04)00038-8

Nakamura M, Suk K, Lee MG, Jang IS (2013) α_{2A} adrenoceptor-mediated presynaptic inhibition of GABAergic transmission in rat tuberomammillary nucleus neurons. J Neurochem 125:832–842. https://doi.org/10.1111/jnc.12259

Nicholson R, Dixon AK, Spanswick D, Lee K (2005) Noradrenergic receptor mRNA expression in adult rat superficial dorsal horn and dorsal root ganglion neurons. Neurosci Lett 380:316–321. https://doi.org/10.1016/j.neulet.2005.01.079

Nörenberg W, Schöffel E, Szabo B, Starke K (1997) Subtype determination of soma-dendritic α_2-autoreceptors in slices of rat locus coeruleus. Naunyn Schmiedebergs Arch Pharmacol 356:159–165. https://doi.org/10.1007/pl00005036

Oates JA, Gillespie L, Udenfriend S, Sjoerdsma A (1960) Decarboxylase inhibition and blood pressure reduction by alpha-methyl-3,4-dihydroxy-DL-phenylalanine. Science 131:1890–1891. https://doi.org/10.1126/science.131.3417.1890

O'Connor SC, Brain KL, Bennett MR (1999) Individual sympathetic varicosities possess different sensitivities to alpha 2 and P2 receptor agonists and antagonists in mouse vas deferens. Br J Pharmacol 128:1739–1753. https://doi.org/10.1038/sj.bjp.0702984

Olave MJ, Maxwell DJ (2004) Axon terminals possessing α_{2C}-adrenergic receptors densely innervate neurons in the rat lateral spinal nucleus which respond to noxious stimulation. Neuroscience 126:391–403. https://doi.org/10.1016/j.neuroscience.2004.03.049

Ortiz de Urbina AV, Davy M, Midol-Monnet M, Heimburger M, Beslot F, Cohen Y (1992) Modulation of noradrenergic transmission in the rat isolated portal vein: role of prejunctional α_2-adrenoceptors and β-adrenoceptors. Gen Pharmacol 23:763–767. https://doi.org/10.1016/0306-3623(92)90163-e

Palazidou E, Papadopoulos A, Sitsen A, Stahl S, Checkley S (1989) An alpha$_2$ adrenoceptor antagonist, Org 3770, enhances nocturnal melatonin secretion in man. Psychopharmacology (Berl) 97:115–117. https://doi.org/10.1007/BF00443424

Paparrigopoulos T, Psarros C, Bergiannaki JD, Varsou E, Dafni U, Stefanis C (2001) Melatonin response to clonidine administration in depression: indication of presynaptic alpha2-adrenoceptor dysfunction. J Affect Disord 65:307–313. https://doi.org/10.1016/s0165-0327(00)00270-6

Parker DA, de la Lande IS, Marino V, Ivar PM (1994) Presynaptic control of noradrenaline release from sympathetic nerves in human dental pulp. Arch Oral Biol 39:35–41. https://doi.org/10.1016/0003-9969(94)90032-9

Parker DA, Marino V, Ivar PM, de la Lande IS (1998) Modulation by presynaptic β-adrenoceptors of noradrenaline release from sympathetic nerves in human dental pulp. Arch Oral Biol 43:949–954. https://doi.org/10.1016/s0003-9969(98)00087-9

Pelayo F, Dubocovich ML, Langer SZ (1977) Regulation of noradrenaline release in the rat pineal through a negative feedback mechanism mediated by presynaptic α-adrenoceptors. Eur J Pharmacol 45:317–318. https://doi.org/10.1016/0014-2999(77)90018-8

Proudman RGW, Akinaga J, Baker JG (2022) The affinity and selectivity of α-adrenoceptor antagonists, antidepressants and antipsychotics for the human α2A, α2B, and α2C-adrenoceptors and comparison with human α1 and β-adrenoceptors. Pharmacol Res Perspect 10:e00936. https://doi.org/10.1002/prp2.936

Pudovkina OL, Kawahara Y, de Vries J, Westerink BH (2001) The release of noradrenaline in the locus coeruleus and prefrontal cortex studied with dual-probe microdialysis. Brain Res 906:38–45. https://doi.org/10.1016/s0006-8993(01)02553-7

Raiteri M, Bonanno G, Maura G, Pende M, Andrioli GC, Ruelle A (1992) Subclassification of release-regulating α_2-autoreceptors in human brain cortex. Br J Pharmacol 107:1146–1151. https://doi.org/10.1111/j.1476-5381.1992.tb13421.x

Rege S (2008) Antipsychotic induced weight gain in schizophrenia: mechanisms and management. Aust N Z J Psychiatry 42:369–381. https://doi.org/10.1080/00048670801961123

Remie R, Zaagsma J (1986) A new technique for the study of vascular presynaptic receptors in freely moving rats. Am J Physiol 251:H463–H467

Remie R, Knot HJ, Kolker HJ, Zaagsma J (1988a) Pronounced facilitation of endogenous noradrenaline release by presynaptic β_2-adrenoceptors in the vasculature of freely moving rats. Naunyn Schmiedebergs Arch Pharmacol 338:215–220. https://doi.org/10.1007/BF00173390

Remie R, Knot HJ, Bos EA, Zaagsma J (1988b) Characterization of presynaptic β-adrenoceptors facilitating endogenous noradrenaline release in the portal vein of permanently cannulated, freely moving rats. Eur J Pharmacol 157:37–43. https://doi.org/10.1016/0014-2999(88)90468-2

Remie R, Van Rossum JXM, Coppes RP, Zaagsma J (1992) Dysfunctional presynaptic α_2-adrenoceptors expose facilitatory β_2-adrenoceptors in the vasculature of spontaneously hypertensive rats. Eur J Pharmacol 211:257–261

Riedl MS, Schnell SA, Overland AC, Chabot-Doré AJ, Taylor AM, Ribeiro-da-Silva A, Elde RP, Wilcox GL, Stone LS (2009) Coexpression of α_{2A}-adrenergic and δ-opioid receptors in substance P-containing terminals in rat dorsal horn. J Comp Neurol 513:385–398. https://doi.org/10.1002/cne.21982. Erratum in: J Comp Neurol 514:674, 2009

Rump LC, Ruff G, Wolk V, Schollmeyer P (1991) α_2-adrenoceptor activation inhibits noradrenaline release in human and rabbit isolated renal arteries. Eur J Pharmacol 196:277–283. https://doi.org/10.1016/0014-2999(91)90440-2

Rump LC, Schwertfeger E, Schaible U, Fraedrich G, Schollmeyer P (1994) β_2-adrenergic receptor and angiotensin II receptor modulation of sympathetic neurotransmission in human atria. Circ Res 74:434–440. https://doi.org/10.1161/01.res.74.3.434

Rump LC, Bohmann C, Schaible U, Schöllhorn J, Limberger N (1995a) α_{2C}-adrenoceptor-modulated release of noradrenaline in human right atrium. Br J Pharmacol 116:2617–2624. https://doi.org/10.1111/j.1476-5381.1995.tb17216.x

Rump LC, Bohmann C, Schaible U, Schultze-Seemann W, Schollmeyer PJ (1995b) β-Adrenergic, angiotensin II, and bradykinin receptors enhance neurotransmission in human kidney. Hypertension 26:445–451. https://doi.org/10.1161/01.hyp.26.3.445

Rutz S, Riegert C, Rothmaier AK, Jackisch R (2007) Presynaptic modulation of 5-HT release in the rat septal region. Neuroscience 146:643–658. https://doi.org/10.1016/j.neuroscience.2007.02.005

Saitow F, Satake S, Yamada J, Konishi S (2000) β-Adrenergic receptor-mediated presynaptic facilitation of inhibitory GABAergic transmission at cerebellar interneuron-Purkinje cell synapses. J Neurophysiol 84:2016–2025. https://doi.org/10.1152/jn.2000.84.4.2016

Saitow F, Suzuki H, Konishi S (2005) β-Adrenoceptor-mediated long-term up-regulation of the release machinery at rat cerebellar GABAergic synapses. J Physiol 565:487–502. https://doi.org/10.1113/jphysiol.2005.084384

Sato H, Ito C, Tashiro M, Hiraoka K, Shibuya K, Funaki Y, Iwata R, Matsuoka H, Yanai K (2013) Histamine H_1 receptor occupancy by the new-generation antidepressants fluvoxamine and mirtazapine: a positron emission tomography study in healthy volunteers. Psychopharmacologia 230:227–234. https://doi.org/10.1007/s00213-013-3146-1

Schäfers RF, Nürnberger J, Herrmann B, Wenzel RR, Philipp T, Michel MC (1999) Adrenoceptors mediating the cardiovascular and metabolic effects of α-methylnoradrenaline in humans. J Pharmacol Exp Ther 289:918–925. PMID: 10215671

Scheibner J, Trendelenburg AU, Hein L, Starke K (2001) Stimulation frequency-noradrenaline release relationships examined in α_{2A}-, α_{2AB}- and α_{2AC}-adrenoceptor-deficient mice. Naunyn Schmiedebergs Arch Pharmacol 364:321–328. https://doi.org/10.1007/s002100100432

Scheibner J, Trendelenburg AU, Hein L, Starke K, Blandizzi C (2002) α_2-adrenoceptors in the enteric nervous system: a study α_{2A}-adrenoceptor-deficient mice. Br J Pharmacol 135:697–704. https://doi.org/10.1038/sj.bjp.0704512

Schelb V, Göbel I, Khairallah L, Zhou H, Cox SL, Trendelenburg AU, Hein L, Starke K (2001) Postnatal development of presynaptic receptors that modulate noradrenaline release in mice. Naunyn Schmiedebergs Arch Pharmacol 364:359–371. https://doi.org/10.1007/s002100100455

Schlicker E, Feuerstein T (2017) Human presynaptic receptors. Pharmacol Ther 172:1–21. https://doi.org/10.1016/j.pharmthera.2016.11.005

Schoffelmeer AN, Wierenga EA, Mulder AH (1986) Role of adenylate cyclase in presynaptic α_2-adrenoceptor- and μ-opioid receptor-mediated inhibition of [^{3}H]noradrenaline release from rat brain cortex slices. J Neurochem 46:1711–1717. https://doi.org/10.1111/j.1471-4159.1986.tb08488.x

Schofield GG (1990) Norepinephrine blocks a calcium current of adult rat sympathetic neurons via an α_2-adrenoceptor. Eur J Pharmacol 180(1):37–47. https://doi.org/10.1016/0014-2999(90)90590-3

Schwartz DD (1997) Activation of alpha-2 adrenergic receptors inhibits norepinephrine release by a pertussis toxin-insensitive pathway independent of changes in cytosolic calcium in cultured rat sympathetic neurons. J Pharmacol Exp Ther 282:248–255

Silva I, Costa AF, Moreira S, Ferreirinha F, Magalhães-Cardoso MT, Calejo I, Silva-Ramos M, Correia-de-Sá P (2017) Inhibition of cholinergic neurotransmission by β3-adrenoceptors depends on adenosine release and A1-receptor activation in human and rat urinary bladders. Am J Physiol Renal Physiol 313:F388–F403. https://doi.org/10.1152/ajprenal.00392.2016

Silva I, Magalhães-Cardoso MT, Ferreirinha F, Moreira S, Costa AF, Silva D, Vieira C, Silva-Ramos M, Correia-de-Sá P (2020) β3 adrenoceptor-induced cholinergic inhibition in human and rat urinary bladders involves the exchange protein directly activated by cyclic AMP 1 favoring adenosine release. Br J Pharmacol 177:1589–1608. https://doi.org/10.1111/bph.14921

Smith DF, Stork BS, Wegener G, Jakobsen S, Bender D, Audrain H, Jensen SB, Hansen SB, Rodell A, Rosenberg R (2007) Receptor occupancy of mirtazapine determined by PET in healthy volunteers. Psychopharmacologia 195:131–138. https://doi.org/10.1007/s00213-007-0877-x

Somogyi GT, de Groat WC (1990) Modulation of the release of [^{3}H]norepinephrine from the base and body of the rat urinary bladder by endogenous adrenergic and cholinergic mechanisms. J Pharmacol Exp Ther 255:204–210

Spafford JD, Zamponi GW (2003) Functional interactions between presynaptic calcium channels and the neurotransmitter release machinery. Curr Opin Neurobiol 13:308–314. https://doi.org/10.1016/s0959-4388(03)00061-8

Stähle H (2000) A historical perspective: development of clonidine. Bailliere's Clin Anaesthesiol 14:237–246. https://doi.org/10.1053/bean.2000.0079

Starke K (1972a) Alpha sympathomimetic inhibition of adrenergic and cholinergic transmission in the rabbit heart. Naunyn Schmiedebergs Arch Pharmacol 274:18–45. https://doi.org/10.1007/BF00501004

Starke K (1972b) Influence of extracellular noradrenaline on the stimulation-evoked secretion of noradrenaline from sympathetic nerves: evidence for an α-receptor-mediated feed-back inhibition of noradrenaline release. Naunyn Schmiedebergs Arch Pharmacol 275:11–23. https://doi.org/10.1007/BF00505064

Starke K (1977) Regulation of noradrenaline release by presynaptic receptor systems. Rev Physiol Biochem Pharmacol 77:1–124. https://doi.org/10.1007/BFb0050157

Starke K (1981) Presynaptic receptors. Annu Rev Pharmacol Toxicol 21:7–30. https://doi.org/10.1146/annurev.pa.21.040181.000255

Starke K (1987) Presynaptic α-autoreceptors. Rev Physiol Biochem Pharmacol 107:73–146

Starke K (2001) Presynaptic autoreceptors in the third decade: focus on α_2-adrenoceptors. J Neurochem 78:685–693. https://doi.org/10.1046/j.1471-4159.2001.00484.x

Starke K, Endo T, Taube HD (1975a) Relative pre- and postsynaptic potencies of α-adrenoceptor agonists in the rabbit pulmonary artery. Naunyn Schmiedebergs Arch Pharmacol 291:55–78. https://doi.org/10.1007/BF00510821

Starke K, Endo T, Taube HD (1975b) Pre- and postsynaptic components in effect of drugs with alpha adrenoceptor affinity. Nature 254:440–441. https://doi.org/10.1038/254440a0

Starke K, Göthert M, Kilbinger H (1989) Modulation of neurotransmitter release by presynaptic autoreceptors. Physiol Rev 69:864–989. https://doi.org/10.1152/physrev.1989.69.3.864

Stephens GJ, Mochida S (2005) G protein βγ subunits mediate presynaptic inhibition of transmitter release from rat superior cervical ganglion neurones in culture. J Physiol 563:765–776. https://doi.org/10.1113/jphysiol.2004.080192

Swedberg K, Hjalmarson A, Waagstein F, Wallentin I (1979) Prolongation of survival in congestive cardiomyopathy by beta-receptor blockade. Lancet 30:1374–1376. https://doi.org/10.1016/s0140-6736(79)92010-5

Swedberg K, Bristow MR, Cohn JN, Dargie H, Straub M, Wiltse C et al (2002) Moxonidine safety and efficacy (MOXSE) investigators. Effects of sustained-release moxonidine, an imidazoline agonist, on plasma norepinephrine in patients with chronic heart failure. Circulation 105:1797–1803. https://doi.org/10.1161/01.cir.0000014212.04920.62

Szabo B (2002) Imidazoline antihypertensive drugs: a critical review on their mechanism of action. Pharmacol Ther 93:1–35. https://doi.org/10.1016/s0163-7258(01)00170-x

Szabo B, Starke K (2021) Synaptic transmission. In: Offermanns S, Rosenthal W (eds) Encyclopedia of molecular pharmacology. Springer, Cham, pp 1476–1484. https://doi.org/10.1007/978-3-030-21573-6

Szabo B, Hedler L, Starke K (1989) Peripheral presynaptic and central effects of clonidine, yohimbine and rauwolscine on the sympathetic nervous system in rabbits. Naunyn Schmiedebergs Arch Pharmacol 340:648–657

Szabo B, Schramm A, Starke K (1992) Effect of yohimbine on renal sympathetic nerve activity and renal norepinephrine spillover in anesthetized rabbits. J Pharmacol Exp Ther 260:780–788

Szabo B, Fröhlich R, Illes P (1996) No evidence for functional imidazoline receptors on locus coeruleus neurons. Naunyn Schmiedebergs Arch Pharmacol 353:557–363. https://doi.org/10.1007/BF00169176

Szabo B, Fritz T, Wedzony K (2001) Effects of imidazoline antihypertensive drugs on sympathetic tone and noradrenaline release in the prefrontal cortex. Br J Pharmacol 134:295–304. https://doi.org/10.1038/sj.bjp.0704237

Szabo B, Than M, Wallmichrath I, Thorn D (2004) Analysis of the effects of cannabinoids on synaptic transmission between basket and Purkinje cells in the cerebellar cortex of the rat. J Pharmacol Exp Ther 310:915–925

Szemeredi K, Zukowska-Grojec Z, Bagdy G, Fekete MI, Kopin IJ (1989) Opposite effects of chronic cortisol treatment on pre- and postsynaptic actions of clonidine in pithed rats. J Auton Pharmacol 9:35–43. https://doi.org/10.1111/j.1474-8673.1989.tb00194.x

Tanda G, Bassareo V, Di Chiara G (1996) Mianserin markedly and selectively increases extracellular dopamine in the prefrontal cortex as compared to the nucleus accumbens of the rat. Psychopharmacology (Berl) 123:127–130. https://doi.org/10.1007/BF02246169

Taube HD, Starke K, Borowski E (1977) Presynaptic receptor systems on the noradrenergic neurones of rat brain. Naunyn Schmiedebergs Arch Pharmacol 299:123–141. https://doi.org/10.1007/BF00498554

Terakado M (2014) Adrenergic regulation of GABA release from presynaptic terminals in rat cerebral cortex. J Oral Sci 56:49–57. https://doi.org/10.2334/josnusd.56.49

Than M, Szabo B (2002) Analysis of the function of $GABA_B$ receptors on inhibitory afferent neurons of Purkinje cells in the cerebellar cortex of the rat. Eur J Neurosci 15:1575–1584

Todorov LD, Clerkin R, Mihaylova-Todorova ST, Khoyi MA, Westfall DP (2001) β_2-adrenoceptor-mediated prejunctional facilitation and postjunctional inhibition of sympathetic neuroeffector transmission in the Guinea pig vas deferens. J Pharmacol Exp Ther 298:623–633

Trendelenburg AU, Starke K, Limberger N (1994) Presynaptic α_{2A}-adrenoceptors inhibit the release of endogenous dopamine in rabbit caudate nucleus slices. Naunyn Schmiedebergs Arch Pharmacol 350:473–481. https://doi.org/10.1007/BF00173016

Trendelenburg AU, Limberger N, Starke K (1996) The presynaptic alpha-2 autoreceptors in pig brain cortex are alpha-2A. J Pharmacol Exp Ther 278:462–467

Trendelenburg AU, Sutej I, Starke K (1997a) Presynaptic $\alpha_{2A/D}$-autoreceptors in the brain cortex of Cercopithecus aethiops. Naunyn Schmiedebergs Arch Pharmacol 355:341–346. https://doi.org/10.1007/pl00004952

Trendelenburg AU, Sutej I, Wahl CA, Molderings GJ, Rump LC, Starke K (1997b) A re-investigation of questionable subclassifications of presynaptic α_2-autoreceptors: rat vena cava, rat atria, human kidney and Guinea-pig urethra. Naunyn Schmiedebergs Arch Pharmacol 356:721–737. https://doi.org/10.1007/pl00005111

Trendelenburg AU, Cox SL, Schelb V, Klebroff W, Khairallah L, Starke K (2000) Modulation of ^{3}H-noradrenaline release by presynaptic opioid, cannabinoid and bradykinin receptors and

β-adrenoceptors in mouse tissues. Br J Pharmacol 130:321–330. https://doi.org/10.1038/sj.bjp.0703305

Trendelenburg AU, Nörenberg W, Hein L, Meyer A, Starke K (2001) α_2-adrenoceptor-mediated inhibition of cultured sympathetic neurons: changes in $\alpha_{2A/D}$-adrenoceptor-deficient mice. Naunyn Schmiedebergs Arch Pharmacol 363:110–119. https://doi.org/10.1007/s002100000331

Trendelenburg AU, Meyer A, Klebroff W, Guimarães S, Starke K (2003) Crosstalk between presynaptic angiotensin receptors, bradykinin receptors and α_2-autoreceptors in sympathetic neurons: a study in α_2-adrenoceptor-deficient mice. Br J Pharmacol 138:1389–1402. https://doi.org/10.1038/sj.bjp.0705223

Tsuda K, Kimura K, Shima H, Nishio I, Masuyama Y (1992) Presynaptic α_2-adrenoceptor-mediated modulation of norepinephrine release from vascular adrenergic neurons in reduced renal mass salt hypertensive rats. Clin Exp Pharmacol Physiol 19:531–535. https://doi.org/10.1111/j.1440-1681.1992.tb00500.x

Umeda E, Satoh T, Nagashima H, Potter PE, Tarkovács G, Vizi ES (1997) α_{2A}-subtype of presynaptic alpha α_2-adrenoceptors modulates the release of [^{3}H]-noradrenaline from rat spinal cord. Brain Res Bull 42:129–132. https://doi.org/10.1016/s0361-9230(96)00223-7

Urban R, Szabo B, Starke K (1994) Is the sympathoinhibitory effect of rilmenidine mediated by alpha-2 adrenoceptors or imidazoline receptors? J Pharmacol Exp Ther 270:572–578

Urban R, Szabo B, Starke K (1995a) Involvement of α_2-adrenoceptors in the cardiovascular effects of moxonidine. Eur J Pharmacol 282:19–28. https://doi.org/10.1016/0014-2999(95)00297-x

Urban R, Szabo B, Starke K (1995b) Involvement of peripheral presynaptic inhibition in the reduction of sympathetic tone by moxonidine, rilmenidine and UK 14304. Eur J Pharmacol 282:29–37. https://doi.org/10.1016/0014-2999(95)00265-m

Vidovic M, Hill CE (1997) Transient expression of α-1B adrenoceptor messenger ribonucleic acids in the rat superior cervical ganglion during postnatal development. Neuroscience 77:841–848. https://doi.org/10.1016/s0306-4522(96)00522-2

Wang SJ (2002) Interaction between FK 506 and isoproterenol in the modulation of glutamate release from cerebrocortical nerve terminals. Neuroreport 13:983–986. https://doi.org/10.1097/00001756-200205240-00017

Wang SJ, Coutinho V, Sihra TS (2002) Presynaptic cross-talk of β-adrenoreceptor and 5-hydroxytryptamine receptor signalling in the modulation of glutamate release from cerebrocortical nerve terminals. Br J Pharmacol 137:1371–1379. https://doi.org/10.1038/sj.bjp.0705045

Wang Y, Yu X, Wang F, Wang Y, Wang Y, Li H, Lv X, Lu D, Wang H (2013) Yohimbine promotes cardiac NE release and prevents LPS-induced cardiac dysfunction via blockade of presynaptic α_{2A}-adrenergic receptor. PLoS One 8:e63622. https://doi.org/10.1371/journal.pone.0063622

Yamaguchi N (1982) Evidence supporting the existence of presynaptic α-adrenoceptors in the regulation of endogenous noradrenaline release upon hepatic sympathetic nerve stimulation in the dog liver in vivo. Naunyn Schmiedebergs Arch Pharmacol 321:177–184. https://doi.org/10.1007/BF00505482

Yamaguchi N, de Champlain J, Nadeau RA (1977) Regulation of norepinephrine release from cardiac sympathetic fibers in the dog by presynaptic α- and β-receptors. Circ Res 41:108–117. https://doi.org/10.1161/01.res.41.1.108

Yamauchi M, Imanishi T, Koyama T (2012) A combination of mirtazapine and milnacipran augments the extracellular levels of monoamines in the rat brain. Neuropharmacology 62:2278–2287. https://doi.org/10.1016/j.neuropharm.2012.01.024

Yavich L, Jäkälä P, Tanila H (2005) Noradrenaline overflow in mouse dentate gyrus following locus coeruleus and natural stimulation: real-time monitoring by in vivo voltammetry. J Neurochem 95:641–650. https://doi.org/10.1111/j.1471-4159.2005.03390.x

Zelaszczyk D, Zakrzeska A, Kwolek G, Malinowska B, Schlicker E (2005) A search for presynaptic β_3-adrenoceptors in the rat. Fundam Clin Pharmacol 19:147–153. https://doi.org/10.1111/j.1472-8206.2005.00318.x

Roles of β-adrenoceptor Subtypes and Therapeutics in Human Cardiovascular Disease: Heart Failure, Tachyarrhythmias and Other Cardiovascular Disorders

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Abstract

β-Adrenoceptors (β-ARs) provide an important therapeutic target for the treatment of cardiovascular disease. Three β-ARs, β_1-AR, β_2-AR, β_3-AR are localized to the human heart. Activation of β_1-AR and β_2-ARs increases heart rate, force of contraction (inotropy) and consequently cardiac output to meet physiological demand. However, in disease, chronic over-activation of β_1-AR is responsible for the progression of disease (e.g. heart failure) mediated by pathological hypertrophy, adverse remodelling and premature cell death. Furthermore, activation of β_1-AR is critical in the pathogenesis of cardiac arrhythmias while activation of β_2-AR directly influences blood pressure haemostasis. There is an increasing awareness of the contribution of β_2-AR in cardiovascular disease, particularly arrhythmia generation. All β-blockers used therapeutically to treat cardiovascular disease block β_1-AR with variable blockade of β_2-AR depending on relative affinity for β_1-AR vs β_2-AR. Since the introduction of β-blockers into clinical practice in 1965, β-blockers with different properties have been trialled, used and evaluated, leading to better understanding of their therapeutic effects and tolerability in various cardiovascular conditions. β-Blockers with the property of intrinsic sympathomimetic activity (ISA), i.e. β-blockers that also activate the receptor, were used in the past for post-treatment of myocardial infarction and had limited use in heart failure. The β-blocker carvedilol continues to intrigue due to numerous properties that differentiate it from other β-blockers and is used successfully in the treatment of heart failure. The discovery of β_3-AR in human heart created interest in the role of β_3-AR in heart failure but has not resulted in therapeutics at this stage.

Keywords

Acute coronary syndrome · Anxiety · Arrestin · Arrhythmia · Cardiac ryanodine receptors · Carvedilol · Chronic coronary artery syndrome · Coronary artery disease · Cyclic AMP · Excitation-contraction coupling · Giα-protein · Gsα-protein · Human heart · Human heart failure · Hypertension · Hypertension in

pregnancy · Hyperthyroidism · Intrinsic sympathomimetic activity · Migraine · Mirabegron · Phaeochromocytomas and paragangliomas · Phosphodiesterase enzymes · Portal hypertension · Protein kinase A · Ventricular arrhythmias · β_1-adrenoceptor · β_2-adrenoceptor · β_3-adrenoceptor · β-adrenoceptors · β-blockers

Abbreviations

AHF	Acute heart failure
ACEI	Angiotensin-converting enzyme inhibitor
ADHF	Acute decompensated heart failure
ARB	AT_1 receptor blocker
BRL37344	(RR + SS)[4-[2-[[2-(3-chlorophenyl)-2-hydroxy-ethyl]amino] propyl]phenoxy]acetic acid
β_1-AR	β_1-adrenoceptor
β_{1H}-AR	High affinity binding site of the β_1-adrenoceptor, blocked by (−)-CGP 12177
β_{1L}-AR	Low affinity binding site of the β_1-adrenoceptor, activated by (−)-CGP 12177
β_2-AR	β_2-adrenoceptor
β_3-AR	β_3-adrenoceptor
βARK	β-adrenergic receptor kinase
CaMKII	Calcium calmodulin-dependent protein kinase II
Cav1.2	L-type Ca^{2+} channel
CGP12177	(7)-4-(3-tertiarybutylamino-2-hydroxypropoxy) benzimidazol-2-one
CGP20712A	(2-hydroxy-S-[2-[[2-hydroxy-3-[4-[methyl-4-(trifluoromethyl)-1H-imidazol-2-yl]phenoxy]propyl]amino]ethoxy]-benzamide);
CL316,243	disodium (R,R)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl-amino] propyl]-1,3-benzodioxole-2,2-dicarboxylate];
DAD	Delayed after depolarization
FRET	Fluorescence resonance energy transfer
GRK	G-protein receptor kinase
Gsα-protein	Heterotrimeric stimulatory G-protein containing the α-subunit. Activates adenylyl cyclase to produce cyclic AMP
Giα-protein	Heterotrimeric inhibitory G-protein containing the α-subunit. Inhibits adenylyl cyclase to decrease cyclic AMP production
HIF-1	Hypoxia-inducible factor
HFimpEF	Heart failure with improved ejection fraction
HFmrEF	Heart failure with either mid-range or mildly reduced ejection fraction
HFrEF	Heart failure with reduced ejection fraction
IBMX	3-isobutyl-1-methylxanthine

ICI 118,551	1-[2,3-dihydro-7-methyl-1H-inden-4-yl] oxy-3-[(1-methylethyl) amino-2-butanol)]
ISA	Intrinsic sympathomimetic activity
$I_{Ca,L}$	L-type Ca^{2+} channel current
L748,347	N-(3-[3-[2-(4-benzenesulphonylamino phenyl)ethylamino]-2-hydroxypropoxy]benzyl acetamide
PDE	Phosphodiesterase
PI3K	Phosphatidylinositol-3 kinase
PKA	Protein Kinase A, cyclic AMP-dependent protein kinase
Po	Open probability of RyR2
RyR2	Ryanodine receptor isoform predominantly expressed in the heart responsible for Ca^{2+} release from the sarcoplasmic reticulum
Ser	Serine
SERCA2A	Sarcoplasmic reticulum Ca^{2+} ATPase pump expressed in heart
SICM	Scanning ion conductance microscopy
SR 58611	ethyl{(7S)-7-[(2R)-2-(3-chlorophenyl)-2-hydroxyethylamino]-5,6,7,8-tetrahydronaphtyl2-yloxy} acetate hydrochloride
SR	Sarcoplasmic reticulum
SVT	Supraventricular tachycardia
TG4	Transgenic mouse line with approximately 200–435-fold overexpression of the wild type β_2-adrenoceptor
Thr	Threonine
Tnc	Troponin C
TnI	Troponin I
VT	Ventricular tachycardia
ZD2079	4-[2-[[(2*R*)-2-Hydroxy-2-phenylethyl]amino]ethoxy]-benzeneacetic acid hydrochloride

1 Introduction

β-ARs provide an important therapeutic target for cardiovascular diseases including heart failure, coronary artery disease, arrhythmias (tachyarrhythmias) and hypertension. There are 3 β-AR subtypes, β_1-AR, β_2-AR and β_3-AR with all cardiovascular therapies to date targeting β_1-AR and β_2-AR. The first β-blocker introduced into clinical practice was propranolol in 1965 but since then, other β-blockers with varying properties have been developed, trialled, replaced or established for different cardiovascular indications reflecting increased knowledge towards obtaining optimal benefit to patients. This chapter describes the current uses of β-AR therapeutics and reflects on the basic principles upon which this is based. Reference to the animal heart is made where complementary to or indicative of the human heart.

2 Clinical Therapeutic Utility of β-ARs in Other Cardiovascular Diseases

Over the past few decades, there has been significant translational research in β-ARs and their subtypes. Numerous discoveries have been made in this field, leading to the development of β-adrenoceptor antagonists (blockers) and agonists that have been adopted in clinical practice. These pharmacological agents have proven to be effective and have played a vital role in the treatment of various medical conditions.

β-Blockers were initially developed to address the need for medicines that could directly counteract the adverse effects of sympathetic nerve activation in patients with conditions such as atrial fibrillation, ventricular tachycardia and angina (Black and Stephenson 1962). Since the early use of propranolol for the treatment of angina in 1968, β-blockers have continued to be used for cardiovascular indications such as angina, as well as for an increasing range of other conditions.

In the human heart both β_1- and β_2-adrenoceptors (β_1-AR and β_2-AR) co-exist in cardiomyocytes, atrium, ventricle, sinoatrial node, atrioventricular node and conducting regions, evidenced by complementary functional, biochemical, molecular and histological/anatomical localization experiments (Bristow et al. 1986; Buxton et al. 1987; Kaumann et al. 1999; Brodde 1991; Elnatan et al. 1994; Brodde and Michel 1999; Chandler et al. 2009, 2011; Greener et al. 2011). Activation of β_1-AR or β_2-AR increases force of contraction (inotropy, Bristow et al. 1986; Buxton et al. 1987; Brodde 1991; Kaumann et al. 1999), shortens the duration of the contraction (lusitropy, Kaumann et al. 1999; Molenaar et al. 2000, 2007b) in atrium and ventricle and increases heart rate (chronotropy, Hall et al. 1989). Activation of β-AR by adrenaline decreases conduction time through the AV node (dromotropy) which is blocked by propranolol (Morady et al. 1988). The β-AR subtypes (β_1-AR or β_2-AR) responsible for decreasing conduction time were not determined (Morady et al. 1988). Functional involvement of both subtypes is however predicted from canine AV node studies (Motomura and Hashimoto, 1992). Adrenaline had no effect on conduction time through the bundle of His–Purkinje system (Morady et al. 1988) reflecting a lower density of β_1-AR (Elnatan et al. 1994).

The early acceptance of the co-existence of β_1-AR and β_2-AR was facilitated by the development of selective antagonists and agonists for the subtypes. Later Chantal Gauthier's group reported a cardiodepressant β_3-AR in human ventricle (Gauthier et al. 1996; Gauthier et al. 1998). Another, 'third heart β-AR' (Kaumann 1989, note the intentional, subtle, but significant difference in terminology to 'β_3-AR') was identified in human heart (Kaumann 1996; Sarsero et al. 2003), which was later elucidated to be a 'propranolol-resistant' binding site of the β_1-AR (Kompa and Summers 1999; Kaumann et al. 2001; β_{1L}-AR low affinity binding site of the β_1-AR, Kaumann and Molenaar 2008). Availability of human non-failing and failing hearts for research allowed important contributions to the understanding of the role of all three β-ARs in heart failure and other heart diseases.

2.1 Coronary Artery Disease

Considering the role of β-blockers in the management of coronary artery disease, it is necessary to distinguish the various clinical manifestations of coronary artery diseases. In brief, coronary artery disease is associated with an inadequate supply of blood to the myocardium due to flow limitation or obstruction of the epicardial coronary arteries. For most patients, atherosclerosis is the fundamental pathophysiologic process leading to abnormal coronary blood flow. Patients may present with chronic (stable) or acute (unstable) symptoms that require different management approaches.

2.1.1 Chronic Coronary Artery Syndrome

Angina pectoris refers to chest discomfort a patient experiences when the myocardial oxygen demand exceeds the coronary oxygen supply. Stable angina can be triggered by a particular level of physical exertion, depending on the severity of luminal stenosis. Myocardial oxygen demand is influenced by myocardial contractility, heart rate and left ventricular wall stress. β-Blockers can reduce each of these factors. However, β-blockers can also negatively affect adrenergically mediated coronary vasodilation, leading to a decrease in coronary blood flow. Nevertheless, such potential drawback is offset by increased diastole that enhances total coronary perfusion, due to the negative chronotropic effect of β-blockers. All of the β-blockers, regardless of receptor subtype affinity, appear to be equally effective in reducing the symptoms of stable angina pectoris. As mentioned, propranolol, a non-selective β-blocker, was initially developed for the treatment of angina in the 1960s, with early clinical studies indicating a 50% or more reduction in episodes (Warren et al. 1976). Similarly, atenolol, termed a 'cardioselective' β-blocker (albeit with only 4.7-fold selectivity for β_1-AR vs β_2-AR, Baker 2005), has been shown to be effective with the additional advantage of once-daily dosing when compared to propranolol (Jackson et al. 1978). Metoprolol, another 'cardioselective' β-blocker (~ 2.5-fold selective for β_1-AR vs β_2-AR see below), was demonstrated in the International Multicenter Angina Exercise (IMAGE) study to be superior in improving exercise tolerance compared to the L-type Ca^{2+} channel blocker (nifedipine) (Savonitto et al. 1996). Carvedilol, with the additional selective α_1-AR antagonism, may be considered in patients where additional blood pressure lowering is desirable and is effective in reducing angina symptoms compared to placebo and L-type Ca^{2+} channel blocker (Hauf-Zachariou et al. 1997; Weiss et al. 1998). Despite the unparalleled advancement in managing coronary artery disease in the past 4–5 decades, the principle of reducing myocardial oxygen requirements (Black and Stephenson 1962) in the presence of cardiac sympathetic nervous system activity (Black and Prichard 1973) remains. β-Blockers continue to be the first-line therapy to reduce angina episodes and improve effort tolerance among patients with stable chronic coronary artery syndrome (Fihn et al. 2012; Chew et al. 2016). Nevertheless, it is important to highlight that β-blockers have not been shown to improve survival among patients with stable chronic angina (Motivala et al. 2016), except for those

with prior myocardial infarction (Andersson et al. 2014) and heart failure (see below).

2.1.2 Acute Coronary Syndrome

Unlike stable coronary artery syndrome, acute coronary syndrome refers to patients who experience a sudden onset of myocardial ischaemia or infarction. This condition is typically caused by acute intracoronary thrombosis associated with plaque rupture, platelet aggregation and subsequent thrombus formation (Libby 2013). Acute coronary syndrome can be further classified into unstable angina pectoris (Braunwald and Morrow 2013) and myocardial infarction with or without ST elevation on ECG (Anderson and Morrow 2017; Thygesen et al. 2018). Potential pharmacodynamic benefits of β-blockers in managing patients for acute coronary syndrome include: (i) reduction in myocardial oxygen consumption, by reducing contractility, afterload and heart rate; (ii) reduction in risk of ventricular arrhythmias and risk of sudden cardiac death (Rydén et al. 1983; Friedman et al. 1986) (iii) prolonging the diastolic phase thereby increasing coronary perfusion. Furthermore, less appreciated potential mechanistic effects of β-blockers include minimization of secondary insult from reperfusion injury, inhibition of platelet aggregation and thromboxane synthesis and reduction in the risk of adverse remodelling of the left ventricle, especially when infarct size is substantial (Mak and Weglicki 1988; Hu et al. 1998; Doughty et al. 2004).

Although early randomized control studies did show mortality benefit of β-blockers in the management of acute myocardial infarction, the absolute risk reduction independently associated with β-blocker use post myocardial infarction has been called into question (Freemantle et al. 1999; Bangalore et al. 2014). This is in part mediated by the significant improvement in the holistic management of patients presenting with acute coronary syndrome with more extensive use of antithrombotic therapy and early reperfusion strategies, resulting in a much smaller infarct size. Consistent with observations from animal studies, the benefit of β-blocker use was strongly associated with infarct size. The most profound long-term beneficial effect of β-blockers was observed in animal models with large infarcts treated with bisoprolol (Hu et al. 1998). Therefore, in current practice, β-blockers (including carvedilol, bisoprolol, nebivolol, metoprolol succinate) are indicated for patients with reduced left ventricular systolic function (LVEF $\leq$40%) for discharge management and secondary prevention of myocardial infarction (Chew et al. 2016). There is stronger evidence for mortality benefit in patients with reduced left ventricular systolic function (Desta et al. 2021). The evidence of whether β-blockers provide mortality benefit to patients with myocardial infarction in the absence of heart failure or left ventricular dysfunction and with treatments including reperfusion, revascularization, antithrombotic and anti-lipidemic is less clear (Bangalore et al. 2014; Desta et al. 2021) and is a subject of the ongoing investigation in several prospective clinical trials. It is also important to note that β-blocker use should be contraindicated in the early management of post myocardial infarction in patients with hemodynamic instability, especially those complicated with cardiogenic shock. Furthermore, patients with acute bronchospasm, significant

first-degree and second-degree heart block or symptomatic bradycardia (for example, heart rate of less than 40 beats per minute), initiation of early β-blocker use should be delayed. Lastly patients with overt acute heart failure symptoms and signs, such as acute pulmonary oedema, early β-blocker use should be avoided until the clinical condition can be stabilized.

2.2 Hypertension

β-Blockers reduce blood pressure primarily by reducing cardiac output and renin release from juxtaglomerular cells in afferent arterioles that enter the glomerulus. Currently β-blockers are not used as first-line medicines for patients with hypertension that is uncomplicated by other co-morbidities as they display a less favourable balance between efficacy and safety (Chew et al. 2016). Other options (ACE inhibitors, ARBs, L-type Ca^{2+} channel antagonists, thiazide diuretics either as monotherapy or combinations) are preferred (Chew et al. 2016). Hypertension is an independent risk factor for occurrence of stroke (Mukete et al. 2015; Feigin et al. 2017). In these patients risk is reduced by treatment with antihypertensive medicines (ACEI, ARB, L-type Ca^{2+} channel blockers, thiazide diuretics) but in contrast risk is increased with β-blockers versus non-β-blocker antihypertensive medicines despite effectiveness in lowering blood pressure (Mukete et al. 2015). On the other hand, β-blockers are recommended for patients with hypertension and previous myocardial infarction and selected β-blockers (carvedilol, bisoprolol, metoprolol, nebivolol) for heart failure patients (Chew et al. 2016).

2.3 Arrhythmias

β-Blockers are commonly used for the management of various heart rhythm disorders. By virtue of their conduction slowing effects in the AV node, they are able to effectively control rapid ventricular rate in patients with atrial fibrillation (AF). However, when used alone they generally have weak rhythm control efficacy for the maintenance of sinus rhythm. An important exception is sotalol which has significant Vaughan Williams class III membrane-active effects to prolong myocardial refractoriness. Sotalol can thus be used as a rhythm control agent to prevent recurrences of AF and other arrhythmias. In view of its proarrhythmic potential due to QT interval prolongation and the significant resulting risk of producing *torsades de pointes*, sotalol should not be used solely for the purposes of controlling ventricular rate in AF patients (Joglar et al. 2024).

β-Blockers may be used for the termination of supraventricular tachycardia (SVT) in the event that adenosine is ineffective. They can also be used for long-term SVT control but in view of the efficacy and safety of catheter ablation of SVT, they are infrequently used for this purpose. For patients without heart failure, focal idiopathic ventricular tachycardia (VT) and ventricular ectopy also represent indications for β-blocker use, both for initial termination of the arrhythmia but also for long-term

rhythm control. Patients with VT in the context of cardiomyopathy have a demonstrated prognostic benefit for specific β-blockers (e.g. bisoprolol) and will usually be on them as part of standard heart failure medical therapy. However, some patients with acutely recurrent VT and electrical storm may benefit from short-term use of propranolol to suppress the repeated VT initiations seen in these life-threatening clinical presentations (Chatzidou et al. 2018). Esmolol is often also considered for this purpose given its ultra-short half-life.

Various genetic heart rhythm disorders (or channelopathies as they are sometimes known) are uniquely responsive to β-blockers and their use can be life-saving for the often very young patients who are diagnosed with these conditions. Foremost among these is long QT syndrome, particularly the LQTS-1 and LQTS-2 subtypes, where the variant potassium channel function is modulated by β-AR-mediated second messenger signalling. Catecholaminergic polymorphic VT is another such β-blocker responsive channelopathy, characteristically caused by mutations in the ryanodine receptor (RyR2) gene. The β-blocker of choice for both CPVT and LQTS is nadolol due to its long half-life and non-selective pharmacodynamics (Peltenburg et al. 2022; Chockalingam et al. 2012).

2.4 Heart Failure

Heart failure is a complex and heterogeneous clinical syndrome with various aetiologies and pathophysiology. As such, the definition of heart failure has evolved over the years and differs among various professional guidelines. Nevertheless, key common features include: (i) the presence of characteristic symptoms, such as dyspnoea, fatigue, oedema and or venous congestion; (ii) secondary to structural changes to cardiac anatomy, such as reduction of ventricular systolic function, chamber dilatation and ventricular wall hypertrophy (either eccentric or concentric hypertrophy); (iii) leading to functional impairment of the heart in either pumping adequate blood flow to meet the metabolic demands of the body (systolic dysfunction), or the inability to fill cardiac chambers without elevated intracardiac pressure (diastolic dysfunction) (NHFA CSANZ Heart Failure Guidelines Working Group 2018; Bozkurt et al. 2021; Heidenreich et al. 2022; McDonagh et al. 2023).

The use of β-blockers for the treatment of heart failure had its origin with the seminal work of Finn Waagstein and colleagues in the mid-1970s (Waagstein et al. 1975). The original idea was that patients with congestive heart failure may benefit from a reduction of tachycardia by administration of β-blockers. It makes fascinating reading given the current use and significance of β-blockers in heart failure treatment. Briefly, chronic administration of alprenolol (1 patient) or practolol (6 patients) for 2–12 months to patients with heart failure resulted in clinical improvement including decreased heart rate, heart size, dyspnoea, exercise capacity and increased stroke volume (Waagstein et al. 1975). There followed a period of scepticism, with Ikram and Fitzpatrick (1981) unable to confirm the results of Waagstein et al. (1975) with another β-blocker, acebutolol using a double-blind cross-over trial design involving 15 patients. It wasn't until the 1990s, following large randomized

controlled clinical trials with metoprolol (Waagstein et al. 1993; MERIT-HF Study Group 1999), carvedilol (Bristow et al. 1996; Colucci et al. 1996; Packer et al. 1996, 2001) and bisoprolol (LeChat et al. 1997; CIBIS-II Investigators and Committees 1999) that reported reduced morbidity and a survival benefit that β-blockers established their place in heart failure treatment. The CAPRICORN trial investigated the effect of carvedilol in patients with left ventricular dysfunction (baseline average LVEF 32.8%) after an acute myocardial infarction. Carvedilol showed benefit for all-cause and cardiovascular mortality, and non-fatal myocardial infarction, additional to benefit obtained with ACE inhibitors (98% patients) and reperfusion intervention (46% patients) (CAPRICORN Investigators et al. 2001). This confirmed the benefit of other β blockers post myocardial infarction (see McMurray et al. 2005). A retrospective analysis of CAPRICORN study patients also revealed atrial and ventricular antiarrhythmic benefit with carvedilol (McMurray et al. 2005). Previously it had been shown other β-blockers provided benefit for ventricular arrhythmias in patients post myocardial infarction but without left ventricular systolic dysfunction (see McMurray et al. 2005 for references).

When considering the clinical use of β-blockers and β-AR agonists, it is necessary to consider the various clinical presentations of symptomatic heart failure. Clinical practice and professional guidelines have conventionally classified left-sided heart failure according to left ventricular ejection fraction. Heart failure with reduced ejection fraction has universally been recognized in patients with left ventricular ejection fraction less than or equal to 40% (HFrEF). In contrast, patients may also present with heart failure despite the presence of preserved LV systolic function, measured by ejection fraction of greater or equal to 50% (HFpEF). Some differences in how professional societies and guidelines address those patients with an ejection fraction between 40–50% exist. Most professional guidelines do consider this group of patients to be heart failure with either mid-range or mildly reduced ejection fraction (HFmrEF) or heart failure with improved ejection fractions (HFimpHF) depending on the prior clinical trajectory (Heidenreich et al. 2022). However, some professional guidelines take a more pragmatic approach in considering patients with left ventricular ejection fraction of less than 50% to be HFrEF (NHFA CSANZ Heart Failure Guidelines Working Group et al. 2018).

In addition to left ventricular ejection fraction, the acuity of heart failure is important when considering β-blockers. Acute and subacute presentation of heart failure often refers to acute heart failure (AHF) or acute decompensated heart failure (ADHF). These patients usually manifest with some hemodynamic instability and clinical signs and features of decompensation. A more severe form of AHF would be those complicated by cardiogenic shock (Naidu et al. 2022), where cardiac output is so severely impaired that end organs hypoperfusion occurs and carries a substantially higher risk of morbidity and mortality. In contrast, chronic heart failure refers to patients who may still be symptomatic but haemodynamically may be compensated adequately to allow outpatient management. Nevertheless, patients often fluctuate between these various states of clinical stability throughout the life span of this chronic condition.

The evidence supporting the use of β-blockers in patients with heart failure is the most robust and well-established in patients with HFrEF and HFimpEF. The initiation of β-blockers should take into consideration hemodynamic compensation, preferably with initiation and up-titration of the β-blockers to be undertaken after the patient has demonstrated haemodynamic stability and resolution of significant pulmonary oedema and hypervolemia resulting from venous congestion. Failure to do so can potentially lead to an increased risk of deterioration of AHF and cardiogenic shock. In contrast, a multitude of studies, albeit mostly observational, have demonstrated the safety and beneficial effects of continuing β-blockers in patients presenting with acute heart failure without cardiogenic shock. Lastly, even in the event of normalization of left ventricular systolic function among patients with HFrEF (i.e. HFimpEF), continuation and adherence to β-blockers are strongly encouraged, due to limited but alarming clinical trials evidence indicating an unacceptable risk of recurrence in the event of withdrawal of guideline-directed medical therapy including β-blockers.

After decades of mixed results in clinical trials among patients with HFrEF, management and clinical outcomes of patients with HFrEF have improved substantially with multiple therapeutics shown to reduce morbidity and prolong survival of this potentially fatal condition. Currently, four classes of pharmacological management form the fundamentals of guideline-recommended medical therapy for patients with chronic HFrEF. Namely, these include 1) renin-angiotensin system inhibitors (angiotensin-converting enzyme [ACE] inhibitor, single-agent angiotensin II receptor blockers [ARB], or angiotensin receptor-neprilysin inhibitor [ARNI]), 2) β-blockers, 3) mineralocorticoid receptor antagonists (MRA) and 4) a sodium-glucose cotransporter 2 (SGLT2) inhibitor – conveniently termed 'the 4 pillars' of HFrEF medical therapy. Patients clearly may need other therapy such as diuretics, hydralazine with nitrates, and digoxin based on individual clinical needs. Therefore, it is important to consider β-blockers in the management of heart failure not in isolation, but in conjunction with the above-mentioned proven therapies.

2.5 Other Important Uses of β-blockers in Cardiovascular and Related Disorders

2.5.1 Portal Hypertension

Portal hypertension is an elevation of the hepatic venous pressure gradient >5 mmHg. An elevation >10 mmHg is 'clinically significant portal hypertension' which predicts the development of gastrooesophageal varices (dilated collateral subepithelial gastrooesophageal veins which connect the portal and systemic circulations, Sharma and Rameshbabu (2012)) and haemorrhage (Groszmann et al. 2005; Bari and Garcia-Tsao 2012; de Franchis et al. 2022). β-Blockers which block both β_1-AR and β_2-AR at clinically used doses were recommended as the treatment of first choice to prevent variceal haemorrhage (de Franchis 1996) and their use has continued (Bari and Garcia-Tsao 2012; de Franchis et al. 2022). Propranolol or nadolol is recommended for primary prophylaxis of variceal bleeding and secondary

prophylaxis to prevent rebleeding in patients with previous event of variceal haemorrhage (Bari and Garcia-Tsao 2012). The rationale for use of β-blockers that block both β_1-AR and β_2-AR was that hepatic portal pressure is reduced by a combination of 1. reduction in cardiac output (β_1-AR blockade) and 2. splanchnic vasoconstriction by blocking vasodilatory β_2-AR (leaving vasoconstrictor α_1-AR unopposed) (Bari and Garcia-Tsao 2012; see Martell et al. 2010 for vascular smooth muscle mechanisms of splanchnic vasodilation in portal hypertension). In updated guidelines, carvedilol has been recommended, 'in contrast with traditional non selective β-blockers, ((i.e. propranolol and nadolol), carvedilol has intrinsic anti-α-adrenergic vasodilatory effects that contribute to its greater portal pressure reducing effect' (de Franchis et al. 2022). Furthermore, carvedilol is preferred for compensated cirrhosis since it is more effective at reducing the hepatic venous gradient (de Franchis et al. 2022, also for definition of compensated cirrhosis and further detail).

2.5.2 Hypertension in Pregnancy

Women can develop hypertension during pregnancy (hypertension ≥20 weeks gestation:- gestational hypertension, transient gestational hypertension, pre-eclampsia, superimposition of pre-eclampsia on chronic hypertension) or it may pre-exist (Magee et al. 2022). Hypertension is a leading cause of maternal and perinatal morbidity and mortality and should be treated irrespective of cause (Magee et al. 2022). Labetalol is a first-line medicine for treatment of hypertension in pregnancy (Magee et al. 2022). In a small (33 patients) prospective, randomized, double-blind placebo-controlled study administration of atenolol to women with mild hypertension towards the end of the first trimester was associated with babies with lower birth weight (Butters et al. 1990). Atenolol is therefore not recommended for treatment of hypertension in the early stages of pregnancy (Butters et al. 1990; Lydakis et al. 1999).

2.5.3 Hyperthyroidism

Hyperthyroidism is associated with sensitization of sympathetic nervous system effects (Aumann and Youmans 1940; Silva and Bianco 2008). Synergy between the sympathetic nervous system and thyroid hormones in patients with thyrotoxicosis causes tachycardia with minimal exercise (Silva and Bianco 2008). Increased sensitivity of effects mediated through β-ARs is attributed at least in part to thyroid hormone-induced upregulation of β-ARs and not a change in catecholamine affinity (Williams et al. 1977) or increased sympathetic nervous system activity as plasma noradrenaline levels are unchanged (Coulombe et al. 1976). Thyroid hormone, T3 is transported into the myocyte, binds to thyroid hormone nuclear receptors. T3 bound thyroid hormone nuclear receptor binds to a thyroid hormone responsive element to increase or decrease gene activity (Lazar and Chin 1990). T3 increased cardiac β_1-AR (but not β_2-AR) mRNA, rate of mRNA transcription and receptor density (Bahouth 1991; Bahouth et al. 1997). The effects of T3 on the heart are more widespread than directly on β_1-ARs and include other proteins that impact on β_1-

AR mediated effects including phospholamban, SERCAIIA and adenylyl cyclase (Klein and Danzi 2007).

Cardiovascular symptoms of hyperthyroidism can include atrial fibrillation and ventricular arrhythmias (Klein and Danzi 2007). The severity of peripheral endocrine symptoms was found to be correlated with the severity of anxiety (Trzepacz et al. 1989). Other symptoms include skeletal muscle tremor. β-Blockers are recommended in all suitable patients with symptomatic thyrotoxicosis to control cardiac symptoms and tremor (see Ross et al. 2016; Kahaly et al. 2018 for more details).

2.5.4 Phaeochromocytomas and Paragangliomas

Phaeochromocytomas and paragangliomas secrete catecholamines causing catecholamine-induced hypertension or a hypertensive crisis with life-threatening cardiovascular effects including tachyarrhythmias, myocardial infarction (secondary to catecholamine-induced coronary artery constriction) and stroke (Nazari et al. 2023). The clinical presentation will depend on the level of individual catecholamines, adrenaline, noradrenaline, dopamine that are secreted (Nazari et al. 2023). Cardioselective β-blockers, in the presence of α-AR blockade, are preferred for the treatment of catecholamine-induced tachyarrhythmias (Nazari et al. 2023). α-AR blockade prevents worsening α_1-AR-mediated hypertension in the presence of β-blockers (Nazari et al. 2023). In theory, cardioselective β-blockers will maintain catecholamine (primarily adrenaline) activation of vasodilatory β_2-AR (Nazari et al. 2023). Labetalol is contraindicated for the treatment of catecholamine-induced hypertensive crises as it blocks β_1-AR (affinity pKi 7.6–8.2) and β_2-AR (pKi 8.0) with higher affinity than α_1-AR (pKi 6.1–6.6) (Harding et al. 2023) and may worsen hypertension (Nazari et al. 2023).

2.5.5 Anxiety

β-Blockers, mostly propranolol (but including oxprenolol (stage fright, James et al. 1977), atenolol (stage fright, Neftel et al. 1982)) have been trialled to treat anxiety of different causes including high trait anxiety, substance disorder and withdrawal symptoms, schizophrenia, exam nerves, stage fright, performance anxiety in musicians, surgeons (Steenen et al. 2016) and anxiety associated with hyperthyroidism (Trzepacz et al. 1989). For the management of anxiety, β-blockers can provide relief from symptoms of anxiety, such as palpitations, increased ventilation (Steenen et al. 2016) and tachycardia. In Australia, propranolol has TGA (Therapeutic Goods Administration) approval to treat patients with anxiety tachycardia.

2.5.6 Migraine

The treatment of migraine is multi-modal and may include lifestyle modification, medicines to treat an acute attack, and medicines to prevent the occurrence of an attack (Ailani et al. 2021). The treatment aims of preventative treatment include reducing the frequency and severity of attacks (Ailani et al. 2021). β-Blockers are one of many options indicated for the prevention of migraine (Ailani et al. 2021). β-Blockers commonly include metoprolol, propranolol, timolol and nadolol, but

currently and previously other β-blockers (Holroyd et al. 2010; Danesh and Gottschalk 2019; Ailani et al. 2021) with different levels of evidence (Danesh and Gottschalk 2019). The mode of action of β-blockers for prevention of migraine continues to be investigated without finding a consistent unifying mechanism and is considered partly understood (Sprenger et al. 2018; Danesh and Gottschalk 2019). Their mechanism may in part include a reduction in cortical excitability (for further discussion, see Sprenger et al. 2018).

3 General Properties of β-blockers: Selectivity and ISA

The successful introduction of propranolol (Black et al. 1965) into clinical medicine for the treatment of angina in 1968 motivated the design and synthesis of chemically related compounds with β-blocker properties. What emerged were β-blockers with heterogenous properties as discussed briefly in this section and circumstantially elsewhere.

Bristow (2000) conveniently identified and categorized 3 classes of β-blockers available for clinical use, Generation/Class:- First/non-selective, for example propranolol and timolol; Second/'selective' β_1-AR, for example metoprolol, bisoprolol; Third/β-blocker-vasodilator, for example carvedilol, bucindolol, nebivolol. Selectivity referred to higher affinity for β_1-AR than β_2-AR. The studies of Baker (2005) and Hoffmann et al. (2004) report affinity values for a large number of antagonists (β-blockers) at β_1-AR, β_2-AR and β_3-AR. Another very helpful resource is the IUPHAR/BPS Guide to Pharmacology (www.guidetopharmacology.org). It should be noted that the β_1-AR selective β blockers currently available are not highly selective and β_2-AR blockade associated with these drugs is a common clinical problem (Baker 2005).

Of note, the consistent property of cardiovascular medicines, e.g. propranolol, atenolol, metoprolol, bisoprolol, carvedilol and nebivolol is the ability to block β_1-AR in the heart. In general, the main idea for the development of β_1-AR selective blockers was to avoid bronchial smooth muscle constriction caused by blockade of β_2-AR, particularly in patients with airways disease such as asthma and chronic obstructive airways disease (COPD). A vasodilator response associated with third generation β-blockers can be produced by different mechanisms (carvedilol α_1-AR blockade, nebivolol nitric oxide production).

β-Blockers have varying levels of intrinsic sympathomimetic activity (ISA). ISA is used to describe and quantitate the magnitude of ligand-induced β-AR agonist effect. A reference full agonist (e.g. isoprenaline) is commonly used to determine intrinsic activity of another β-AR ligand in experimental situations (McPherson et al. 1984; Molenaar et al. 1985). Where the test agonist produces a response that is less than that of the full agonist (isoprenaline), it is referred to as a 'partial agonist'. John Blink's early interest in β-blockers included detection of 'sympathomimetic effects at lower concentrations' (Blinks 1967). Interestingly in isolated tissue studies, slight increases in rate or force were observed for propranolol at concentrations of 0.01–1 μM, but were considered minimal (Blinks 1967). In the same study, other

compounds had more pronounced sympathomimetic activity at concentrations that 'have an appreciable adrenergic blocking effect' (Blinks 1967).

β-Blockers with ISA (e.g. pindolol) were introduced into the clinic for patients with angina or acute myocardial infarction (Heikkilä and Nieminen 1982). The concept was that the β-blocker with ISA caused less cardiodepression and therefore provided a 'safety factor' not provided by β-blockers without ISA (e.g. metoprolol) (Heikkilä and Nieminen 1982). β-Blockers are administered clinically as racemic mixtures of two enantiomers, sometimes designated (±), indicating a (−)-enantiomer and (+)-enantiomer with differing chemistry at the chiral carbon. For pindolol, it was later found that the (+)- enantiomer caused cardiostimulation through activation of β_2-AR (Walter et al. 1984; Kaumann and Molenaar 2008). (−)-Pindolol activated the β_1-AR at both the high (β_{1H}-AR) and low affinity binding sites (β_{1L}-AR) (Walter et al. 1984; Joseph et al. 2003; Kaumann and Molenaar 2008). (−)-Pindolol was classified as a 'non-conventional partial agonist' through its ability to activate β_{1L}-AR, at considerably higher concentrations (~ 100 fold) than those required to block the effects of catecholamines at the β_1-AR (Kaumann 1989; Joseph et al. 2003; Kaumann and Molenaar 2008). Other β-blockers with ISA including oxprenolol and alprenolol (Barrett and Carter 1970) were also introduced into the clinic.

The presence of ISA in β-blockers used for management of patients' postmyocardial infarction on mortality was investigated (Freemantle et al. 1999). Overall, long-term (6–48 months) administration of β-blockers without ISA gave a survival benefit with propranolol, timolol and metoprolol (Freemantle et al. 1999). β-Blockers with ISA, including xamoterol, pindolol and oxprenolol did not result in a survival benefit (Freemantle et al. 1999). The meta-analysis of Freemantle et al. (1999) included searches of databases from 1974–1997 and the data, according to Desta et al. (2021) was obtained when reperfusion or revascularization was not implemented and co-administration of antithrombotics or statins was limited.

Clinical trials of the β-AR blockers carvedilol, metoprolol and bisoprolol established a mortality benefit for patients with heart failure (Molenaar and Parsonage 2005). This is not the case for all β-blockers, for example bucindolol and xamoterol. Bucindolol is a non-selective β-AR blocker (Hershberger et al. 1990) that in some (Maack et al. 2000) but not all studies (Hershberger et al. 1990) displays measurable ISA on contractility of the human heart. The ISA (Maack et al. 2000) was dependent on optimized conditions produced by administering bucindolol in the presence of forskolin that provided pre-activation of adenylyl cyclase. Under those conditions, bucindolol increased (3 out of 8 preparations) or decreased (5 out of 8 preparations) contractility of left ventricular myocardium (Maack et al. 2000). It was concluded that for ISA bucindolol > carvedilol > metoprolol, with metoprolol being classified as an inverse agonist. In both studies (Hershberger et al. 1990; Maack et al. 2000) guanine nucleotide-sensitive binding in radioligand binding experiments was observed for bucindolol, supporting the presence of a high affinity agonist binding site. The BEST clinical trial that investigated chronic administration of bucindolol in patients with advanced heart failure, with left ventricular ejection fraction <35%, was stopped following interim analysis showing no significant difference in mortality in the overall study population compared with placebo (The

Beta-Blocker Evaluation of Survival Trial Investigators 2001). At the time of termination it was speculated that a possible reason for the lack of survival benefit from bucindolol, in contrast to that found for other β-blockers, could be different pharmacological properties (The Beta-Blocker Evaluation of Survival Trial Investigators 2001) but interestingly this study group described bucindolol as not having ISA.

Xamoterol is a selective (~ 100-fold β_1-AR vs β_2-AR) partial agonist at β_1-AR (ISA range 0–0.55 relative to isoprenaline 1.0, observed in a range of animal isolated cardiac preparations, Malta et al. 1985). The clinical trial, 'Xamoterol in severe heart failure', compared patients treated with xamoterol with placebo (The Xamoterol in severe heart failure study group 1990). The study had exclusion criteria including drugs with activity at β-AR. The trial was terminated after ~3 months due to excess deaths in the xamoterol group. It was concluded that xamoterol must be avoided in patients with severe heart failure. One suggested possible explanation for the excess mortality was β-AR agonist activity observed at night (The Xamoterol in severe heart failure study group 1990).

Maack et al. (2000) concluded that in general β-blockers with low ISA have better outcomes on mortality in heart failure patients compared with β-blockers with high ISA.

The foregoing discussion of β-blockers with ISA marks a point in time in their development and use in the context of heart failure and myocardial infarction. There is benefit with long-term use of β-blockers in patients with reduced LVEF (≤ 40%) (Desta et al. 2021); however, the question of whether long-term administration of β-blockers gives survival benefit to patients without heart failure remains to be determined (Desta et al. 2021).

4 Heart Failure: In Detail

For the rest of this chapter, we are going to focus on heart failure, β-AR, β-blockers and potential future improvements.

4.1 Human Heart Failure β_1-AR and β_2-AR: Expression Levels and Signalling

The emergence of β-AR radioligands with high affinity for β-ARs ([^{3}H] Dihydroalprenolol (DHA); [^{125}I]cyanopindolol (CYP), [^{3}H]CGP 12177; note [^{3}H] DHA has been superseded as a radioligand) and others (Bristow et al. 1982, 1986; Brodde 1991; Sarsero et al. 2003) made it possible to determine β-AR receptor densities in the heart. Competition binding experiments between radioligand and selective β_1-AR (betaxolol, bisoprolol, CGP 20712A) or β_2-AR antagonists (ICI 118,551) allowed the proportion (and therefore density) of β_1-AR and β_2-ARs to be determined (Brodde 1991). This work has been the focus of numerous laboratories that taken together helped towards understanding some effects of heart failure on

β-ARs, summarized and reviewed in detail (Brodde 1991; Brodde and Michel 1999). With access to non-failing (brain stem death, cadaver, pulmonary hypertension heart lung transplant with normal left ventricle) and end-stage heart failure hearts (idiopathic cardiomyopathy, ischemic cardiomyopathy), Mike Bristow's laboratory compared β-AR densities in the left ventricle of the two groups. There was ~50% lower density of β-ARs in the failing heart group compared to non-failing hearts. Later (Bristow et al. 1986), down-regulation of β-AR was attributed to selective down-regulation of β_1-AR in the failing left ventricle from patients with idiopathic dilated cardiomyopathy and failing *right* ventricle from patients with primary pulmonary hypertension. β_1-ARs were not down-regulated in the left ventricle of patients with isolated right ventricular failure due to primary pulmonary hypertension. The ratio of β_1-AR:β_2-AR in non-failing ventricle membrane homogenate preparations was 77:23 and in failing ventricle 60:38 (Bristow et al. 1986). Whilst absolute values may vary between laboratories, likely due to differences between hearts and experimental conditions, the concept of down-regulation of β_1-ARs associated with failure has been confirmed in a large number of laboratories (Brodde 1991).

In the study of Bristow et al. (1982), isolated right ventricular papillary muscles and left ventricular trabeculae were used to assess inotropic responsiveness to the non-selective β-AR agonist isoprenaline (Bristow et al. 1986; Brodde 1991). The maximal effect of isoprenaline was reduced in both left and right ventricular preparations (Bristow et al. 1982) whereas in the same preparations, maximal inotropic responses to histamine were not reduced so giving early insight into selective regulation and impairment of the β-AR signalling pathway compared to another G-protein coupled receptor. This assessment was further supported by comparing isoprenaline and histamine stimulated adenylyl cyclase activity that showed reduced potency and maximal responses to isoprenaline but maintenance of effects to histamine (Bristow et al. 1982).

This seminal study (Bristow et al. 1982) provided direction for future research into β-AR mechanisms in the failing human heart, facilitated by the expansion of heart transplant centres with the availability of human failing heart and on occasion 'donor' 'non-failing' hearts not transplanted but made available for research. Subsequently, determination of the contribution of β_1-AR and β_2-AR to inotropic responses in failing and non-failing hearts was made by the use of the full agonist isoprenaline, β_1-AR and β_2-AR subtype 'selective' partial agonists (denopamine β_1-AR 'selective'; zinterol β_2-AR 'selective') and antagonists (betaxolol β_1-AR 'selective'; ICI 118,551 β_2-AR 'selective') (Bristow et al. 1986). With these tools, it was shown that activation of both β_1-AR and β_2-AR mediated inotropic responses and that β_1-AR but not β_2-AR-mediated responses were reduced in heart failure (Bristow et al. 1986).

Subsequently, the mechanisms involved in β-AR desensitization were elucidated with the identification of G-protein receptor kinases (GRKs) and β-arrestins (Benovic et al. 1989; Lohse et al. 1990). The agonist-occupied β-AR is phosphorylated by GRK and subsequently bound by β-arrestins to uncouple it from Gsα-protein (Lohse et al. 1990). GRK2 (β-ARK1) mRNA and activity is increased in human heart failure (Ungerer et al. 1993, 1994). These events were

used to explain the loss of responsiveness (desensitization) of β_1-AR observed in human heart failure (Bristow et al. 1986; Ungerer et al. 1993, 1994). Table 1 provides a summary of β-AR subtype signalling in human heart and changes in heart failure.

The failing heart creates an environment of arrhythmogenicity associated with increased sympathetic nervous system tone, release of catecholamines and activation of β-ARs. These effects promote abnormal sarcoplasmic reticulum (SR) Ca^{2+} release through ryanodine receptors (RyR2) that is a major cause of cardiac dysfunction, lethal arrhythmias and remodelling in heart failure (Denniss et al. 2020). A brief description of cardiac excitation-contraction coupling is given to enable context for the involvement of β_1-AR and β_2-ARs in the human non-failing and failing heart and their role in arrhythmogenesis.

4.2 Excitation-Contraction Coupling, β-ARs and Heart Failure

Cardiac excitation-contraction coupling is the highly coordinated linking of electrical excitation of the myocyte to mechanical contraction (Bers 2002; Mayourian et al. 2018; Dashwood et al. 2020; Denniss et al. 2020). Excitation-contraction coupling in human atrium and ventricle is modulated by activation of β_1-AR and β_2-ARs producing an increased force of contraction and hastening of relaxation in failing and non-failing heart (Kaumann et al. 1999; Molenaar et al. 2000, 2007b).

Initiation of the action potential in the sinoatrial node (Chandler et al. 2009, 2011) and propagation through both atria, the atrioventricular conducting system (Dobrzynski et al. 2011; Greener et al. 2011) and finally to the ventricular free walls promotes coordinated contraction of atrium and then ventricle to pump blood through the body (Dashwood et al. 2020). During the action potential, Ca^{2+} enters the cell primarily through long-lasting depolarization activated L-type Ca^{2+} channels (Cav1.2), $I_{Ca,L}$ located in microdomains at sarcolemmal–sarcoplasmic reticulum (SR) junctions (Pelzmann et al. 1998; Bers and Perez-Reyes 1999; Bodi et al. 2005). Influx of Ca^{2+} through Cav1.2 raises local Ca^{2+} concentration to initiate Ca^{2+} release from the SR through Ca^{2+} release channels called ryanodine receptors (RyR2) (Bers 2002). Release of Ca^{2+} from the SR into the junctional space leads to regenerative RyR2 activation in a process classically described as 'Ca^{2+}-induced Ca^{2+} release' (Fabiato and Fabiato 1975, 1977, 1979; Fabiato 1983; Cannell et al. 2013). Consequently, cytosolic [Ca^{2+}] increases, diffuses through the myocyte to contractile sarcomeric proteins where it binds to Troponin C to initiate a signalling cascade causing force generation through actin-myosin cross-bridge cycling (Solaro and Rarick 1998; Morano 1999; Layland et al. 2005; Malik et al. 2011; Woody et al. 2018), the systolic phase of the contractile cycle. Relaxation of the myocyte occurs by diffusion of Ca^{2+} from the contractile proteins (TnC), transport into SR by ATP-dependent SR Ca^{2+} ATPase pump (SERCA2A) and transport out of the myocyte by the Na^+/Ca^{2+} exchanger to return Ca^{2+} concentrations in the SR and cytosol to precontractile levels (diastole) (Bers 2002). Disruption of this highly coordinated process results in arrhythmias (below).

Table 1 $\beta_{1,2,3}$AR signalling in heart and changes in heart failure

Signalling pathway	Effects	Heart failure
β_1AR		
Gsα-cyclic AMP-PKA	[a]PLB Ser16 phosphorylation (Molenaar et al. 2000, 2007b) PLB Thr17 phosphorylation (via CaMKII, Molenaar et al. 2000, 2007b) TnI phosphorylation (Molenaar et al. 2000, 2007b) C-protein phosphorylation (Molenaar et al. 2000, 2007b) Cav1.2 phosphorylation (Weiss et al. 2013 deduced)	↓β_1AR density (Bristow et al. 1986; Brodde 1991) Desensitization (Bristow et al. 1986, Brodde 1991) ↑ GRK2 (βARK1) mRNA and activity (Ungerer et al. 1993, 1994) ↓Inotropy (Bristow et al. 1986; Brodde 1991)
	RyR2 Ser 2808 phosphorylation (failing heart Molenaar et al. 2019) RyR2 Ser 2814 (via CaMKII failing heart Molenaar et al. 2019) ↑ $I_{Ca,L}$ (Molenaar et al. 2006) ↑Inotropy (Molenaar et al. 2000, 2007b) ↑lusitropy (Molenaar et al. 2000, 2007b) ↑dromotropy(Molenaar et al. 2000, 2007b) Arrhythmias (right atrium, Kaumann and Sanders 1993 Ventricle failing heart Lang et al. 2015) ↑RyR2 Po (failing heart Molenaar et al. 2019)	Arrhythmia only observed in failing heart (Lang et al. 2015)
β_2AR		
Gsα-cyclic AMP-PKA	PLB Ser16 phosphorylation (Molenaar et al. 2000, 2007b) TnI phosphorylation (Molenaar et al. 2000, 2007b) C-protein phosphorylation (Molenaar et al. 2000, 2007b) Cav1.2 phosphorylation (Weiss et al. 2013 deduced) RyR2 Ser 2814 (via CaMKII failing heart Molenaar et al. 2019) ↑ $I_{Ca,L}$ (Molenaar et al. 2006) ↑inotropy (Molenaar et al. 2000, 2007b) ↑lusitropy (Molenaar et al. 2000, 2007b) ↑dromotropy(Molenaar et al. 2000, 2007b) Arrhythmias (right atrium, Kaumann and Sanders 1993; Ventricle failing heart DeSantiago et al. 2008; Lang et al. 2015) ↑inotropy (mouse, rat heart) ↑apoptosis (mouse heart)	Arrhythmia only observed in failing heart (Lang et al. 2015)

(continued)

Table 1 (continued)

Signalling pathway	Effects	Heart failure
Giα (Human heart, Kilts et al. 2000) Giα	Human heart (Kilts et al. 2000) ↓inotropy (mouse, rat heart) ↓arrhythmia (rat heart, Xiao et al. 1995)	
Giα-Gβγ -PI3K-Akt	↓apoptosis (mouse, rat heart, Communal et al. 1999; Chesley et al. 2000; Zhu et al. 2001)	
β_3AR		
Gi/Go-protein – NO – PKG	↓inotropy (Gauthier et al. 1996, 1998) ↑ Na^+-K^+ ATPase pump current (*I*p) (rabbit heart Bundgaard et al. 2010)	↑β_3AR (monoclonal antibody, Moniotte et al. 2001)

[a] Studies quoted are human except where indicated

4.3 Human Heart β_1-AR and β_2-AR Modulation of Excitation-Contraction Coupling

In the human heart, activation of β_1-AR and β_2-ARs causes increases in force of contraction and hastening of relaxation (Fig. 1, Kaumann et al. 1999; Molenaar et al. 2000, 2007a, b). The duration of contraction is shorter following activation of either β_1-AR or β_2-AR (Kaumann et al. 1999; Molenaar et al. 2000, 2007b). These changes are due to increased Ca^{2+} cycling; increased Ca^{2+} influx into the myocyte through Cav1.2, increased release of Ca^{2+} from the SR through RyR2 resulting in greater binding to TnC and force of contraction and accelerated transport of Ca^{2+} into SR by SERCA2A. These effects result from coupling of β_1-AR and β_2-AR to the Gs-α-cyclic AMP-PKA pathway with PKA-dependent phosphorylation of proteins responsible for increasing the force of contraction and hastening relaxation. Activation of either β_1-AR or β_2-AR enhances $I_{Ca,L}$ in human atrium (Molenaar et al. 2006). β-AR enhancement of $I_{Ca,L}$ through Cav1.2 is mediated by PKA (reviewed Weiss et al. 2013), although the specific PKA phosphorylation site(s) critical for enhanced $I_{Ca,L}$ have proved difficult to identify (for further reading, see Weiss et al. 2013; Minobe et al. 2014; Roybal et al. 2020). Inhibition of Cav1.2 with L-type Ca^{2+} channel antagonists reduces β-AR-mediated inotropic responses in human heart (Sarsero et al. 1998; Angus et al. 2000). β-AR-mediated effects on RyR2 are due at least in part to enhanced $I_{Ca,L}$ (to cause Ca^{2+}-induced RyR2 Ca^{2+} release) and phosphorylation of RyR2 (PKA, Ser2808 and possibly 2830; CaMKII, Ser 2814; Denniss et al. 2020). Li et al. (2013) observed that acute activation of β-ARs with isoprenaline in rat heart increased open probability of RyR2 (Po), 20-fold during diastole and three-fold during systole. These studies provide important mechanistic information as increased RyR2 open probability during diastole would be expected to increase risk of delayed after depolarization (DAD) arrhythmias during β-AR activation (see below). In human heart, activation of either β_1-AR or β_2-AR

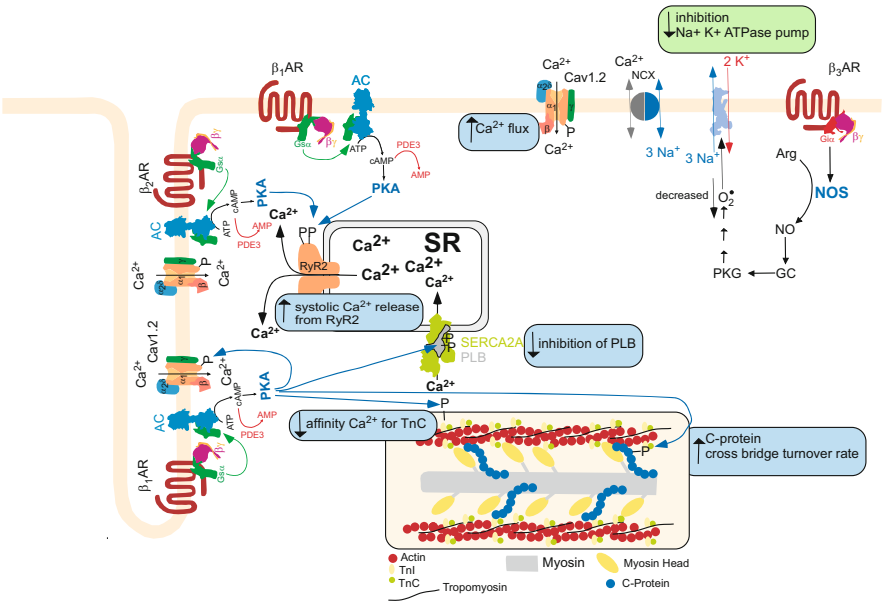

Fig. 1 β_1-AR, β_2-AR, β_3-AR signalling in human heart. Shown is a stylized cartoon of a human cardiac myocyte with t-tubule. There is strong evidence that both β_1-AR and β_2-AR are coupled to the Gsα-protein – cyclic AMP – PKA pathway with phosphorylation of key proteins responsible for mediating increases in contractility and hastening of relaxation in atrium and ventricle of failing and non-failing hearts. Key proteins phosphorylated by PKA by both β_1-AR and β_2-AR indicated by representative blue lines and effects in blue boxes:- L-type calcium channel (Cav1.2) increases Ca^{2+} flux which in turn causes greater Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum (SR) through the ryanodine receptor (RyR2); RyR2 increases systolic Ca^{2+} release; Troponin I (TnI) which reduces the affinity of Ca^{2+} for Troponin C (TnC); C-protein accelerates cross-bridge turnover rate, increases rate of force development and hastens relaxation; phospholamban (PLB) reduces inhibition of SERCA2A, the ATP-dependent Ca^{2+} pump which pumps Ca^{2+} from the cytosol to SR. Note activation of β_1-AR and β_2-AR causes CaMKII-mediated phosphorylation of RyR2 and PLB. β_1-AR and β_2-AR are shown localized to the T-tubule but only β_1-AR to the plasma membrane in non-failing heart as described by Nikolev et al. (refer to text for further explanation on localization and redistribution of β_2-AR in failing heart). PDE3 controls ventricular inotropic and lusitropic effects mediated through activation of β_1-AR and β_2-AR in patients treated with metoprolol or carvedilol but not in heart failure patients not treated with a β-blocker. β_3-AR is coupled to the inhibitory Giα-protein–NOS–NO–PKG pathway to reverse cardiac Na^+-K^+ ATPase pump inhibition by decreasing pump glutathionylation and reduce high cytosolic Na^+ concentrations in heart failure. β_3-AR information from the laboratories of Gauthier, Bundgaard and Rassmussen (for references and further explanation, see text)

phosphorylates sarcomeric proteins, C-protein (Kaumann et al. 1999; Molenaar et al. 2007b) and troponin I. C-protein is a sarcomeric protein that interacts with titin, myosin and actin to regulate sarcomeric protein assembly, structure and function (Barefield and Sadayappan 2010). Phosphorylation of C-protein accelerates cross-bridge turnover rate (Barefield and Sadayappan 2010), increases rate of force development and hastens relaxation (McNamara et al. 2019). Activation of β_1-AR

and β_2-ARs phosphorylates troponin I in human heart (Kaumann et al. 1999; Molenaar et al. 2007b). Troponin I is an inhibitory protein and part of the troponin protein complex (Layland et al. 2005). PKA phosphorylation of troponin I (Ser22, Ser23) decreases affinity of Ca^{2+} for troponin C, increases the dissociation of Ca^{2+} from troponin C and decreases myofilament Ca^{2+} sensitivity to hasten relaxation (Layland et al. 2005). Phosphorylation may also contribute to a β-AR-mediated positive inotropic effect by enhancing cross-bridge cycling rate and shortening velocity (Layland et al. 2005). Activation of both β_1-AR and β_2-AR in the human heart phosphorylates phospholamban Ser16 (PKA) and Thr17 (CaMKII) (Molenaar et al. 2000, 2007b). Phospholamban is an inhibitor of Ca^{2+} transport into the SR by SERCA2A and phosphorylation reduces the inhibitory effect (Koss and Kranias 1996).

Considerable progress in the knowledge of β-ARs and their signalling pathways has been made from research carried out in animals, isolated organs, tissues and cells. This is considered briefly in the next section with emphasis on specific examples of β_2-AR signalling in the heart that are of interest for human heart disease.

4.4 β_2-AR Signalling in Mammalian Heart

In failing and non-failing human hearts β_2-ARs are tightly coupled to the Gs-α-protein–cyclic AMP–PKA pathway with maximal β_2-AR-mediated inotropic and lusitropic effects equal to or nearly equal to maximal β_1-AR mediated effects. However, this is at variance to some other species including rat, mouse and guinea-pig (Molenaar and Summers 1987; Xiao et al. 1994, 1995; Xiao et al. 1999). In rat ventricular cardiomyocytes, the striking features of the contractile response to activation of β_2-AR were the absence of shortening of the duration of contraction, no acceleration of the Ca^{2+} transient or phosphorylation of phospholamban unlike that observed for activation of β_1-AR (Xiao et al. 1994, 1995). In mouse heart, activation of β_2-AR mediates a small inotropic effect (mouse left atrium, Bond et al. 1995) or no increase (mouse ventricular cardiomyocytes, wild type and TG4 (~ 200-fold β_2-AR overexpression) Xiao et al. 1995) and no increase in $I_{Ca,L}$ (wild type, TG4, Xiao et al. 1999; Heubach et al. 2001). These results were interpreted as the β_2-AR (but not β_1-AR) simultaneously coupling to both Gsα-protein and pertussis toxin-sensitive Giα-proteins (Xiao et al. 1995; Xiao et al. 1999), specifically Giα2 and Giα3 in mouse ventricle (Xiao et al. 1999). Simultaneous coupling causes an increase in cyclic AMP via Gsα-protein and decrease via Giα-protein resulting in opposing effects (increase/decrease) on contractility, that is the β_2-AR Giα-protein signalling pathway prevents β_2-AR-Gsα-protein-mediated cardiostimulation. In left atrium and right ventricle from TG4 hearts (260–435-fold overexpression of human β_2-adrenoceptors), low concentrations (nM) of adrenaline increased contractility but 1,000-fold higher concentrations (μM) decreased contractility in a pertussis toxin-sensitive manner (Heubach et al. 2004). In the same study, noradrenaline increased contractility at low concentrations, but did not cause cardiodepression at higher concentrations indicating a lack of ability of noradrenaline to stabilize the β_2-AR-

Giα-protein signalling pathway in TG4 mouse heart (Heubach et al. 2004). Furthermore, concentrations of adrenaline that reduce contractility (1 and 10 μM) antagonized the positive inotropic effects of noradrenaline in left atrium and right ventricle (Heubach et al. 2004). This also reveals a critical structure-activity relationship with noradrenaline differing from adrenaline by only one N-substituted methyl group. However, this property of adrenaline in the TG4 mouse heart β_2-AR differs from that in human non-failing and failing ventricle where β_2-AR-mediated increases in inotropic responses are stable at concentrations up to 600 μM (Kaumann et al. 1999; Molenaar et al. 2000, 2013).

Does the ability of β_2-AR to couple to Giα-protein have any effect on arrhythmia generation? Spontaneous (arrhythmic) contractions in rat ventricular cardiomyocytes mediated by activation of β_2-AR in the presence of pertussis toxin were more frequent than in the absence of pertussis toxin (Xiao et al. 1995) giving rise to the suggestion of a β_2-AR-cyclic AMP-mediated arrhythmia pathway of relevance to human heart (see below).

Apoptosis occurs in ischaemia-reperfusion and in heart failure (Foo et al. 2005). Low rates of cardiomyocyte apoptosis that occur in human heart failure may have a causal role (Foo et al. 2005). In adult rat (Communal et al. 1999) and hypoxic (Chesley et al. 2000) or non-hypoxic murine ventricular myocytes (Zhu et al. 2001), activation of β_1-AR was pro-apoptotic whilst activation of β_2-AR caused inhibition of apoptosis through a pertussis toxin-sensitive (Communal et al. 1999; Chesley et al. 2000) increase in PI3K activity and phosphorylation of Akt (Chesley et al. 2000). Stimulation of the β_2-AR-Gi-Gβγ -PI3K-Akt prosurvival signalling pathway opposed β_1-AR-Gsα-mediated apoptosis (Communal et al. 1999; Chesley et al. 2000; Zhu et al. 2001). Furthermore, activation of β_2-ARs prevented H_2O_2 (oxidation)-induced apoptosis and ventricular myocyte loss (Chesley et al. 2000). Activation of β_2-ARs did however induce myocyte loss and apoptosis, but only after pertussis toxin inhibition of Giα (Zhu et al. 2001).

Taken together, the investigation of the role of β_2-AR signalling pathways in apoptosis in mouse and rat heart was consistent with simultaneous coupling of β_2-AR to Gsα and Giα. Discovery of the β_2-AR anti-apoptotic (survival) pathway in isolated cardiomyocytes was tested in the more complex rat model of dilated ischaemic cardiomyopathy induced by coronary artery ligation (Ahmet et al. 2004). Following coronary artery ligation, chronic administration of the β_2-AR agonists zinterol or fenoterol attenuated the progression of heart failure, reduced apoptosis, attenuated left ventricular dilation and improved diastolic and systolic function (Ahmet et al. 2004). It was pointed out by the authors that fenoterol did not promote coupling of the β_2-AR to Giα that indicates complexity of β_2-AR signalling and poses further questions about the identification of signalling pathways responsible for overall benefit and their applicability to human heart failure.

In studies carried out in rat cardiomyocytes, it was shown that β_1-AR and β_2-AR signalling is compartmentalized and modified by heart failure (Nikolaev et al. 2010). Using a combination of high resolution live cell SICM (scanning ion conductance microscopy) with localized measurement of cyclic AMP production using FRET it was shown that activation of β_1-AR increased cyclic AMP in both the 'cell crest' and

T-tubule whereas activation of β_2-AR only increased cyclic AMP in the T-tubule. Myocardial infarction-induced heart failure in rats changed β_2-AR signalling resulting in more uniform increases in cyclic AMP in both the cell crest and T-tubule, interpreted as being due to redistribution of β_2-AR from the T-tubules to the crest (Nikolaev et al. 2010). This study nicely demonstrates β_1-AR, β_2-AR signalosomes in the heart and the effect of heart failure.

An interesting, detailed review and perspective on β_2-AR signalling in heart, mostly based on animal studies is given in the context of heart disease (Woo et al. 2015). Of significance is the early finding of Kilts et al. (2000) in human atrium that β_2-AR (but not the β_1-AR) can couple to Giα-protein.

4.5 Human Heart β_3-AR

Historically, knowledge and understanding of human heart β-ARs changed with the report of a 'functional' cardiodepressant β_3-AR (Gauthier et al. 1996). At the same time, it created interest in a possible role for β_3-AR in heart failure (Gauthier et al. 1996). On the basis of early studies (Gauthier et al. 1996, 1998; Moniotte et al. 2001), it was proposed that a β_3-AR antagonist could provide a therapeutic option for heart failure (Moniotte et al. 2001; Moniotte and Balligand 2002), but it was a β_3-AR agonist, mirabegron, that eventually and opportunistically progressed to clinical trials (see below) after more research over the ensuing decades.

The report of a 'functional' β_3-AR that mediated cardiodepressant effects in human heart was made from experiments carried out in right ventricular endomyocardial biopsies obtained from patients who had received a heart transplant or were undergoing open heart surgery (Gauthier et al. 1996, 1998). A key strategy in experimental design was to carry out functional experiments testing agonist responses in the presence of the β-AR antagonist nadolol (Gauthier et al. 1996, 1998). At the time, there was an understanding that nadolol blocked β_1-AR and β_2-AR but had 'no β_3-AR antagonist properties' (Gauthier et al. 1996) or 'low affinity for β_3-AR' (Gauthier et al. 1998). Nadolol was reported to have an affinity (pA_2) of 4.3–4.7 for an adrenoceptor other than β_1-AR or β_2-AR in guinea-pig ileum (Bond and Clarke 1988) and have no antagonist effect at a concentration of 100 μM for the cloned human β_3-AR (Emorine et al. 1989). With this information for nadolol, observed agonist responses in human heart that could not be accounted for by competitive antagonism of nadolol at β_1-AR or β_2-AR would be caused by a receptor other than β_1-AR or β_2-AR (Gauthier et al. 1996, 1998). However later it was determined that the affinity of nadolol at the human β_3-AR expressed in CHO cells was pK 6.2–6.3 (Candelore et al. 1999; Baker 2005). Thus, the concentration of nadolol (10 μM) used (Gauthier et al. 1996, 1998) would block ~100% β_1-AR, β_2-AR and ~ 95% β_3-AR.

Micro Molar concentrations of isoprenaline and noradrenaline in the presence of 10 μM nadolol unexpectedly reduced contractile force of right ventricular endomyocardial biopsies (Gauthier et al. 1996, 1998). The 'β_3-AR agonists', BRL 37344, SR 58611, CL 316243 and CGP 12177 also reduced contractile force. The

β-blockers nadolol and metoprolol (blockade of β_1-AR, β_2-AR, Baker 2005; Molenaar et al. 2013) did not affect the response to BRL 37344 but 1 μM bupranolol which blocks β_1-AR, β_2-AR and β_3-AR (Baker 2005) caused competitive blockade (Gauthier et al. 1996). The action potential was shortened and the duration of contraction reduced, suggesting the possibility of increased K^+ channel activity, decreased L-type Ca^{2+} channel activity or activation of a Cl^- repolarizing current through CFTR channels (Kaumann and Molenaar 1997; Moniotte and Balligand 2002). The cardiodepressant effect of BRL 37344 was reduced in the presence of pertussis toxin indicating coupling through Gi/o protein (Gauthier et al. 1996). It was then shown that the negative inotropic effects of BRL 37344 and noradrenaline were mediated through the endothelial nitric oxide synthase (NOS3)–NO–cyclic GMP pathway (Gauthier et al. 1998). On a puzzling note, confirmation of a cardiodepressant effect mediated through β_3-AR was unable to be made using intact right ventricular trabeculae and the same β_3-AR agonists used by Gauthier including BRL 37344, SR 58611, CL 316243 and additionally ZD2079 (Kaumann and Molenaar 1997; Molenaar et al. 1997) or in human ventricular myocytes (Harding 1997).

At this point it may be helpful and informative to comment briefly on the pharmacology of the 'β_3-AR agonists', BRL 37344 and CGP 12177 used to support the classification of 'β_3-AR' that mediated the cardiodepressant effect of noradrenaline and isoprenaline (Gauthier et al. 1996, 1998). BRL 37344 is 0–21-fold selective for human β_3-AR vs β_2-AR and 15–88-fold selective for human β_3-AR vs β_1-AR on the basis of affinity (Sennitt et al. 1998; Hoffmann et al. 2004). In human right atrium, in the presence of 10 μM IBMX, a non-selective PDE inhibitor, BRL 37344 increased contractile force through β_1-AR and β_2-AR but not β_3-AR (Christ et al. 2011). Therefore, the use of BRL 37344 to unambiguously identify effects in human heart mediated by β_3-AR is somewhat complicated by its ability to activate β_2-AR and β_1-AR. It requires experiments in the absence and presence of antagonists to block β_1-AR and β_2-AR at lower concentrations than those required to block β_3-AR (Lönnqvist et al. 1993). In separate experiments (Gauthier et al. 1996), bupranolol was used to block β_1-AR, β_2-AR and β_3-AR and a rightward shift of the concentration-effect curve for BRL 37344 was observed corresponding to an affinity of bupranolol for β_3-AR (pK_B 6.88 Gauthier et al. 1996 vs pK_D 7.04 Baker 2005). Whilst this is the case, it is important to note that the affinity of bupranolol for β_3-AR is similar to its affinity for the low affinity binding site of the β_1-AR (β_{1L}-AR, Kaumann and Molenaar 2008). Low concentrations of CGP 12177 block β_1-AR and β_2-AR and much higher concentrations (~ 100-fold higher) activate a low affinity, relatively propranolol-resistant binding site of the β_1-AR (β_{1L}-AR) (Kaumann and Molenaar 2008). The concentrations that activate β_{1L}-AR are similar to those that activate human β_3-AR, for example in CHO cells expressing β_3-AR or adipose tissue (Lönnqvist et al. 1993; Sennitt et al. 1998), where CGP 12177 is a partial agonist. In human right atrium, (−)-CGP 12177 (2–20 nM) reduced contractile force, most likely due to blockade of the effects of endogenously released (−)-noradrenaline on β_1-AR (Sarsero et al. 2003). Gauthier et al. (1996) also observed a cardiodepressant effect of CGP 12177 at 10 nM (and up to 100 μM) but attributed it to β_3-AR. In

human right atrium, higher concentrations of 60 nM–200 nM caused a small increase in contractile force due to activation of β_{1L}-AR, after which it continued to reduce contractile force (Sarsero et al. 2003). The lack of confirmation for a cardiodepressant effect mediated through activation of β_3-AR (Kaumann and Molenaar 1997; Molenaar et al. 1997) was not simply due to the use of endomyocardial biopsies vs trabeculae since trabeculae (left ventricular trabeculae) were used in the study of Moniotte et al. (2001).

In studies comparing inotropic responses in human failing and non-failing hearts it was observed that both positive inotropic responses to isoprenaline (β_1-AR, β_2-AR) and negative inotropic responses to BRL 37344 (β_3-AR, confirmed with the β_3-AR antagonist L748,337) were reduced in failing vs non-failing hearts but the reduction was more 'prominent' for isoprenaline. These data were associated with a decrease in β_1-AR mRNA, no change in β_2-AR mRNA and an increase in β_3-AR detected by monoclonal antibody in the failing heart (Moniotte et al. 2001). The density of β_3-AR in heart relative to β_1-AR and β_2-AR is very low. Michel et al. (2020) reported that β_3-AR comprise approximately 3% of the total population of β-ARs in human heart 'under physiological conditions'. Similarly, in rat heart, β_3-AR comprise 8% of the total population of β-ARs, based on Western blot data (Dincer et al. 2001). Other techniques used for the measurement of β_1-AR, β_2-AR and β_3-AR (mRNA measurements for β_1-AR and β_2-AR, Western blotting for β_3-AR) do not allow the determination of the relative percentage of β_3-ARs to the total population of β-ARs in the human failing heart (Moniotte et al. 2001). However, based on Western blot data, the density of β_3-ARs was increased by ~three-fold and 1½ – fold in ischaemic and idiopathic dilated cardiomyopathy failing hearts, respectively (Moniotte et al. 2001), although Fischer et al. (2008), using a radioligand binding assay found no difference in densities in human non-failing and failing hearts. The β_3-AR is upregulated in heart in animal models of disease including heart failure and diabetes (Dal Monte et al. 2020). A very interesting and unifying hypothesis for upregulation of β_3-AR across multiple tissues and cell lines, i.e. not only heart, is the presence of hypoxia (Dal Monte et al. 2020). It was proposed that hypoxia could cause activation and translocation of HIF-1 to the nucleus and stimulate β_3-AR transcription (Dal Monte et al. 2020). Alternatively, under hypoxic conditions, the β_3-AR may undergo reduced proteasomal degradation (Dal Monte et al. 2020). It is intriguing to understand the purpose of upregulation of β_3-AR in cardiac ischaemia and whether it confers benefit.

The studies of Moniotte et al. (2001) formed the basis of speculation that a β_3-AR antagonist could correct the 'disordered adrenergic regulation of the failing heart'.

The discovery that β_3-AR agonists could reverse cardiac Na^+-K^+ ATPase pump inhibition and potentially abnormally high cytosolic Na^+ concentrations has consequences for heart failure (Fig. 1, Bundgaard et al. 2010). In rabbit ventricular cardiomyocytes, β_3-AR agonists (BRL37344, CL316,243) and noradrenaline increased Na^+-K^+ ATPase pump current in the presence of nadolol (β_1-AR, β_2-AR antagonist, used at a concentration of 1 μM which would block ~60% β_3-AR, Baker (2005)) but not L748,337 (β_3-AR antagonist) or NOS inhibition (L-NAME) (Bundgaard et al. 2010). Together, with additional complementary approaches a

β_3-AR-NOS-NO-guanylyl cyclase mechanism was validated (Bundgaard et al. 2010), consistent with the cardiac β_3-AR signalling pathway elucidated by Gauthier et al. (1998). Activation of β_3-ARs decreased Na^+-K^+ ATPase β_1 subunit glutathionylation, an oxidative post-translational modification that inhibits the Na^+-K^+ ATPase pump (Bundgaard et al. 2010). It was argued that upregulation of β_3-AR in heart failure may serve a beneficial purpose to transport excess Na^+ out of the myocyte (Bundgaard et al. 2010). Reduced Na^+-K^+ ATPase pump current in a rabbit model of heart failure (coronary artery ligation) was restored by 3-day infusion of β_3-AR agonist CL316,243 (Fry et al. 2020). Furthermore CL316,243 reduced heart failure-induced organ congestion (Fry et al. 2020). Elevated $[Na^+]$ is a pathological characteristic of human heart failure (Pieske and Houser 2003). Elevated $[Na^+]i$ increases Na^+/Ca^{2+} exchanger reverse mode (Ca^{2+} in/Na^+ out) and contributes to increased diastolic $[Ca^{2+}]i$, together with diastolic RyR2 Ca^{2+} leak from the SR in the failing heart (Pieske and Houser 2003; Walweel et al. 2017, 2019; Denniss et al. 2020). An increase in diastolic Ca^{2+} is likely to increase risk of delayed after depolarizations and arrhythmias (Walweel et al. 2017; Denniss et al. 2020).

4.6 β_3-AR Therapeutics

The approved use of the β_3-AR agonist mirabegron for management of overactive bladder syndrome is facilitating its use in heart failure trials. The BEAT-HF (Beta 3 Agonists Treatment in HF) was the first randomized trial of a β_3-AR agonist in patients with chronic heart failure (Bundgaard et al. 2017). 70 patients taking β-AR blockers (to prevent cardiostimulant effects mediated through the β_1-AR (above, Mo et al. 2017) with NHYA II-III ischaemic or non-ischaemic stable heart failure, LVEF <40% on echocardiography were enrolled with a primary endpoint of increase in LVEF after 6 months treatment with escalating mirabegron dosing to target 150 mg twice daily. The primary endpoint, an increase in LVEF was not met.

With the approval of the use of the β_3-AR agonist mirabegron for the management of overactive bladder syndrome it was imperative to understand its effects on human heart. The availability of mirabegron provided another opportunity, using a new agonist to investigate the effects of activation of β_3-AR directly on human heart. In human atrium mirabegron increased contractile force through β_1-AR but not β_3-AR, prevented by neuronal uptake blockers, desipramine or phenoxybenzamine (Mo et al. 2017) suggesting a neuronal but not β_3-AR mechanism. Furthermore, a non-specific cardiodepressant effect observed in the presence of β_1-AR blockade was not mediated through β_3-ARs (Mo et al. 2017). An informative and detailed review of β_3-AR inotropic effects in human heart is given, noting unresolved discrepancies (Michel et al. 2020).

4.7 Heart Failure Arrhythmias

Heart failure fosters a rich environment for generation of potentially lethal ventricular arrhythmias. Ventricular arrhythmias account for approximately 50% of deaths in

patients with heart failure (Mozaffarian et al. 2007). Sudden, unexpected death in clinically stable heart failure patients without a clear non-cardiovascular cause is presumed to be caused by a cardiac arrhythmia (Mozaffarian et al. 2007). Activation of the sympathetic nervous system contributes to the genesis of dangerous ventricular arrhythmias. Patients with a history of sustained ventricular tachycardia or fibrillation, at high risk of recurrent ventricular arrhythmias and sudden death, had elevated cardiac noradrenaline spillover due to activation of cardiac sympathetic nerves (Meredith et al. 1991).

Increased sympathetic nervous system activity (Esler et al. 1997; Grassi et al. 2004), noradrenaline concentration (Cohn et al. 1984; Esler et al. 1997) and activation of β-ARs in the failing heart all contribute to increased risk of arrhythmias (Packer 1985; Pogwizd et al. 2001; Pogwizd and Bers 2004; Lang et al. 2015). Sympathetic nervous system activity in heart failure increases with decreasing left ventricular ejection fraction (Seravalle et al. 2019). In addition, adrenaline levels are elevated in heart failure (Kaye et al. 1995; Esler et al. 1997) raising the prospect of activation of β_2-AR as well as β_1-AR in the failing heart.

The possibility that β_2-ARs could mediate arrhythmias in human failing heart was supported by observations of β_2-AR-mediated arrhythmias in an in vitro human right atrial model of arrhythmia (Kaumann and Sanders 1993) and coupling of β_2-AR to the Gsα-cyclic AMP-PKA pathway in human failing ventricle (Kaumann et al. 1999). It was then demonstrated that both β_1-AR (Lang et al. 2015) and β_2-AR (DeSantiago et al. 2008; Lang et al. 2015) are arrhythmogenic in human failing heart. Activation of β_1-AR or β_2-AR increased the frequency of ectopic activity in failing but not in non-failing (donor) hearts (Lang et al. 2015). Both β_1-AR and β_2-AR induced premature ventricular contractions originating mostly from Purkinje fibres in the endocardium (Lang et al. 2015). Interestingly Lang et al. (2015) concluded that activation of β_2-AR is more arrhythmogenic than β_1-ARs in the left ventricle of the failing heart. In a rabbit aortic constriction model of heart failure, DeSantiago et al. (2008) showed that activation of β_2-AR by zinterol (selective β_2-AR agonist) caused ventricular arrhythmias (in vivo). In left ventricular myocytes from rabbit failing heart zinterol caused after contractions and increased cell shortening, Ca^{2+} transient amplitude, SR Ca^{2+} load, Ca^{2+} transient decline and phosphorylation of PLB Ser16. Zinterol was more arrhythmogenic in the failing than in the non-failing heart. In human left ventricular myocytes from failing hearts, zinterol induced after contractions, increased SR Ca^{2+} load and hastened the Ca^{2+} transient consistent with rabbit heart failure studies (DeSantiago et al. 2008). The rabbit model of heart failure used in these studies replicates the sudden death observed in human heart failure (DeSantiago et al. 2008).

The increased ability of β_2-AR to cause arrhythmias, increase cell shortening, Ca^{2+} transients, SR Ca^{2+} load, hasten the Ca^{2+} transient and phosphorylate (Ser16) phospholamban in failing but not non-failing hearts (DeSantiago et al. 2008) may in part be due to reduced regulatory PDE4 (phosphodiesterase) control of cyclic AMP accumulated in the localized region of RyR2 (Berisha et al. 2021). The later finding of Berisha et al. (2021) that β_2-AR mediated increases in cyclic AMP localized to RyR2 in human failing but not non-failing ventricular myocytes attributed to

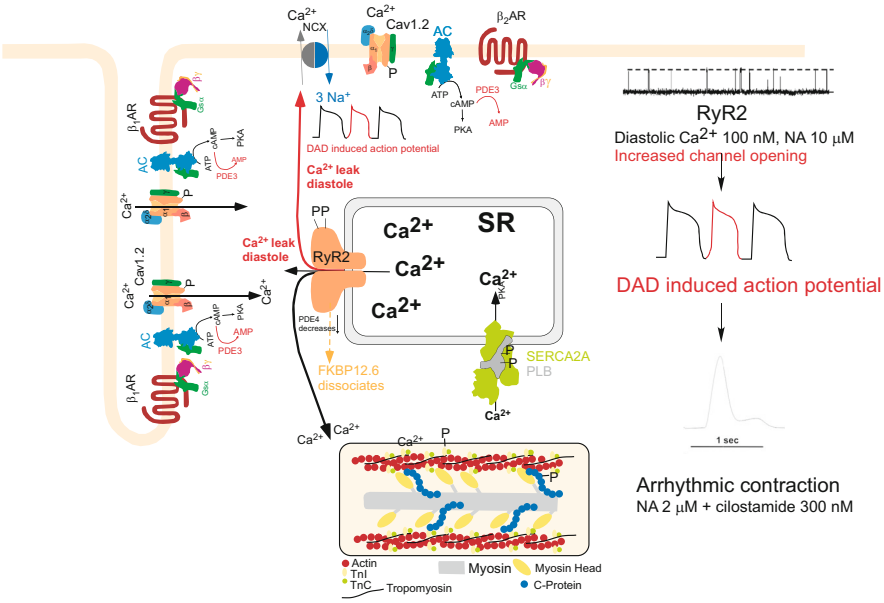

Fig. 2 The arrhythmic human failing heart in diastole. Rhythmic contraction relies on coordinated cycling of Ca^{2+} in and out of the myocyte and sarcoplasmic reticulum. RyR2 becomes remodelled in the failing heart, characterized by spontaneous diastolic Ca^{2+} 'leak' (red arrow from RyR2), generation of DADs in phase 4 and action potential (shown in red) causing an arrhythmic contraction (shown as an original recording in the presence of (−)-noradrenaline 2 μM and PDE3 inhibitor cilostamide (300 nM)). Black arrows from RyR2 represent Ca^{2+} release in response to DAD. The failing human heart RyR2 is characterized by reduced levels of bound FKBP12.0 12.6, PP1, PP2a, reduced PDE4 and hyperphosphorylation of Ser2808. RyR2 shows increased probability (P_o) of RyR2 diastolic channel opening. Activation of β_1-AR and β_2-AR causes phosphorylation of Ser2808 (and CaMKII phosphorylation of Ser2814), increased oxidation, channel opening and arrhythmic contractions in a human ventricular trabeculae model of arrhythmia

reduced PDE4 localized to RyR2 may provide another explanation for *increased* β_2-AR arrhythmogenesis in human failing ventricle.

β_2-AR activation increases the difference between the duration of the Ca^{2+} transient duration and duration of the action potential in failing heart epicardium and midmyocardium (but not endocardium) causing cytosolic Ca^{2+} concentration to remain high after complete repolarization, creating conditions for DADs (Lang et al. 2015). PKA Ser-16 and CaMKII phosphorylation of phospholamban (Kaumann et al. 1999) and increased SERCA activity contribute to SR Ca^{2+} overload (DeSantiago et al. 2008) which in turn may trigger store overload-induced Ca^{2+} release from heart failure modified RyR2s (DeSantiago et al. 2008; Zhou et al. 2011; Walweel et al. 2017; Denniss et al. 2020) leading to DADs and triggered arrhythmias (Schlotthauer and Bers 2000; Pogwizd and Bers 2004). In human failing right ventricular trabeculae, activation of either β_1-AR or β_2-AR causes arrhythmic contractions in a model of ventricular arrhythmia, RyR2 channel opening and

phosphorylation at RyR2 Ser2808 and 2814 (Molenaar et al. 2019). Figure 2 summarizes β_1-AR, β_2-AR arrhythmia mechanisms in the human failing heart.

RyR2 forms a macromolecular complex with SR luminal and cytoplasmic proteins which modulate function in the non-failing and failing human heart (Marx et al. 2000; Denniss et al. 2020). The RyR2 complex includes the FK506 binding proteins, FKBP12.0, FKBP12.6, mAKAP bound PKA, protein phosphatases, PP1 and PP2a (Marx et al. 2000; Reiken et al. 2003; Denniss et al. 2020). RyR2 bound levels of FKBP12.0, 12.6, PP1, PP2a were reduced in human failing heart (Marx et al. 2000; Walweel et al. 2017). RyR2 Ser2808 is hyperphosphorylated by PKA in failing human hearts compared to non-failing hearts (Marx et al. 2000; Reiken et al. 2003; Walweel et al. 2017). Phosphorylation levels were increased further in heart failure patients receiving β-AR agonist (dobutamine) treatment (Marx et al. 2000), indicating that RyR2 whilst hyperphosphorylated in failing hearts, not all 4 Ser2808 of the RyR2 tetramer were phosphorylated (Marx et al. 2000; Reiken et al. 2003). Phosphorylation of RyR2 Ser2808 causes dissociation of FKBP12.6 from RyR2 and increased probability (P_o) of RyR2 diastolic channel opening (Marx et al. 2000; Reiken et al. 2003; Walweel et al. 2017).

The effect of implantation of left ventricular assist devices (LVADs) into heart failure patients provided further information of RyR2 regulation in heart failure. LVADs are increasingly used as a bridge to heart transplantation in patients with advanced heart failure. LVADs result in a lowering of left ventricular pressures. The surgical LVAD procedure involves removal of a left ventricular apex 'core' at the time of implantation, giving unique opportunity for comparisons between the left ventricle at the time of implantation (core) vs the explanted heart after a period of unloading of the heart. Interestingly RyR2 Ser2808 phosphorylation levels and open channel probability (P_o at diastolic [Ca^{2+}], 50 nM) in the explanted heart at the time of heart transplantation were returned to non-failing heart values (Marx et al. 2000). Correspondingly, β-AR-mediated contractile responses in left ventricular trabeculae were greater in the explanted LVAD heart (~ 2 months LVAD) compared to left ventricular trabeculae taken from the 'core' (pre LVAD sample), intriguingly interpreted at least in part due to normalization of RyR2 (Marx et al. 2000).

Whilst β_2-AR have the capability to mediate ventricular arrhythmias in the human failing heart, the relevance of β_2-AR in heart failure therapy has been questioned (Bristow et al. 2003). The context at the time of writing (Bristow et al. 2003) was the Carvedilol Or Metoprolol European Trial (COMET, Poole-Wilson et al. 2003) that compared carvedilol and metoprolol tartrate in heart failure patients, New York Heart Association Class II-IV, LVEF <35% optimally treated with diuretics and angiotensin-converting enzyme inhibitors (Poole-Wilson et al. 2003). The primary endpoints were all-cause mortality (Poole-Wilson et al. 2003). A contention of COMET was that carvedilol blocks β_1-AR, β_2-AR and α_1-AR whilst metoprolol has high specificity for β_1-AR (Poole-Wilson et al. 2003), but later studies showed metoprolol is only ~2.5-fold selective for β_1-AR vs β_2-AR (Baker 2005; Molenaar et al. 2013). Using the mass action equation to determine occupancy of β_1-AR, β_2-AR and examples of different concentrations of noradrenaline and adrenaline that occur in heart failure, together with affinities of noradrenaline and adrenaline it was

concluded that the occupancy of β_2-AR would be <10% (7.2% with highest concentrations of noradrenaline 50 nM and adrenaline 0.4 nM). It was concluded that it would be difficult to detect differences between selective β_1-AR blockers and carvedilol when administered at doses that cause equal blockade (occupancy) of β_1-AR. The question of possible relevance of β_2-AR was further considered hypothetically for heart failure patients where it was predicted that patients receiving a PDE3 inhibitor acutely and metoprolol during the presence of adverse stress-induced surges of adrenaline (Molenaar et al. 2013). In this specific circumstance, metoprolol sensitizes β_2-AR and PDE3 inhibition potentiates β_2-AR mediated responses (Molenaar et al. 2013).

4.8 Therapeutic Role of β-adrenoceptor Antagonists (β-blockers) and Agonists in the Management of Heart Failure: A Focus on Carvedilol

The unifying feature of β-blockers used for heart failure treatment is blockade of β_1-ARs; however, interesting and intriguing pharmacological differences between them have emerged, particularly with carvedilol. For this purpose, the next section discusses carvedilol in more depth.

4.8.1 Carvedilol

Chronic administration of carvedilol reduces the risk of hospitalization and death in patients with mild, moderate or severe chronic heart failure. Carvedilol caused dose-dependent increases in left ventricular ejection fraction in patients with stable mild to moderate chronic heart failure (Bristow et al. 1996). In another group of relatively stable, outpatient-treated heart failure patients with nonischaemic dilated cardiomyopathy, carvedilol-mediated increases in left ventricular ejection fraction were variable over the study population (−10–47% change, Chen et al. 2007). It was found that carvedilol caused greater increases in left ventricular ejection fraction in patients carrying the Arg389 allele (Arg389Arg 18% increase; Arg389Gly 9% increase; Gly389Gly 6% increase), confirming earlier studies in patients with ischaemic or dilated cardiomyopathy (Mialet Perez et al. 2003). A possible explanation for clinically observed differences in outcomes for carvedilol in patients with heart failure may be because of differences in coupling between Arg389- and Gly389- β_1-AR (Mason et al. 1999). In cell lines it was shown that Arg389-β_1AR couples more efficiently than Gly389-β_1AR to Gsα-protein, resulting in higher basal and catecholamine (noradrenaline, adrenaline, isoprenaline) induced adenylate cyclase activity, maximal isoprenaline stimulated guanine nucleotide binding and guanine nucleotide sensitive agonist binding at Arg389-β_1AR (Mason et al. 1999). In heart failure patients, cardiostimulation by endogenous catecholamines (noradrenaline and adrenaline) may be greater at Arg389-β_1AR than Gly389-β_1AR resulting in greater effects of carvedilol (Chen et al. 2007). In practice, an appreciation of polymorphic differences may better inform heart failure clinicians of expected outcomes of treatment with carvedilol (Chen et al. 2007).

There is agreement that carvedilol is selective for human β_2-AR vs β_1-AR (affinities pK_D or pK_B β_2-AR 9.0–10.1; β_1-AR 8.8–9.0, Hoffmann et al. 2004; Baker 2005 and Molenaar et al. 2006). However, β_2-AR/β_1-AR selectivity is something of a moot point as clinically it is used at concentrations that block both β_1-AR and β_2-AR. Additionally carvedilol blocks α-ARs (pK_D human α_{1A}-AR 8.35, α_{1B}-AR 7.84, α_{1D}-AR 7.87, Proudman et al. 2020) with lower affinity for α_2-ARs (pK_D human α_{2A}-AR 6.6, α_{2B}-AR 6.5, α_{2C}-AR 7.5, Proudman et al. 2022). The blockade of α_1-ARs lowers blood pressure. There is no evidence from clinical trials to indicate blockade of α_1-ARs confers benefit in the treatment of heart failure. ALLHAT (The ANTIHYPERTENSIVE ANT Lipid-Lowering Treatment to Prevent Heart Attack Trial) clinical trial compared the α_1-AR blocker doxazosin with diuretic chlorthalidone as part of a bigger trial which also included other vasodilators amlodipine and lisinopril (ALLHAT Collaborative Research Group 2000). The doxazosin arm of the trial was discontinued in part due to a doubling of congestive heart failure risk compared to chlorthalidone (ALLHAT 2000). In another clinical study with three arms, 1 prazosin, 2 combined hydralazine and isosorbide dinitrate and 3 placebo, which compared chronic 'vasodilator' therapy on mortality in patients with chronic congestive heart failure, prazosin had no effect on mortality, whereas combined hydralazine and isosorbide dinitrate reduced mortality (Cohn et al. 1986). In a 3-month trial comparing metoprolol vs combination metoprolol + doxazosin in patients with congestive heart failure, both groups produced similar haemodynamic, exercise and neurohormonal benefits (Kukin et al. 1996).

Occupancy of β_1-AR and β_2-AR by carvedilol in human heart abrogates endogenous noradrenaline and adrenaline signalling through the β_1-AR, β_2-AR–Gsα-protein–adenylyl cyclase–cyclic AMP–PKA pathway and phosphorylation of target proteins, physiologically to reduce heart rate, conduction and contractility, the original idea that inspired the use of β-blockers for the treatment of heart failure (Waagstein et al. 1975). The interaction of carvedilol with β_1-AR and β_2-AR is however far more complex.

Chronic administration of β-blockers carvedilol and metoprolol to patients with heart failure induces PDE3 control of inotropic and lusitropic responses mediated through activation of β_1-AR and β_2-AR by noradrenaline and adrenaline, respectively, in isolated ventricular trabeculae (Molenaar et al. 2013, 2014). The effects of carvedilol and metoprolol on PDE3 differ. Carvedilol has greater ability to facilitate control by PDE3 of β_2-AR-mediated responses than metoprolol in the failing heart (Molenaar et al. 2013, 2014). Thus, carvedilol may be able to provide greater protection than metoprolol against β_2-AR-mediated arrhythmias in part through greater PDE3 control together with occupancy of the β_2-AR (Molenaar et al. 2006, 2013, 2014).

Carvedilol was shown to be a strong inverse agonist at human β_2-AR through the Gsα-protein–adenylyl cyclase signalling pathway (Wisler et al. 2007). The property was shown in optimized conditions in HEK-293 cells expressing high levels of β_2-AR (2 pmol/mg, presumably referenced to protein), pretreated with IBMX to prevent PDE metabolism of cyclic AMP (Wisler et al. 2007). Carvedilol stimulated β_2-AR phosphorylation at GRK phosphorylation sites, recruited β-arrestin2, internalized β_2-

AR and signalled in a β-arrestin2-dependent manner to activate ERK (Wisler et al. 2007). Although the other heart failure β-blockers, metoprolol and bisoprolol were shown to be inverse agonists in the same study, in contrast to carvedilol, they were unable to activate ERK through the β_2-AR (Wisler et al. 2007). Chronic administration of carvedilol, but not nadolol or metoprolol, to mice enhanced contractility of the extensor digitorum longus muscle, dependent on the presence of β-arrestin 1 (Kim et al. 2020). This property may be of value to patients with muscle loss associated with heart failure.

Classically the β_1-AR couples to the Gsα-protein signalling pathway. However, it was reported that carvedilol-bound β_1-AR has the ability to stabilize a conformation that favourably couples to a β_1-AR-Giα-arrestin signalling pathway (Wang et al. 2017). The effects of carvedilol were concentration-dependent with threshold ~1 nM (Wang et al. 2017). The ability to stabilize a β_1-AR-Giα-arrestin signalling pathway was not replicated by β_2-AR or shared by β-blockers (ligands) metoprolol, acebutolol, alprenolol, propranolol, carazolol or agonists noradrenaline, adrenaline or isoprenaline (Wang et al. 2017). Recruitment of Giα-protein by carvedilol is not dependent on GRK or PKA-mediated β_1-AR phosphorylation and can be prevented by propranolol binding to the orthosteric binding site of the β_1-AR (Wang et al. 2017). Carvedilol-β_1-AR-Giα-arrestin signalling is an intriguing possibility, pending evaluation in human heart and disease.

However, with the motivation to clearly define mechanisms through which β-blockers produce favourable clinical outcomes, in the light of accumulating evidence of carvedilol–β_2-AR – arrestin signalling and possible significance for heart disease treatment, Benkel et al. (2022) utilized cell lines with selective deletion of either G-proteins or arrestins to re-investigate and clarify carvedilol – β_2-AR signalling mechanisms. In their studies, carvedilol–β_2-AR-mediated effects could be explained entirely through activation of the β_2-AR–Gsα pathway, through low efficacy Gsα-protein activation but not through arrestins (Benkel et al. 2022). Notably ERK phosphorylation, a characteristic of arrestin-biased signalling, was observed only in cells expressing Gsα but not other G-proteins or arrestins (Benkel et al. 2022); cyclic AMP accumulation, a characteristic of human heart β_1-AR, β_2-AR– Gsα coupling (Kaumann et al. 1989; Molenaar et al. 2007b), was increased in HEK cells and cardiomyocytes (Benkel et al. 2022). Carvedilol-induced cAMP accumulation was associated with a positive chronotropic response in murine neonatal ventricular myocytes (Benkel et al. 2022). Guanine nucleotide-sensitive binding in radioligand binding experiments indicates receptor – G-protein interaction. Detectable guanine nucleotide-sensitive carvedilol binding to β_2-AR was detected in human lymphocytes which have a homogenous population of β_2-AR (no β_1-AR) and in human ventricle, notably more in failing vs non-failing ventricle which has a higher proportion of β_2-AR (Bristow et al. 1992) but in another study, guanine nucleotide-sensitive binding for carvedilol in human ventricle was not observed at β_2-AR, but was at β_1-AR (Maack et al. 2000). Does the guanine nucleotide sensitive binding for carvedilol translate to inotropic responses in human heart? For carvedilol it is extremely difficult to detect if at all. Carvedilol did not increase contractility, even in the presence of the adenylyl cyclase activator forskolin to facilitate

contractility (Bristow et al. 1992) but did in one out of seven ventricular trabeculae in another study, again in the presence of forskolin (Maack et al. 2000). In cell lines, of interest, where concentration-effect curves were established for carvedilol at β_2-AR for various effects (Benkel et al. 2022), the potency (-logEC_{50} M) varied and in some cases was ~7 or even less whereas the affinity is much greater at human β_2-AR (Hoffmann et al. 2004; Baker 2005; Molenaar et al. 2006) or as determined from their radioligand binding assay (Benkel et al. 2022, Supplement). It was concluded that carvedilol stabilizes a conformation of the β_2-AR that only poorly targets GRK phosphorylation and arrestin binding (Benkel et al. 2022). The concept that ISA explains β-blocker superiority in heart failure trials (Benkel et al. 2022) was strongly refuted by a combined group of well-known leading scientists and heart failure cardiologists (Lefkowitz et al. 2023). It is worthwhile to read the papers and comments (Benkel et al. 2022; Kostenis et al. 2023; Lefkowitz et al. 2023) on this matter together to gain further insight into the clinical context.

The antioxidant properties of carvedilol and at least one of its main cytochromeP450 metabolites (CYP450 1A2, 8-OH-carvedilol, Oldham and Clarke 1997) may contribute to its benefit in heart failure (Yue et al. 1992). The hydroxy metabolite, 8-OH-carvedilol is a more potent antioxidant than carvedilol (Yue et al. 1992). The antioxidant effects of carvedilol occur at μM concentrations which at face value might be difficult to reconcile with affinities at β-ARs (nM for carvedilol, above) or plasma concentrations (~ 500 nM in patients with severe heart failure taking carvedilol 50 mg twice daily, Ogawa et al. 2014). However, carvedilol is highly lipophilic (logP = 3.91, Mannhold 2005) and likely accumulates in tissues to increase local concentration (Yue et al. 1992). Tissue retention is consistent with persistent β-blockade in the heart (Kindermann et al. 2004; Molenaar et al. 2006). The antioxidant properties of carvedilol distinguish it from other β-blockers, such as atenolol, pindolol, propranolol, celiprolol and labetalol that have little (at much higher concentrations) or no effect (Yue et al. 1992). The distinguishing feature is the presence of the carbazole moiety in carvedilol but not in other β-blockers, responsible for the antioxidant activity (Yue et al. 1992). In an animal model of heart failure (canine, ventricular pacing, 250 bpm for 28 days), carvedilol prevented heart failure induced oxidation of the ryanodine receptor (RyR2, prevented the loss of free RyR2 thiol groups caused by oxidation) (Mochizuki et al. 2007). In line with its direct antioxidant properties, carvedilol was able to 'scavenge' peroxynitrite (Mochizuki et al. 2007). Chronic administration of carvedilol (in vivo) prevented protein kinase A phosphorylation of RyR2 (RyR2-Ser2808), FKBP12.6 dissociation and Ca^{2+} leak from RyR2 (Mochizuki et al. 2007). Incubation of myocytes from the failing hearts with carvedilol (30 nM for 12 hours) increased sarcoplasmic reticulum (SR) Ca^{2+} content, improved the Ca^{2+} transient and myocyte shortening (Mochizuki et al. 2007). On the other hand, metoprolol at an 'equivalent' β-blocking concentration (100 nM) had no effect on myocyte transient and cell shortening (Mochizuki et al. 2007). Taken together, carvedilol prevention of PKA phosphorylation, oxidation and FKBP12.6 dissociation from RyR2 indirectly prevent critical changes in RyR2 structure, 'unzipping' of N-terminal and central domains observed in heart failure responsible for Ca^{2+} leak (Mochizuki et al. 2007). A direct effect of carvedilol

on RyR2 has been described to reduce store overload induced Ca^{2+} release, responsible for ventricular arrhythmias in heart failure (Zhou et al. 2011). At μM concentrations, carvedilol, but not other β-blockers, including those used for heart failure treatment (bisoprolol, metoprolol) inhibited store overload induced Ca^{2+} release (Zhou et al. 2011). From single RyR2 channel studies it was shown that carvedilol reduced open probability (Po), mean open and closed times (Zhou et al. 2011). This property appears to be dependent on the carbazole moiety within carvedilol, as structural derivatives based on carvedilol with little or no β-blocking activity (VK-II-86, CS-I-34, CS-I-59) inhibited store overload induced Ca^{2+} release (Zhou et al. 2011). The direct effect on RyR2 was observed at 300 nM that could be achieved through carvedilol accumulation and retention in tissues (Yue et al. 1992; Kindermann et al. 2004; Molenaar et al. 2006; Zhou et al. 2011).

In human failing hearts, phosphorylation of RyR2 Ser2808 was lower in a group of patients chronically treated, mostly with carvedilol (total pool 10 patients, 8 treated with carvedilol, one with atenolol, one with metoprolol, experiments conducted on 5 of those patients, β-blocker not specified) compared to phosphorylation levels in non-β-blocker treated patients (Reiken et al. 2003). Furthermore, chronic administration of β-blockers restored levels of RyR2 complexed proteins, PP1, PP2A and FKBP12.6 towards that of levels observed in non-failing hearts, and RyR2 channel function (open channel probability Po) was restored to normal (Reiken et al. 2003).

In practice it may be difficult to quantify benefit of one β-blocker used for heart failure treatment over another, particularly if benefit has a small increment. Nevertheless, recognition of additional molecular properties of carvedilol over other heart failure β-blockers raises the possibility of further advances in β-blocker therapy. The availability of carvedilol with properties offering potential benefit over other β-blockers for use in heart failure unfortunately may be limited by its action to cause α_1-adrenoceptor-mediated vasodilation, lowering of blood pressure causing haemodynamic compromise. To maintain important survival benefit of β-adrenoceptor blockade, another less vasodilating β-blocker could be substituted for carvedilol, for example bisoprolol (LeChat et al. 1997; CIBIS-II Investigators and Committees 1999) or metoprolol (Waagstein et al. 1993; MERIT-HF Study Group 1999). Another possibility, but not tested in large-scale survival trials, is to appreciate affinity data of carvedilol and, for example, metoprolol, decrease the dose of carvedilol to reduce α_1-AR mediated dilation and use a combination of carvedilol and metoprolol to fully block β_1-AR and β_2-AR. For example, based on affinity values for carvedilol (pKi β_2-AR 10.13; β_1-AR 9.02 Molenaar et al. 2006; α_{1a}-AR 8.35 Proudman et al. 2020) a concentration of 0.74 nM would occupy 85% β_2-AR, 32% β_1-AR, 9% α_1-AR. Critical β_1-AR and β_2-AR blockade can be supplemented by metoprolol (affinities, β_1-AR 7.40, β_2-AR 7.01, Molenaar et al. 2013), for example at a concentration of 100 nM giving metoprolol occupancies of 51% β_2-AR, 72% β_1-AR. Overall carvedilol + metoprolol combination ('ComBeta') occupancies in this example would be β_1-AR 100%, β_2-AR 100%, α_1-AR 9%. A similar approach could be used with the combination of carvedilol and bisoprolol.

5 Conclusions

β-ARs have a fundamental role in the pathogenesis and therapeutics of cardiovascular diseases. The pattern of use of β-blockers in therapeutics has evolved towards optimization. As basic and clinical research continues, further optimization and patient benefit is possible, testament to nearly 60 years of clinical use of β-blockers and dedicated researchers and funding organizations. Ongoing research suggests further improvements in β-blocker treatment of heart failure may be possible. Thus, there should be optimism that further advances can be made towards better β-AR therapeutics for the treatment of cardiovascular diseases.

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References

Ahmet I, Krawczyk M, Heller P, Moon C, Lakatta EG, Talan MI (2004) Beneficial effects of chronic pharmacological manipulation of β-adrenoceptor subtype signalling in rodent dilated ischemic cardiomyopathy. Circulation 110:1083–1090

Ailani J, Burch RC, Robbins MS, Board of Directors of the American Headache Society (2021) The American Headache Society Consensus Statement: update on integrating new migraine treatments into clinical practice. Headache 61:1021–1039

ALLHAT Collaborative Research Group (2000) Major cardiovascular events in hypertensive patients randomized to doxazosin vs chlorthalidone. The antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). JAMA 283:1967–1975

Anderson JL, Morrow DA (2017) Acute Myocardial Infarction. N Engl J Med 376:2053–2064

Andersson C, Shilane D, Go AS, Chang TI, Kazi D, Solomon MD, Boothroyd DB, Hlatky MA (2014) β-Blocker therapy and cardiac events among patients with newly diagnosed coronary heart disease. J Am Coll Cardiol 64:247–252

Angus JA, Sarsero D, Fujiwara T, Molenaar P, Xi Q (2000) Quantitative analysis of vascular to cardiac selectivity of L- and T-type voltage-operated calcium channel antagonists in human tissues. Clin Exp Pharmacol Physiol 27:1019–1021

Aumann KW, Youmans WB (1940) Differential sensitization of adrenergic neuro-effector systems by thyroid hormone. J Physiol 131:394-4011

Bahouth SW (1991) Thyroid hormones transcriptionally regulate the β_1-adrenergic receptor gene in cultured ventricular myocytes. J Biol Chem 266:15863–15869

Bahouth SW, Cui X, Beauchamp MJ, Park EA (1997) Thyroid hormone induces β_1-adrenergic receptor gene transcription through a direct repeat separated by five nucleotides. J Mol Cell Cardiol 29:3223–3237

Baker JG (2005) The selectivity of β-adrenoceptor antagonists at human β1, β2 and β3 adrenoceptors. Br J Pharmacol 14:317–322

Bangalore S, Makani H, Radford M, Thakur K, Toklu B, Katz SD, DiNicolantonio JJ, Devereaux PJ, Alexander KP, Wetterslev J, Messerli FH (2014) Clinical outcomes with β-blockers for myocardial infarction: a meta-analysis of randomized trials. Am J Med 127:939–953

Barefield D, Sadayappan S (2010) Phosphorylation and function of cardiac myosin binding protein-C in health and disease. J Mol Cell Cardiol 48:866–875

Bari K, Garcia-Tsao G (2012) Treatment of portal hypertension. World J Gastroenterol 18:1166–1175

Barrett AM, Carter J (1970) Comparative chronotropic activity of β-adrenoceptive antagonists. Br J Pharmacol 40:373–381

Benkel T, Zimmermann M, Zeiner J, Bravo S, Merten N, Lim V, Matthees E, Drube J, Miess-Tanneberg E, Malan D, Szpakowska M, Monteleone S, Grimes J, Koszegi Z, Lanoiselée Y, O'Brien S, Pavlaki N, Dobberstein N, Inoue A, Nikolaev V, Calebiro D, Chevigné A, Sasse P, Schulz S, Hoffmann C, Kolb P, Waldhoer M, Simon K, Gomeza J, Kostenis E (2022) How carvedilol activates β_2-adrenoceptors. Nat Commun 13:7109

Benovic JL, DeBlasi A, Stone WC, Caron MG, Lefkowitz RJ (1989) Beta-adrenergic receptor kinase: primary structure delineates a multigene family. Science 246:235–240

Berisha F, Götz KR, Wegener JW, Brandenburg S, Subramanian H, Molina CE, Rüffer A, Petersen J, Bernhardt A, Girdauskas E, Jungen C, Pape U, Kraft AE, Warnke S, Lindner D, Westermann D, Blankenberg S, Meyer C, Hasenfuß G, Lehnart SE, Nikolaev VO (2021) cAMP imaging at ryanodine receptors reveals β_2-adrenoceptor driven arrhythmias. Circ Res 129:81–94

Bers DM (2002) Cardiac excitation-contraction coupling. Nature 415:198–205

Bers DM, Perez-Reyes E (1999) Ca channels in cardiac myocytes: structure and function in Ca influx and intracellular Ca release. Cardiovasc Res 42:339–360

Black JW, Prichard BNC (1973) Activation and blockade of β adrenoceptors in common cardiac disorders. Br Med Bull 29:163–167

Black JW, Stephenson JS (1962) Pharmacology of a new adrenergic beta-receptor-blocking compound (nethalide). Lancet 2:311–314

Black JW, Duncan WA, Shanks RG (1965) Comparison of some properties of pronethalol and propranolol. Br J Pharmacol Chemother 25:577–591

Blinks JR (1967) Evaluation of the cardiac effects of several beta adrenergic blocking agents. Ann N Y Acad Sci 139:673–685

Bodi I, Mikala G, Koch SE, Akhter SA, Schwartz A (2005) The L-type calcium channel in the heart: the beat goes on. J Clin Invest 115:3306–3317

Bond RA, Clarke DE (1988) Agonist and antagonist characterization of a putative adrenoceptor with distinct pharmacological properties from the α- and β -subtypes. Br J Pharmacol 95:723–734

Bond RA, Leff P, Johnson TD, Milano CA, Rockman HA, McMinn TR, Apparsundaram S, Hyek MF, Kenakin TP, Allen LF, Lefkowitz RJ (1995) Physiological effects of inverse agonists in transgenic mice with myocardial overexpression of the β_2-adrenoceptor. Nature 374:272–276

Bozkurt B, Coats A, Tsutsui H (2021) Universal definition and classification of heart failure. J Card Fail 27:387–413

Braunwald E, Morrow DA (2013) Unstable angina: is it time for a requiem? Circulation 127:2452–2457

Bristow MR (2000) β-Adrenergic receptor blockade in chronic heart failure. Circulation 2000(101): 558–569

Bristow MR, Ginsburg R, Minobe W, Cubicciotti RS, Sageman WS, Lurie K, Billingham ME, Harrison DC, Stinson EB (1982) Decreased catecholamine sensitivity and β-adrenergic-receptor density in failing human hearts. N Engl J Med 307:205–211

Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R, Zera P, Menlove R, Shah P, Jamieson S (1986) Beta1- and beta2-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective beta 1-receptor down-regulation in heart failure. Circ Res 59:297–309

Bristow MR, Larrabee P, Minobe W, Roden R, Skerl L, Klein J, Handwerger D, Port J, Müller-Beckmann B (1992) Receptor pharmacology of carvedilol in the human heart. J Cardiovasc Pharmacol 19(Supplement 1):S68–S80

Bristow MR, Gilbert EM, Abraham WT, Adams KR, Fowler MB, Hershberger RE, Kubo SH, Narahara KA, Ingersoll H, Krueger S, Young S, Shusteman N, for the MOCHA Investigators (1996) Carvedilol produces dose-related improvements in left ventricular function and survival in subjects with chronic heart failure. Circulation 94:2807–2816

Bristow MR, Feldman AM, Adams KF, Goldstein S (2003) Selective versus nonselective β-blockade for heart failure therapy: are there lessons to be learned from the COMET trial? J Cardiac Fail 9:444–453

Brodde O-E (1991) β_1- and β_2-adrenoceptors in the human heart: properties, function, and alterations in chronic heart failure. Pharmacol Rev 43:203–242

Brodde O-E, Michel MC (1999) Adrenergic and muscarinic receptors in the human heart. Pharmacol Rev 51:651–689

Bundgaard H, Liu C-C, Garcia A, Hamilton EJ, Huang Y, Chia KKM, Hunyor SN, Figtree GA, Rasmussen HH (2010) Adrenergic stimulation of the cardiac Na^+-K^+ pump by reversal of an inhibitory oxidative modification. Circulation 122:2699–2708

Bundgaard H, Axelsson A, Thomsen JH, Sørgaard M, Kofoed KF, Hasselbalch R, Fry NAS, Valeur N, Boesgaard S, Gustafsoon F, Køber L, Iversen K, Rasmussen HH (2017) The first-in-man randomized trial of a beta3 adrenoceptor agonist in chronic heart failure: the BEAT-HF trial. Eur J Heart Fail 19:566–575

Butters L, Kennedy S, Rubin PC (1990) Atenolol in essential hypertension during pregnancy. BMJ 301:587–589

Buxton BF, Jones CR, Molenaar P, Summers RJ (1987) Characterization and autoradiographic localization of β-adrenoceptor subtypes in human cardiac tissues. Br J Pharmacol 92:299–310

Candelore MR, Deng L, Tota L, Guan XM, Amend A, Liu Y, Newbold R, Cascieri MA, Weber AE (1999) Potent and selective human β_3-adrenergic receptor antagonists. J Pharmacol Exp Ther 290:649–655

Cannell MB, Kong CHT, Imtiaz MS, Laver DR (2013) Control of sarcoplasmic reticulum Ca^{2+} release by stochastic RyR gating within a 3D model of the cardiac dyad and importance of induction decay for CICR termination. Biophys J 104:2149–2159

CAPRICORN investigators (2001) Effect of carvedilol on outcome after myocardial infarction in patients with left-ventricular dysfunction: the CAPRICORN randomised trial. Lancet 357: 1385–1390

Chandler NJ, Greener ID, Tellez JO, Shin Inada S, Musa H, Molenaar P, di Francesco D, Baruscotti M, Longhi R, Anderson RH, Billeter R, Vinod Sharma V, Sigg DC, Boyett MR, Dobrzynski H (2009) Molecular architecture of the human sinus node. Insights into the function of the cardiac pacemaker. Circulation 119:1562–1575

Chandler N, Aslanidi O, Buckley D, Inada S, Birchall S, Atkinson A, Kirk D, Monfredi O, Molenaar P, Anderson R, Sharma V, Sigg D, Zhang H, Boyett M, Dobrzynski H (2011) Computer three-dimensional anatomical reconstruction of the human sinus node and a novel paranodal area. Anat Rec 294:970–979

Chatzidou S, Kontogiannis C, Tsilimigras DI, Georgiopoulos G, Kosmopoulos M, Papadopoulou E, Vasilopoulos G, Rokas S (2018) Propranolol versus metoprolol for treatment of electrical storm in patients with implantable cardioverter-defibrillator. J Am Coll Cardiol 71: 1897–1906

Chen L, Meyers D, Javorsky G, Burstow D, Lolekha P, Lucas M, Semmler ABT, Savarimuthu SM, Fong KM, Yang IA, Atherton J, Galbraith AJ, Parsonage WA, Molenaar P (2007) Arg389Gly-β 1-adrenergic receptors determine improvement in left ventricular systolic function in nonischemic cardiomyopathy patients with heart failure after chronic treatment with carvedilol. Pharmacogenet Genomics 17:941–949

Chesley A, Lundberg MS, Asai T, Xiao R-P, Ohtani S, Lakatta EG, Crow MT (2000) The β_2-adrenergic receptor delivers an antiapoptotic signal to cardiac myocytes through G_i-dependent coupling to phosphatidylinositol 3′-kinase. Circ Res 87:1172–1179

Chew DP, Scott IA, Cullen L, French JK, Briffa TG, Tideman PA, Woodruffe S, Kerr A, Branagan M, Aylward PE (2016) NHFA/CSANZ ACS Guideline 2016 Executive Working Group: National Heart Foundation of Australia & Cardiac Society of Australia and New Zealand: Australian clinical guidelines for the management of acute coronary syndromes 2016. Heart Lung Circ 25:895–951

Chockalingam P, Crotti L, Girardengo G, Johnson JN, Harris KM, van der Heijden JF, Hauer RN, Beckmann BM, Spazzolini C, Rordorf R, Rydberg A, Clur SA, Fischer M, van den Heuvel F, Kääb S, Blom NA, Ackerman MJ, Schwartz PJ, Wilde AA (2012) Not all beta-blockers are equal in the management of long QT syndrome types 1 and 2: higher recurrence of events under metoprolol. J Am Coll Cardiol 60:2092–2099

Christ T, Molenaar P, Klenowski PM, Ravens U, Kaumann AJ (2011) Human atrial β_{1L}-adrenoceptor but not β_3-adrenoceptor activation increases force and Ca^{2+} current at physiological temperature. Br J Pharmacol 162:823–839

CIBIS-II Investigators and Committees (1999) The cardiac insufficiency bisoprolol study II (CIBIS-II) a randomised trial. Lancet 353:9–13

Cohn JN, Levine TB, Olivari MT, Garberg V, Lura D, Francis GS, Simon AB, Rector T (1984) Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. N Engl J Med 311:819–823

Cohn JN, Archibald DG, Ziesche S, Franciosa JA, Harston WE, Tristani FE, Dunkman WB, Jacobs W, Francis GS, Flohr KH, Goldman S, Cobb FR, Shar PM, Saunders R, Fletcher RD, Loeb HS, Hughes VC, Baker B (1986) Effect of vasodilator therapy on mortality in chronic congestive heart failure. Results of a veterans administration cooperative study. N Engl J Med 314:1547–1552

Colucci WS, Packer M, Bristow MR, Gilvert M, Cohn JN, Fowler MB, Krueger SK, Hershberger R, Uretsky BF, Bowers JA, Sackner-Bernstein JD, Young ST, Holcslaw TL, Lukas MA, for the US Carvedilol Heart Failure Study Group (1996) Carvedilol inhibits clinical progression in patients with mild symptoms of heart failure. Circulation 94:2800–2806

Communal C, Singh K, Sawyer DB, Colucci WS (1999) Opposing effects of β_1- and β_2-adrenergic receptors on cardiac myocyte apoptosis. Role of a pertussis toxin-sensitive G protein. Circulation 100:2210–2212

Coulombe P, Dussault JH, Walker P (1976) Plasma catecholamine concentrations in hyperthyroidism and hypothyroidism. Metabolism 25:973–979

Dal Monte M, Evans BA, Arioglu-Inan E, Michel MC (2020) Upregulation of β3-adrenoceptors—a general marker of and protective mechanism against hypoxia? Naunyn Schmiedeberg's Arch Pharmacol 393:141–146

Danesh A, Gottschalk PCH (2019) Beta-blockers for migraine prevention: a review article. Curr Treat Options Neurol 21:20

Dashwood A, Cheesman E, Beard N, Haqqani H, Wong YW, Molenaar P (2020) Understanding how phosphorylation and redox modifications regulate cardiac ryanodine receptor type 2 activity to produce an arrhythmogenic phenotype in advanced heart failure. ACS Pharmacol Transl Sci 3:563–582

de Franchis R (1996) Developing consensus in portal hypertension. J Hepatol 25:390–394

de Franchis R, Bosch J, Garcia-Tsao G, Reiberger T, Ripoll C (2022) Baveno VII faculty. Baveno VII - renewing consensus in portal hypertension. J Hepatol 76:959–974

Denniss AL, Dashwood AM, Molenaar P, Beard NA (2020) Sarcoplasmic reticulum calcium mishandling: central tenet in heart failure? Biphys Rev 12:865–878

DeSantiago J, Ai X, Islam M, Acuna G, Ziolo MT, Bers DM, Pogwizd SM (2008) Arrhythomogenic effects of β_2-adrenergic stimulation in the failing heart are attributable to enhanced sarcoplasmic reticulum Ca load. Circ Res 102:1389–1397

Desta L, Raposeiras-Roubin S, Ibanez B (2021) The art of prescribing β-blockers after myocardial infarction. Circ Cardiovasc Interv 14:e010720

Dincer ÜD, Bidasee KR, Güner S, Tay A, Özcelikay AT, Altan VM (2001) The effects of diabetes on expression of β_1-, β_2-, and β_3-adrenoceptors in rat hearts. Diabetes 50:455–461

Dobrzynski H, Monfredi O, Greener ID, Atkinson A, Inada S, Taube M-A, Yanni J, Fedorenko O, Molenaar P, Anderson RH, Efimov IR, Boyett MR (2011) Molecular basis of the electrical activity of the atrioventricular junction and Purkinje fibres. In: Heart rate and rhythm. Springer, Berlin, Heidelberg, pp 211–230

Doughty RN, Whalley GA, Walsh HA, Gamble GD, López-Sendón J, Sharpe N, CAPRICORN Echo Substudy Investigators (2004) Effects of carvedilol on left ventricular remodeling after acute myocardial infarction: the CAPRICORN Echo Substudy. Circulation 109:201–206

Elnatan J, Molenaar P, Rosenfeldt FL, Summers RJ (1994) Autoradiographic localization and quantitation of beta1- and beta2-adrenoceptors in the human atrioventricular conducting system: a comparison of patients with idiopathic dilated cardiomyopathy and ischemic heart disease. J Mol Cell Cardiol 26:313–323

Emorine LJ, Marullo S, Briend-Sutren MM, Patey G, Tate K, Delavier-Klutchko C, Strosberg AD (1989) Molecular characterization of the human β_3-adrenergic receptor. Science 245:1118–1121

Esler M, Kaye D, Lambert G, Esler D, Jennings G (1997) Adrenergic nervous system in heart failure. Am J Cardiol 80(11A):7L–14L

Fabiato A (1983) Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. Am J Phys 245:C1–C14

Fabiato A, Fabiato F (1975) Contractions induced by a calcium-triggered release of calcium from the sarcoplasmic reticulum of single skinned cardiac cells. J Physiol 249:469–495

Fabiato A, Fabiato F (1977) Calcium release from the sarcoplasmic reticulum. Circ Res 40:119–129

Fabiato A, Fabiato F (1979) Calcium and cardiac excitation-contraction coupling. Annu Rev Physiol 41:473–484

Feigin VL, Norrving B, Mensah GA (2017) Global burden of stroke. Circ Res 120:439–448

Fihn SD, Gardin JM, Abrams J, Berra K, Blankenship JC, Dallas AP, Douglas PS, Foody JM, Gerber TC, Hinderliter AL, King SB 3rd, Kligfield PD, Krumholz HM, Kwong RY, Lim MJ, Linderbaum JA, Mack MJ, Munger MA, Prager RL, Sabik JF, Shaw LJ, Sikkema JD, Smith CR Jr, Smith SC Jr, Spertus JA, Williams SV (2012) American College of Cardiology Foundation. 2012 ACCF/AHA/ACP/AATS/PCNA/SCAI/STS guideline for the diagnosis and management of patients with stable ischemic heart disease: executive summary: a report of the American College of Cardiology Foundation/American Heart Association task force on practice guidelines, and the American College of Physicians, American Association for Thoracic Surgery, Preventive Cardiovascular Nurses Association, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. Circulation 126:3097–3137

Fischer V, Gabauer I, Tillinger A, Novakova M, Pechan I, Krizanova O, Kvetňanský R, Myslivecekc J (2008) Heart adrenoceptor gene expression and binding sites in the human failing heart. Ann N Y Acad Sci 1148:400–408

Foo RS-Y, Mani K, Kitsis RN (2005) Death begets failure in the heart. J Clin Invest 115:565–571

Freemantle N, Cleland J, Young P, Mason J, Harrison J (1999) β blockade after myocardial infarction: systematic review and meta regression analysis. BMJ 318:1730–1737

Friedman LM, Byington RP, Capone RJ, Furberg CD, Goldstein S, Lichstein E (1986) Effect of propranolol in patients with myocardial infarction and ventricular arrhythmia. J Am Coll Cardiol 7:1–8

Fry NAS, Liu CC, Garcia A, Hamilton EJ, Galougahi KK, Kim YJ, Whalley DW, Bundgaard H, Rasmussen HH (2020) Targeting cardiac myocyte Na^+-K^+ pump function with β3 adrenergic agonist in rabbit model of severe congestive heart failure. Circ Heart Fail 13:e006753

Gauthier C, Tavernier G, Charpentier F, Langin D, Le Marec H (1996) Functional β_3-adrenoceptor in the human heart. J Clin Invest 98:556–562

Gauthier C, Leblais V, Kobzik L, Trochu J-N, Khandoudi N, Bril A, Balligand J-L, Le Marec H (1998) The negative inotropic effect of β_3-adrenoceptor stimulation is mediated by activation of a nitric oxide synthase pathway in human ventricle. J Clin Invest 102:1377–1384

Grassi G, Seravalle G, Dell'Oro R, Facchini A, Ilardo V, Mancia G (2004) Sympathetic and baroreflex function in hypertensive or heart failure patients with ventricular arrhythmias. J Hypertens 22:1747–1753

Greener ID, Monfredi O, Inada S, Chandler NJ, Tellez JO, Atkinson A, Taube M-A, Billeter R, Anderson RH, Efimov IR, Molenaar P, Sigg DC, Sharma V, Boyett MR, Dobrzynski H (2011) Molecular architecture of the human specialised atrioventricular conduction axis. J Mol Cell Cardiol 50:642–651

Groszmann RJ, Garcia-Tsao G, Bosch J, Grace ND, Burroughs AK, Planas R, Escorsell A, Garcia-Pagan JC, Patch D, Matloff DS, Gao H, Makuch R (2005) Portal Hypertension Collaborative Group. Beta-blockers to prevent gastroesophageal varices in patients with cirrhosis. N Engl J Med 353:2254–2261

Hall JA, Petch MC, Brown MJ (1989) Intracoronary injections of salbutamol demonstrate the presence of functional beta 2-adrenoceptors in the human heart. Circ Res 65(3):546–553. https://doi.org/10.1161/01.res.65.3.546

Harding SE (1997) Lack of evidence for β3-adrenoceptor modulation of contractile function in human ventricular myocytes [abstract]. Circulation 96(Suppl. S):284

Harding SD, Armstrong JF, Faccenda E, Southan C, Alexander SPH, Davenport AP, Spedding M, Davies JA (2023) The IUPHAR/BPS guide to PHARMACOLOGY in 2024. Nucleic Acids Res: gkad944

Hauf-Zachariou U, Blackwood RA, Gunawardena KA, O'Donnell JG, Garnham S, Pfarr E (1997) Carvedilol versus verapamil in chronic stable angina: a multicentre trial. Eur J Clin Pharmacol 52:95–100

Heidenreich PA, Bozkurt B, Aguilar D, Allen LA, Byun JJ, Colvin MM, Deswal A, Drazner MH, Dunlay SM, Evers LR, Fang JC, Fedson SE, Fonarow GC, Hayek SS, Hernandez AF, Khazanie P, Kittleson MM, Lee CS, Link MS, Milano CA, Nnacheta LC, Sandhu AT, Stevenson LW, Vardeny O, Vest AR, Yancy CW (2022) 2022 AHA/ACC/HFSA guideline for the Management of Heart Failure: a report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. J Am Coll Cardiol 79:e263–e421

Heikkilä J, Nieminen MS (1982) Cardiac safety of acute beta blockade: intrinsic sympathomimetic activity is superior to beta-1 selectivity. Am Heart J 104:464–472

Hershberger RE, Wynn JR, Sundberg L, Bristow MR (1990) Mechanism of action of bucindolol in human ventricular myocardium. J Cardiovasc Pharmacol 15:959–967

Heubach JF, Graf EM, Molenaar P, Jäger A, Schröder F, Herzig S, Harding SE, Ravens U (2001) Murine ventricular L-type Ca^{2+} current is enhanced by zinterol via β_1-adrenoceptors, and is reduced in TG4 mice overexpressing the human β_2-adrenoceptor. Br J Pharmacol 133:73–82

Heubach JF, Ravens U, Kaumann AJ (2004) Epinephrine activates both Gs and Gi pathways, but norepinephrine activates only the Gs pathway through human β_2-adrenoceptors overexpressed in mouse heart. Mol Pharmacol 65:1313–1322

Hoffmann C, Leitz MR, Oberdorf-Maass S, Lohse MJ, Klotz K-N (2004) Comparative pharmacology of human β-adrenergic receptor subtypes—characterization of stably transfected receptors in CHO cells. Naunyn Schmiedeberg's Arch Pharmacol 369:151–159

Holroyd KA, Cottrell CK, O'Donnell FJ, Cordingley GE, Drew JB, Carlson BW, Himawan L (2010) Effect of preventive (β blocker) treatment, behavioural migraine management, or their combination on outcomes of optimised acute treatment in frequent migraine: randomised controlled trial. BMJ 341:c4871

Hu K, Gaudron P, Ertl G (1998) Long-term effects of beta-adrenergic blocking agent treatment on hemodynamic function and left ventricular remodeling in rats with experimental myocardial infarction: importance of timing of treatment and infarct size. J Am Coll Cardiol 31:692–700

Ikram H, Fitzpatrick D (1981) Double-blind trial of chronic oral beta blockade in congestive cardiomyopathy. Lancet 2(8245):490–493

Jackson G, Harry JD, Robinson C, Kitson D, Jewitt DE (1978) Comparison of atenolol with propranolol in the treatment of angina pectoris with special reference to once daily administration of atenolol. Br Heart J 40:998–1004

James IM, Griffith DN, Pearson RM, Newbury P (1977) Effect of oxprenolol on stage-fright in musicians. Lancet 2:952–954

Joglar JA, Chung MK, Armbruster AL, Benjamin EJ, Chyou JY, Cronin EM, Deswal A, Eckhardt LL, Goldberger ZD, Gopinathannair R, Gorenek B, Hess PL, Hlatky M, Hogan G, Ibeh C, Indik JH, Kido K, Kusumoto F, Link MS, Linta KT, Marcus GM, McCarthy PM, Patel N, Patton KK, Perez MV, Piccini JP, Russo AM, Sanders P, Streur MM, Thomas KL, Times S, Tisdale JE,

Valente AM, Van Wagoner DR (2024) 2023 ACC/AHA/ACCP/HRS guideline for the diagnosis and management of atrial fibrillation: a report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. Circulation 149: e1–e156

Joseph SS, Lynham JA, Molenaar P, Grace AA, Colledge WH, Kaumann AJ (2003) Intrinsic sympathomimetic activity of (−)-pindolol mediated through a (−)-propranolol-resistant site of the β_1-adrenoceptor in human atrium and recombinant receptors. Naunyn Schmiedeberg's Arch Pharmacol 368:496–503

Kahaly GJ, Bartalena L, Hegedüs L, Leenhardt L, Poppe K, Pearce SH (2018) 2018 European thyroid association guideline for the Management of Graves' hyperthyroidism. Eur Thyroid J 7: 167–186

Kaumann AJ (1989) Is there a third heart β-adrenoceptor? Trends Pharmacol Sci 10:316–320

Kaumann AJ (1996) (−)-CGP 12177-induced increase of human atrial contraction through a putative third β-adrenoceptor. Br J Pharmacol 117:93–98

Kaumann AJ, Molenaar P (1997) Modulation of human cardiac function through 4 β-adrenoceptor populations. Naunyn Schmiedeberg's Arch Pharmacol 355:667–681

Kaumann AJ, Molenaar P (2008) The low-affinity site of the β_1-adrenoceptor and its relevance to cardiovascular pharmacology. Pharmacol Ther 118:303–336

Kaumann AJ, Sanders L (1993) Both β_1- and β_2-adrenoceptors mediate catecholamine-evoked arrhythmias in isolated human right atrium. Naunyn Schmiedeberg's Arch Pharmacol 348:536–540

Kaumann AJ, Hall JA, Murray KJ, Wells FC, Brown MJ (1989) A comparison of the effects of adrenaline and noradrenaline on human heart: the role of β_1- and β_2-adrenoceptors in the stimulation of adenylate cyclase and contractile force. Eur Heart J 10(Supplmenet B):29–37

Kaumann A, Bartel S, Molenaar P, Sanders L, Burrell K, Vetter D, Hempel P, Karczewski P, Krause E-G (1999) Activation of β_2-adrenergic receptors hastens relaxation and mediates phosphorylation of phospholamban, troponin I, and C-protein in ventricular myocardium from patients with terminal heart failure. Circulation 99:65–72

Kaumann AJ, Engelhardt S, Hein L, Molenaar P, Lohse M (2001) Abolition of (−)-CGP 12177-evoked cardiostimulation in double β_1/β_2-adrenoceptor knockout mice. Obligatory role of β_1-adrenoceptors for putative β_4-adrenoceptor pharmacology. Naunyn Schmiedeberg's Arch Pharmacol 363:87–93

Kaye DM, Lefkovits J, Cox H, Lambert G, Jennings G, Turner A, Esler MD (1995) Regional epinephrine kinetics in human heart failure: evidence for extra-adrenal, nonneural release. Heart Circ Physiol 38:H182–H188

Kilts JD, Gerhardt MA, Richardson MD, Sreeram G, Mackensen GB, Grocott JP, White WD, Davis RD, Newman MF, Reves JG, Schwinn DA, Kwatra MM (2000) β_2-adrenergic and several other G protein-coupled receptors in human atrial membranes activate both G_s and G_i. Circ Res 87: 705–709

Kim J, Grotegu CA, Wisler JW, Mao L, Rosenberg PB, Rockman HA, Lefkowitz RJ (2020) The β-arrestin-biased β-adrenergic receptor blocker carvedilol enhances skeletal muscle contractility. Proc Natl Acad Sci USA 117:12435–12443

Kindermann M, Maack C, Schaller S, Finkler N, Schmidt K, Läer S, Wuttke H, Schäfers H-J, Böhm M (2004) Carvedilol but not metoprolol reduces β-adrenergic responsiveness after complete elimination from plasma in vivo. Circulation 109:3182–3190

Klein I, Danzi S (2007) Thyroid disease and the heart. Circulation 116:1725–1735

Kompa AR, Summers RJ (1999) Desensitization and resensitization of β_1- and putative β_4-adrenoceptor mediated responses occur in parallel in a rat model of cardiac failure. Br J Pharmacol 128:1399–1406

Koss KL, Kranias EG (1996) Phospholamban: a prominent regulator of myocardial contractility. Circ Res 79:1059–1063

Kostenis E, Gomeza J, Miess-Tanneberg E, Blum NK, Benkel T, Chevigné A, Hoffmann C, Kolb P, Nikolaev V, Waldhoer M, Szpakowska M, Inoue A, Schulz S (2023) Reply to: how carvedilol does not activate β_2-adrenoceptors. Nat Commun 14:7867

Kukin ML, Kalman J, Mannino M, Freudenberger R, Buchholz C, Ocampo O (1996) Combined alpha-beta blockade (doxazosin plus metoprolol) compared with beta blockade alone in chronic congestive heart failure. Am J Cardiol 77:486–491

Lang D, Holzem K, Kang C, Xial M, Hwang HJ, Ewald GA, Yamada KA, Efimov IR (2015) Arrhythmogenic remodeling of β_2 versus β_1 adrenergic signalling in the human failing heart. Circ Arrhythm Electrophysiol 8:409–419

Layland J, Solaro RJ, Shah AM (2005) Regulation of cardiac contractile function by troponin I phosphorylation. Cardiovasc Res 66:12–21

Lazar MA, Chin WW (1990) Nuclear thyroid hormone receptors. J Clin Invest 86:1777–1782

LeChat P, Escoloano S, Golmard JL, Lardoux H, Witchitz S, Henneman JA, Maisch B, Jaillon P, Boissel J-P, Mallet A, on behalf of the CIBIS Investigators (1997) Prognostic value of bisoprolol-induced hemodynamic effects in heart failure during the cardiac insufficiency Bisoprolol Study (CIBIS). Circulation 96:2197–2205

Lefkowitz RJ, Rockman HA, Shim PJ, Liu S, Ahn S, Pani B, Rajagopal S, Shenoy SK, Bouvier M, Benovic JL, Liggett SB, Ruffolo RR, Bristow MR, Packer M (2023) How carvedilol does not activate β_2-adrenoceptors. Nat Commun 14:7866

Li J, Imtiaz MS, Beard NA, Dulhunty AF, Thorne R, vanHelden DF, Laver DR (2013) β-adrenergic stimulation increases RyR2 activity via intracellular Ca^{2+} and Mg^{2+} regulation. PLoS One 8: e58334

Libby P (2013) Mechanisms of acute coronary syndromes and their implications for therapy. N Engl J Med 368:2004–2013

Lohse MJ, Benovic JL, Codina J, Caron MG, Lefkowitz RJ (1990) β-Arrestin: a protein that regulates β-adrenergic receptor function. Science 248:1547–1550

Lönnqvist F, Krief S, Strosberg D, Nyberg B, Emorine LJ, Amer P (1993) Evidence for a functional β_3-adrenoceptor in man. Br J Pharmacol 110:929–936

Lydakis C, Lip GY, Beevers M, Beevers DG (1999) Atenolol and fetal growth in pregnancies complicated by hypertension. Am J Hypertens 12:541–547

Maack C, Cremers B, Flesch M, Höper A, Südkamp M, Böhm M (2000) Different intrinsic activities of bucindolol, carvedilol and metoprolol in human failing myocardium. Br J Pharmacol 130:1131–1139

Magee LA, Brown MA, Hall DR, Gupte S, Hennessy A, Karumanchi SA, Kenny LC, McCarthy F, Myers J, Poon LC, Rana S, Saito S, Staff AC, Tsigas E, von Dadelszen P (2022) The 2021 International Society for the Study of hypertension in pregnancy classification, diagnosis & management recommendations for international practice. Pregnancy Hypertens 27:148–169

Mak IT, Weglicki WB (1988) Protection by β-blocking agents against free radical-mediated sarcolemmal lipid peroxidation. Circ Res 63:262–266

Malik FI, Hartman JJ, Elias KA, Morgan BP, Rodriguez H, Brejc K, Anderson RL, Sueoka SH, Lee KH, Finer JT JT, Sakowicz R, Baliga R, Cox DR, Garard M, Godinez G, Kawas R, Kraynack E, Lenzi D, Lu PP, Muci A, Niu G, Qian X, Pierce DW, Pokrovskii M, Suehiro I, Sylvester S, Tochimoto T, Valdez C, Wang W, Katori T, Kass DA, Shen Y-T, Vatner SF, Morgans DJ (2011) Cardiac myosin activation: a potential therapeutic approach for systolic heart failure. Science 331:1439–1443

Malta E, Mian MA, Raper C (1985) The in vitro pharmacology of xamoterol (ICI 118,587). Br J Pharmacol 85:179–187

Mannhold R (2005) The impact of lipophilicity in drug research: a case report on β-blockers. Mini Rev Med Chem 5:197–205

Martell M, Coll M, Ezkurdia N, Raurell I, Genescà J (2010) Physiopathology of splanchnic vasodilation in portal hypertension. World J Hepatol 27:208–220

Marx SO, Reiken S, Hisamatsu Y, Jayaraman T, Burkhoff D, Rosemblit N, Marks AR (2000) PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. Cell 101:365–376

Mason DA, Moore JD, Green SA, Liggett SB (1999) A gain-of-function polymorphism in a G-protein coupling domain of the human β_1-adrenergic receptor. J Biol Chem 274:12670–12674

Mayourian J, Ceholski DK, Gonzalez DM, Cashman TJ, Sahoo S, Hajjar RJ, Costa KD (2018) Physiologic, pathologic, and therapeutic paracrine modulation of cardiac excitation-contraction coupling. Circ Res 122:167–183

McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, Burri H, Butler J, Čelutkienė J, Chioncel O, Cleland JGF, Crespo-Leiro MG, Farmakis D, Gilard M, Heymans S, Hoes AW, Jaarsma T, Jankowska EA, Lainscak M, Lam CSP, Lyon AR, McMurray JJV, Mebazaa A, Mindham R, Muneretto C, Francesco Piepoli M, Price S, Rosano GMC, Ruschitzka F, Skibelund AK (2023) ESC Scientific Document Group. 2023 focused update of the 2021 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J 44:3627–3639

McMurray J, Køber L, Robertson M, Dargie H, Colucci W, Lopez-Sendon J, Remme W, Sharpe N, Ford I (2005) Antiarrhythmic effect of carvedilol after acute myocardial infarction. J Am Cardiol Soc 45:525–530

McNamara JW, Singh RR, Sadayappan S (2019) Cardiac myosin binding protein-C phosphorylation regulates the super-relaxed state of myosin. Proc Natl Acad Sci USA 116:11731–11736

McPherson GA, Malta E, Molenaar P, Raper C (1984) The affinity and efficacy of the selective β_1-adrenoceptor stimulant RO363 at β_1- and β_2-adrenoceptor sites. Br J Pharmacol 82:897–904

Meredith IT, Broughton A, Jennings GL, Esler MD (1991) Evidence of a selective increase in cardiac sympathetic activity in patients with sustained ventricular arrhythmias. N Engl J Med 325:618–624

MERIT-HF Study Group (1999) Effect of metoprolol CR/XL in chronic heart failure: Metoprolol CR/XL randomised intervention trial in congestive heart failure (MERIT-HF). Lancet 353: 2001–2007

Mialet Perez J, Rathz DA, Petrashevskaya NN, Hanh H, Wagoner LE, Schwartz A, Dorn GW II, Liggett SB (2003) β_1-adrenergic receptor polymorphisms confer differential function and predisposition to heart failure. Nat Med 9:1300–1305

Michel LYM, Farah C, Balligand JL (2020) The Beta3 adrenergic receptor in healthy and pathological cardiovascular tissues. Cells 9:2584

Minobe E, Maeda S, Xu J, Hao L, Kameyama A, Kameyama M (2014) A new phosphorylation site in cardiac L-type Ca^{2+} channels (Cav1.2) responsible for its cAMP-mediated modulation. Am J Physiol Cell Physiol 307:C999–C1009

Mo W, Michel MC, Lee XW, Kaumann AJ, Molenaar P (2017) The β3-adrenoceptor agonist mirabegron increases human atrial force through β_1-adrenoceptors: an indirect mechanism? Br J Pharmacol 174:2706–2715

Mochizuki M, Yano M, Oda T, Tateishi H, Kobayashi S, Yamamoto T, Ikeda Y, Ohkusa T, Ikemoto N, Matsuzaki M (2007) Scavenging free radicals by low-dose carvedilol prevents redox-dependent Ca^{2+} leak via stabilization of ryanodine receptor in heart failure. J Am Coll Cardiol 49:1722–1732

Molenaar P, Parsonage WA (2005) Fundamental considerations of β-adrenoceptor subtypes in human heart failure. Trends Pharmacol Sci 26:368–375

Molenaar P, Summers RJ (1987) Characterization of Beta-1 and Beta-2 adrenoceptors in Guinea pig atrium: functional and receptor binding studies. J Pharmacol Exp Ther 241:1041–1047

Molenaar P, McPherson GA, Malta E, Raper C (1985) The influence of molecular structure on the affinity and efficacy of some β-adrenoceptor agonists. Naunyn Schmiedeberg's Arch Pharmacol 331:240–246

Molenaar P, Sarsero D, Kaumann AJ (1997) Proposal for the interaction of non-conventional partial agonists and catecholamines with the 'putative β4-adrenoceptor' in mammalian heart. Clin Exp Pharmacol Physiol 24:647–656

Molenaar P, Bartel S, Cochrane A, Vetter D, Jalali H, Pohlner P, Burrell K, Karczewski P, Krause E-G, Kaumann A (2000) Both β_2- and β_1-adrenergic receptors mediate hastened relaxation and phosphorylation of phospholamban and troponin I in ventricular myocardium of Fallot infants, consistent with selective coupling of β_2-adrenergic receptors to G_s-protein. Circulation 102: 1814–1821

Molenaar P, Christ T, Ravens U, Kaumann A (2006) Carvedilol blocks β_2- more than β_1-adrenoceptors in human heart. Cardiovasc Res 69:128–139

Molenaar P, Chen L, Semmler ABT, Parsonage WA, Kaumann AJ (2007a) Human heart β-adrenoceptors: β_1-adrenoceptor diversification through 'affinity states' and polymorphism. Clin Exp Pharmacol Physiol 34:1020–1028

Molenaar P, Savarimuthu SM, Sarsero D, Chen L, Semmler ABT, Carle A, Yang I, Bartel S, Vetter D, Beyerdörfer I, Georg Krause E-G, Kaumann AJ (2007b) (−)-Adrenaline elicits positive inotropic, lusitropic, and biochemical effects through β_2-adrenoceptors in human atrial myocardium from nonfailing and failing hearts, consistent with Gs coupling but not with Gi coupling. Naunyn Schmiedeberg's Arch Pharmacol 375:11–28

Molenaar P, Christ T, Hussain RI, Engel A, Berk E, Gillette KT, Chen L, Galindo-Tovar A, Krobert KA, Ravens U, Levy FO, Kaumann AJ (2013) PDE3, but not PDE4, reduces β_1- and β_2-adrenoceptor-mediated inotropic and lusitropic effects in failing ventricle from metoprolol-treated patients. Br J Pharmacol 169:528–538

Molenaar P, Christ T, Berk E, Engel A, Gillette KT, Galindo-Tovar A, Ravens U, Kaumann AJ (2014) Carvedilol induces greater control of β_2- than β_1-adrenoceptor-mediated inotropic and lusitropic effects by PDE3, while PDE4 has no effect in human failing myocardium. Naunyn Schmiedeberg's Arch Pharmacol 387:629–640

Molenaar P, Mo, W, Cheesman E, Dashwood AM, Kaumann AJ, Beard NA (2019) Both β_1- and β_2-adrenoceptors mediate arrhythmic contractions, ryanodine receptor opening and phosphorylation in human failing hearts: control by PDE3. Australasian Society of Clinical and Experimental Pharmacologists Scientific Meeting, Queenstown, Abstract 101

Moniotte S, Balligand J-L (2002) Potential use of β_3-adrenoceptor antagonists in heart failure therapy. Cardiovasc Drug Rev 20:19–26

Moniotte S, Kobzik L, Feron O, Trochu J-N, Gauthier C, Balligand J-L (2001) Upregulation of β3-adrenoceptors and altered contractile response to inotropic amines in human failing myocardium. Circulation 103:1649–1655

Morady F, Nelson SD, Kou WH, Pratley R, Schmaltz S, De Buitleir M, Halter JB (1988) Electrophysiologic effects of epinephrine in humans. J Am Coll Cardiol 11(6):1235–1244. https://doi.org/10.1016/0735-1097(88)90287-2

Morano I (1999) Tuning the human heart molecular motors by myosin light chains. J Mol Med 77: 544–555

Motivala AA, Parikh V, Roe M, Dai D, Abbott JD, Prasad A, Mukherjee D (2016) Predictors, trends, and outcomes (among older patients ≥65 years of age) associated with beta-blocker use in patients with stable angina undergoing elective percutaneous coronary intervention: insights from the NCDR registry. JACC Cardiovasc Interv 9:1639–1648

Motomura S, Hashimoto K (1992) Beta 2-adrenoceptor-mediated positive dromotropic effects on atrioventricular node of dogs. Am J Phys Jan;262(1 Pt 2):H123-H129. https://doi.org/10.1152/ajpheart.1992.262.1.H123.

Mozaffarian D, Anker SD, Anand I, Linker DT, Sullivan MD, Cleland JGF, Carson PE, Maggioni AP, Mann DL, Pitt B, Poole-Wilson PA, Levy WC (2007) Prediction of mode of death in heart failure. The Seattle heart failure model. Circulation 116:392–398

Mukete BN, Cassidy M, Ferdinand KC, Le Jemtel TH (2015) Long-term anti-hypertensive therapy and stroke prevention: a meta-analysis. Am J Cardiovasc Drugs 15:243–257

Naidu SS, Baran DA, Jentzer JC, Hollenberg SM, van Diepen S, Basir MB, Grines CL, Diercks DB, Hall S, Kapur NK, Kent W, Rao SV, Samsky MD, Thiele H, Truesdell AG, Henry TD (2022) SCAI SHOCK stage classification expert consensus update: a review and incorporation of validation studies: this statement was endorsed by the American College of Cardiology (ACC), American College of Emergency Physicians (ACEP), American Heart Association (AHA), European Society of Cardiology (ESC) Association for Acute Cardiovascular Care (ACVC), International Society for Heart and Lung Transplantation (ISHLT), Society of Critical Care Medicine (SCCM), and Society of Thoracic Surgeons (STS) in December 2021. J Am Coll Cardiol 79:933–946

Nazari MA, Hasan R, Haigney M, Maghsoudi A, Lenders JWM, Carey RM, Pacak K (2023) Catecholamine-induced hypertensive crises: current insights and management. Lancet Diabetes Endocrinol 11:942–954

Neftel KA, Adler RH, Käppeli L, Rossi M, Dolder M, Käser HE, Bruggesser HH, Vorkauf H (1982) Stage fright in musicians: a model illustrating the effect of beta blockers. Psychosom Med 44: 461–469

NHFA CSANZ Heart Failure Guidelines Working Group, Atherton JJ, Sindone A, De Pasquale CG, Driscoll A, MacDonald PS, Hopper I, Kistler PM, Briffa T, Wong J, Abhayaratna W, Thomas L, Audehm R, Newton P, O'Loughlin J, Branagan M, Connell C (2018) National Heart Foundation of Australia and Cardiac Society of Australia and New Zealand: guidelines for the prevention, detection, and Management of Heart Failure in Australia 2018. Heart Lung Circ 27: 1123–1208

Nikolaev VO, Moshkov A, Lyon AR, Miragoli M, Novak P, Paur H, Lohse MJ, Korchev YE, Harding SE, Gorelik J (2010) β_2-adrenergic receptor redistribution in heart failure changes cAMP compartmentation. Science 237:1653–1657

Ogawa R, Stachnik JM, Echizen H (2014) Clinical pharmacokinetics of drugs in patients with heart failure: an update (part 2, drugs administered orally). Clin Pharmacokinet 53:1083–1114

Oldham HG, Clarke SE (1997) *In vitro* identification of the human cytochrome P450 enzymes involved in the metabolism of R(+)- and S(−)-carvedilol. Drug Metab Dispos 25:970–977

Packer M (1985) Sudden unexpected death in patients with congestive heart failure: a second frontier. Circulation 72:681–685

Packer M, Bristow MR, Cohn JN, Colucci WS, Fowler MB, Gilvert EM, Shusterman HH, for the U.S. Carvedilol Heart Failure Study Group (1996) The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. N Engl J Med 334:1349–1355

Packer M, AJS C, Fowler MB, Katus HA, Krum H, Mohacsi P, Rouleau JL, Tendera M, Castaigne A, Roecker EB, Schultz MK, De Mets DL, for the Carvedilol Prospective Randomized Cumulative Survival Study Group (2001) Effect of carvedilol on survival in severe chronic heart failure. N Engl J Med 344:1651–1658

Peltenburg PJ, Kallas D, Bos JM, Lieve KVV, Franciosi S, Roston TM, Denjoy I, Sorensen KB, Ohno S, Roses-Noguer F, Aiba T, Maltret A, LaPage MJ, Atallah J, Giudicessi JR, Clur SB, Blom NA, Tanck M, Extramiana F, Kato K, Barc J, Borggrefe M, Behr ER, Sarquella-Brugada-G, Tfelt-Hansen J, Zorio E, Swan H, Kammeraad JAE, Krahn AD, Davis A, Sacher F, Schwartz PJ, Roberts JD, Skinner JR, van den Berg MP, Kannankeril PJ, Drago F, Robyns T, Haugaa K, Tavacova T, Semsarian C, Till J, Probst V, Brugada R, Shimizu W, Horie M, Leenhardt A, Ackerman MJ, Sanatani S, van der Werf C, Wilde AAM (2022) An international multicenter cohort study on β-blockers for the treatment of symptomatic children with catecholaminergic polymorphic ventricular tachycardia. Circulation 145:333–344

Pelzmann B, Schaffer P, Bernhart E, Lang P, Mächler H, Rigler B, Koidl B (1998) L-type calcium current in human ventricular myocytes at a physiological temperature from children with tetralogy of Fallot. Cardiovasc Res 38:424–432

Pieske B, Houser SR (2003) $[Na^+]i$ handling in the failing human heart. Cardiovasc Res 57:874–886

Pogwizd SM, Bers DM (2004) Cellular basis of triggered arrhythmias in heart failure. Trends Cardiovasc Med 14:61–66

Pogwizd SM, Schlotthauer K, Li L, Yuan W, Bers DM (2001) Arrhythmogenesis and contractile dysfunction in heart failure. Roles of sodium-calcium exchange, inward rectifier potassium current, and residual β-adrenergic responsiveness. Circ Res 88:1159–1167

Poole-Wilson PA, Swedberg K, Cleland JGF, Di Lenarda A, Hanrath P, Komajda M, Lubsen J, Lutiger B, Metra M, Remme WJ, Torp-Pedersen C, Scherhag A, Skene A, for the COMET Investigators (2003) Comparison of carvedilol and metoprolol on clinical outcomes in patients with chronic heart failure in the carvedilol or metoprolol European trial (COMET): randomised controlled trial. Lancet 362:7–13

Proudman RGW, Pupo AS, Baker JG (2020) The affinity and selectivity of α-adrenoceptor antagonists, antidepressants, and antipsychotics for the human α1A, α1B, and α1D-adrenoceptors. Pharmacol Res Perspect 8(4):e00602

Proudman RGW, Akinaga J, Baker JG (2022) The affinity and selectivity of α-adrenoceptor antagonists, antidepressants and antipsychotics for the human α2A, α2B and α2C-adrenoceptors and comparison with human α1 and β-adrenoceptors. Pharmacol Res Perspect 10:300936

Reiken S, Wehrens XHT, Vest JA, Barbone A, Klotz S, Mancini D, Burkhoff D, Marks AR (2003) β-blockers restore calcium release channel function and improve cardiac muscle performance in human heart failure. Circulation 107:2459–2466

Ross DS, Burch HB, Cooper DS, Greenlee MC, Laurberg P, Maia AL, Rivkees SA, Samuels M, Sosa JA, Stan MN, Walter MA (2016) 2016 American Thyroid Association guidelines for diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. Thyroid 26: 1343–1421

Roybal D, Hennessey JA, Marx SO (2020) The quest to identify the mechanism underlying adrenergic regulation of cardiac Ca^{2+} channels. Channels 14:123–131

Rydén L, Ariniego R, Arnman K, Herlitz J, Hjalmarson A, Holmberg S, Reyes C, Smedgård P, Svedberg K, Vedin A, Waagstein F, Waldenström A, Wilhelmsson C, Wedel H, Yamamoto M (1983) A double-blind trial of metoprolol in acute myocardial infarction. Effects on ventricular tachyarrhythmias. N Engl J Med 308:614–618

Sarsero D, Fujiwara T, Molenaar P, Angus JA (1998) Human vascular to cardiac tissue selectivity of L- and T-type calcium channel antagonists. Br J Pharmacol 125:109–119

Sarsero D, Russell FD, Lynham JA, Rabnott G, Yang I, Fong KM, Li L, Kaumann AJ, Molenaar P (2003) (−)-CGP 12177 increases contractile force and hastens relaxation of human myocardial preparations through a propranolol-resistant state of the β_1-adrenoceptor. Naunyn Schmiedeberg's Arch Pharmacol 367:10–21

Savonitto S, Ardissiono D, Egstrup K, Rasmussen K, Bae EA, Omland T, Schjelderup-Mathiesen PM, Marraccini P, Wahlqvist I, Merlini PA, Rehnqvist N (1996) Combination therapy with metoprolol and nifedipine versus monotherapy in patients with stable angina pectoris. Results of the international multicenter angina exercise (IMAGE) study. J Am Coll Cardiol 27:311–316

Schlotthauer K, Bers DM (2000) Sarcoplasmic reticulum Ca^{2+} release causes myocyte depolarization. Underlying mechanism and threshold for triggered action potentials. Circ Res 87:774–780

Sennitt MV, Kaumann AJ, Molenaar P, Beeley LJ, Young PW, Kelly J, Chapman H, Henson SM, Berge JM, Dean DK, Kotecha NR, Morgan HKA, Rami HK, Ward RW, Thompson M, Wilson S, Smith SA, Cawthorne MA, Stock ML, Arch JRS (1998) The contribution of classical ($\beta_{1/2}$-) and atypical β- adrenoceptors to the stimulation of human white adipocyte lipolysis and right atrial appendage contraction by novel β_3-adrenoceptor agonists of differing selectivities. J Pharmacol Exp Ther 285:1084–1095

Seravalle G, Quarti-Trevano F, Dell'Oro R, Gronda E, Spaziani D, Facchetti R, Cuspidi C, Mancia G, Grassi G (2019) Sympathetic and baroreflex alterations in congestive heart failure with preserved, midrange and reduced ejection fraction. J Hypertens 37:443–448

Sharma M, Rameshbabu CS (2012) Collateral pathways in portal hypertension. J Clin Exp Hepatol 2:338–352

Silva JE, Bianco SD (2008) Thyroid-adrenergic interactions: physiological and clinical implications. Thyroid 18:157–165

Solaro RJ, Rarick HM (1998) Troponin and Tropomyosin. Proteins that switch on and tune in the activity of cardiac myofilaments. Circ Res 83:471–480

Sprenger T, Viana M, Tassorelli C (2018) Current prophylactic medications for migraine and their potential mechanisms of action. Neurotherapeutics 15:313–323

Steenen SA, van Wijk AJ, van der Heijden GJ, van Westrhenen R, de Lange J, de Jongh A (2016) Propranolol for the treatment of anxiety disorders: systematic review and meta-analysis. J Psychopharmacol 30:128–139

The Beta-Blocker Evaluation of Survival Trial Investigators (2001) A trial of the beta-blocker bucindolol in patients with advanced chronic heart failure. N Engl J Med 344:1659–1667

The Xamoterol in Severe Heart Failure Study Group (1990) Xamoterol in severe heart failure. Lancet 336:1–6

Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, White HD, Executive Group on behalf of the Joint European Society of Cardiology (ESC), American College of Cardiology (ACC), American Heart Association (AHA), World Heart Federation (WHF), Task Force for the Universal Definition of Myocardial Infarction (2018) Fourth universal definition of myocardial infarction. J Am Coll Cardiol 72:2231–2264

Trzepacz PT, Klein I, Roberts M, Greenhouse J, Levey GS (1989) Graves' disease: an analysis of thyroid hormone levels and hyperthyroid signs and symptoms. Am J Med 87:558–561

Ungerer M, Böhm M, Elce JS, Erdmann E, Lohse MJ (1993) Altered expression of β-adrenergic receptor kinase and β_1-adrenergic receptors in the failing human heart. Circulation 87:454–463

Ungerer M, Parruti G, Bohm M, Puzicha M, DeBlasi A, Erdmann E, Lohse MJ (1994) Expression of beta-arrestins and beta-adrenergic receptor kinases in the failing human heart. Circ Res 74: 206–213

Waagstein F, Hjalmarson Å, Varnauskas E, Wallentin I (1975) Effect of chronic beta-adrenergic receptor blockade in congestive cardiomyopathy. Br Heart J 37:1022–1036

Waagstein F, Bristow MR, Swedberg K, Camerini F, Fowler MB, Silver MA, Gilbert EM, Johnson MR, Goss FG, Hjalmarson A, for the Metoprolol in Dilated Cardiomyopathy (MDC), Trial Study Group (1993) Beneficial effects of metoprolol in idiopathic dilated cardiomyopathy. Lancet 342:1441–1446

Walter M, Lemoine H, Kaumann AJ (1984) Stimulant and blocking effects of optical isomers of pindolol on the sinoatrial node and trachea of Guinea pig. Role of β-adrenoceptor subtypes in the dissociation between blockade and stimulation. Naunyn Schmiedeberg's Arch Pharmacol 327:159–175

Walweel K, Molenaar P, Imtiaz MS, Denniss A, dos Remedios C, van Helden DF, Dulhunty AF, Laver DR, Beard NA (2017) Ryanodine receptor modification and regulation by intracellular Ca^{2+} and Mg^{2+} in healthy and failing human hearts. J Mol Cell Cardiol 140:53–62

Walweel K, Gomez-Hurtado N, Rebbeck RT, Oo YW, Beard NA, Molenaar P, dos Remedios C, van Helden DF, Cornea RL, Knollmann BC, Laver DR (2019) Calmodulin inhibition of human RyR2 channels requires phosphorylation of RyR2-S2808 or RyR2-S2814. J Mol Cell Cardiol 130:96–106

Wang J, Hanada K, Staus DP, Makara MA, Dahal GR, Chen Q, Ahles A, Engelhardt S, Rockman HA (2017) Gαi is required for carvedilol-induced β_1 adrenergic receptor β-arrestin biased signalling. Nat Commun 8:1706

Warren SG, Brewer DL, Orgain ES (1976) Long-term propranolol therapy for angina pectoris. Am J Cardiol 37:420–426

Weiss R, Ferry D, Pickering E, Smith LK, Dennish G 3rd, Krug-Gourley S, Lukas MA (1998) Effectiveness of three different doses of carvedilol for exertional angina. Carvedilol-Angina Study Group. Am J Cardiol 82:927–931

Weiss S, Oz S, Benmocha A, Dascal N (2013) Regulation of cardiac L-type Ca^{2+} channel $Ca_v1.2$ via the β-adrenergic-cAMP-protein kinase a pathway. Old dogmas, advances, and new uncertainties. Circ Res 113:617–631

Williams LT, Lefkowitz RJ, Watanabe AM, Hathaway DR, Besch HR Jr (1977) Thyroid hormone regulation of β-adrenergic receptor number. J Biol Chem 25:2787–2279

Wisler JW, DeWire SM, Whalen EJ, Violin JD, Drake MT, Ahn S, Shenoy SK, Lefkowitz RJ (2007) A unique mechanism of β-blocker action: Carvedilol stimulates β-arrestin signalling. PNAS 104:16657–16662

Woo AY-H, Song Y, Xiao R-P, Zhu W (2015) Biased β_2-adrenoceptor signalling in heart failure: pathophysiology and drug discovery. Br J Pharmacol 172:5444–5456

Woody MS, Greenberg MJ, Barua B, Winkelmann DA, Goldman YE, Ostap EM (2018) Positive cardiac inotrope omecamtiv mecarbil activates muscle despite suppressing the myosin working stroke. Nat Commun 9:3838

Xiao RP, Hohl C, Altschuld R, Jones L, Livingston B, Ziman B, Tantini B, Lakatta EG (1994) β_2-adrenergic receptor-stimulated increase in cAMP in rat heart cells is not coupled to changes in Ca^{2+} dynamics, contractility, or phospholamban phosphorylation. J Biol Chem 269:19151–19156

Xiao R-P, Ji X, Lakatta EG (1995) Functional coupling of the β_2-adrenoceptor to a pertussis toxin-sensitive G protein in cardiac myocytes. Mol Pharmacol 47:322–329

Xiao R-P, Avdonin P, Zhou Y-Y, Cheng H, Akhter SA, Eschenhagen T, Lefkowitz RJ, Koch WJ, Lakatta EG (1999) Coupling of β2-adrenoceptor to Gi proteins and its physiological relevance in murine cardiac myoctyes. Circulation 84:43–52

Yue T-L, Cheng H-Y, Lysko PG, McKenna PJ, Feuerstein R, Gu J-L, Lysko KA, Davis LL, Feuerstein G (1992) Carvedilol, a new vasodilator and Beta adrenoceptor antagonist, is an antioxidant and free radical scavenger. J Pharmacol Exp Ther 263:92–98

Zhou Q, Xiao J, Jiang D, Wang R, Vembaiyan K, Wang A, Smith CD, Xie C, Chen W, Zhang J, Tian X, Jones PP, Zhong X, Guo A, Chen H, Zhang L, Zhu W, Yang D, Li X, Chen J, Gillis AM, Henry J, Duff HJ, Cheng H, Feldman AM, Song L-S, Fill M, Thomas G, Back TG, Chen SRW (2011) Carvedilol and its new analogs suppress arrhythmogenic store overload–induced Ca^{2+} release. Nat Med 17:1003–1009

Zhu W-Z, Zheng M, Koch WJ, Lefkowitz RJ, Kobilka BK, Xiao R-P (2001) Dual modulation of cell survival and cell death by β_2-adrenergic signalling in adult mouse cardiac myocytes. Proc Natl Acad Sci U S A 98:1607–1612

Adrenoceptors and Hypertension

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Contents

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Abstract

Hypertension is a very prevalent condition associated with high mortality and morbidity, secondary to changes resulting in blood vessels and resultant end-organ damage. Haemodynamic changes, including an initial rise in cardiac output followed by an increase in total peripheral resistance, denote the early changes associated with borderline or stage 1 hypertension, especially in young men. Increased sodium reabsorption leading to kidney damage is another mechanism proposed as one of the initial triggers for essential hypertension. The underlying pathophysiological mechanisms include catecholamine-induced α_1- and $ß_1$-adrenoceptor stimulation, and renin–angiotensin–aldosterone system activation leading to endothelial dysfunction which is believed to lead to persistent blood pressure elevation.

α_1 blockers, α_2 agonists, and ß blockers were among the first oral antihypertensives. They are no longer first-line therapy after outcome trials did not demonstrate any benefits over and above other agents, despite similar blood pressure reductions. Angiotensin-converting enzyme inhibitors (or angiotensin receptor blockers), calcium channel blockers, and thiazide-like diuretics are now considered the first line of therapy, although adrenoceptor agents still have a role as second- or third-line therapy. The chapter also highlights hypertension in specific medical conditions such as pregnancy, phaeochromocytoma, hyperthyroidism, portal hypertension, pulmonary arterial hypertension, and ocular hypertension, to provide an overview for clinicians and researchers interested in the role of adrenoceptors in the pathophysiology and management of hypertension.

Keywords

Adrenoceptors · Essential Hypertension · Pathophysiology

Abbreviations

ABPM	Ambulatory blood pressure monitoring
ACEi	Angiotensin-converting enzyme inhibitor
AR	Adrenoceptor
ARB	Angiotensin receptor blocker
ASCOT	The Anglo-Scandinavian cardiovascular outcomes trial
BP	Blood pressure
BZD	Benzodiazepine
CCB	Calcium channel blocker
CVD	Cardiovascular disease
CO	Cardiac output
DBP	Diastolic blood pressure
EH	Essential hypertension
EOD	End organ damage
FDA	Food and Drug Administration (FDA)

GFR	Glomerular filtration rate
HR	Heart rate
HVPG	Hepatic venous pressure gradient
LDL	Low-density lipoprotein
LIFE	Losartan Intervention for Endpoint reduction
LV	Left ventricle
MAP	Mean arterial pressure
MRC	Medical Research Council
NANC	Nonadrenergic and noncholinergic neurons
NICE	National Institute for Health and Care Excellence
NO	Nitric oxide
NSBB	Non-selective ß-blockers
OSA	Obstructive sleep apnoea
PH	Pulmonary hypertension
PAH	Pulmonary arterial hypertension
PPGL	Paragangliomas
RAAS	Renin–angiotensin–aldosterone-system
RCT	Randomised control trial
SBP	Systolic blood pressure
SNS	Sympathetic nervous system
TPR	Total peripheral resistance
TPRI	Total peripheral resistance index

1 Introduction

Hypertension (raised blood pressure (BP)) was recognised as a specific risk factor for cardiovascular disease (CVD) more than 100 years ago. BP measurement itself began just over 100 years ago, and treatment was more generally available about 60 years ago. The first modern BP cuff was invented in 1896 by Riva-Rocci, and Korotkoff described the 'Korotkoff sounds' in 1905. The turning point for research in hypertension and CVD management followed the death of President Franklin Roosevelt in 1945 – presumed secondary to cerebral haemorrhage, as his BP readings in days preceding his death were higher than 300/190 mmHg (Moser 2006).

Hypertension is estimated to affect about 1/3rd of the adult population (Mills et al. 2020) making it one of the most common medical diagnoses. Left untreated, hypertension increases the risk of serious damage to end-organs and leads to life-threatening states that include heart disease (heart failure, coronary heart disease), cerebrovascular accidents (stroke), and chronic kidney disease. It remains the leading cause of death and morbidity worldwide and has been linked to more than 10.4 million deaths annually (Collaborators GRF 2018). A systolic BP (SBP) of ≥140 mmHg is associated with 14% of all-cause deaths (Forouzanfar et al. 2017). Apart from high rates of mortality and morbidity, hypertension also results in a huge

economic burden to healthcare services across the globe as a result of the devastating CVD events, i.e. the cost of treating strokes, heart failure, and coronary heart disease (Agency UHS 2021). Fortunately, pharmacological treatment of hypertension reduces all-cause mortality, and the incidence of CVD events (Turnbull & Blood Pressure Lowering Treatment Trialists Collaboration 2003). However, among people diagnosed with hypertension, only about 50% are optimally treated. Various factors contribute to this apparent failure. First and foremost is the fact that hypertension is a silent killer. In comparison to a rise in temperature, heart rate, or respiratory rate, there are no corresponding direct symptoms or signs that make elevated BP obvious to the patient or to the clinician, other than by measuring with a sphygmomanometer.

1.1 Diagnosis of Hypertension

Hypertension is usually defined as a sustained blood pressure of 140/90 mmHg or higher after repeated measurements in clinic. The term white-coat hypertension, coined by Thomas Pickering, indicates individuals who have elevated BP measured in a clinical environment, but normal daytime BP measurements on ambulatory blood pressure monitoring (ABPM). ABPM is offered to all patients whose BP has been noted to be high for the very first time. If this is not feasible, home BP measurements (HBPM) are a practical and reasonable alternative. ABPM or HBPM daytime average of 135/85 mmHg or higher is considered a confirmed diagnosis of hypertension as per current National Institute for Health and Care Excellence (NICE) guidelines for hypertension diagnosis and management (NG136) (NICE 2022). The definition and categories of hypertension have evolved over the years. More recently, intensive BP targets (SBP $<$ 120 mmHg) have been shown to reduce cardiovascular and all-cause death in randomised control trials (RCTs) (Group et al. 2015) making the case for using a lower BP as a target for diagnosis. Indeed, other international guidelines recommend a therapeutic target of 130/80 mmHg or less at least in some scenarios (Mancia et al. 2023). However, at the time of writing, the benefits of intensive reduction in BP are not perceived to be homogenous across all populations of hypertension.

Hypertension is typically defined as essential or idiopathic hypertension (EH) when there are no clear reasons detectable for the persistently elevated BP. EH (90–95% cases) is the most common cause of hypertension, even in young-onset (defined as the onset of hypertension at $\leq$40 years of age) (Hinton et al. 2020; Rison et al. 2022). EH has been noted to run in families, however, genetic studies have failed to show a clear single genetic variant as a causal factor. Thus, it is viewed as a polygenic condition involving the inheritance of susceptibility genes with environmental factors contributing. Obesity is considered a major modifiable contributor with an estimated 65–75% of the risk of EH is associated with excess weight gain (Garrison et al. 1987). Secondary hypertension is hypertension that has an underlying detectable pathological cause, the true prevalence of which is unknown (Mancia et al. 2023) but amounts to ~5–10% of all cases. It includes

conditions such as primary aldosteronism, thyroid disorders, vasculitis, obstructive sleep apnoea (OSA), phaeochromocytoma, coarctation of the aorta, and fibromuscular dysplasia affecting renal arteries. OSA in most scenarios cannot be considered as a true secondary cause, as it is usually associated with obesity and metabolic syndrome that are considered contributory factors associated with EH. Secondary hypertension is often underdiagnosed and affects younger patients and those with resistant hypertension, which is defined as hypertension that remains refractory to the three first-line anti-hypertensive medications that include a diuretic (thiazide-like usually) at maximum tolerated doses.

1.2 Haemodynamics of Chronic Hypertension

Mean arterial pressure (MAP) is the product of cardiac output (CO) and total peripheral vascular resistance (TPR). The latter is dependent on the cross-sectional area of resistance vessels (diameter 100–300 μM) that are controlled by smooth muscle tone but also depend to a lesser extent on blood viscosity. Thus, vasoconstriction reduces blood vessel diameter and raises BP. Arterial pressure is tightly regulated via CO and TPR to intricately maintain blood flow within organs, which ensures tissues receive appropriate blood flow across a wide range of pressures. Normotensive people autoregulate flow across a relatively wide range of 'usual' pressures between 50 and 150 mmHg of mean arterial pressure (MAP) (Lassen 1959), although this has been questioned more recently (Lucas et al. 2010). Baseline resistance and autoregulatory capacity, or behaviour, vary across different organs. Renal and cerebral circulations are low resistance and have a high capacity for autoregulation, whereas the splanchnic circulation and skeletal muscles are higher resistance and only have moderate capacity for autoregulation. In contrast, cutaneous and mesenteric circulation is largely devoid of autoregulatory capacity (Clifford 2011). This is much more discernible when faced with a flight or fight scenario when skin and gut vessels contract (resulting in pallor or white with fright and a queasy feeling) whereas vessels in muscles dilate to maximise blood delivery to organs most needed in that response.

BP can vary quite rapidly in states such as stress and cardiac dysrhythmias. The nocturnal dipping pattern of BP (normally ~10–20%) is a physiological phenomenon but may be either absent ('non-dippers' $<10\%$ reduction), or reversed, and such a classification is based on ambulatory BP monitoring (ABPM) only (O'Brien et al. 2018). Various factors including poor quality of sleep, OSA, higher day or average ABPM, low glomerular filtration rate (GFR), and male gender are associated with loss of nighttime dip. A sharp rise in BP on waking, referred to as the 'morning surge', is another well-studied physiological occurrence. Other factors that affect BP are age, body morphology, smoking, effects of meals, sleep-wake cycle, patient awareness, and the white coat effect (Parati et al. 2015). It is well known that even in relaxed subjects, placing a BP cuff over the arm for measurement can increase BP by 10 mmHg, and in the past, the sight of a physician during a ward round increased BP by 10–20 mmHg, although with better monitoring methods, the advent of

telemedicine, availability of home BP monitors, and changing doctor–patient relationships it is not known if this observation persists.

Fortunately, tackling EH and its deleterious effects has become much easier in the last few decades with a well-established armamentarium of evidence-based anti-hypertensive therapies. In other words, the management of hypertension has been a pharmacological success story with well-tolerated, effective anti-hypertensive drugs that are readily available, backed by a plethora of high-quality evidence from RCTs (NICE 2022; Mancia et al. 2023). Current national and international guidelines for the management of chronic hypertension recommend angiotensin-converting enzyme inhibitors (ACEi)/angiotensin receptor blockers (ARB), calcium channel blockers (CCB), and thiazide-like diuretics as first-line therapies with few considerations based on age and ethnicity (NICE 2022). Concomitant lifestyle change recommendations such as increased physical activity, reduction in salt and alcohol intake, and cutting down smoking are recommended for all patients. ß-blockers, α-blockers, and mineralocorticoid antagonists are considered as options in specific indications only (Please see the section on Management of Hypertension for further details and evidence base).

2 Pathophysiology of Essential Hypertension with a Focus on Adrenoceptors

Adrenoceptors mediate the biological effects of the sympathetic nervous system (SNS) at the organ and cellular levels by interactions with endogenous neurotransmitters or ligands, namely adrenaline and noradrenaline.

α-and ß-adrenoceptors (AR) are G-protein-coupled receptors of which there are 9 AR subtypes with a distribution that varies throughout the vasculature and the heart (Gambardella et al. 2023). The subtypes of ARs include α-AR and ß-AR, which can be further broadly characterised into α_1 (α_{1A}, α_{1B}, α_{1D}), α_2 (α_{2A}, α_{2B}, and α_{2C}) (Proudman et al. 2022), and $ß_1$, $ß_2$, $ß_3$ (Baker et al. 2023). The AR subtypes are different from each other primarily in their affinity for ligands and the type of G-protein they are coupled to. This coupling generates different intracellular second messengers. α_1-ARs are typically coupled to the Gq protein, leading to the production of inositol triphosphate and calcium. On the other hand, α_2-ARs are coupled to the Gi protein, which inhibits adenylyl cyclase and activates hyperpolarising K+ currents (Bylund 2007). The classical common pathway for $ß_1$-, $ß_2$-, and $ß_3$-ARs is coupling to Gs protein resulting in increased second messenger, cyclic adenosine monophosphate (cAMP) levels that in turn can have different cellular outputs depending on the location of the AR (Holthoff et al. 2012). α_2-ARs were first characterised as presynaptic receptors on central and peripheral nerves inhibiting the release of noradrenaline, forming a negative feedback loop. Later it was appreciated that these α_2-ARs also had post-synaptic function. α_2-ARs are widely distributed throughout the central nervous system ultimately regulating sympathetic nervous system (SNS) activity. Interestingly, α_2-ARs are not only autoreceptors that inhibit the release of their own neurotransmitter but they can regulate the release of

other neurotransmitters such as serotonin and dopamine in different parts of the brain (Philipp et al. 2002).

Understanding the differential effects of stimulatory and inhibitory effects of ARs in the brain, blood vessels, heart, and kidney will help delineate the role of SNS in hypertension.

Human vascular smooth muscle expresses both vasoconstrictor α_1- and α_2-ARs, and vasodilatory ß-ARs. Vascular AR subtypes may be further differentially distributed between various organ vascular beds and the effects depend on the cell type. Typically, α_1-AR activation in vascular smooth muscle causes contraction leading to vasoconstriction. ARs in the cutaneous vascular smooth cells in the skin are predominantly of the α_1-AR subtype whereas in the coronary blood vessels ß-AR are predominant. Vascular α_2-AR subtypes are also differentially distributed between vascular beds and across species. For example, the hypertensive response seen with α_2-AR agonist injection in the carotid artery was shown to be mediated by the α_{2B}-AR in mice (Link et al. 1996). In some arteries and in certain species, environmental conditions such as temperature may determine the downstream signalling, so for example at cooler temperatures, α_2-AR mediated vasoconstriction may predominate over α_{1A}-AR induced vasoconstriction. Cold-induced sensitivity of peripheral vascular α_2-ARs is implicated in vasospastic attacks in Raynaud's disease (Freedman et al. 1995). There is evidence that receptor distribution changes in disease states, so for example in diabetic neuropathy the α_1-AR in human blood vessels are upregulated and associated with neuropathic pain (Schlereth et al. 2021). Thus, the clinical impact of the stimulation and inhibition of AR depends on the organs and the blood vessels themselves but also on the distribution of the AR subtypes.

The inner monolayer of blood vessels, the endothelium, expresses several different ARs including $ß_2$, $ß_3$, α_1, and α_2 all of which contribute to vasodilatation and vascular angiogenesis, in part by the release of nitric oxide (NO) from endothelial cells (Gambardella et al. 2023). Additionally, there is growing evidence that the vasculature is also innervated by non-adrenergic and non-cholinergic (NANC) neurons that modulate vascular tone by releasing neurotransmitters such as neuropeptide Y and NO (via neuronal NO synthase). Neuronal NO has been shown to play an important role in the physiological regulation of systemic vascular resistance and BP in humans in healthy states (Shabeeh et al. 2017).

In the healthy human heart, cardiomyocytes express all three ß-AR subtypes. $ß_1$-ARs predominate in the heart, followed by $ß_2$ (Bristow et al. 1986) along with minimal expression of $ß_3$-AR. $ß_1$-ARs are present in all cardiomyocytes. The $ß_2$-AR is found in the myocardial and endothelial cells and even cardiac fibroblasts. The ratio of $ß_1$-to $ß_2$-ARs varies across different regions of the human heart (Stiles et al. 1983; Brodde et al. 2001). The $ß_2$ ARs in human cardiomyocytes, although fewer in number are better coupled to adenylyl cyclase and cAMP production and have been shown to have a functional role, stimulation leading to an increase in heart rate. $ß_2$- and $ß_3$-ARs can under certain circumstances couple to Gi protein inhibiting adenylyl cyclase leading to an alternate pathway and additionally $ß_3$-ARs can also couple to NO synthase (do Vale et al. 2019). There is also a clear difference in the cellular

distribution of ß$_1$- and ß$_2$-AR that influences where the cAMP is produced within the cell.

Overall, activation of both ß$_1$ and ß$_2$ ARs in the heart leads to an increase in force (inotropy) and the rate (chronotropy) of systolic contraction, and a more rapid rate of relaxation (lusitropy) once in diastole. Persistent ß$_1$ stimulation is associated with the development of cardiac hypertrophy and is implicated in the pathogenesis of heart failure. However, persistent ß$_2$-AR activation leads to a reversal of these effects, thus having both stimulatory and inhibitory effects on the heart. ß$_3$ ARs are believed to exhibit cardio-depressant activity in contrast to ß$_1$ and ß$_2$ ARs (Skeberdis 2004). α$_1$-ARs are found in the heart; however, it is unknown whether stimulation resulting in vasoconstriction contributes directly towards pathological cardiac hypertrophy. Presynaptic α$_2$-ARs have been found in the right atrium and inhibit noradrenaline release (Brodde et al. 2001).

The coronary blood flow is modulated by AR by a complex balance between vasoconstrictive and vasodilatory forces. ß-AR (all three subtypes are present) in coronary blood vessels mediate vasodilatation, whereas α-AR tends to have vasoconstrictive effects. Increased SNS activity, for example in exercise, leads to vasodilation, which stimulates both cardiac metabolism and coronary vasodilation. This mechanism is known as 'feedforward sympathetic vasodilatation' (Miyashiro and Feigl 1993). This has clinical implications in disease states such as atherosclerosis where vasodilatory components (ß-ARs, endothelial α$_2$-ARs) are impaired while unmasking vasoconstrictive components (vascular smooth muscle α-ARs), contributing to the precipitation of myocardial ischaemia (Barbato 2009). The vasodilatory effect of ß$_2$-ARs observed in the microcirculation mostly occurs through mechanisms involving NO release and vessel hyperpolarisation.

In addition, genetic variations across various ARs may contribute to inter-subject variability in the strength of response resulting from the ligand-receptor interaction (see Genetic Variants of Adrenoceptors chapter in this volume) (Ahles and Engelhardt 2023).

2.1 Haemodynamics at the Onset of Hypertension: Role of the Sympathetic Nervous System

Various models have been studied to delineate the initial triggers that lead to the onset of essential hypertension (EH). In the 1940s and 1950s, increased total peripheral vascular resistance (TPR) was regarded as the primary reason for the rise in arterial BP with a reduction in CO as a secondary effect based on pre-clinical renal models of hypertension. Lund-Johansen challenged this analogy (Lund-Johansen 1979, 1983, 1991; Lund-Johansen and Omvik 1991; Omvik and Lund-Johansen 1990; Lund-Johansen and Bakke 1979) by undertaking a series of cohort-styled clinical studies by recording changes in rest- and exercise-induced haemodynamic parameters over a 20-year follow-up period in hypertensive and normotensives young participant cohorts. The most interesting finding was the exercise-induced initial increase in stroke index in the hypertensive group compared

to the normotensive cohort. This was followed by a transition period of a raised CO and low TPR phase before changing to a lower CO and high TPR phase followed by the persistence of elevated TPR over time despite good BP control. At first, the rise in CO was thought to be due to increased blood volume but blood volume is usually normal in EH if not hypovolaemic secondary to pressure natriuresis (Weidmann et al. 1977). The young borderline and hypertensive patients in these studies tended to have higher levels of catecholamines in comparison to normotensive age-matched controls demonstrating excessive sympathetic drive and decreased parasympathetic inhibition to the heart. Similarly, Julius et al. showed that young patients with borderline hypertension had statistically higher heart rates (HR) accompanied by relatively elevated levels of plasma noradrenaline (with levels below the cut-offs for diagnosis of neuroendocrine tumour) in comparison to normotensive individuals (Julius et al. 1991). The marked sympathetic activation in early hypertension was also demonstrated by more robust methods including noradrenaline spill-over studies and microneurography (Esler et al. 1988). Young adults with a higher resting tachycardia had higher rates of development of hypertension over the years compared to age-matched controls as shown in the Framingham Heart study (Levy et al. 1945). In a longitudinal study, increased responses to stress measured by initial BP values (even within normal BP ranges) associated with higher plasma noradrenaline (within normal ranges) were found to predict the development of hypertension underlining the role of the SNS in the onset of EH especially in young-onset (Gudmundsdottir et al. 2008). The transition to a lower CO and sustained rise in TPR is thought to be driven by the hypothalamic cardiovascular neurons becoming sensitised by repeated exposure to mental stress. Neurogenic pressor responses with pressure-related negative feedback on SNS activity have been used to explain why these young patients then go on to develop hypertension without subsequent elevation in sympathetic drive, i.e. without persistently raised noradrenaline levels (Julius 1988). The haemodynamic and autonomic outflow in borderline EH patients reveal an early tonic increase in SNS activity (Anderson et al. 1989), resembling a stress-induced hypothalamic defence response and when that is persistent, gradual structural and functional changes occur in the peripheral vasculature (Matthews et al. 2006). Complementary evidence in support of the role of the SNS at the onset of EH are the early studies demonstrating only complete autonomic blockade (α and ß sympathetic and parasympathetic blockade) leads to the resolution of BP in borderline hypertension with raised renin (Esler et al. 1977). This study also suggested that markers for SNS activity on psychometric testing correlated with plasma renin activity. However, the resolution in BP was not mimicked in another early study conducted in Japan in a much younger age group with a higher dietary salt intake, higher vascular damage, and normal plasma renin activity and dissimilar to the cohort of 'hyperkinetic EH' (Tanaka et al. 1978). Thus, the differences in environmental triggers and downstream effects including RAAS activation may affect the outcomes of the initial SNS activation.

Based on the above, one may conclude that the initial haemodynamic abnormalities in EH can occur in a stepwise fashion by SNS activation leading to an initial phase of high CO and normal TPR followed by a subsequent increase in TPR with normalisation of CO (Julius 1988). Of note, most of the haemodynamic

Table 1 Early changes in cardiovascular haemodynamics (data and table adapted from Lund-Johansen et al. (1983))

Stages	MAP (mmHg)	CI (l/min/m^2)	TPRI (dyn * s/cm^5 * m^2)	HR (beats/min)
Normotensives	83–91	3.05–3.45	1950–2440	63–74
Borderline or mild hypertension	100–108	3.53–4.14	1970–2400	75–81

HR heart rate
Cardiac output (CO) = HR × stroke volume
Cardiac index (CI) = CO/body surface area (l/min/m^2)
Mean arterial pressure (MAP) = Diastolic blood pressure + 1/3(Systolic blood pressure − diastolic blood pressure)
Total peripheral resistance index (TPRI) = Mean arterial pressure * 80/Cardiac index (dyn * s/cm^5 * m^2)

data in these early studies were conducted in the 1970s are in men (18–40 years) only (Table 1).

The next section explores the contrary evidence to this analogy.

2.2 Role of the Kidney in the Onset of Hypertension

Richard Bright introduced the concept of the renal origin of hypertension in the nineteenth century (Harlos and Heidland 2008; Mahomed 1874). Guyton developed the theory of hypertension several years later, assigning all raised BP primarily to chronic salt loading (Guyton et al. 1969). This theory was derived from Lewis Dahl's concept that assumed faulty renal sodium handling (Dahl 1972). Guyton's theory is the most well-cited and is right in parts, in that most types of chronic (i.e. long-term) hypertension are associated with renal dysfunction (Guyton 1991). Transplant studies in animals and humans demonstrate that kidneys from hypertensive donors lead to a rise in BP in normotensive recipients and vice versa. The main factors supporting Guyton's theory include the fact that BP does correlate with sodium intake and can be improved by sodium restriction. However, normal kidneys can excrete sodium at high MAP. Hence, there must be more stimuli beyond the salt that trigger impairment of pressure natriuresis leading to hypertension and hypertensive kidney disease. Could there be a link to explain the interlinking mechanisms between the salt related and SNS pathways (central and local) activation leading to hypertension?

The kidney is richly innervated by sympathetic nerves. Renal noradrenaline spillover is increased in untreated patients with EH, suggesting enhanced renal SNS activity (Esler et al. 1990). Noradrenaline through α_1- and α_2-ARs stimulation leads to a rise in renal vascular resistance and a reduction in glomerular filtration rate (GFR). In renal tubules, stimulation of α_1-, α_2-, and $ß_2$-ARs leads to accelerated salt and water reabsorption by effects via various sodium transporters including the epithelial sodium channel. Efferent renal sympathetic nerve regulates renal blood

flow, glomerular filtration rate, and reabsorption of sodium and water. The renal afferent nerves complete the loop as part of the bidirectional neural network to and from the brain and modulate the SNS outflow and thus a self-regulated renorenal reflex loop is established. The second level regulation of renal SNS activity is by the pre-junctional α2-ARs that inhibit noradrenaline release serving as autoreceptors (Hering et al. 2020) as noted elsewhere. Under healthy conditions, stimulation of afferent renal chemo-mechano-sensitive nerves (in the renal pelvis) leads to a decrease in efferent renal sympathetic nerve activity, which leads to increased natriuresis (Kopp 2011). Noradrenaline also stimulates the renin–angiotensin–aldosterone-system (RAAS) causing the release of renin from the juxtaglomerular apparatus (a type of modified vascular smooth muscle cell in afferent arterioles) via $ß_1$-ARs that then attempts to restore blood flow through increased sodium and water reabsorption which in physiological conditions switches off the noradrenaline release. However, these regulatory reflexes are disturbed in chronic hypertension and chronic kidney disease.

Based on the above, renal sympathetic denervation (i.e. blocking the SNS activity) is postulated to reduce BP by targeting this raised regional SNS and causing a left shift in the renal pressure-natriuresis curve (DiBona and Esler 2009). However, phase 3 RCTs trialling renal denervation as a therapy failed to show a statistically significant reduction in BP (Bhatt et al. 2014), with a multitude of factors contributing to failure and as such renal denervation remains an experimental tool.

Are there specific clinical phenotypes that may help identify patients who are most affected by SNS activity and/or salt sensitivity?

2.3 Obesity–Hypertension Phenotype

The association between hypertension and obesity is well known, with obesity accounting for more 2/3rd cases of newly diagnosed hypertension as noted in the old Framingham offspring study (Garrison et al. 1987). The excessive dietary energy load in obese individuals stimulates the SNS leading to elevated BP giving rise to the hypertensive–obesity phenotype. The phenotype is characterised by increased CO, HR and concomitant insulin-induced vasodilatation with hyperinsulinaemia being a well-recognised effect of obesity and metabolic syndrome. Patients with obesity exhibit raised SNS activity in comparison to non-obese individuals, but pathophysiological differences also exist between lean–hypertensive and obese–hypertensive phenotypes. There are fundamental differences in sympathetic nerve firing rates in the two groups of hypertensives (Lambert et al. 2007) along with regional differences (renal and cardiac) in SNS activity (Vaz et al. 1997) as seen in noradrenaline spill-over studies. SNS activation seems to play a greater role in lean-hypertensives in comparison to obese-hypertensives, although there is higher SNS activation in all obese patients irrespective of BP, and pharmacological blockade of SNS activity leads to a much greater reduction in BP in the obese phenotype (Wofford et al. 2001), thus pointing to a distinct and complicated role of the SNS in these patients.

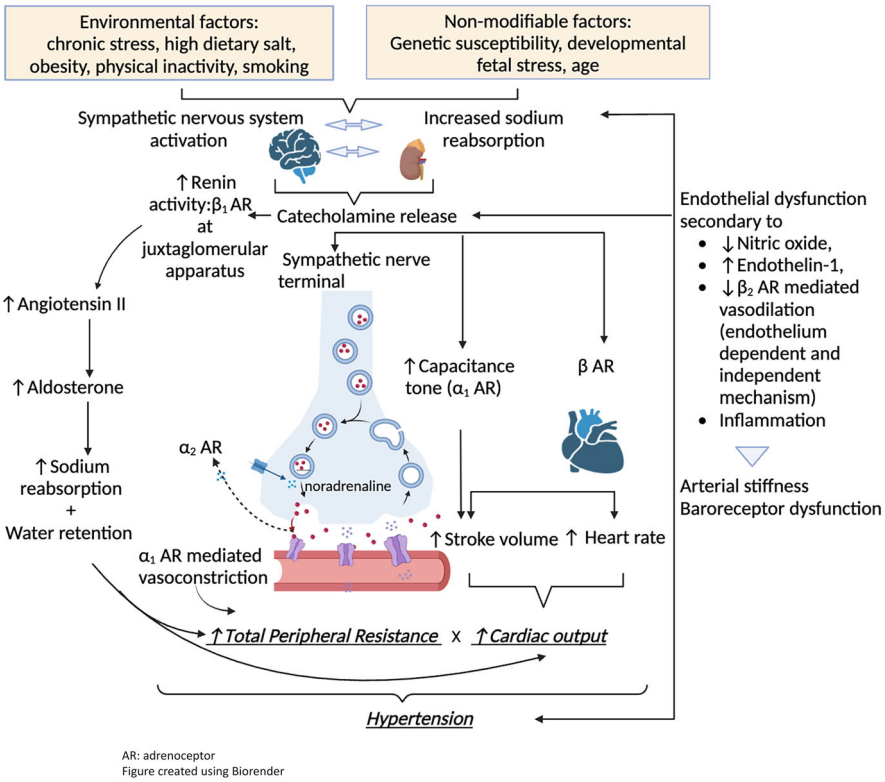

Fig. 1 Pathophysiology of essential hypertension: focus on the role of adrenoceptors and haemodynamic changes at the onset

In non-obese individuals, and possibly in the initial stages of metabolic syndrome, insulin causes peripheral vasodilatation that counteracts the vasoconstriction resulting from SNS and RAAS activation. However, hyperinsulinaemia-induced vasodilatation is eventually impaired as insulin resistance develops and the SNS activation makes the situation worse by concomitant vasoconstriction. In keeping with the principle, a combination therapy of α and ß blockers was shown to improve glucose metabolism in insulin-resistant obese patients in a small study (Gamboa et al. 2014). Obstructive sleep apnoea (OSA), a prevalent condition in obesity and metabolic syndrome, in which the SNS is stimulated in response to repeated nocturnal hypoxia (and sometimes consequent hypercapnia), and loss of nocturnal dip in BP which further adds to the maintenance of hypertension (Bisogni et al. 2016).

Hypertension results from a mosaic of factors: interaction between baroreceptor reflexes, SNS, RAAS, endothelial dysfunction with alteration in endothelin and NO signalling and behavioural disposition (Fig. 1) subsequently leading to structural changes in blood vessels and EOD. The deleterious effects of hypertension are

confounded by the impact of ageing. Exercise-induced haemodynamic changes, including right and left ventricular filling pressure and reduced left ventricular (LV) compliance, are associated with age. Similarly, a decrease in ß$_1$-AR responsiveness leads to a smaller increase in HR (Christou and Seals 2008). Though the exact impact of age on clinical responses is conflicting, a reduced number of receptors in some tissues, a decreased affinity, and reduced hormone-mediated adenylyl cyclase activity are in support of true receptor down-regulation in some tissues (in response to persistent SNS stimulation) (Scarpace et al. 1991). A decreased baroreceptor sensitivity is also another pathophysiological change that is confounded by ageing (Jones et al. 2001).

3 Management of Hypertension

The first attempt to treat hypertension by sympathectomy was in 1934 (Allen 1952), at a time when no medical therapy was established and malignant hypertension had a survival span of about 8 months. Drastic times called for drastic measures. Systemic recording of surgical outcomes in case series fashion demonstrated variable outcomes and seemed to halt progression at least temporarily. Smithwick et al. showed a 50% reduction in mortality in their case series (Smithwick and Thompson 1953) in comparison to standard therapy which was thin at the time. Patients, however, were debilitated by severe orthostatic hypotension and significant risks of surgery (Findlay 1936). Ganglion-blocking agents, such as hexamethonium, were introduced into clinical practice in the 1950s and became the first effective treatment for hypertension. Guanethidine which depletes noradrenaline storage peripherally by inhibition of the re-uptake and reserpine depleted both peripherally and central noradrenaline storage sites (now withdrawn) (Rosenthal 2004) due to similar side effects as the surgical counterparts. Reserpine was also associated with depression. Centrally acting sympatholytics such as methyldopa and clonidine, which is a potent α_2 agonist, were effectively used in the 1960s (DeQuattro and Li 2002) and continue to be used in today's clinical practice in specific scenarios highlighted later on in the chapter.

Thiazide diuretics were the first evidence-based drugs for hypertension which continue to be extremely effective even today. Propranolol, a non-selective β blocker (NSBB), was used for hypertension in the 1960s (Moser 2006) and the first α_1 blockers were approved for clinical use in the 1970s and 1980s.

3.1 Current Approach to Hypertension Management

In clinical practice, hypertension management involves broadly three steps undertaken in parallel or in some cases sequentially as below:

1. Diagnosis or determination of whether and by how much the BP is permanently raised and delineating acute and chronic end organ damage (EOD). Subclinical

EOD such as the presence of retinopathy without eye symptoms, and left ventricular hypertrophy on electrocardiogram or echocardiogram helps to identify the impact of hypertension beyond the actual value of the BP level itself. Management of clinically relevant EODs such as stroke, heart failure, or chronic kidney disease is undertaken in conjunction with specialist teams based on the organ system involved.

2. Non-pharmacological and pharmacological treatment with goal setting. Most patients require anti-hypertensive pharmacotherapy along with lifestyle changes. Broad lifestyle changes such as increasing physical activity, reduction in dietary salt intake, and weight optimisation through a balanced diet are recommended to all patients. NICE guidelines recommend a target BP of ≤135/85 mmHg home BP readings or day average values of ABPM or ≤140/90 mmHg in the clinic, with modifications in patients above 80 years of age.
3. Exclusion of secondary causes of hypertension.

A meta-analysis of RCTs demonstrated that every 10 mmHg systolic BP reduction leads to a substantial reduction in major CVD events (RR: 0.80, 95% CI, 0.77–0.83), heart failure (RR: 0.72, 95% CI, 0.67–0.78), stroke (RR: 0.73, 95% CI, 0.68–0.77), coronary and CV and all-cause mortality (RR: 0.87, 95% CI, 0.84–0.91) (Ettehad et al. 2016). Among available pharmacotherapies, currently, ACEi, angiotensin receptor blocker (ARB), calcium channel blocker (CCB), and thiazide-like diuretics are considered first-line anti-hypertensive agents for the management of hypertension (NICE 2022). ACEi/ARBs are preferred as first-line treatments in younger patients and CCBs are used as first-line agents in older patients and select populations such as patients with Afro-Caribbean ethnicity (Fig. 2). If BP remains above target, despite dose titrations, CCBs are added on in younger patients and ACEi/ARBs are added to other cohorts of patients. Thiazide-like diuretics are used as third-line agents and ß blockers, selective α_1 blockers, and anti-mineralocorticoid agents such as spironolactone constitute fourth-line agents. Medications in these classes are resorted to if resistant hypertension is established and in specific scenarios that would be best suited for the mechanism of action of pharmacotherapy detailed in subsequent sections.

ABPM: Ambulatory blood pressure monitoring
ACEi: Angiotensin-converting enzyme inhibitor
ARB: Angiotensin receptor blocker
CCB: Calcium channel blocker
CV: Cardiovascular
BP: Blood pressure
BIHS: British and Irish Hypertension Society
MRA: Mineralocorticoid antagonist
SNS: Sympathetic nervous system
SHEP: The Systolic Hypertension in the Elderly Program
ASCOT: The Anglo-Scandinavian Cardiovascular Outcomes Trial
LIFE: The Losartan Intervention for Endpoint Reduction

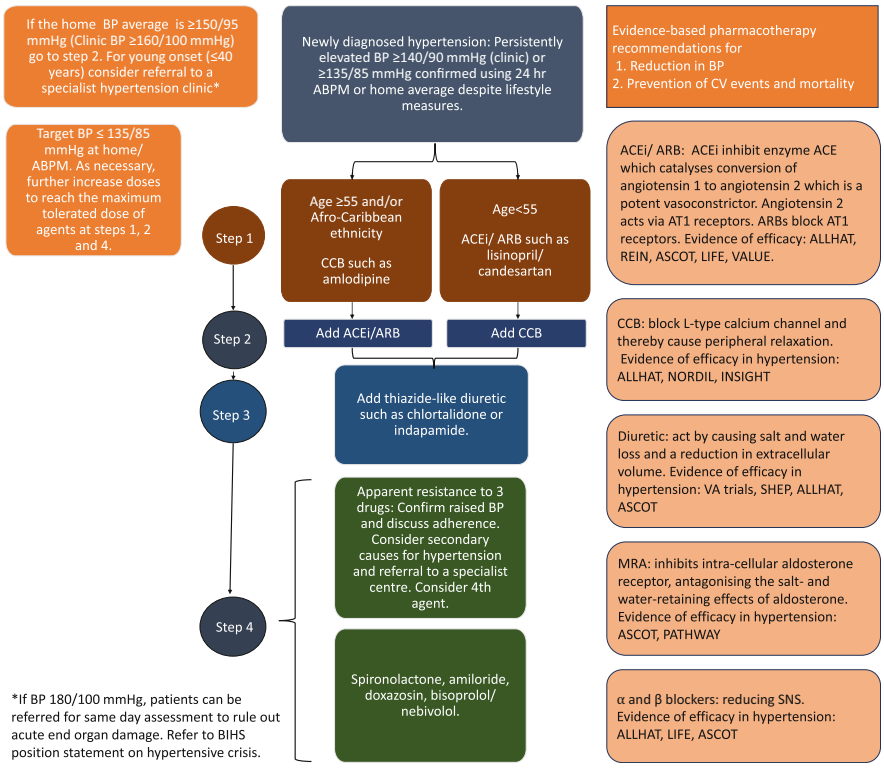

Fig. 2 Approach to hypertension management, based on BIHS position statement (Lewis et al. 2024) and NICE guidelines (NICE 2022) along with a summary of mechanism of action and RCT evidence-base demonstrating the efficacy of anti-hypertensive drug classes (van Vark et al. 2012; Savarese et al. 2013; Thomopoulos et al. 2015; Wei et al. 2020)

ALLHAT: Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Trial

VALUE: Valsartan Antihypertensive Long-term Use Evaluation

REIN: Ramipril in non-diabetic renal failure

PATHWAY: Prevention and Treatment of Hypertension with Algorithm-based Therapy

The goal of achieving adequate BP control on a population level largely relates to the management strategies, awareness and education of the population, and better screening strategies for early diagnosis. Data from May Measurement Month (2018) a population-level hypertension screening strategy undertaken across many countries, showed that 33.4% of patients who had their BP measured had hypertension: 59.5% of whom were aware of their diagnosis, and 55.3% were taking medications. Among the treated group, only 60% had achieved target BP control (Beaney et al. 2019). Several factors may be responsible for this, varying from inadequate monitoring, physician inertia to prescribe medication, poor efficacy of

the current therapeutic algorithm as a blanket strategy and poor adherence to therapy. The most important and controllable factor is possibly due to poor adherence (intentional and unintentional) to prescribed therapy. It is estimated that approximately 1 year after initiation, <50% of patients take medications as prescribed (Beaney et al. 2019). Poor tolerance to standard therapy is one of the many contributory causes of poor adherence.

A consistent and combined focus towards better screening strategies for secondary hypertension, and personalisation of therapy may help streamline therapy for at least a subset of patients. Cure of hypertension by rectifying the secondary cause is a far less common scenario and most patients diagnosed with hypertension are subjected to lifelong therapy without having a fair chance at diagnosis of a secondary cause. Personalised action towards the prevention of hypertension and tools to predict and prognosticate the development of hypertension and related end organ damage (EOD) have not received enough momentum into translate to clinical practice. In summary, implementation of guidelines/treatment in the first place, improving patient adherence and personalisation of therapy are key areas that can impact BP optimisation.

In the next few sections, the anti-hypertensive therapies that act on ARs are discussed.

3.2 α_1-AR Blockers

Clinically used α_1 blockers, such as prazosin, doxazosin, and terazosin, lower BP mainly by blocking post-synaptic α_1-ARs on vascular smooth muscle cells. This prevents activation by endogenous catecholamines thus resulting in vasodilatation, and lowering TPR. These agents reduce the vascular tone in both capacitance and resistance vessels leading to a balance in cardiac preload and afterload (peripheral resistance). There are minor changes in the HR that can lead to a significant reduction in CO at higher doses. The favourable effect on cardiovascular haemodynamics has been demonstrated during exercise with preservation of cardiac performance, in contrast to ß blockers. α_1 blockers are efficacious in the treatment of mild to moderate hypertension. The early studies with α_1 blockers showed a diastolic BP reduction, like that produced by NSBB such as propranolol and centrally acting sympatholytic such as methyldopa, of approximately 10 mmHg. The drop is higher for diastolic than SBP consistent with a vasodilatory action (Stokes and Weber 1974). α_1 blockers are also thought to be metabolically beneficial with a mild insulin-sensitising effect and some also have a beneficial impact on lipid profile by inhibition of oxidation of LDL cholesterol. The in vitro studies show that the α_1 blockers bind to all three subtypes of α_1 _ARs with high affinity. α_1 blockers used for benign prostatic hyperplasia such as tamsulosin also block all three subtypes of α_1 -ARs (Proudman et al. 2020). Thus, it can be expected that the latter can result in hypotension like α_1 blockers used for hypertension.

The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT), a major hypertension trial in the 1990s, tested various medications

including doxazosin (2–8 mg daily) in a head-to-head comparison with diuretics and ACEi. This study showed no difference in the primary endpoint of myocardial infarction or overall mortality between doxazosin and other drug classes including ACEis, CCBs, and chlorthalidone. However, there was an excess rate of heart failure (66%) and CVD (25%) in the doxazosin arm, that was subsequently terminated (Group ACR 2000). This led most international and national hypertension management guidelines to relegate α_1 blockers to the status of add-on therapy rather than first-line treatment. They continue to find a place in hypertension management in specific scenarios including resistant hypertension (Mancia et al. 2023). Patients with lower urinary tract symptoms secondary to the presence of benign prostatic hyperplasia and concomitant hypertension present as a compelling dual indication, where α_1 blockers can be effectively employed (Guthrie and Siegel 1999). As α_1 blockers do not affect the RAAS pathway directly, they are preferred at the screening stage of primary aldosteronism, and patients are prescribed α_1 blockers to ensure plasma levels for renin and aldosterone are measured in the absence of confounding agents (Funder et al. 2016; Faconti et al. 2024).

Usually, doxazosin is initiated at the lowest dose possible, typically 1 mg/day. Long-acting doxazosin formulation is tolerated better and should be preferred over other formulations. The main side effect, especially in the elderly, is orthostatic hypotension. In patients prone to orthostatic intolerance, careful initiation at the lowest dose and careful up-titration are undertaken. If possible, dosing at night is preferred. Fluid retention is a commonly noted side effect and using concomitant diuretics may help combat this adverse effect.

Nonspecific α blockade causes tachycardia induced by ß-AR stimulation, enhanced renin secretion and RAAS activation. Thus, nonspecific α blockers are less successful in treating patients with EH but have a higher benefit versus risk ratio and are considered appropriate in states of excess catecholamine states. The competitive antagonist phentolamine and the non-competitive antagonist phenoxybenzamine are nonspecific α blockers that were discovered first. They are approved by the Food and Drug Administration (FDA) for the management of phaeochromocytoma and paraganglioma (adrenal and extra-adrenal catecholamine-secreting tumours). Phentolamine is exclusively used as an intravenous (IV) drug for hypertensive emergencies associated with increased SNS activation such as phaeochromocytoma or sympathomimetic drug overdose with drugs such as amphetamine or cocaine. Phenoxybenzamine is used in the pre-operative management of phaeochromocytoma and cases of inoperable metastatic phaeochromocytoma (see section on Phaeochromocytoma, Paragangliomas and Adrenergic Crisis).

3.3 α_2-AR Agonists

These agents attenuate noradrenaline release centrally (brainstem) reducing the output of vasoconstrictor signals to the peripheral SNS leading to hypotension and bradycardia. The classical example in this class is clonidine. This class of agents are

used only in exceptional clinical cases and circumstances, mainly due to the limiting side effects of sedation, dry mouth, rebound effects after stopping, and depression. The side effects are due to a reduction in circulating noradrenaline levels and are minimised by gradually tapering doses when discontinuing. As such, the property of reduction in noradrenaline with clonidine is harnessed in testing for phaeochromocytoma where noradrenaline levels are not suppressed. Clonidine may be used in resistant hypertension and hypertensive crisis particularly in patients in intensive care with concomitant agitation.

Methyldopa is metabolised to α-methyl-noradrenaline that acts as an agonist at presynaptic α_2-ARs but is less active at post-synaptic α_1-ARs. The efficacy of methyldopa in reducing BP has been shown in controlled trials, but there are no CVD outcome data (Mah et al. 2009). As a widely available and cheap pharmacotherapy with an established safety profile and no evidence of risk of teratogenicity in pregnancy, it is one of the most common drugs prescribed for pregnancy-associated hypertension worldwide (Al Khaja et al. 2014). It is recommended that it is stopped after delivery to avoid risking contributing to post-partum depression.

α_2-AR antagonists such as yohimbine have the exact opposite effects: they raise BP and as such have been trialled in neurogenic orthostatic hypotension (Shibao et al. 2010).

3.4 ß Blockers

In 1965, propranolol was introduced as the first clinically useful ß blockers for which James Black was awarded the Nobel Prize in 1988. The mechanism of action of ß blockers is complex, and they are thought to reduce BP by lowering CO, inhibiting renin release, and resetting baroreceptor thresholds. The reduction in renin activity and central sympathetic outflow varies with different ß blockers (Stokes et al. 1974). The BP lowering impact seems to be better in younger patients, without any major effect on TPR. ß blockers may work particularly well in young obese patients with high SNS activity. However, long-term use is associated with dyslipidaemia and diabetes.

ß blockers can be categorised into three generations:

The first generation are non-selective ß blockers (NSBB) and include propranolol and labetalol. The early mechanistic studies in the 1960s demonstrated the haemodynamic effects of propranolol, with a reduction in HR and CO, both at rest and on exercise, with acute or chronic administration (Prichard and Gillam 1969). NSBBs are prescribed in patients with migraine, anxiety states, and adrenergic crises after adequate alpha blockade and portal hypertension. Labetalol, an NSBB with mild α_1 blocking properties is used for acute IV and oral administration in hypertensive emergency states.

Second-generation ß blockers are agents supposedly with higher $ß_1$ selectivity (Smith and Teitler 1999). Clinically used medications in this class include atenolol, metoprolol, and bisoprolol. Currently, second-generation ß-blockers are prescribed in patients with hypertension with compelling dual indications such as a history of

myocardial infarction, heart failure, angina, chronic aortic dissection, and atrial fibrillation. Although this class of medications are intended to have higher $ß_1 > ß_2$ selectivity, in vitro studies show little $ß_1$ selectivity in living cells (Baker 2005). There are intrinsic differences between the medications in this class beyond AR selectivity and affinity, including pharmacokinetic properties such as elimination, duration of action, and genetic polymorphic variants that impact efficacy in humans. This also implies the efficacy of medications such as bisoprolol for heart failure cannot be assumed to be a class effect. Most of the second-generation ß blockers tend to cause hypotension and bradycardia through $ß_1$ blockade, they are contraindicated in patients with asthma as they also block β_2-AR increasing the risk of bronchoconstriction and preventing the actions of bronchodilating β_2-AR agonists (Baker and Wilcox 2017).

Third-generation ß blockers such as carvedilol and nebivolol possess additional vasodilating effects – carvedilol by blocking α_1-AR and nebivolol by activating $ß_3$-AR to induce endothelial NO synthase-mediated vasodilatation, leading to a reduction in vascular resistance with preservation of skeletal muscle blood flow and is thought to have a better metabolic profile (Cockcroft et al. 1995; McEniery et al. 2004; Kamp et al. 2010).

Overall, the second-generation ß blockers have fewer adverse effects, but fatigue and dizziness due to on-target effects may affect tolerability and adherence. The third-generation ß-blockers seem to be largely devoid of the increased risk of dyslipidaemia, diabetes mellitus, and insulin resistance.

Overall, in selected patients with uncomplicated hypertension, ß blockers might still be useful as first-line agents, especially the third-generation ß blocker nebivolol that possesses the advantage of vasodilatation. Nebivolol is also the only ß blocker known to reduce central BP clinically (Vaz-de-Melo et al. 2014; Bowman et al. 1994). The superiority of nebivolol over placebo, comparable efficacy to other classes of anti-hypertensives and advantages over other ß-blockers in hypertension has been demonstrated in clinical studies (Mazza et al. 2002; Cockcroft and Pedersen 2012; Van Nueten et al. 1998). However, a recent meta-analysis showed no statistical difference in SBP and DBP between nebivolol and other second-generation ß blockers although nebivolol had significantly better tolerability with fewer adverse events (RR: 0.52, 95% CI, 0.34–0.79; $I^2 = 48\%$) (Liu et al. 2020).

There are no direct head-to-head studies demonstrating a reduction in mortality with ß-blockers compared with placebo. The Medical Research Council (MRC) elderly study (Party 1992), the Losartan Intervention For Endpoint Reduction (LIFE) (Dahlöf et al. 2002), and The Anglo-Scandinavian Cardiovascular Outcomes Trial (ASCOT) (Dahlöf et al. 2005) suggest that ß blockers are inferior to other first-line therapies including ACEi, ARB, CCBs, and diuretics despite similar reductions in BP. A Cochrane review assessed the effects of ß blockers as first-line/initial therapy on cardiovascular mortality and morbidity (Wiysonge et al. 2017). The review included 13 RCTs that included intake of ß-blockers for at least a month and a total study duration of at least 1 year. This review compared the outcomes of ß blockers against placebo and other anti-hypertensive medications. Of note, most of the trials utilised the ß blocker atenolol as the intervention. There was no difference

in all-cause mortality between ß blockers and placebo (4 studies: RR: 0.99, 95% CI, 0.88–1.11), diuretics or RAAS inhibitors but the mortality was higher for ß blockers compared to CCBs (4 studies: RR: 1.07, 95% CI, 1.00–1.14). The total CVD was lower for ß blockers versus placebo (RR: 0.88, 95% CI, 0.79–0.97), but worse than that of CCBs (RR: 1.18, 95% CI, 1.08–1.29). However, most RCTs included in this review have a high risk of bias and the authors note moderate to low confidence in the findings. There were no outcome trials comparing the newer generation ß blockers such as nebivolol.

These findings may be impacted by numerous factors including the subtype of ß blocker, and the age of participants in the RCTS. Overall, ß blockers have less effect on central (aortic) BP due to bradycardia, increased risk of DM when used with diuretics, and poor impact on BP variability. The main conditions that preclude the initiation of ß blockers include asthma, severe heart failure, and high-grade heart block, mainly due to the deleterious effects of blocking $ß_2$ AR. ß blockers are also relatively less useful in older, 'low renin' patients and patients of Afro-Caribbean descent (Materson et al. 1993), with the latter possibly due to a higher prevalence of non-responsive single nucleotide polymorphisms (Brodde and Stein 2003; Kurnik et al. 2008). Thus, NICE guidelines do not recommend ß blockers as first-line therapy (NICE 2022).

Overall, in selected patients with uncomplicated hypertension, there may still be a place for ß blockers as a first-line agent, especially the third-generation ß blocker nebivolol. Nebivolol is also the only ß blocker known to reduce central BP clinically (Vaz-de-Melo et al. 2014; Bowman et al. 1994). The superiority of nebivolol over placebo, comparable efficacy to other classes of anti-hypertensives and advantages over other ß blockers in hypertension has been demonstrated in clinical studies (Mazza et al. 2002; Cockcroft and Pedersen 2012; Van Nueten et al. 1998). However, a recent meta-analysis showed no statistical difference in SBP and DBP between nebivolol and other second-generation ß blockers in the BP measurements, although nebivolol had significantly better tolerability with fewer adverse events (RR: 0.52, 95% CI, 0.34–0.79; $I^2 = 48\%$) (Liu et al. 2020). Future larger outcomes studies might shed on the effectiveness of this drug.

4 Hypertension in Specific Medical Conditions

In this section, hypertension in specific populations and/or diagnosis relevant to ARs are highlighted.

4.1 Pregnancy-Associated Hypertension

Hypertensive disorders in pregnancy are relatively common and affect 8–10% of all pregnancies with substantial complications for the woman and the baby. Women with a diagnosis of EH or secondary hypertension before pregnancy or who are diagnosed with hypertension in the first 20 weeks are referred to as having chronic

hypertension. Onset of hypertension that occurs after 20 weeks of pregnancy without any organ involvement and no proteinuria is referred to as gestational hypertension. Preeclampsia is the onset of hypertension in the second half of pregnancy (after 20 weeks of gestation) with organ dysfunction typically in the form of proteinuria (>300 mg/day) and symptoms such as headaches and epigastric pain. Preeclampsia may occur during pregnancy superimposed on chronic hypertension. For all the categories of pregnancy-associated hypertension, a consistently elevated BP of ≥140/90 mmHg is used as a clinical cut-off and together constitutes a hypertension of pregnancy. Presentation with BP ≥ 160/100 mmHg along with proteinuria (>0.3 g/24 h) after 20 weeks of gestation is defined as severe preeclampsia. Severe preeclampsia can progress to the more life-threatening eclampsia (1 in 4000 pregnancies), with features of preeclampsia and new seizures.

The concepts used to define these diagnoses and the underlying pathophysiology are constantly expanding. The traditional definitions used to categorize the hypertensive disorders of pregnancy mostly rely on the presence of proteinuria. In 2014, the International Society for the Study of Hypertension in Pregnancy introduced new definitions to include other organ dysfunction beyond proteinuria such as liver, haematological, renal or neurological organ dysfunction, uteroplacental insufficiency and/or foetal growth restriction that better reflect the adverse features of hypertensive disorders in pregnancy and the associated mortality and morbidity. More recently, the broader definition of preeclampsia has been adopted by multiple international guidelines and the definition has proven to be more sensitive in detecting maternal and foetal complications (Lai et al. 2021).

The risk of preeclampsia in pregnancies is 3–5%. Amongst women with chronic hypertension, 17% to 25% develop superimposed preeclampsia, i.e. a patient with chronic hypertension develops worsening BP along with organ involvement after 20 weeks. Maternal hypertension is the second most common cause of maternal deaths in the UK (Webster et al. 2019). The deleterious effects of preeclampsia and eclampsia not only include maternal and foetal perinatal mortality and morbidity, but also an increased risk of CVD later in life (Seely and Ecker 2014).

4.1.1 Pathophysiology of Preeclampsia and Eclampsia

The underlying pathophysiology of preeclampsia and eclampsia is poorly understood. It is thought to be due to abnormal placentation (i.e. dysfunctional trophoblast invasion) and/or inherent maternal cardiovascular dysfunction (Masini et al. 2022). Impaired perfusion of the placenta leads to a state of oxidative stress and release of proinflammatory cytokines that in turn triggers endothelial dysfunction. In severe preeclampsia, there is increased capillary permeability and movement of fluid to interstitial spaces which leads to hyperperfusion and associated cerebral oedema. This leads to further vasoconstriction and reduction in blood flow across vascular beds.

Several physiological changes in the cardiovascular system take place in pregnancy leading to an altered haemodynamic state to meet the metabolic demands of the mother and foetus. The blood volume increases by 150%, CO increases by 50% in the first 20 weeks of gestation, and TPR falls until mid-gestation. This pattern of

haemodynamic changes is different in patients who develop hypertension during pregnancy and varies depending on the nature of the underlying condition.

Apart from raised BP, a multitude of symptoms are associated with severe preeclampsia. The most common include headaches, vomiting, or visual disturbance. Hyperreflexia and clonus indicate ongoing cerebral oedema that if unchecked can lead to seizures. Thus, it is crucial to recognise and treat preeclampsia quickly. The main aim of treating hypertension is to prevent the development of severe hypertension, reduce the risk of stroke (the largest cause of maternal death in severe preeclampsia and eclampsia), development of eclampsia and other EODs including renal failure.

4.1.2 Pregnancy-Safe Anti-Hypertensive Agents and Management of Preeclampsia

All hypertensive women of childbearing age who are planning a pregnancy are generally prescribed agents with proven safety for the treatment of pre-existing hypertension. As pregnancy poses potential adverse effects for both mother and foetus, the balance of benefit versus risk for any drug prescribed is of utmost importance. Unsurprisingly, most anti-hypertensive agents are not licensed for use in pregnancy primarily because the required efficacy and safety studies have not been undertaken. The older agents tend to be considered as the only agents with 'proven' safety records as these have been widely and safely used, even before the alarm of teratogenicity was raised.

For BP control, ß blockers such as labetalol, CCBs such as long-acting nifedipine and methyldopa are among the most prescribed drugs. If BP remains uncontrolled, other agents that are considered safe include hydralazine, a directly acting arterial vasodilator, and α_1 blockers such as doxazosin. If other anti-hypertensive agents are chosen, there must be a discussion around pregnancy planning and potential switching of drugs.

Screening for pregnancy should be undertaken in women of childbearing age presenting with hypertension. NICE guidelines (NG 133) (National Institute for Health and Care Excellence (NICE) 2019) recommend a tighter BP target of 135/85 mmHg for all patients with hypertensive disorders in pregnancies whilst acknowledging that the evidence base for this target is modest.

Other strategies are recommended to ensure preeclampsia is detected and managed appropriately. Measurement of BP and urinalysis for protein is undertaken at each antenatal visit and risk factors for preeclampsia are determined at the first antenatal appointment. Patients deemed to be at high risk of preeclampsia are usually prescribed aspirin 75–150 mg/day from 12 weeks until delivery (National Institute for Health and Care Excellence (NICE) 2019).

Treatment of severe preeclampsia, eclampsia or severe hypertension in pregnancy should be undertaken in a critical care setting. Magnesium sulphate infusion and anti-hypertensive agents such as oral or IV labetalol, and oral nifedipine and/or IV hydralazine are recommended (NG133). Ultimately severe preeclampsia is treated by delivering the baby and hypertension management does not have much impact on

complications of preeclampsia such as HELLP (haemolysis, elevated liver enzymes, low platelet) syndrome.

Further research on biomarkers, the role of the anti-angiogenic pathway, and the involvement of the RAAS pathway might pave the way for newer therapeutic options.

4.2 Phaeochromocytoma, Paragangliomas, and Catecholamine Excess

Phaeochromocytomas and paragangliomas (PPGL) are neuroendocrine tumours that arise from catecholamine-producing chromaffin cells. Phaeochromocytoma usually refers to a tumour in the adrenal medulla that typically produces adrenaline and/or noradrenaline with a few exceptions whereas a paraganglioma refers to an extra-adrenal location of tumours that produce excess noradrenaline but both can secrete either hormone. Genetic mutations are reported in more than 1/3rd of cases and genetic testing can help define prognosis in certain cases. Childhood-onset, pregnancy-associated cases in older people have higher morbidity and mortality risk. The plasma concentrations of catecholamines in PPGL tissues are enormous (commonly >3 times of upper limit of the normal limit of plasma catecholamines), and these tumours are akin to a volcano that can erupt.

Symptoms can range from that of a catecholamine storm which presents as paroxysms of massive surges in BP associated with sweating, headaches, tremors, and palpitations that represent significant eruptions whilst smaller and/or continuous eruptions usually lead to the clinical presentation of hypertension and palpitations (the most common symptom) or symptoms of sustained hypertension, palpitations, sweating, and pallor. Other reported symptoms include a pounding headache, anxiety, tremulousness, a feeling of impending death, nausea, vomiting, and abdominal pain. Rarely, some PPGLs may be asymptomatic. The excess catecholamines lead to the activation of other pathophysiological pathways; for example, a secondary activation of RAAS which leads to an exaggerated pressure natriuresis and intravascular volume depletion giving rise to a vicious cycle, paradoxically exacerbating the hypertension.

Patients may describe episodes or attacks that build up over a few minutes and then subside over 15–60 min. These patients may have a normal BP at baseline or may have co-existent EH. Rarely, do patients progress to develop other EODs leading to hypertensive emergency states such as hypertensive encephalopathy. The (ab)use of cocaine or amphetamine, or several prescription drugs (e.g. tricyclic anti-depressants) may present with a sympathetic crisis and display similar symptoms. Adrenergic crisis may be manifested by a short-lived rise in BP; hence, the BP can be safely reduced to the normal range within hours. The rise in BP may even resolve spontaneously in some cases by the time medical attention has been sought and medication sourced. Incidental identification on imaging, followed by biochemical testing is frequent as symptoms may be nonspecific as noted above.

The first step in confirmation of diagnosis is measurement of plasma-free metanephrines or 24-h urine fractionated metanephrines, which is usually followed by imaging tests including magnetic resonance imaging, computed tomography, and functional imaging with meta-iodobenzylguanidine (MIBG or Gallium-68 DOTATATE) and positron emission tomography.

The definitive management is surgical removal of the tumour. Management until then is focussed on reversing the effects of sympathetic stimulation and correcting intravascular volume depletion with resultant dehydration and is usually planned within a multidisciplinary team setting. The rarity of these sympathetic syndromes makes RCTs for treatment strategies non-existent and treatment is based on the underlying pathophysiology. Full oral α blockade, with phenoxybenzamine, is the first-line therapy. Doxazosin may be used if phenoxybenzamine is unavailable. The addition of a CCB may be beneficial in some cases (Mazza et al. 2014). Adequate fluid replacement is essential (oral and/or IV) to correct intravascular volume depletion. β blockade is contraindicated before adequate α blockade. However, selective or non-selective β blockade may be used to limit tachycardia, or prophylactically in patients with pre-existing ischaemic heart disease or dysrhythmias once adequate α blockade is achieved. Just before the planned surgery, α blockade is maximised to induce controlled orthostatic hypotension, followed by higher replacement of saline replacement. This is to ensure that in the event there is a catecholamine surge when the tumour is surgically handled, the α blockade prevents an iatrogenic storm (Mazza et al. 2014).

In a crisis, IV phentolamine may be useful for acute control of BP administered in the setting of an intensive care unit. If phentolamine is unavailable, labetalol may be used, though α blockade with labetalol can be incomplete. Adequate fluid replacement is followed by oral phenoxybenzamine as above if the crisis is controlled.

For drug toxicity-induced hypertension mediated by cocaine or amphetamines, IV benzodiazepines are the first-line treatment. Benzodiazepines (BZD) act on gamma-aminobutyric acid receptors to reduce and prevent neurological complications such as seizures. If BP remains high despite BZDs, CCBs such as IV nicardipine, ß blockers such as IV labetalol or IV glyceryl trinitrate may be considered.

4.3 Hypertension Associated with Hyperthyroidism

Thyroid disorders account for approximately 1% of cases with hypertension (Rivas et al. 2021). Hypertension is known to be present in about 25% of patients with hyperthyroidism (Hurxthal 1931), with higher rates in younger patients (Baker et al. 2023; Bylund 2007; Holthoff et al. 2012; Philipp et al. 2002; Link et al. 1996; Freedman et al. 1995; Schlereth et al. 2021; Shabeeh et al. 2017; Bristow et al. 1986; Stiles et al. 1983; Brodde et al. 2001; do Vale et al. 2019; Skeberdis 2004; Miyashiro and Feigl 1993; Barbato 2009; Ahles and Engelhardt 2023; Lund-Johansen 1979, 1983, 1991; Lund-Johansen and Omvik 1991; Omvik and Lund-Johansen 1990; Lund-Johansen and Bakke 1979; Weidmann et al. 1977; Julius et al. 1991; Esler

et al. 1988; Levy et al. 1945; Gudmundsdottir et al. 2008; Julius 1988; Anderson et al. 1989; Matthews et al. 2006; Saito et al. 1985), although limited data exists to confirm the accuracy of the estimates.

Pathophysiologically, excess thyroid hormone causes a clinical picture resembling a catecholamine surge stimulating ß-ARs with a rise in HR and CO except that the effects without a rise in blood or urine catecholamine levels or altered sensitivity to catecholamines. Peripheral vascular resistance is reduced by the direct action of thyroid hormone T3 on vascular smooth muscle cells. This indirectly stimulates the RAAS pathway causing increased plasma renin activity. T3 also directly stimulates renin release by increasing the expression of ß-ARs in the renal cortex. Thus, when untreated and severe, hyperdynamic circulation ensues due to reduced systemic vascular resistance, leading to high CO (50–300% higher than normal) and systolic heart failure (Mazza et al. 2011; Klein and Ojamaa 2001).

Clinically, patients have systolic hypertension with a wide pulse pressure due to underlying increased CO, HR, and plasma volume, whilst the reverse happens in hypothyroidism leading to a diastolic hypertension phenotype. Other prominent clinical features include tremors and anxiety. Hyperthyroidism is a risk factor for atrial fibrillation.

Initial treatment with ß blockers is useful in controlling BP and symptoms, usually with non-selective ß-blockers such as propranolol, while definitive management is established. Usually, BP reverts to the normal range once hyperthyroidism is corrected.

5 Organ-Specific Hypertension

5.1 Portal Hypertension

Portal hypertension is defined as a sustained increased BP within the portal venous system. A rise in BP in any vascular system results from either an increase in the blood flow to the organ and/or an increase in resistance to blood flow. Portal hypertension is clinically determined by an increase in portal pressure above the normal range of 6–10 mmHg or a hepatic venous pressure gradient (HVPG) above 5 mmHg. HVPG above 10–12 mmHg is associated with complications of portal hypertension.

Portal hypertension can result from various causes, but cirrhosis of the liver characterised by fibrosis and nodule formation is the most common cause. Hepatic stellate cells that are essentially peri-sinusoidal fat-storing cells transform into myofibroblastic-like cells in cirrhosis leading to structural alterations. The activated hepatic stellate cells express several proinflammatory and fibrotic genes and become contractile in this phenotypic transformation (Reynaert et al. 2002). Liver sinusoidal endothelial cell dysfunction leads to impaired vasodilatation, increased endothelin-1 and inflammation, and impaired vasomotor control. Together these changes lead to a rise in intrahepatic resistance and portal pressure. This leads to the activation of compensatory neurohormonal mechanisms that include SNS activation (as shown by

noradrenaline spill-over studies), the RAAS, and the arginine vasopressin system which causes sodium and water retention and an increase in plasma volume, leading to splanchnic vasodilatation and systemic vasoconstriction. The rise in portal pressure leads to a few complications: these include opening up of portal-systemic collateral blood vessels (the most hazardous of which are oesophageal varices that can cause sudden life-threatening bleeding); the combination of increased pressure and compensatory vasodilation in the mesenteric circulation leads to a reduction in oncotic pressure due to decreased protein production by liver to cause fluid leak into the peritoneal cavity causing ascites; and substances normally cleared by the liver are distributed back into the systemic circulation leading to encephalopathy and enhance the hyperdynamic vascular state (Bloom et al. 2015). The hyperdynamic state is associated with increased CO and extracellular fluid accumulation, although the effective circulating volume is low, that leads to a vicious perpetuation of neurohormonal activation. Finally, the compensatory mechanisms fail and the increase in CO is insufficient to maintain effective circulating volume despite maximal activation of the neurohormonal systems. The patient decompensates and irreversible multiorgan dysfunction ensues with refractory ascites, hyponatraemia, decreased renal perfusion, and hepatorenal syndrome (Bosch et al. 2015).

Very few treatments can reverse portal hypertension. Current clinical practice relies on pharmacotherapy with ß blockers that act by blocking the effects of catecholamines on ß-AR, loop diuretics that reduce ascites and mineralocorticoids that block the deleterious action of aldosterone until a liver transplant becomes available.

5.1.1 Non-selective β Blockers in Portal Hypertension

A high HR and high basal cardiac index are well-known consequences of cirrhosis both in the compensated and decompensated state, which is a direct consequence of splanchnic vasodilatation and a fall in TPR leading to a decrease in effective circulating volume. Several small mechanistic studies conducted in the 1980s and 1990s showed that NSBB such as propranolol (in doses that reduced the HR by 25%) reduced the risk of gastrointestinal bleeding (primarily variceal in origin) by producing a sustained reduction in portal pressure in cirrhotic patients (Lebrec et al. 1980, 1981, 1982; Bihari et al. 1984; Hillon et al. 1982). The reduction in hepatic blood flow with NSBB is due to the blockade of β_2-AR in the splanchnic circulation (Mastai et al. 1989), that in turn leads to vasoconstriction in the splanchnic bed due to the unopposed action of catecholamines on α-AR. Variceal blood flow is much more effectively reduced with NSBB, greater than hepatic blood flow, courtesy of β_2-AR within the collateral blood vessels. Thus, a reduction in the risk of repeated episodes of variceal bleeding in cirrhotic patients is achieved with NSBB (Bosch et al. 1984). Carvedilol is considered more effective than propranolol due to additional α_1-AR blocking properties (α_1-AR are present in splanchnic and systemic vascular smooth muscle cells) and a direct inhibitory action on hepatic stellate cell activation (Ling et al. 2019). A recent RCT in compensated cirrhosis confirmed the efficacy of HVPG-response-led propranolol or carvedilol in the long-term reduction of risk of hepatic decompensation events including ascites, bleeding overt

encephalopathy, and death in comparison to placebo (hazard ratio of 0.51 (95% CI, 0.26–0.97, $p = 0.041$) (Villanueva et al. 2019). In contrast, in decompensated cirrhosis, where extremely high circulating catecholamines lead to unopposed α-AR mediated vasoconstriction in the portal vein (that only has α-ARs) thus increasing portal pressure, NSBB are ineffective (Colman et al. 1982). In the past, α_1 blockers have been shown to reduce the HVPG, but are accompanied by high rates of systemic hypotension and adverse events (Albillos et al. 1994). Other vasodilators such as NO donors also provided a similar picture.

β blockers are thought to be particularly unsafe in patients with decompensated cirrhosis and refractory ascites (Tellez et al. 2020) due to β_1 antagonism in the heart leading to a further reduction in CO. Effective β blockade is not sustained in many patients (40% maintained the response beyond 3 months) (Vorobioff et al. 1987) which may be due to intolerance to the cardiac haemodynamic effects including hypotension, bradycardia, and fatigue that affects long-term adherence to β blockers or progression of underlying haemodynamic effects and possibly due to receptor desensitisation (Banares et al. 2002). Yet, until new therapies are proven effective for this unmet need, ß blockers are the only approved pharmacotherapy for portal hypertension (de Franchis et al. 2022).

5.2 Pulmonary Arterial Hypertension

Pulmonary hypertension (PH) is defined as an abnormal elevation in pulmonary arterial BP. The World Health Organization (WHO) classifies PH into five groups based on pathophysiology and histology. Here we explore in brief the role of ARs in group 1 PH, i.e. pulmonary arterial hypertension (PAH), a rarer form of PH that is characterised by elevated pulmonary vascular resistance (PVR) secondary to vascular remodelling. This sustained elevation of PVR leads to right ventricular failure, and severe reduction in CO. PAH is a life-threatening condition with a poor prognosis. There are multiple causes for group PAH including idiopathic, familial, collagen vascular disease, infections, etc., making pathogenesis complex and diverse. Vascular smooth muscle proliferation, endothelial dysfunction and inflammation together result in the remodelling of the pulmonary vasculature and as such excessive vasoconstriction is a hallmark. Current PAH therapies target vasodilatory pathways such as NO, endothelial-1 inhibition, and prostacyclin pathways (Mayeux et al. 2021), despite which there remains a significant unmet need.

As noted in the previous sections, α_1-ARs are expressed in most vascular smooth muscle cells and their subtypes are distributed in a pattern that is specific for functionally distinct vessel types. Compared to other arteries, α_1-AR in the medium-sized pulmonary vessels display a high affinity for noradrenaline (Salvi 1999). In physiological states, this facilitates local regulation of vascular tone in response to hypoxia thereby helping match ventilation and perfusion. α_1-AR expression in pulmonary vascular smooth muscle cells is known to be upregulated in vivo and in vitro under hypoxic conditions (Faber et al. 2007; Eckhart et al. 1997) leading to hypertrophy. In addition, hypoxia causes a concomitant downregulation of the

β-AR density in the pulmonary vessels unfavourably tilting the vasodilator/vasoconstrictor balance. There is also evidence for increased sympathetic nerve activity in advanced PAH (Velez-Roa et al. 2004). It is not surprising therefore that α_1 blockers have been tried for PH (Alpert et al. 1994) in animal models. There are several in vitro and animal studies demonstrating a reduction in pulmonary artery pressure and right ventricular systolic pressure and antiproliferative effects on vascular smooth cells with AR antagonists that block both α_1- and β-AR such as carvedilol (Ishikawa et al. 2009; Fujio et al. 2006). In other cell culture studies, nebivolol improved endothelium-dependent and NO-dependent relaxation of the pulmonary vasculature (Al-Sharefi et al. 2019). The main concern in attempting β blockade in PAH in human studies is the deleterious effects of a reduction in exercise capacity and systemic hypotension resulting from negative inotropic and chronotropic effects in an already haemodynamically unstable condition. However, most vasodilators currently employed cause a reduction of CO and indeed may cause reflex activation of the SNS as a compensatory mechanism. Given the unclear benefit versus risk of AR blockade in PAH in humans, blockade of ARs is not recommended. However, there is a need to better understand the role of ARs especially α blockers in a pulmonary vessel-specific, PAH context to tap any beneficial effects.

5.3 Ocular Hypertension (Glaucoma)

Glaucoma is a neurodegenerative eye disease characterised by damage to the optic nerve head, secondary to increased intraocular pressure (IOP). It is the second leading cause of blindness worldwide (Quigley and Broman 2006). AR including α_1-, α_2-, and β-AR play a pivotal role in the pathophysiology of glaucoma, ultimately leading to progressive selective retinal ganglion cell damage that leads to visual field loss (Cvenkel and Kolko 2020). Diagnosis is based on a comprehensive eye examination that includes measurement of IOP, examination of the optic nerve, and assessment of the visual field. Treatment aims to reduce IOP to prevent further damage to the optic nerve. Since the late 1970s, topical β blockers have been used in treating glaucoma. Prostaglandin analogues are currently the first line of therapy. Both α_2-AR agonists (brimonidine) and β blockers (betaxolol and timolol) are used in the management of this and will be discussed in the chapter on eye disease.

6 Summary

In summary, in this review, we summarise the role of AR in the pathophysiology of the onset of EH, delineating current therapies employed in the management of hypertension. We highlight the role of both α_1- and β-AR blocking agents and α_2-AR agonists in EH and in special situations such as phaeochromocytoma, pregnancy-associated hypertension, hyperthyroidism, and portal hypertension.

References

Agency UHS (2021) Blood pressure: are your pipes in good working order? https://www.gov.uk/government/organisations/uk-health-security-agency. https://ukhsa.blog.gov.uk/2021/09/06/blood-pressure-are-your-pipes-in-good-working-order/

Ahles, A., Engelhardt, S. (2023). Genetic Variants of Adrenoceptors. In: Handbook of Experimental Pharmacology. Springer, Berlin, Heidelberg. https://doi.org/10.1007/164_2023_676

Al Khaja KAJ, Sequeira RP, Alkhaja AK, Damanhori AHH (2014) Drug treatment of hypertension in pregnancy: a critical review of adult guideline recommendations. J Hypertens 32(3)

Albillos A, Lledó JL, Bañares R, Rossi I, Iborra J, Calleja JL et al (1994) Hemodynamic effects of α-adrenergic blockade with prazosin in cirrhotic patients with portal hypertension. Hepatology 20(3):611–617

Allen EV (1952) Sympathectomy for essential hypertension. Circulation 6(1):131–140

Alpert MA, Concannon MD, Mukerji B, Mukerji V (1994) Pharmacotherapy of chronic pulmonary arterial hypertension: value and limitations: part I: primary pulmonary hypertension. Angiology 45(8):667–676

Al-Sharefi A, Javaid U, Perros P, Ealing J, Truran P, Nag S et al (2019) Clinical presentation and outcomes of phaeochromocytomas/paragangliomas in neurofibromatosis type 1. Eur Endocrinol 15(2):95–100

Anderson EA, Sinkey CA, Lawton WJ, Mark AL (1989) Elevated sympathetic nerve activity in borderline hypertensive humans. Evidence from direct intraneural recordings. Hypertension 14(2):177–183

Baker JG (2005) The selectivity of beta-adrenoceptor antagonists at the human beta1, beta2 and beta3 adrenoceptors. Br J Pharmacol 144(3):317–322

Baker JG, Wilcox RG (2017) β-Blockers, heart disease and COPD: current controversies and uncertainties. Thorax 72(3):271–276

Baker J, Balaji P, Bond R, Bylund D, Eikenburg D, Graham R et al (2023) Adrenoceptors in GtoPdb 2023(1):2023

Banares R, Moitinho E, Matilla A, Garcia-Pagan JC, Lampreave JL, Piera C et al (2002) Randomized comparison of long-term carvedilol and propranolol administration in the treatment of portal hypertension in cirrhosis. Hepatology 36(6):1367–1373

Barbato E (2009) Role of adrenergic receptors in human coronary vasomotion. Heart 95(7): 603–608

Beaney T, Burrell LM, Castillo RR, Charchar FJ, Cro S, Damasceno A et al (2019) May measurement month 2018: a pragmatic global screening campaign to raise awareness of blood pressure by the International Society of Hypertension. Eur Heart J 40(25):2006–2017

Bhatt DL, Kandzari DE, O'Neill WW, D'Agostino R, Flack JM, Katzen BT et al (2014) A controlled trial of renal denervation for resistant hypertension. N Engl J Med 370(15):1393–1401

Bihari D, Westaby D, Gimson A, Crossley I, Harry J, Williams R (1984) Reductions in portal pressure by selective beta 2-adrenoceptor blockade in patients with cirrhosis and portal hypertension. Br J Clin Pharmacol 17(6):753–757

Bisogni V, Pengo MF, Maiolino G, Rossi GP (2016) The sympathetic nervous system and catecholamines metabolism in obstructive sleep apnoea. J Thorac Dis 8(2):243–254

Bloom S, Kemp W, Lubel J (2015) Portal hypertension: pathophysiology, diagnosis and management. Intern Med J 45(1):16–26

Bosch J, Masti R, Kravetz D, Bruix J, Gaya J, Rigau J, Rodes J (1984) Effects of propranolol on azygos venous blood flow and hepatic and systemic hemodynamics in cirrhosis. Hepatology 4(6):1200–1205

Bosch J, Groszmann RJ, Shah VH (2015) Evolution in the understanding of the pathophysiological basis of portal hypertension: how changes in paradigm are leading to successful new treatments. J Hepatol 62(1 Suppl):S121–S130

Bowman AJ, Chen CP, Ford GA (1994) Nitric oxide mediated venodilator effects of nebivolol. Br J Clin Pharmacol 38(3):199–204

Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R et al (1986) Beta 1- and beta 2-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective beta 1-receptor down-regulation in heart failure. Circ Res 59(3):297–309

Brodde O-E, Stein CM (2003) The Gly389Arg β1-adrenergic receptor polymorphism: a predictor of response to β-blocker treatment? Clin Pharmacol Ther 74(4):299–302

Brodde O-E, Bruck H, Leineweber K, Seyfarth T (2001) Presence, distribution and physiological function of adrenergic and muscarinic receptor subtypes in the human heart. Basic Res Cardiol 96(6):528–538

Bylund DB (2007) Alpha- and beta-adrenergic receptors: Ahlquist's landmark hypothesis of a single mediator with two receptors. Am J Physiol Endocrinol Metab 293(6):E1479–E1481

Christou DD, Seals DR (2008) Decreased maximal heart rate with aging is related to reduced β-adrenergic responsiveness but is largely explained by a reduction in intrinsic heart rate. J Appl Physiol 105(1):24–29

Clifford PS (2011) Local control of blood flow. Adv Physiol Educ 35(1):5–15

Cockcroft JR, Pedersen ME (2012) β-Blockade: benefits beyond blood pressure reduction? J Clin Hypertens (Greenwich) 14(2):112–120

Cockcroft JR, Chowienczyk PJ, Brett SE, Chen CP, Dupont AG, Nueten LV et al (1995) Nebivolol vasodilates human forearm vasculature: evidence for an L-arginine/NO-dependent mechanism. J Pharmacol Exp Ther 274(3):1067

Collaborators GRF (2018) Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392(10159) 1474-547X (Electronic):1923–1994

Colman JC, Jennings GL, McLean AJ, Mignot PR, Dudley FJ (1982) Propranolol in decompensated alcoholic cirrhosis. Lancet 2(8306):1040–1041

Cvenkel B, Kolko M (2020) Current medical therapy and future trends in the management of glaucoma treatment. J Ophthalmol 2020(2090-004X (Print)):6138132

Dahl LK (1972) Salt and hypertension. Am J Clin Nutr 25(2):231–244

Dahlöf B, Devereux RB, Kjeldsen SE, Julius S, Beevers G, de Faire U et al (2002) Cardiovascular morbidity and mortality in the losartan intervention for endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. Lancet 359(9311):995–1003

Dahlöf B, Sever PS, Poulter NR, Wedel H, Beevers DG, Caulfield M et al (2005) Prevention of cardiovascular events with an antihypertensive regimen of amlodipine adding perindopril as required versus atenolol adding bendroflumethiazide as required, in the Anglo-Scandinavian cardiac outcomes trial-blood pressure lowering arm (ASCOT-BPLA): a multicentre randomised controlled trial. Lancet 366(9489):895–906

de Franchis R, Bosch J, Garcia-Tsao G, Reiberger T, Ripoll C, Abraldes JG et al (2022) Baveno VII – renewing consensus in portal hypertension. J Hepatol 76(4):959–974

DeQuattro V, Li D (2002) Sympatholytic therapy in primary hypertension: a user friendly role for the future. J Hum Hypertens 16(1):S118–SS23

DiBona GF, Esler M (2009) Translational medicine: the antihypertensive effect of renal denervation. Am J Physiol Regul Integr Comp Physiol 298(2):R245–RR53

do Vale GT, Ceron CS, Gonzaga NA, Simplicio JA, Padovan JC (2019) Three generations of beta-blockers: history, class differences and clinical applicability. Curr Hypertens Rev 15(1):22–31

Eckhart AD, Yang N, Xin X, Faber JE (1997) Characterization of the α1B-adrenergic receptor gene promoter region and hypoxia regulatory elements in vascular smooth muscle. Proc Natl Acad Sci 94(17):9487–9492

Esler M, Julius S, Zweifler A, Randall O, Harburg E, Gardiner H, DeQuattro V (1977) Mild high-renin essential hypertension. N Engl J Med 296(8):405–411

Esler M, Jennings G, Korner P, Willett I, Dudley F, Hasking G et al (1988) Assessment of human sympathetic nervous system activity from measurements of norepinephrine turnover. Hypertension 11(1):3–20

Esler M, Jennings G, Lambert G, Meredith I, Horne M, Eisenhofer G (1990) Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. Physiol Rev 70(4):963–985

Ettehad D, Emdin CA, Kiran A, Anderson SG, Callender T, Emberson J et al (2016) Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis. Lancet 387(10022):957–967

Faber JE, Szymeczek CL, Cotecchia S, Thomas SA, Tanoue A, Tsujimoto G, Zhang H (2007) Alpha1-adrenoceptor-dependent vascular hypertrophy and remodeling in murine hypoxic pulmonary hypertension. Am J Physiol Heart Circ Physiol 292(5):H2316–H2323

Faconti L, Kulkarni S, Delles C, Kapil V, Lewis P, Glover M et al (2024) Diagnosis and management of primary hyperaldosteronism in patients with hypertension: a practical approach endorsed by the British and Irish hypertension society. J Hum Hypertens 38(1):8–18

Findlay FM (1936) Hypertension: its surgical approach. Cal West Med 45(4):334–340

Forouzanfar MH, Liu P, Roth GA, Ng M, Biryukov S, Marczak L et al (2017) Global burden of hypertension and systolic blood pressure of at least 110 to 115 mmHg, 1990-2015. JAMA 317(2):165–182

Freedman RR, Baer RP, Mayes MD (1995) Blockade of vasospastic attacks by α2-adrenergic but not α1-adrenergic antagonists in idiopathic Raynaud's disease. Circulation 92(6):1448–1451

Fujio H, Nakamura K, Matsubara H, Kusano KF, Miyaji K, Nagase S et al (2006) Carvedilol inhibits proliferation of cultured pulmonary artery smooth muscle cells of patients with idiopathic pulmonary arterial hypertension. J Cardiovasc Pharmacol 47(2)

Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H et al (2016) The management of primary aldosteronism: case detection, diagnosis, and treatment: an endocrine society clinical practice Guideline. J Clin Endocrinol Metabol 101(5):1889–1916

Gambardella J, Fiordelisi A, Avvisato R, Buonaiuto A, Cerasuolo FA, Sorriento D, Iaccarino G (2023) Adrenergic receptors in endothelial and vascular smooth muscle cells. Curr Opin Physio 36:100721

Gamboa A, Okamoto LE, Arnold AC, Figueroa RA, Diedrich A, Raj SR et al (2014) Autonomic blockade improves insulin sensitivity in obese subjects. Hypertension 64(4):867–874

Garrison RJ, Kannel WB, Stokes J, Castelli WP (1987) Incidence and precursors of hypertension in young adults: the Framingham offspring study. Prev Med 16(2):235–251

Group ACR (2000) Major cardiovascular events in hypertensive patients randomized to doxazosin vs chlorthalidone: the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). ALLHAT Collaborative Research Group. JAMA 283(15):1967–1975

Group SR, Wright JT Jr, Williamson JD, Whelton PK, Snyder JK, Sink KM et al (2015) A randomized trial of intensive versus standard blood-pressure control. N Engl J Med 373(22): 2103–2116

Gudmundsdottir H, Strand AH, Høieggen A, Reims HM, Westheim AS, Eide IK et al (2008) Do screening blood pressure and plasma catecholamines predict development of hypertension? Twenty-year follow-up of middle-aged men. Blood Press 17(2):94–103

Guthrie RM, Siegel RL (1999) A multicenter, community-based study of doxazosin in the treatment of concomitant hypertension and symptomatic benign prostatic hyperplasia: the hypertension and BPH intervention trial (HABIT). Clin Ther 21(10):1732–1748

Guyton AC (1991) Blood pressure control – special role of the kidneys and body fluids. Science 252(5014):1813–1816

Guyton AC, Fau-Coleman TG, Coleman TG (1969) Quantitative analysis of the pathophysiology of hypertension. J Am Soc Nephrol 10(10):2248–2258. 1046–6673 (Print)

Harlos J, Heidland A (2008) Hypertension as cause and consequence of renal disease in the 19th century. Am J Nephrol 14(4–6):436–442

Hering L, Rahman M, Hoch H, Markó L, Yang G, Reil A et al (2020) α2A-Adrenoceptors modulate renal sympathetic neurotransmission and protect against hypertensive kidney disease. J Am Soc Nephrol 31(4):783–798

Hillon P, Blanchet L, Lebrec D (1982) Effect of propranolol on hepatic blood flow in normal and portal hypertensive rats. Clin Sci 63(1):29–32

Hinton TC, Adams ZH, Baker RP, Hope KA, Paton JFR, Hart EC, Nightingale AK (2020) Investigation and treatment of high blood pressure in young people. Hypertension 75(1):16–22

Holthoff HP, Zeibig S, Jahns-Boivin V, Bauer J, Lohse MJ, Kääb S et al (2012) Detection of anti-β 1-AR autoantibodies in heart failure by a cell-based competition ELISA. Circ Res 111(6): 675–684

Hurxthal LM (1931) Blood pressure before and after operation in hyperthyroidism. Arch Intern Med 47(2):167–181

Ishikawa M, Sato N, Asai K, Takano T, Mizuno K (2009) Effects of a pure alpha/beta-adrenergic receptor blocker on monocrotaline-induced pulmonary arterial hypertension with right ventricular hypertrophy in rats. Circ J 73(12):2337–2341

Jones PP, Shapiro LF, Keisling GA, Jordan J, Shannon JR, Quaife RA, Seals DR (2001) Altered autonomic support of arterial blood pressure with age in healthy men. Circulation 104(20): 2424–2429

Julius S (1988) Transition from high cardiac output to elevated vascular resistance in hypertension. Am Heart J 116(2, Part 2):600–606

Julius S, Krause L, Schork NJ, Mejia AD, Jones KA, van de Ven C et al (1991) Hyperkinetic borderline hypertension in Tecumseh, Michigan. J Hypertens 9(1)

Kamp O, Metra M, Bugatti S, Bettari L, Dei Cas A, Petrini N, Dei CL (2010) Nebivolol: haemodynamic effects and clinical significance of combined beta-blockade and nitric oxide release. Drugs 70(1):41–56

Klein I, Ojamaa K (2001) Thyroid hormone and the cardiovascular system. N Engl J Med 344(7): 501–509

Kopp UC (2011) Integrated systems physiology: from molecule to function to disease. Neural Control of renal function. Morgan & Claypool Life Sciences Copyright © 2011 by Morgan & Claypool Life Sciences, San Rafael

Kurnik D, Li C, Sofowora GG, Friedman EA, Muszkat M, Xie H-G et al (2008) Beta-1-adrenoceptor genetic variants and ethnicity independently affect response to beta-blockade. Pharmacogenet Genomics 18(10)

Lai J, Syngelaki A, Nicolaides KH, von Dadelszen P, Magee LA (2021) Impact of new definitions of preeclampsia at term on identification of adverse maternal and perinatal outcomes. Am J Obstet Gynecol 224(5):518.e1–518e11

Lambert E, Straznicky N, Schlaich M, Esler M, Dawood T, Hotchkin E, Lambert G (2007) Differing pattern of sympathoexcitation in normal-weight and obesity-related hypertension. Hypertension 50(5):862–868

Lassen NA (1959) Cerebral blood flow and oxygen consumption in man. Physiol Rev 39(2): 183–238

Lebrec D, Nouel O, Corbic M, Benhamou JP. Propranolol – a medical treatment for portal hypertension? Lancet 1980;2(8187):180–182

Lebrec D, Poynard T, Hillon P, Benhamou JP (1981) Propranolol for prevention of recurrent gastrointestinal bleeding in patients with cirrhosis: a controlled study. N Engl J Med 305(23): 1371–1374

Lebrec D, Hillon P, Munoz C, Goldfarb G, Nouel O, Benhamou JP (1982) The effect of propranolol on portal hypertension in patients with cirrhosis: a hemodynamic study. Hepatology 2(5): 523–527

Levy RL, White PD, Stroud WD, Hillman CC (1945) Transient tachycardia: prognostic significance alone and in association with transient hypertension. JAMA 129(9):585–588

Lewis P, George J, Kapil V, Poulter NR, Partridge S, Goodman J et al (2024) Adult hypertension referral pathway and therapeutic management: British and Irish hypertension society position statement. J Hum Hypertens 38(1):3–7

Ling L, Li G, Wang G, Meng D, Li Z, Zhang C (2019) Carvedilol improves liver cirrhosis in rats by inhibiting hepatic stellate cell activation, proliferation, invasion and collagen synthesis. Mol Med Rep 20(2):1605–1612
Link RE, Desai K, Hein L, Stevens ME, Chruscinski A, Bernstein D et al (1996) Cardiovascular regulation in mice lacking α2-adrenergic receptor subtypes b and c. Science 273(5276):803–805
Liu JY, Guo LN, Peng WZ, Jiang Y, Wang AL, Guo XM, Xu ZS (2020) Efficacy and safety of nebivolol in hypertensive patients: a meta-analysis of randomized controlled trials. J Int Med Res 48(10):300060520931625
Lucas SJE, Tzeng YC, Galvin SD, Thomas KN, Ogoh S, Ainslie PN (2010) Influence of changes in blood pressure on cerebral perfusion and oxygenation. Hypertension 55(3):698–705
Lund-Johansen P (1979) Comparative haemodynamic effects of labetalol, timolol, prazosin and the combination of tolamolol and prazosin. Br J Clin Pharmacol 8(Suppl 2):107s–111s
Lund-Johansen P (1983) Haemodynamics in essential hypertension. JIS R (ed)
Lund-Johansen P (1991) Twenty-year follow-up of hemodynamics in essential hypertension during rest and exercise. Hypertension 18(5_supplement):III54
Lund-Johansen P, Bakke OM (1979) Haemodynamic effects and plasma concentrations of labetalol during long-term treatment of essential hypertension. Br J Clin Pharmacol 7(2):169–174
Lund-Johansen P, Omvik P (1991) Acute and chronic hemodynamic effects of drugs with different actions on adrenergic receptors: a comparison between alpha blockers and different types of beta blockers with and without vasodilating effect. Cardiovasc Drugs Ther 5(3):605–615
Mah GT, Tejani AM, Musini VM (2009) Methyldopa for primary hypertension. Cochrane Database Syst Rev 4
Mahomed FA (1874) The etiology of Bright's disease and the Prealbuminuric stage. Med Chir Trans 57(0959-5287 (Print))):197–228
Mancia G, Kreutz R, Brunström M, Burnier M, Grassi G, Januszewicz A et al (2023) ESH guidelines for the management of arterial hypertension the task force for the management of arterial hypertension of the European Society of Hypertension: endorsed by the International Society of Hypertension (ISH) and the European Renal Association (ERA). J Hypertens 41(12)
Masini G, Foo LF, Tay J, Wilkinson IB, Valensise H, Gyselaers W, Lees CC (2022) Preeclampsia has two phenotypes which require different treatment strategies. Am J Obstet Gynecol 226(2): S1006–S1S18
Mastai R, Bosch J, Bruix J, Navasa M, Kravetz D, Rodes J (1989) Beta-blockade with propranolol and hepatic artery blood flow in patients with cirrhosis. Hepatology 10(3):269–272
Materson BJ, Reda DJ, Cushman WC, Massie BM, Freis ED, Kochar MS et al (1993) Single-drug therapy for hypertension in men – a comparison of six antihypertensive agents with placebo. N Engl J Med 328(13):914–921
Matthews KA, Zhu S, Tucker DC, Whooley MA (2006) Blood pressure reactivity to psychological stress and coronary calcification in the coronary artery risk development in young adults study. Hypertension 47(3):391–395
Mayeux JD, Pan IZ, Dechand J, Jacobs JA, Jones TL, McKellar SH et al (2021) Management of pulmonary arterial hypertension. Curr Cardiovasc Risk Rep 15(1):2
Mazza A, Gil-Extremera B, Maldonato A, Toutouzas T, Pessina AC (2002) Nebivolol vs amlodipine as first-line treatment of essential arterial hypertension in the elderly. Blood Press 11(3):182–188
Mazza A, Beltramello G, Armigliato M, Montemurro D, Zorzan S, Zuin M et al (2011) Arterial hypertension and thyroid disorders: what is important to know in clinical practice? Ann Endocrinol 72(4):296–303
Mazza A, Armigliato M, Marzola MC, Schiavon L, Montemurro D, Vescovo G et al (2014) Anti-hypertensive treatment in pheochromocytoma and paraganglioma: current management and therapeutic features. Endocrine 45(3):469–478
McEniery CM, Schmitt M, Qasem A, Webb DJ, Avolio AP, Wilkinson IB, Cockcroft JR (2004) Nebivolol increases arterial distensibility in vivo. Hypertension 44(3):305–310

Mills KT, Stefanescu A, He J (2020) The global epidemiology of hypertension. Nat Rev Nephrol 16(4):223–237

Miyashiro JK, Feigl EO (1993) Feedforward control of coronary blood flow via coronary beta-receptor stimulation. Circ Res 73(2):252–263

Moser M (2006) Historical perspectives on the management of hypertension. J Clin Hypertens 8 (s8):15–20

National Institute for Health and Care Excellence (NICE) (2019) Hypertension in pregnancy: diagnosis and management [Internet]

NICE (2022) National Institute for Health and Care Excellence. Hypertension in adults: diagnosis and management. NICE Guideline [NG136]. https://www.niceorguk/guidance/ng136

O'Brien E, Kario K, Staessen JA, de la Sierra A, Ohkubo T (2018) Patterns of ambulatory blood pressure: clinical relevance and application. J Clin Hypertens (Greenwich) 20(7):1112–1115

Omvik P, Lund-Johansen P (1990) The initial hemodynamic response to newer antihypertensive agents at rest and during exercise: review of visacor, doxazosin, nisoldipine, tiapamil, perindoprilat, pinacidil, dilevalol, and carvedilol. Cardiovasc Drugs Ther 4(4):1135–1143

Parati G, Ochoa JE, Lombardi C, Bilo G (2015) Blood pressure variability: assessment, predictive value, and potential as a therapeutic target. Curr Hypertens Rep 17(4):23

Party MW (1992) Medical Research Council trial of treatment of hypertension in older adults: principal results. MRC Working Party. BMJ. Clinical research ed 304(6824):405–412

Philipp M, Brede M, Hein L (2002) Physiological significance of α2-adrenergic receptor subtype diversity: one receptor is not enough. Am J Physiol Regul Integr Comp Physiol 283(2):R287–RR95

Prichard BN, Gillam PM (1969) Treatment of hypertension with propranolol. Br Med J 1(5635): 7–16

Proudman RGW, Pupo AS, Baker JG (2020) The affinity and selectivity of α-adrenoceptor antagonists, antidepressants, and antipsychotics for the human α1A, α1B, and α1D-adrenoceptors. Pharmacol Res Perspect 8(4):e00602

Proudman RGW, Akinaga J, Baker JG (2022) The affinity and selectivity of α-adrenoceptor antagonists, antidepressants and antipsychotics for the human α2A, α2B, and α2C-adrenoceptors and comparison with human α1 and β-adrenoceptors. Pharmacol Res Perspect 10(2):e00936

Quigley HA, Broman AT (2006) The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 90(3):262

Reynaert H, Thompson MG, Thomas T, Geerts A (2002) Hepatic stellate cells: role in microcirculation and pathophysiology of portal hypertension. Gut 50(4):571

Rison SC, Carvalho C, Rull G, Robson J (2022) Investigating hypertension in younger patients. BMJ. Clinical research ed 376(1756–1833 (Electronic)):e067924

Rivas AM, Pena C, Kopel J, Dennis JA, Nugent K (2021) Hypertension and hyperthyroidism: association and pathogenesis. Am J Med Sci 361(1):3–7

Rosenthal T (2004) Contemplating the history of drug therapy for hypertension. Blood Press 13(5): 262–271

Saito I, Ito K, Saruta T (1985) The effect of age on blood pressure in hyperthyroidism. J Am Geriatr Soc 33(1):19–22

Salvi SS (1999) Alpha1-adrenergic hypothesis for pulmonary hypertension. Chest 115(6): 1708–1719

Savarese G, Costanzo P, Cleland John George F, Vassallo E, Ruggiero D, Rosano G, Perrone-Filardi P (2013) A meta-analysis reporting effects of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers in patients without heart failure. J Am Coll Cardiol 61(2): 131–142

Scarpace PJ, Turner N, Mader SL (1991) β-Adrenergic function in aging. Drugs Aging 1(2): 116–129

Schlereth T, Morellini N, Lismont NCAM, Lemper C, Birklein F, Drummond PD (2021) Alpha 1 adrenoceptor expression in skin, nerves and blood vessels of patients with painful diabetic neuropathy. Auton Neurosci 234:102814

Seely EW, Ecker J (2014) Chronic hypertension in pregnancy. Circulation 129(11):1254–1261

Shabeeh H, Khan S, Jiang B, Brett S, Melikian N, Casadei B et al (2017) Blood pressure in healthy humans is regulated by neuronal NO synthase. Hypertension 69(5):970–976

Shibao C, Okamoto LE, Gamboa A, Yu C, Diedrich A, Raj SR et al (2010) Comparative efficacy of Yohimbine against pyridostigmine for the treatment of orthostatic hypotension in autonomic failure. Hypertension 56(5):847–851

Skeberdis VA (2004) Structure and function of beta3-adrenergic receptors. Medicina (Kaunas) 40(5):407–413

Smith C, Teitler M (1999) Beta-blocker selectivity at cloned human Beta1- and Beta2-adrenergic receptors. Cardiovasc Drugs Ther 13(2):123–126

Smithwick RH, Thompson JE (1953) Splanchnicectomy for essential hypertension: results in 1,266 cases. JAMA 152(16):1501–1504

Stiles GL, Taylor S, Lefkowitz RJ (1983) Human cardiac beta-adrenergic receptors: subtype heterogeneity delineated by direct radioligand binding. Life Sci 33(5):467–473

Stokes GS, Weber MA (1974) Prazosin: preliminary report and comparative studies with other antihypertensive agents. Br Med J 2(5914):298–300

Stokes GS, Weber MA, Thornell IR (1974) Beta-blockers and plasma renin activity in hypertension. Br Med J 1(5897):60–62

Tanaka S, Fau-Takeshita A, Takeshita A, Fau-Tomoike H, Tomoike H, Fau-Nakamura M, Nakamura M (1978) Role of autonomic and non-autonomic circulatory components in borderline hypertension in young men. Jpn Heart J 19:66–73. 0021-4868 (Print)

Tellez L, Ibanez-Samaniego L, Perez Del Villar C, Yotti R, Martinez J, Carrion L et al (2020) Non-selective beta-blockers impair global circulatory homeostasis and renal function in cirrhotic patients with refractory ascites. J Hepatol 73(6):1404–1414

Thomopoulos C, Parati G, Zanchetti A (2015) Effects of blood pressure-lowering on outcome incidence in hypertension: 5. Head-to-head comparisons of various classes of antihypertensive drugs – overview and meta-analyses. J Hypertens 33(7)

Turnbull F, Blood Pressure Lowering Treatment Trialists Collaboration (2003) Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials. Lancet 362(9395):1527–1535

Van Nueten L, Taylor FR, Robertson JIS (1998) Nebivolol vs atenolol and placebo in essential hypertension: a double-blind randomised trial. J Hum Hypertens 12(2):135–140

van Vark LC, Bertrand M, Akkerhuis KM, Brugts JJ, Fox K, Mourad J-J, Boersma E (2012) Angiotensin-converting enzyme inhibitors reduce mortality in hypertension: a meta-analysis of randomized clinical trials of renin–angiotensin–aldosterone system inhibitors involving 158 998 patients. Eur Heart J 33(16):2088–2097

Vaz M, Jennings G, Turner A, Cox H, Lambert G, Esler M (1997) Regional sympathetic nervous activity and oxygen consumption in obese normotensive human subjects. Circulation 96(10): 3423–3429

Vaz-de-Melo RO, Giollo-Júnior LT, Martinelli DD, Moreno-Júnior H, Mota-Gomes MA, Cipullo JP et al (2014) Nebivolol reduces central blood pressure in stage I hypertensive patients: experimental single cohort study. Sao Paulo medical journal =. Rev Paul Med 132(5):290–296

Velez-Roa S, Ciarka A, Najem B, Vachiery JL, Naeije R, van de Borne P (2004) Increased sympathetic nerve activity in pulmonary artery hypertension. Circulation 110(10):1308–1312

Villanueva C, Albillos A, Genesca J, Garcia-Pagan JC, Calleja JL, Aracil C et al (2019) Beta blockers to prevent decompensation of cirrhosis in patients with clinically significant portal hypertension (PREDESCI): a randomised, double-blind, placebo-controlled, multicentre trial. Lancet 393(10181):1597–1608

Vorobioff J, Picabea E, Villavicencio R, Puccini V, Rossi O, Bordato J, Audano M (1987) Acute and chronic hemodynamic effects of propranolol in unselected cirrhotic patients. Hepatology 7(4):648–653

Webster K, Fishburn S, Maresh M, Findlay SC, Chappell LC, Guideline C (2019) Diagnosis and management of hypertension in pregnancy: summary of updated NICE guidance. BMJ. Clinical research ed 366(1756–1833 (Electronic)):l5119

Wei J, Galaviz KI, Kowalski AJ, Magee MJ, Haw JS, Narayan KMV, Ali MK (2020) Comparison of cardiovascular events among users of different classes of antihypertension medications: a systematic review and network meta-analysis. JAMA Netw Open 3(2):e1921618

Weidmann P, Hirsch D, Beretta-Piccoli C, Reubi FC, Ziegler WH (1977) Interrelations among blood pressure, blood volume, plasma renin activity and urinary catecholamines in benign essential hypertension. Am J Med 62(2):209–218

Wiysonge CS, Bradley HA, Volmink J, Mayosi BM, Opie LH (2017) Beta-blockers for hypertension. Cochrane Database Syst Rev 1(1):Cd002003

Wofford MR, Anderson DC, Brown CA, Jones DW, Miller ME, Hall JE (2001) Antihypertensive effect of alpha- and beta-adrenergic blockade in obese and lean hypertensive subjects. Am J Hypertens 14(7 Pt 1):694–698

Adrenoceptors in the Lower Urinary Tract

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Abstract

Adrenoceptors importantly contribute to the physiological regulation of lower urinary tract (LUT) function and have become a target of several clinically successful treatments for major LUT diseases. In the bladder dome, β-adrenoceptor subtypes are found in multiple cell types and mediate relaxation of detrusor smooth muscle, perhaps partly indirectly by acting on afferent nerves and cells of the mucosa. β_3-adrenoceptor agonists such as mirabegron and vibegron are used to treat overactive bladder syndrome. In the bladder trigone and urethra, α_1-adrenoceptors cause contraction and thereby physiologically contribute to bladder outlet resistance. α_1-adrenoceptors in the prostate also cause contraction and pathophysiologically elevate bladder outlet resistance leading to voiding dysfunction in benign prostatic hyperplasia. α_1-adrenoceptor antagonist such as tamsulosin is widely used as a first-line option to treat LUT symptoms in men, but it remains unclear to which extent and how smooth muscle relaxation contributes to symptom relief.

Keywords

α_1-Adrenoceptor · β-Adrenoceptor · Prostate · Ureter · Urethra · Urinary bladder

Abbreviations

AR	Adrenoceptor
BPH	Benign prostatic hyperplasia
DO	Detrusor overactivity
EFS	Electrical field stimulation
GRK	G protein-coupled receptor kinase
IP_3	Inositol 1,4,5-trisphosphate
IUP	Intra-urethral pressure

LUT	Lower urinary tract
LUTD	Lower urinary tract dysfunction
OAB	Overactive bladder syndrome
PKC	Protein kinase C
PLC	Phospholipase C

1 Introduction

The lower urinary tract (LUT) comprises the urinary bladder, the urethra and, in males, the prostate. It is functionally supported by the striated muscle of the pelvic floor. While the kidneys produce urine continuously, the major function of the LUT is to store urine and, when appropriate circumstances occur, to expel it. A detailed discussion of the overall anatomy, physiology, and pharmacology of the urinary tract has been provided in a previous volume of the Handbook of Experimental Pharmacology (Andersson and Michel 2011).

LUT dysfunction (LUTD) occurs when elements of the LUT fail to fulfill their roles in the storage or extrusion of urine. In a simplified approach, LUTD can be divided into disturbances of filling and storage and of voiding and emptying. Reasons for a disturbed filling and storage of the bladder include detrusor overactivity (DO) and may result in overactive bladder syndrome (OAB). On the other hand, increased bladder outlet resistance can be caused by enlargement of the prostate and result in disturbed voiding, whereas insufficient resistance from the bladder neck and urethra can result in an inability to hold urine, leading to stress urinary incontinence. Of note, other causes of LUTD and other resulting clinical manifestations exist but are beyond the scope of this chapter.

Adrenoceptors (ARs) play a key role in the physiological regulation of LUT function. Conversely, the LUT has played a key role in the history of AR pharmacology. For instance, α_1-AR antagonists not discriminating between the α_1-AR subtypes such as terazosin were originally used for the treatment of prostate disorders. After it was apparent that multiple α_1-AR subtypes exist and that contraction of the human prostate is primarily mediated by the α_{1A} subtype (see Sect. 5), this led to the development of the α_{1A}-selective antagonist tamsulosin, which became the first clinically used drug that exploited the existence of α_1-AR subtypes to provide similar efficacy to other antagonists with fewer cardiovascular side effects (Michel and de la Rosette 2004). Similarly, the urinary bladder played an important role in recognizing that more than two subtypes of β-AR exist (Nergardh et al. 1977) and diseases of the bladder became the first and until now only indication for the use of β_3-AR selective agonists such as mirabegron (Chapple et al. 2014). Accordingly, AR ligands have become the cornerstone of treatment of LUTD (Fig. 1). Therefore, it is very appropriate that this volume of the Handbook includes a chapter on the LUT.

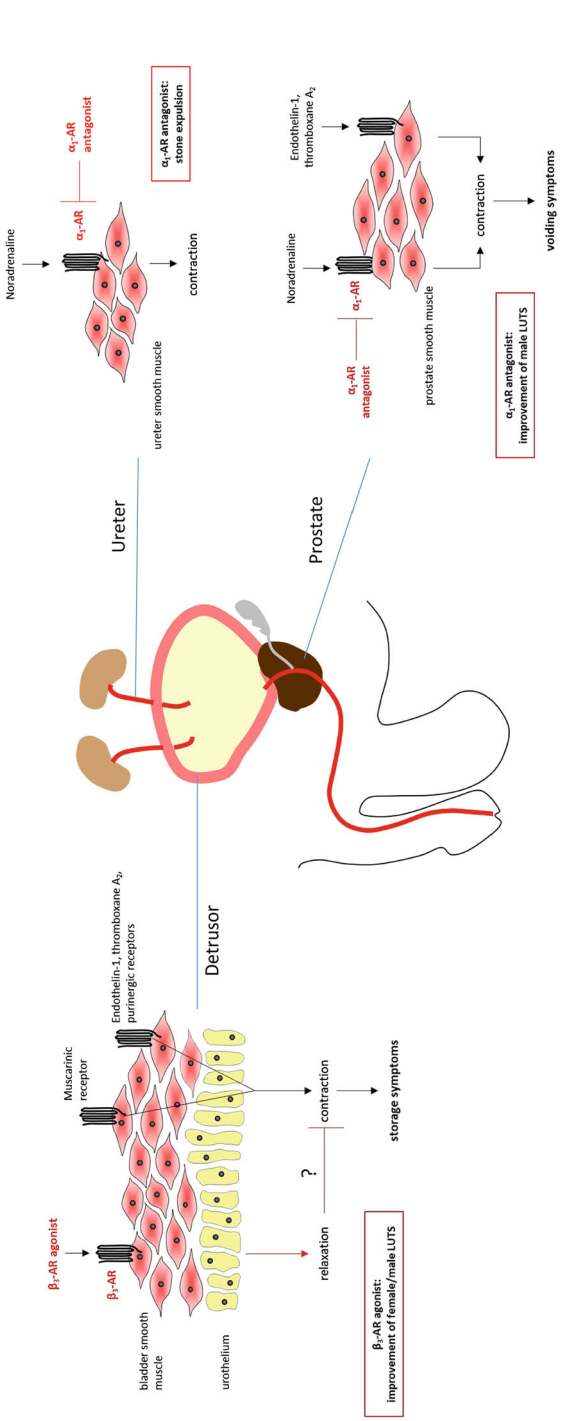

Fig. 1 Available drug classes targeting AR for medical treatment of LUTD. Available drugs include β_3-AR agonists for the treatment of storage symptoms in OAB; α_1-AR antagonists for treatment of voiding symptoms in BPH; and α_1-AR antagonists for expulsion of ureteral stones. Initial concepts suggesting improvement of storage symptoms by β_3-AR agonists were based on β_3-AR mediated relaxation of detrusor smooth muscle but are increasingly challenged. Meanwhile, an involvement of β_3-AR on non-smooth muscle cells, including afferent nerves appears more likely to account for the clinical effects of β_3-AR agonists in OAB. Improvements in voiding symptoms in BPH by α_1-AR antagonists were attributed to relaxation of prostate smooth muscle for a long time, but the causative role of relaxation and symptom relief is in fact uncertain. In addition to the detrusor, prostate, and ureter, adrenergic regulation has been examined in the urethra and trigone. As explained in the text, contributions of adrenergic regulation in these tissues to LUT function are poorly understood and in the case of the trigone underexplored

2 Ureter

The ureters are hollow tubes through which urine flows from the kidneys to the urinary bladder. Like blood vessels, AR contributes to ureteral function by promoting contraction of smooth muscle via α_1-AR and relaxation via β-AR. A role for α_2-AR in control of ureteral function has yet to be defined; however, it is expected on theoretical grounds that they will inhibit transmitter release from sympathetic fibers innervating the ureter. Interestingly, the ureter also contains pacemaker cells that coordinate peristalsis, and their function may at least partly be regulated by AR (Oostendorp et al. 2000). The overall physiology and pharmacology of the ureter has been reviewed (Canda et al. 2007). The main pathology related to ureters is not directly related to the flow of urine but rather to the expulsion of renal and ureteral stones. AR ligands have been tested as means to facilitate stone expulsion.

The mRNA expression of α_1-AR subtypes in the ureter has been studied in rats (Scofield et al. 1995) and mice (Kobayashi et al. 2009). While α_{1A}-AR accounted for about 75% of α_1-AR mRNA in mice, α_{1B}-AR were most prominent in rats (about 45%, with the other two subtypes accounting equally for the remaining 55%). Presence of mRNA for all three β-AR subtypes has been reported in the human ureter without quantification (Park et al. 2000; Matsumoto et al. 2013). Similarly, immunostaining for all three β-AR subtypes was found in both smooth muscle and urothelial cells (Matsumoto et al. 2013). Radioligand binding studies in human ureter homogenates detected mostly β_2-AR (Park et al. 2000), albeit with a ligand that cannot detect β_3-AR in typically applied concentrations.

Early in vivo studies in dogs reported that noradrenaline increases and isoprenaline decreases ureteral contraction, with responses inhibited by phenoxybenzamine and propranolol, respectively (McLeod et al. 1973). In vitro studies in rabbits and dogs confirmed that noradrenaline primarily causes ureteral constriction; that becomes relaxation in the presence of phentolamine (Weiss et al. 1978). Contraction to noradrenaline and relaxation to β-AR agonists were also confirmed in horses (Labadia et al. 1987) and humans (Villa et al. 2013). Contraction to noradrenaline in rat ureter was not detected in one study (Mastrangelo and Iselin 2007) but was in another (Villa et al. 2013). The latter study also showed antagonism by α_{1A}-AR selective antagonists such as silodosin and tamsulosin in both rat and human ureter, indicating that the response is largely mediated by α_{1A}-AR in both species. Thus, the primary role of the endogenous transmitter noradrenaline appears to be smooth muscle contraction, with β-AR-mediated relaxation present but having a smaller role. In vivo administration of the α_1-AR agonist phenylephrine concentration-dependently increased not only amplitude but also frequency of contraction (Danuser et al. 2001), thus providing indirect evidence for an adrenergic control of ureteral pacemaker cells.

Several studies explored the relative roles of β-AR subtypes, which apparently differ between species. These suggest a primary role for β_2-AR in rabbits (Tomiyama et al. 2003a), a major role for β_3-AR in dogs (Tomiyama et al. 2003a, b), and a mixed role for β_2- and β_3-AR in humans (Park et al. 2000; Matsumoto et al. 2013). Studies in pigs are inconclusive: in vitro experiments suggested a combined role of β_2- and

β_3-AR (Wanajo et al. 2004), but another study found that relaxation by mirabegron occurred not via its β_3-AR agonism but rather via its α_1-AR antagonism (Lim and Chess-Williams 2022). In vivo studies identified primarily β_2-AR but did not test agents selective for β_3-AR (Danuser et al. 2001). The latter report was also interesting in that isoprenaline and fenoterol reduced contraction frequency with systemic (i.v.) and local administration, whereas topical administration reduced only contraction amplitude (Danuser et al. 2001). Effects on contraction frequency point to a role of β-AR in the regulation of pacemaker cell activity. Another study reported that CGP 12177 similarly reduced the activity of ureteral pacemaker cells in wild-type and β_3-AR knock-out mice (Oostendorp et al. 2000), suggesting that this may be a β_1-AR-mediated effect.

Therapeutic interest in ureteral AR relates mainly to expulsion of ureteral stones (Fig. 1). In vivo studies in rabbits indicate that β-AR agonism may have beneficial effects on stone expulsion (Miyatake et al. 2001), but commercial research and development programs in this area have been discontinued. In contrast, various investigators explored whether clinically available α_1-AR antagonists improve stone expulsion as reviewed elsewhere (Michel and de la Rosette 2006; Tzortzis et al. 2009; Campschroer et al. 2018). Meanwhile, more than 60 studies, including 15 placebo-controlled trials have been reported. According to a meta-analysis, administration of an α-blocker increased stone clearance (odds ratio 1.45 [1.36; 1.55] corresponding to 278 [223; 340] additional stone clearances) and decreased expulsion time by 3.4 days [−4.2; −2.6) (Campschroer et al. 2018)]. The success rate appears to be related to stone size; earlier reports that more distal stones were associated with a greater success rate of clearance were not confirmed in this meta-analysis. Based on the acute character of a ureteral colic, drugs of choice are α-blockers that do not require dose-titration such as alfuzosin, silodosin, and tamsulosin.

3 Bladder Dome

This section will focus on the bladder dome whereas the trigone will be discussed in the next section because it differs from the dome in anatomy, innervation, and function. While the bladder dome relaxes during urine storage and contracts during voiding, the opposite happens in the trigone. The bladder wall comprises multiple layers including the urothelium/mucosa, smooth muscle layer, and adventitia. The expression and function of AR can differ across those layers, and most studies in the field have examined the entire detrusor or even the total bladder. The expression and function of AR subtypes in the bladder has been reviewed extensively (Michel and Vrydag 2006; Sellers et al. 2018; Igawa et al. 2019). Briefly, α_1-AR are poorly expressed in the detrusor (in humans), in contrast to the trigone (see below). α_2-AR are present in considerable quantities (Goepel et al. 1997) with some on pre-junctional nerve endings where they mediate inhibition of neurotransmitter release. These pre-junctional receptors most likely account for only a small fraction

of the α_2-AR, with the functional role of the remainder largely unclear. The main interest regarding AR in the bladder relates to β-AR.

3.1 β-Adrenoceptor Expression and Function

There are major species differences in expression and function of β-AR subtypes (Michel and Vrydag 2006). At the mRNA level, β_1-AR are most prominent in the mouse bladder, with all three subtypes similarly present in the rat (Barendrecht et al. 2009). In the human detrusor more than 95% of the mRNA is β_3-AR although all three subtypes are present in entire human bladder, similar to rats (Uhlen et al. 2015). Thus, all three β-AR subtypes are present in rat and human bladder, but their relative contributions are difficult to assess due to technical limitations (Michel and Vrydag 2006). A study in rat bladder detected comparable amounts of β_2- and β_3-AR protein but emphasized the technical challenges (Schneider and Michel 2010). Interestingly, layers within the bladder appear to differ with regard to β-AR protein expression with β_3-AR apparently more prominent in the detrusor and β_2-AR more prominent in the mucosa (Sellers et al. 2018). Expression of β-AR subtypes is regulated by disease, and upregulation of β_2-AR mRNA occurs in a mouse model of cystitis (Saban et al. 2002). While none of the β-AR subtypes was regulated in one rat model of bladder outlet obstruction (Barendrecht et al. 2009), severe obstruction caused β_3-AR upregulation (Kurizaki et al. 2013). Short-term (6 h) exposure of rat bladder strips caused more pronounced desensitization of relaxation by the β_2-AR rather than the β_3-AR component (Michel 2014). More generally, regulation of bladder AR by disease and treatment appears to be an under-investigated area.

The main AR-mediated function in the urinary bladder is smooth muscle relaxation by β-AR agonists. While this is almost exclusively mediated by β_3-AR in humans, it is a mixed β_2/β_3-AR response in rat bladder (Michel and Vrydag 2006;

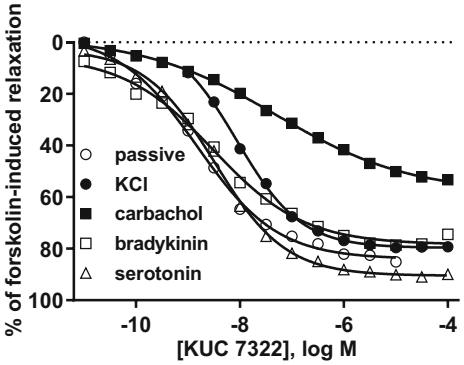

Fig. 2 Relaxation of rat urinary bladder by the β_3-adrenoceptor agonist KUC 7322 against basal tone (passive tension) and that induced receptor-independently by KCl or by agonism at muscarinic (carbachol), bradykinin, or serotonin receptors. Generated from data reported in (Cernecka et al. 2014). Error bars have been omitted for clarity

Igawa et al. 2019) and largely a β_2-AR response in mouse bladder (Wuest et al. 2009). β-AR agonists, including those selective for β_2- or β_3-AR, are less potent relaxants against tone induced by muscarinic receptor stimulation than against other types of contractile stimulus (Cernecka et al. 2014; Erdogan et al. 2022a) (Fig. 2), a feature that has also been observed in other tissues such as airways or gut (Dale et al. 2014). While detrusor relaxation by direct effects on smooth muscle is well documented, it remains unclear whether this explains the clinical response to β_3-AR agonists in patients. The main reason is that the potency of mirabegron causing relaxation of human detrusor strips in vitro is much less than maximum plasma levels (Igawa et al. 2019). Therefore, it is of interest that β-AR including β_3-AR are also found in structures other than detrusor smooth muscle cells including urothelium, suburothelial interstitial cells, pre-junctional endings of efferent nerves, afferent nerves, bladder vasculature, and major pelvic ganglion (Igawa et al. 2019). Whether and to what extent these contribute to clinically observed β_3-AR agonist effects, remains to be determined.

3.2 β_3-Adrenoceptor Agonists as Treatments for Bladder Dysfunction

β_3-AR agonists have become a guideline-recommended treatment of OAB (Lightner et al. 2019) (Fig. 1). Such recommendations were initially based on clinical studies with mirabegron (Chapple et al. 2014) but meanwhile vibegron has also been approved in some countries for the treatment of OAB (Yoshida et al. 2018; Staskin et al. 2020). Other β_3-AR agonists such as solabegron were found to be effective in phase II studies (Ohlstein et al. 2012) but discontinued either for commercial reasons, or having missed the primary endpoint in phase III (Thiagamoorthy et al. 2016).

Mirabegron and vibegron were effective compared to placebo and generally well tolerated (Chapple et al. 2014; Yoshida et al. 2018; Staskin et al. 2020). Of note, they produced less dry mouth than muscarinic receptor antagonists (Chapple et al. 2014). There have been cases of serious cardiovascular side effects with mirabegron (Michel and Gravas 2016), possibly related to off-target effects on sympathetic nerve endings (Mo et al. 2017), but it remains unclear whether these considerations apply to the entire drug class.

Somewhat surprisingly, the clinical efficacy of β_3-AR agonists did not exceed that of muscarinic receptor antagonists. Therefore, some studies explored the effects of combinations of β_3-AR agonists and muscarinic antagonists and found that the combination had greater efficacy than monotherapy at the group level (Michel et al. 2023). Whether this is due to a less than additive effect in most subjects or to some patients primarily responding to one or other drug class remains unclear (Michel and Staskin 2022). Some studies explored whether polymorphisms of the β_3-AR affect clinical status and/or response, but have largely remained inconclusive (Michel 2023).

4 Bladder Trigone

The trigone forms the region between the ureteral orifices and bladder neck, and comprises large parts of the bladder base (Fry et al. 2010a). Although it is part of the bladder wall, together with the detrusor located above the ureteral openings and in the dome, the pharmacological and functional differences between the trigone and detrusor are fundamental. The anatomical terminology used in the bladder outlet region is not always consistent. The trigone joins the bladder outlet, or bladder neck or internal sphincter, and there may be a seamless transition from the trigone (bladder) to the urethra (Fry et al. 2010a). The trigone is composed of two smooth muscle layers, including the "superficial" trigone (inner, intravesical layer) and the "deeper" trigone (outer layer) (Fry et al. 2010a). Like the urethra and ureters, contractile responses of the superficial trigone are mediated by α_1-AR, whereas detrusor smooth muscle contractions occur via cholinergic and in the deeper trigone via both adrenergic and cholinergic receptors (Fry et al. 2010a). A shared mesodermal origin of the superficial trigone layer and the ureters was suggested, but has since been questioned (Fry et al. 2010a). The deeper trigone is considered a continuation of the detrusor (Fry et al. 2010a). Thus, the trigone shows a clear separation into two layers and has a dual, adrenergic and cholinergic innervation (Fry et al. 2010a). However, the few available studies that address trigone pharmacology and physiology do not acknowledge zonation or different functional units within the trigone during tissue sampling, experimental designs, or in their descriptions, that in any case may be challenging with trigone tissues from small animals.

In the storage phase of the voiding cycle (bladder filling), α_1-AR-mediated contraction of the trigone following sympathetic activation contributes to closure of the bladder outlet and to maintenance of continence, together with smooth muscle contraction in the urethra and/or bladder neck (Fry et al. 2010a). During voiding, trigone smooth muscle relaxes, probably by parasympathetically mediated release of nitric oxide, allowing passage of urine into the urethra and bladder emptying by muscarinic detrusor contractions (Fry et al. 2010a). The number of studies addressing trigone pharmacology and physiology, that is surprisingly low compared to other lower tract regions, may reflect a common underestimation of its functional relevance for urodynamic regulation and micturition. α_1-AR are reasonably characterized in the trigone, while α_2- and β-AR have been rarely examined.

4.1 α_1-Adrenoceptors

4.1.1 Expression

In human trigone tissues, all three subtypes are detectable by reverse-transcription polymerase chain reaction (RT-PCR), with signals for α_{1A} being stronger than α_{1B} and α_{1D}. Expression of α_{1A}-AR was higher in female than male and higher in the trigone compared to the detrusor in male (Sigala et al. 2004). At protein level, expression of α_1-AR, and higher levels in female than in male tissues was confirmed

by α_1-subtype-unselective radioligand binding (Sigala et al. 2004). In the trigone of primates and rats, α_{1A}-AR may be the predominant or single subtype of α-AR at mRNA and protein level. In situ hybridization with subtype-selective, radiolabeled oligonucleotide probes and autoradiographic competition studies with ligands consistently revealed α_{1A}-AR in trigone tissues from rhesus monkeys and rats, while α_{1B}- and α_{1D}-AR were undetectable (Walden et al. 1997). A comparison of ^{3}H-prazosin binding across different LUT tissues from rats suggested a lower density of α_1-AR in the trigone than the urethra, but still substantially higher than the bladder dome (Monneron et al. 2000).

4.1.2 Function

α_1-AR-mediated contractions have been demonstrated using phenylephrine for human trigone, and using different agonists and antagonists for non-human trigone. Phenylephrine caused concentration-dependent contractions in tissues from the male, superficial trigone, with an EC_{50} of 6 μM and similar to maximum contractions induced by carbachol in the same study and to 200% of KCl-induced contractions (Hennenberg et al. 2017a). In human tissues, 1 μM phenylephrine-induced slight but obvious contractions in male, superficial trigone tissues, but no or neglectable contractions in tissues from male deep trigone and from female superficial trigone (Walther et al. 2018). A synergistic effect was observed if carbachol was applied after precontraction with 1 μM phenylephrine, resulting in tensions exceeding those following addition of either phenylephrine or carbachol (Walther et al. 2018). Potentiation of phenylephrine contractions by carbachol was limited to the male superficial trigone and was not observed with tissues from the female superficial or male deeper trigone (Walther et al. 2018). Two studies with human tissues allowed direct comparison of adrenergic and cholinergic contractions in the superficial trigone, showing similar maximum responses to adrenergic and cholinergic agonists, or to adrenergic responses doubling the responses to cholinergic agonists (Hennenberg et al. 2017a; Speakman et al. 1988).

Contractions in rabbit and rat isolated trigone tissues were induced using noradrenaline and phenylephrine (Azuma et al. 1989; Deplanne and Galzin 1996; Honda and Nakagawa 1986; Lefevre-Borg et al. 1993; Tatemichi et al. 2012; Teixeira et al. 2007; Van der Graaf et al. 1997). Two studies allowed comparisons between different agonists, or of trigone with other tissues. Maximum contractions to noradrenaline and phenylephrine in rabbit trigone tissues were similar and exceeded contractions of aortic tissues, where contractions by noradrenaline and phenylephrine were 10–25% lower (Azuma et al. 1989). Agonist-induced contractions in rat tissues compared to those to KCl showed that maximum phenylephrine-induced contractions were around 60% of KCl in trigone, around 125% of KCl in urethral smooth muscle, whereas maximum carbachol-induced contractions were around 125% of KCl in detrusor (Teixeira et al. 2007). Further data from guinea-pig trigone showed phenylephrine-induced contractions (Roosen et al. 2008, 2009a), and from porcine trigone, where phenylephrine induced concentration-dependent contractions (Wuest et al. 2011), whereas noradrenaline caused relaxations instead of contractions (Markiewicz et al. 2014, 2017).

Effects of α_1-AR antagonists on trigone smooth muscle have been shown for neurogenic contractions of human tissues, and for agonist-induced contractions of non-human tissues. Most data are available for rabbit, where a number of α_1-AR antagonists, including alfuzosin, prazosin, terazosin, indoramin, 5-methylurapidil, WB-4101, BMY 7378, and tamsulosin right-shift concentration-response curves for phenylephrine and noradrenaline, suggesting competitive antagonism (Azuma et al. 1989; Deplanne and Galzin 1996; Honda and Nakagawa 1986; Lefevre-Borg et al. 1993; Van der Graaf et al. 1997). Even though some of these antagonists may show some subtype selectivity, e.g. tamsulosin for α_{1A} or BMY 7378 for α_{1D}, findings and pK_B values from rabbit tissues do not support a predominance of α_{1A}-AR in these contractions (Van der Graaf et al. 1997). However, in rat trigone, evidence for α_{1A}-AR in trigone smooth muscle contraction was suggested by sub-nanomolar EC_{50} values for inhibition of noradrenaline-induced contractions for silodosin and tamsulosin (α_{1A}-AR selective) but ~100 nM for alfuzosin and >500 nM for naftopidil (Tatemichi et al. 2012).

EFS-induced contractions in trigone were not observed in all studies. In castrated male pigs, EFS failed to induce contractions unless endogenous nitric oxide production was inhibited (Noda et al. 2002). In contrast, in human superficial trigone, EFS-induced contractions were reported at about half of phenylephrine-induced contractions assessed in the same study (Hennenberg et al. 2017a) and were inhibited to half by combined phentolamine and atropine (Speakman et al. 1988).

In rat trigone, α_1-AR-mediated contractions were inhibited by Rho kinase inhibitors (Teixeira et al. 2007), and in guinea-pig by protein kinase C (PKC) inhibitors (Roosen et al. 2009a). In guinea-pig trigones, phenylephrine also caused elevation of cytosolic Ca^{2+} in smooth muscle cells (Roosen et al. 2009a). These findings suggest involvement of Ca^{2+}-, PKC-, and Rho kinase-dependent mechanisms in α_1-AR-mediated contraction of trigone smooth muscle, possibly shared by contractile receptors in any type of smooth muscle.

4.1.3 Physiological Functions In Vivo

Even though a role for α_1-AR-mediated trigone contractions in maintaining closure of the bladder outlet region during filling appears plausible, supporting evidence is low, and a similar function has been proposed for urethral smooth muscle. Smooth muscle in the trigone and urethra both contract in response to α_1-AR stimulation and could be regarded as a functional unit. Based on experimental or clinical evidence, contributions of the urethra and trigone to intraurethral resistance or to continence are not easily separated. Clearly, α_1-AR antagonists improve bladder emptying and voiding, particularly in male patients with voiding symptoms and benign prostatic hyperplasia (BPH), where the prostate critically contributes to intraurethral resistance (see Sect. 5). In female or prostate-ablated male subjects, intraurethral pressure still responds to α_1-AR agonists or antagonists. Accordingly, (see Sect. 6.1.3), α_1-AR antagonists reduce urethral pressure and voiding dysfunction (e.g., underactive bladder) in female humans. These effects in vivo have been attributed to the urethra, with the trigone not taken into account. Together, a role of the trigone in voiding, maintenance of continence, and prevention of vesico-ureteral reflux has been

proposed, but not well proven (Fry et al. 2010b; Roosen et al. 2009b). New conclusions regarding trigone function in urodynamic regulation may emerge from onabotulinum toxin injection into the bladder wall for treatment of storage symptoms. These neurotoxins are recommended by urological guidelines as an alternative to conservative and oral drug treatment in OAB and neurogenic bladder. Improvements were large and initially attributed to inhibition of cholinergically mediated detrusor contractions. However, it became apparent that trigone-including injections are superior to trigone-sparing injections (i.e., to detrusor), explained by disruption of sensory neuronal function during bladder filling, signals that usually initiate contraction and finally voiding (Cui et al. 2021). Within the bladder, the trigone may be the region with the densest innervation by sensory nerves, which may be critically involved in the voiding cycle, in addition to trigone smooth muscle tone (Cui et al. 2021). Whether this involves adrenoceptors remains to be shown, but involvement of neuronal α_1-AR in voiding regulation has been suggested (Barendrecht et al. 2008; Michel and Vrydag 2006).

4.2 α_2-Adrenoceptors

α_2-AR protein expression has been confirmed for rat and guinea-pig trigone by binding of ^{3}H-rauwolscine, with 6.7 fold higher binding in guinea-pig than in rat tissues (Monneron et al. 2000). Whether this reflects a true species difference is another matter, as similar differences were seen for all other LUT tissues examined in this study. Effects of the α_2-AR agonist clonidine were examined in rabbit trigone tissues, with contractions ~ half of those to noradrenaline and phenylephrine (Honda et al. 1985; Ueda et al. 1984). Concentration-response curves for clonidine, but for phenylephrine as well were right-shifted by yohimbine and prazosin, respectively (Honda et al. 1985; Ueda et al. 1984).

4.3 β-Adrenoceptors

Porcine isolated trigone precontracted with K^+ was relaxed by the non-selective β-AR agonist isoprenaline, and the β_2-AR selective agonist salbutamol (Yamanishi et al. 2003). Maximum relaxations to salbutamol were ~80% of isoprenaline, possibly reflecting partial agonism by salbutamol and/or relaxations by β_2-AR and another subtype (Yamanishi et al. 2003). Participation of β_2- and perhaps β_3-AR was confirmed by the β_2-AR antagonist ICI118551 and the mixed β-AR antagonist SR59230A, that both right-shifted concentration-response curves to isoprenaline with low affinity, whereas the β_1-AR antagonist CGP20712A failed to alter isoprenaline responses (Yamanishi et al. 2003). Relaxation of dog trigone by β-AR agonists was examined after precontraction with endothelin-1 and suggested that the β_3-AR was a major contributor to relaxation (Takeda et al. 2003). Relaxations were maximal with isoprenaline and submaximal with the partial β_3-AR agonist CL316243, whereas the β_1-AR selective dobutamine and the β_2-AR agonist

procaterol caused only minor relaxations at concentrations up to 1 μM (Takeda et al. 2003). In human trigone without precontraction, no responses to isoproterenol were observed (Benson et al. 1976).

4.4 Available Drugs and Possible Targets

The limited understanding of the trigone in urodynamic regulation and voiding has inhibited its development as a drug target. Picotamide, initially described as a thromboxane A_2 receptor antagonist, inhibited α_1-AR-mediated, neurogenic and non-adrenergic contractions in human superficial trigone and the prostate (Hennenberg et al. 2017a). Non-adrenergic contractions in the LUT are believed to account for limitations of α_1-AR antagonists (see Sect. 5), so that picotamide may be a promising drug for treatment of voiding symptoms, but proof-of-concept studies in vivo are not available.

5 Prostate

The prostate surrounds the urethra in primates and dogs and is arranged as paired lobes protruding into the abdomen in all other mammals. In primates, it is surrounded by a fibromuscular band, the prostate capsule. This encapsulation, prostate enlargement and increased prostate smooth muscle tone in BPH can cause urethral obstruction in primates, resulting in voiding symptoms due to impaired bladder emptying. The prostate comprises follicles lined by glandular epithelial cells for production of prostate secretions, which are embedded in the prostate stroma, where smooth muscle cells are the predominant cell type. Similar to humans, BPH can develop in dogs, but results in undirected growth into the abdomen, so that symptoms of BPH as observed in humans typically do not develop but they can be experimentally induced by surrounding the prostate with an artificial capsule. Rodents are often used for experimental induction of BPH but the lack of encapsulation and lobal arrangement does not recapitulate human BPH. Even though urethral obstruction may be mimicked by surgical urethral obstruction in rats causing symptoms similar to voiding symptoms, improvements by pharmacological intervention in these models mostly result from effects on the bladder rather than the prostate.

Voiding symptoms due to BPH include delayed initiation of micturition (hesitancy), intermittent and/or weak urinary flow, splitting or spraying of the urinary stream, and terminal dribbling (Chapple 2011). Post-micturition dribbling, and a feeling of incomplete bladder emptying are often/increasingly regarded as a single symptom complex, referred to as post-micturition symptoms, but commonly assigned to BPH-related LUTS (Chapple 2011). In the advanced stages, incomplete bladder emptying shows as post-void residual urine (PVR). Complications include recurrent infections and urinary retention, that require immediate intervention and represent indications for surgery (Oelke et al. 2013). Urethral obstruction in BPH

may be explained by prostate growth and increased prostate smooth muscle tone in the hyperplastic prostate (Lepor 2004). As contraction of prostate smooth muscle can be induced by α_1-AR, α_1-AR antagonists may improve symptoms and represent the first-line option for medical treatment of voiding symptoms (Oelke et al. 2013). In fact, α_1-AR in the prostate are well characterized, whereas α_2- and β-AR were less studied, and the function of β-AR is increasingly afflicted with new questions.

5.1 α_1-Adrenoceptors

5.1.1 Expression

Expression and function of α_1-AR in the prostate has been comprehensively reviewed (Michel and Vrydag 2006). Briefly, all three subtypes of α_1-AR mRNA are present in the human prostate with no difference in expression of subtypes between the periurethral, central, and peripheral zone, from anterior to posterior direction or different lobes. Similar findings have been reported in the monkey, but rodents are less consistent varying between predominantly α_{1A}-AR mRNA to about equal mRNA expression of all three subtypes.

Studies at the protein level, based on radioligand binding, autoradiography, or immunodetection reported mostly α_{1A}-AR and some α_{1B}-AR but failed to detect relevant amounts of α_{1D}-AR in human, monkey, or rat prostate (Michel and Vrydag 2006; Hennenberg et al. 2011a). Lower percentages of α_{1A}-AR were found in dogs and rabbits. α_1-AR were largely found in the stroma, and poor or absent in glands. In line with mRNA expression, no differences were found between different zones of the human prostate.

5.1.2 Function

Prostate Smooth Muscle Contraction

α_1-AR stimulation causes contraction of the prostate, mediated predominantly by α_{1A}-AR, while stimulation of β-AR may cause relaxation (Michel and Vrydag 2006). Later studies using the subtype-selective antagonists L-771,688 (SNAP 6383), SNAP 7915, B 8805-033 or silodosin confirmed this finding in the prostate of humans, dogs, and rats (Buono et al. 2014; Wang et al. 2020). In humans and rats, contractile responses induced by α_1-AR agonists are similar between different prostate regions (Michel and Vrydag 2006).

While antagonists caused the expected right-shifts of concentration-response curves for α_{1A}-AR agonists, some antagonists also reduced maximum contractions by 75–93% for tamsulosin (Wang et al. 2020; Hennenberg et al. 2017b), 76–100% for silodosin (Buono et al. 2014; Wang et al. 2020), <25% for alfuzosin (Oger et al. 2010), or 42–50% for doxazosin (Oger et al. 2009). Thus, the effects of competitive α_1-AR antagonists appear to have a non-competitive component in the prostate.

The physiological stimulus for prostate smooth muscle contraction is generally believed to be neuronally released noradrenaline acting on α_1-AR, although evidence is limited. Maximum inhibition of human prostate contraction induced by EFS amounted to 48, 50, or 76% with tamsulosin (Wang et al. 2020; Herlemann et al.

2018; Spek et al. 2021), 34–47% with silodosin (Buono et al. 2014; Wang et al. 2020), ~17% with doxazosin (Oger et al. 2009), and <10% with alfuzosin (Oger et al. 2010). EFS-induced contractions of human prostate are largely of neurogenic origin, with ~80%inhibition by tetrodotoxin (Spek et al. 2021; Li et al. 2020a).

It needs to be emphasized that α_1-AR are not the sole receptors responsible for smooth muscle contraction in the prostate, although this has been assumed (Oelke et al. 2013) (Fig. 1). Contraction of the human prostate is also induced by endothelin-1 and thromboxane A_2 receptor stimulation (Hennenberg 2022). Thus, maximum prostate smooth muscle tone can be induced even in the presence of α-AR antagonists, that may limit their efficacy in treatment of voiding symptoms (Hennenberg et al. 2013a, 2017b; Hennenberg 2022). Other non-adrenergic mediators may be important in animal models but have failed to induce relevant contractions in human prostate (Hennenberg 2022).

Regulation of Expression and Posttranslational Regulation

Regulation of prostate α_1-AR has been widely studied at the mRNA level (Michel and Vrydag 2006; Hennenberg et al. 2014). Age, the degree of BPH, and medical interventions may all affect expression of α_1-AR in the prostate. While expression of α_{1A}-AR was increased in BPH, castration decreased it although these findings are not always consistent with protein or functional findings that have repeatedly suggested unaltered receptor densities in BPH and unchanged or even decreased potencies of α_1-AR agonists in functional experiments. These discrepancies may point to a limited conclusiveness of mRNA data and point to possible posttranslational regulation.

The function of G protein-coupled receptors (GPCR) may be regulated by accessory interaction partners affecting receptor function (Hennenberg et al. 2014). Molecular and functional principles for posttranslational regulation are highly diverse and have been shown for a panel of receptors. Although posttranslational regulation of clinical relevance likely occurs at prostate α_1-AR, it has been addressed by few studies. The abbreviation GPCR has now been added at first mention of "G protein-coupled receptor" the abbreviation GRK had already been explained upon first mentioning (Hennenberg et al. 2014).

GRK2 and β-arrestin-2 are expressed in the stroma of the human prostate (Hennenberg et al. 2011a, b). Stimulation of human prostate with phenylephrine resulted in phosphorylation of β_2-AR (Hennenberg et al. 2011b). Assuming that phenylephrine preferentially binds to α_1-AR, this could be explained by GRK2-mediated phosphorylation of β_2-AR at positions that are decisive for desensitization (Hennenberg et al. 2011b). Simultaneous phosphorylation of α_1-AR is possible, but not examined due to lack of phospho-specific antibodies against α_{1A}-AR. Another study that examined the interactions between α_{1A}-AR and β-arrestin-2 in human prostate by coimmunoprecipitation suggested that a fraction of the prostatic α_{1A}-AR is bound to β-arrestin-2 (Hennenberg et al. 2011a) and unavailable to induce contractions.

β-Arrestin-bound receptors can subsequently be internalized following binding of clathrins (Hennenberg et al. 2014). Formation of a full receptor-clathrin complex

requires binding of clathrin heavy chains (HC) and two light chains (LCA, LCB) to receptors. Coimmunoprecipitation studies with human prostate suggested that some prostate α_{1A}-AR are bound to clathrin HC and LCB even under resting conditions, while stimulation with noradrenaline caused additional interaction with clathrin LCA (Hennenberg et al. 2013b). Thus, α_1-AR in the prostate may show different patterns of interaction with binding partners, including β-arrestin-2 and clathrins, that may critically determine α_1-adrenergic contractility.

Finally, α_{1A}-AR may interact with the cysteine-rich epidermal growth factor-like domain 1α (CRELD1α) that may account for the α_{1L} phenotype, characterized by a lower affinity for prazosin (White et al. 2019). The α_{1L}-AR is encoded by the α_{1A}-AR gene and is probably the α_{1A}-AR bound to CRELD1α (Hennenberg et al. 2014) since the α_{1L}-phenotype in cells transfected with α_{1A}-AR cDNA depends on expression of CRELD1α (Hennenberg et al. 2014). However, other explanations for the occurrence of the α_{1L}-phenotype have been proposed (White et al. 2019).

Intracellular Signaling

Agonist-induced contraction by G_q-linked GPCRs is mediated by three canonical pathways that are shared by all smooth muscle-rich tissues and many contraction-promoting receptors. These include (1) activation of phospholipase C (PLC) with subsequent formation of inositol 1,4,5-trisphosphate (IP_3) and IP_3-mediated increases in cytosolic Ca^{2+}, (2) PLC-mediated formation of diacylglycerol, followed by diacylglycerol-mediated activation of PKC, and (3) activation of the monomeric GTPase RhoA and RhoA-mediated activation of Rho kinase (Hennenberg et al. 2014). Each of these pathways contributes to contraction by increasing the phosphorylation state of myosin light chains, that is essentially required for smooth muscle contraction (Hennenberg et al. 2014). Increases in myosin light chain phosphorylation occur by Ca^{2+}/calmodulin-dependent activation of myosin light chain kinase, and by inhibition of myosin light chain phosphatase by PKC and Rho kinase (Hennenberg et al. 2014). Some evidence is available for an involvement of all three pathways in α_1-AR-mediated smooth muscle contraction. In guinea-pig prostates, activation of α_1-AR caused inositol phosphate formation, while stimulation with α_1-AR agonists elevated cytosolic Ca^{2+} concentrations in rat prostatic neuroendocrine cells, that was sensitive to the PLC inhibitor U 73,122 (Michel and Vrydag 2006). Contractions to the thromboxane A_2 analog U46619 were inhibited by the calmodulin inhibitor W7 in human prostate (Strittmatter et al. 2011). Inhibition of α_1-AR-mediated contractions by PKC and Rho kinase inhibitors has been reported from human and mouse isolated prostate tissues (Takahashi et al. 2007; White et al. 2013; Kitazawa 2013; Huang et al. 2022). These findings with PKC and Rho kinase inhibitors have to be carefully interpreted, as activation of RhoA, Rho kinase or PKC by α_1-AR agonists has yet to be shown. Similarly, inhibition of α_1-AR-mediated contractions in human prostate by a panel of inhibitors for different kinases and GTPases has been reported (Li et al. 2020b). While these findings may reflect a role of these targets in prostate smooth muscle contraction, their true relevance and relationships to α_1-AR remain uncertain, due to notorious off-target effects of kinase and GTPase inhibitors.

Proliferation

Involvement of α_1-AR in prostate growth and hyperplasia has been repeatedly suggested, but the physiological and clinical relevance is questionable and limited. α_1-AR agonists induce proliferation in cultured prostate stromal cells, and systemic administration caused prostate growth and hyperplasia in rodent models (Hennenberg et al. 2014). α-AR antagonists induce apoptosis and negative regulation of the cell cycle in cell culture models and in human prostate (Hennenberg et al. 2014). A panel of growth-promoting signaling pathways, including MAP kinases and transcription factors, was activated by α_1-AR in stromal cells, and in human prostate (Hennenberg et al. 2014). Later findings suggested that α-AR antagonist-induced apoptosis of prostate cells, particularly those induced by compounds with a quinazoline background is receptor-independent (Hennenberg et al. 2014; Kyprianou et al. 2009). However, reduction of prostate volume during long-term treatment with α_1-AR antagonists for voiding symptoms was never apparent in clinical trials, or during widespread routine use (Sakalis et al. 2021). Meanwhile, there is broad consensus that α-AR antagonists do not reduce prostate size (Oelke et al. 2013).

5.1.3 Physiological Functions In Vivo

The primary physiological function of the prostate is production of secretions and emission from glandular follicles by smooth muscle contractions in the stroma. However, the focus of clinical and experimental in vivo studies has been the involvement of α_1-AR on intraurethral pressure (IUP). Increased IUP in BPH may impair urinary flow and thus bladder emptying, resulting in bladder outlet obstruction and voiding symptoms (Lepor 2004). The in vivo regulation of IUP by α_1-AR has been shown in several species, where administration of α_1-AR agonists increased, and α-AR antagonists decreased IUP. However, IUP represents the sum of contributions from the prostate and the urethra, at least in primates, where the prostate is encapsulated and surrounds the urethra. It is believed that contributions of prostate smooth muscle tone to IUP are larger than contributions from the urethra (Michel and Vrydag 2006). Thus, even in rats, where the prostate is not encapsulated, it may account for 80% of the IUP, suggested by comparison of phenylephrine-induced increases of IUP in castrated and prostate-ablated male rats, female rats, and prostate intact-rats (Akiyama et al. 1999). Increases in IUP by systemic administration of α_1-AR agonists have been shown in rats, cats and in particular dogs, without separating responses from the prostate and the urethra (Michel and Vrydag 2006). Complementary, inhibition of neurogenic or agonist-induced IUP elevation by different α-AR antagonists including alfuzosin, doxazosin, tamsulosin, terazosin, and silodosin has been demonstrated in animal models (Michel and Vrydag 2006; Akiyama et al. 2001; Tatemichi et al. 2006), providing the basis for clinical studies and for the widespread treatment of LUTS suggestive of BPH by α_1-AR antagonists.

More conclusive are findings in primates, owing to their unique prostate anatomy. However, even though α-AR antagonists clearly improve voiding symptoms in BPH (as described below), presumably by relaxation of prostate smooth muscle, evidence

supporting decreases in IUP by α_1-AR antagonists in humans is lacking. Findings reporting decreases in bladder outlet resistance by non-selective α_1-AR antagonists, tamsulosin or the α_{1A}-selective RO700004 agree with findings from animal models (Michel and Vrydag 2006), but involvements of the trigone have not been excluded. In fact, bladder outlet resistance may depend on smooth muscle tone in the trigone as well as IUP, and the relationship between urethral obstruction, bladder outlet resistance, and voiding symptoms in BPH has been questioned (Michel and Vrydag 2006; Fusco et al. 2016).

5.2 α_2-Adrenoceptors

α_2-AR in the prostate have attracted little attention yet all three subtypes of α_2-AR mRNA are found in the human prostate (Michel and Vrydag 2006). Autoradiographic detection suggested a prevailing location in intraprostatic blood vessels, and to lesser extent in the glandular epithelium. In dogs and rats, autoradiographic signals and immunoreactivity for α_2-AR were strongest in the prostatic epithelium. Competition radioligand binding identified α_{2A}-AR as the predominant subtype.

In the human prostate, pre-junctional release of noradrenaline was inhibited by α_2-AR agonists and enhanced by α_2-AR antagonists (Michel and Vrydag 2006). The α_2-AR agonist clonidine inhibited EFS-induced neurogenic contractions of human prostate. Coincident with their lack of stromal localization, α_2-AR are not involved in adrenergic prostate smooth muscle contractions in humans, dogs, or horses (Michel and Vrydag 2006). In a single in vivo study performed with dogs, systemic administration of clonidine caused increases of IUP, or ~50% of the response observed for adrenaline, and responses to both agonists were abolished by the α_2-AR antagonist, yohimbine (Shapiro et al. 1987). Consequently, it was concluded that the effects seen in vivo are imparted by urethral and not prostatic α_2-AR (Michel and Vrydag 2006; Shapiro et al. 1987).

5.3 β-Adrenoceptors

5.3.1 Expression

All three subtypes of β-AR mRNA were detected in human prostate (Michel and Vrydag 2006; Suzuki et al. 2016). β_3-AR mRNA was increased in prostates from patients undergoing ablative surgery for BPH, compared to prostates from patients without diagnosed BPH, whereas expression levels of β_1- and β_2-AR mRNA were similar in both groups (Suzuki et al. 2016). Apart from detection of β_2-AR mRNA in rat prostate no findings have been reported from other species (Michel and Vrydag 2006). Complementary, immunohistochemical studies reported immunoreactivity with antibodies selective for β_1-, β_2-, and β_3-AR in the stroma of human prostate (Suzuki et al. 2016). Early radioligand binding suggested predominantly β_2-AR in prostates of humans, pigs, and rats (Michel and Vrydag 2006). However, ligand

concentrations probably excluded detection of β_3-AR in these studies, and later data are not available (Michel and Vrydag 2006).

5.3.2 Function

Relaxation of prostate smooth muscle by β-AR follows activation of adenylyl cyclase, as shown in human, rat, and guinea-pig prostate (Michel and Vrydag 2006). Inhibition of contractions to α_1-AR agonists or EFS or for receptor-independent contractions by β-AR agonists was observed in prostate from humans, dogs, rats, guinea-pigs, and horses (Michel and Vrydag 2006; Suzuki et al. 2016; Hennenberg et al. 2016). Involvement of β_2-AR in prostate smooth muscle relaxation has been shown for each species, whereas findings suggesting prostate smooth muscle relaxation by β_1-AR are limited to guinea pigs and rats (Michel and Vrydag 2006), and to one study with human prostate (Suzuki et al. 2016).

Inhibition of prostate smooth muscle contraction by β_3-AR agonists has also been reported but is inconclusive and was largely mediated by off-target effects. Interest in effects of β_3-AR agonists on prostate smooth muscle contraction followed approval of the β_3-AR agonist mirabegron for treatment of storage symptoms in OAB, that often occur with voiding symptoms in BPH. Findings are inconclusive since inhibition of agonist-induced or neurogenic contractions required mirabegron concentrations in excess of K_i values for β-AR (2.5, 383, and 977 nM for human β_3-, β_1-, and β_2-adrenoceptors) (Tasler et al. 2012). In prostate from patients with BPH, mirabegron (1 and 10 μM) reduced E_{max} values in concentration-response curves for phenylephrine, that was paralleled by increases in EC_{50} values for phenylephrine by mirabegron (10 μM), but not 1 μM (Calmasini et al. 2015). Right-shifts of concentration-response curves for phenylephrine by 10–100 μM mirabegron, with full recovery at high concentrations were reported from rat prostate (Alexandre et al. 2016). Binding of mirabegron to α_1-AR was confirmed by competition assays, pointing to K_i values of 0.437, 1.8, and 26 μM for α_{1A}-, α_{1D}-, and α_{1B}-AR (Alexandre et al. 2016). Finally, mirabegron right-shifted concentration-response curves for noradrenaline, phenylephrine, and methoxamine and inhibited EFS-induced contractions in human prostate at concentrations of 5 and 10 μM, that was resistant to the β_3-AR antagonist L-748,377 and not observed with 1 μM mirabegron (Huang et al. 2021). Similarly, inhibition of EFS-induced contractions in rabbit prostate was limited to 10 μM mirabegron, but largely lacking at 1 μM, while even the EC_{50} for mirabegron-induced relaxation of phenylephrine-precontracted rabbit prostate tissues was 977 nM (Calmasini et al. 2015). Since mirabegron did not affect contractions induced by endothelin-1 or by a thromboxane A_2 analog in human prostate (Huang et al. 2021), as expected following β-AR-mediated cAMP formation, a significant β_3-AR contribution to regulation of prostate smooth muscle tone appears unlikely, and findings obtained with mirabegron are probably imparted by antagonism of α_1-AR.

Findings with other β_3-AR agonists also suggest a minor role for β_3-AR in regulation of prostate smooth muscle tone. BRL37344 is a commonly used β_3-AR agonist, with K_i values of 420–430 nM for β_3-, 1.1–2.9 μM for β_2-, and 11–38 μM for β_1-ARs (Dallanoce et al. 2007; Hoffstedt et al. 1996; Yanagisawa et al. 2000),

and 649 nM or 1.6 μM for β_3 in other studies (Feve et al. 1991; Mehta et al. 2000), implying that some of its effects can be attributed to β_2-AR. Nonetheless, even 10 μM BRL37344 did not affect EFS-induced contractions of rat prostate (Kalodimos and Ventura 2001). In prostate from patients with BPH, contractions by a single concentration of phenylephrine (20 μM) were equally inhibited around 40% by 3 and 30 μM BRL37344 (Haynes 2007), which may involve direct effects on the β-AR and on α_1-AR. Latter findings may again be explained by antagonism of α_1-AR, as BRL37344 replaced prazosin with K_i values of 3.16 or 178 μM in rat cortical membranes (Brahmadevara et al. 2004; Leblais et al. 2004). TRK-380 is another, supposed β_3-AR agonist, inducing cAMP formation in CHO cells transfected with human β_3-AR with an EC_{50} of 174 nM, but not or weakly in β_1- and β_2-transfected cells, while precise K_i values are obviously missing (Kanie et al. 2012). In human prostate, 100 nM TRK-380 inhibited EFS-induced contractions of human prostate tissues only around 10% and relaxed KCl-precontracted tissues around 10–30%, while both effects increased to 47–50% by 10 μM in normal prostate tissues, and to 32% relaxation and 11% EFS inhibition in tissues from patients with BPH (Suzuki et al. 2016). Apart from prostate tissues, antagonism of α_1-AR has been suggested for different β_3-adrenergic agonists using arteries and other smooth muscle-rich tissues in organ bath experiments, and by binding assays (Michel 2020).

5.3.3 Physiological Functions In Vivo

Binding of radioligands and isoprenaline-induced relaxations were reduced in prostate from patients with BPH, compared to tissues from patients without BPH, suggesting reduced β-AR density and function in BPH (Michel and Vrydag 2006). In vivo studies in rats suggested increases of prostatic β-AR expression, density, and function with androgens (Michel and Vrydag 2006). In rats and rabbits, impaired β-AR function was seen in prostate from aged animals, associated with changes in G proteins, as receptor binding and expression were increased with age (Michel and Vrydag 2006). Three studies addressed prostatic AR in a rat model of type 1 diabetes, and consistently demonstrated reduced β-AR density and function, mostly attributed to a reduction of β_2-AR in prostate from diabetic rats (Erdogan et al. 2022b). However, none of these preclinical findings proceeded to meaningful clinical trials.

Inspired by the introduction of mirabegron for treatment of storage symptoms and encouraged by the first in vitro findings reporting its effects on prostate smooth muscle contraction, a possible application of mirabegron for treatment of voiding symptoms in BPH has been addressed (see Sect. 5.3.2). Although the two trials available suggested a lack of effects of mirabegron on voiding symptoms (Liao and Kuo 2018; Nitti et al. 2013a), add-on of mirabegron to α-AR antagonists may improve symptom scores in patients with mixed LUTS, by improvement of α-AR antagonist-resistant storage symptoms (Kwon et al. 2020). The use of mirabegron in BPH was rated as safe and has been recommended for treatment of male LUTS (Oelke et al. 2013), even though it caused small increases in post-void urine and may slightly increase the risk of acute urinary retention (Herschorn et al. 2017; Nitti et al. 2013b). The lack of effects on voiding symptoms is not surprising in the light of

in vitro findings, suggesting that anticontractile effects are mediated by off-target activity and require μM concentrations, much higher than maximum plasma levels in men that do not exceed 137 nM after standard dosing (Krauwinkel et al. 2012).

5.4 Urological Use of α_1-Adrenoceptor Antagonists

The primary use of α-AR antagonists is in the treatment of male LUTS suggestive of BPH is recommended in various guidelines. Although this is mainly used to obtain rapid symptomatic relief, α-AR antagonists can also inhibit progression of LUTS but have limited impact on the long-term prevention of complications such as acute urinary retention and prostate surgery (Oelke et al. 2013). Alfuzosin, doxazosin, terazosin, tamsulosin, and silodosin are routinely used α-AR antagonists with similar efficacy in equivalent doses (Oelke et al. 2013). Naftopidil and indoramin are α-AR antagonists that are available in some countries (Oelke et al. 2013). In controlled studies with placebo run-in, α-AR antagonists reduce the International Prostate Symptom Score (IPSS) by 30–50%, and increase the maximum urinary flow rate (Q_{max}) by 20–40% (Oelke et al. 2013). In open-label studies without a run-in period, improvements approached 50% reduction of IPSS with increases of Q_{max} up to 40% (Oelke et al. 2013; Djavan et al. 2004; Michel et al. 1998). Large-scaled seminal trials reported decreases in IPSS of −3.8, −6.6, or around −7 points, and increases in Q_{max} of 0.7, 2.5, or 3.53–3.77 ml/s with different α_1-AR antagonists (Chapple et al. 2011; McConnell et al. 2003; Roehrborn et al. 2010). However, α-AR antagonists did not reduce prostate size (Sakalis et al. 2021) or prevent acute urinary retention in studies of >2 years duration (McConnell et al. 2003; Roehrborn et al. 2008, 2010; Roehrborn 2006). The extent of symptom improvement is similar across age groups (Oelke et al. 2013; Michel et al. 1998), but appeared higher in patients with smaller prostates (prostate volumes ≤40 ml) in long-term studies, while the efficacy was independent of prostate size in studies with follow-up periods of <1 year (Oelke et al. 2013; McConnell et al. 2003; Roehrborn et al. 2008, 2010; Roehrborn 2006; Boyle et al. 2001).

Undoubtedly, α_1-AR antagonists improve the situation in many patients, although improvements are limited, and placebo effects are substantial. It is clear from these generalized and representative studies that on average full improvements never occur. Even placebos may reduce IPSS scores by 30% or more, or enhance Q_{max} by up to 15% (Hennenberg 2022). Decreases in IPSS were −7.0, −6.7, and −4.7 points with silodosin, tamsulosin, and placebo, while Q_{max} was improved by 3.77, 3.53, and 2.53 ml/s by silodosin, tamsulosin, and placebo, within a trial including 1,228 men (Chapple et al. 2011). Similarly, a meta-analysis of 25 randomized clinical trials pointed to decreases in IPSS of −4.4 points and increases in Q_{max} of 0.8 ml/s in the placebo group, that was highest in the studies where the highest effect of the treatment group was expected (Eredics et al. 2017). In 30–35% of patients, decreases in IPSS did not exceed 25%, so that up to 69% of patients are disappointed, contributing to discontinuation rates of around 65% within 12 months (Chapple et al. 2011). Finally, this low adherence results in hospitalization and a

high rate of surgery due to BPH (Chapple et al. 2011). It has been assumed, that this limited efficacy is attributable to smooth muscle contraction in the lower urinary tract by non-adrenergic mediators, that continue to cause contractions in the presence of α-AR antagonists, maintaining urethral obstruction and/or symptoms (Hennenberg 2022).

While symptom improvements are similar with all available α-AR antagonists, side effects differ, probably due to differences in subtype selectivity. The most common adverse effects are (orthostatic) hypotension, asthenia, and dizziness (Oelke et al. 2013). Hypotension is caused by inhibition of α_1-AR-mediated vasocontraction. In contrast to the prostate, where smooth muscle contraction is driven by α_{1A}-AR, the subtypes involved in α_1-AR-mediated vasocontraction differ between vessel types, vascular beds, and species (Schwinn and Roehrborn 2008). Thus, development of α-AR antagonists for the treatment of voiding symptoms was driven by the observation that minimization of α_{1B}-AR antagonism reduces overall blood pressure changes. Treatment evolved from compounds with little subtype selectivity (alfuzosin, doxazosin, terazosin), to $\alpha_{1A/D}$-selective antagonists (tamsulosin) to the highly α_{1A}-selective silodosin (Oelke et al. 2013; Schwinn and Roehrborn 2008; Lepor et al. 2012). Hypotension is most pronounced with doxazosin and terazosin, less common with tamsulosin (but also with alfuzosin), while hypotension with silodosin is similar to placebo (Oelke et al. 2013; Chapple et al. 2011; Nickel et al. 2008). In general, patients with cardiovascular comorbidity and/or cardiovascular co-medication are most prone to α-AR antagonist-induced hypotension (Oelke et al. 2013; Barendrecht et al. 2005).

Another side effect of α-AR antagonists is abnormal ejaculation, caused by a reduction expulsion of seminal fluid during orgasm. There is no adverse effect on libido and a small improvement of erectile function (Oelke et al. 2013; van Dijk et al. 2006). These effects are highest with silodosin, followed by tamsulosin, and lowest with doxazosin and terazosin (Oelke et al. 2013; Gacci et al. 2014). Finally, intra-operative floppy iris syndrome (IFIS) was reported as an adverse event, that may occur with all available α_1-AR antagonists and affect cataract surgery (Oelke et al. 2013). Consequently, guidelines recommend informing the ophthalmologist about treatment with α-AR antagonists and stopping treatment prior to cataract surgery.

5.4.1 Possible Targets

Stimulated by the approval of mirabegron for treatment of storage symptoms, together with a presumed function of β_3-AR in smooth muscle relaxation, the use of mirabegron for treatment of voiding symptoms has been suggested. However, effects of β_3-AR agonists on prostate smooth muscle tone appear to be limited at best, if applied in vitro at concentrations in the range of known plasma levels. Even though antagonism of α_1-AR was observed in vitro and looks promising, this requires concentrations exceeding known plasma levels that are unlikely to occur in vivo (see Sects. 5.3.2 and 5.3.3).

A number of studies reported inhibitory effects of small molecule inhibitors on adrenergic prostate smooth muscle contractions, targeting different kinases and monomeric GTPases (Hennenberg 2022). Several of these compounds inhibited

non-adrenergic contractions or the growth of prostate cells (Hennenberg 2022). However, the impact on urodynamic regulation in vivo has yet to be demonstrated, and the translational value needs to be questioned, in the light of well-known side effects of kinase inhibitors and a lack of proof of tolerability in vivo. Findings suggesting inhibition of both adrenergic and non-adrenergic contractions by picotamide and thalidomide and its derivates may be considered more promising, as these compounds are clinically available for other applications (Hennenberg et al. 2017a; Herlemann et al. 2018; Tamalunas et al. 2021a, b). However, new compounds specifically addressing AR in the prostate, such as receptor ligands are unlikely to emerge as new drug candidates. Even silodosin, the latest approved α-AR antagonist with high α_{1A}-AR selectivity, did not substantially change the options of medical treatment in BPH. Novel drugs may be successful should they simultaneously address adrenergic and non-adrenergic contractions in the LUT and control prostate growth to avoid combination therapy.

6 Urethra

The urethra contains striated muscle ("external urethral sphincter") in addition to smooth muscle ("internal urethral sphincter") and contributes to bladder outlet resistance in females and maintenance of bladder continence during the storage/filling phase (Michel and Vrydag 2006). In men, its contribution to bladder outlet resistance and continence is limited and surpassed by regulation of bladder outlet resistance by the prostate (Michel 2011). Apart from AR in the urethral smooth muscle sections reviewed below, striated urethral muscle also contains AR that may be regulated by central AR by modulation of the activity of somatic pelvic nerves (Michel et al. 2005).

6.1 α_1-Adrenoceptors

6.1.1 Expression

The α_{1A}-AR accounts for at least 90% of all α_1-AR in the human urethra (Michel and Vrydag 2006). While mostly localized to smooth muscle in humans, additional α_{1A}-AR mRNA is present in the striated muscle of rhesus monkey urethra (Michel and Vrydag 2006). The α_{1A}-AR is also prominent in rats, but less so than in primates. Radioligand binding studies also suggest that the α_{1A}-AR protein is the predominant subtype in humans, dogs, rats, and rabbits (Michel and Vrydag 2006). In rabbits, proximal and distal portions of the urethra showed similar densities of α_1-AR, and the levels in the urethra were similar to the trigone, and higher than the detrusor.

6.1.2 Function

Phenylephrine and noradrenaline caused contractions of female and male urethra, with high efficacy and potency (Nishimatsu et al. 1999; Kedia et al. 2013). NS-49, a potent non-selective α_1-AR partial agonist-induced contractions of human urethra

suggesting a role of α_1-AR (Nishimatsu et al. 1999). Earlier findings do not allow conclusions regarding subtypes involved (Michel and Vrydag 2006). Most non-human data are from rabbit, where subtype composition and α_1-adrenergic function are similar in proximal and distal portions of the urethra, and α_{1A} is the predominant subtype (Michel and Vrydag 2006). Limited studies are available of urethral contractions by α_1-AR agonists in dogs and rats.

6.1.3 Physiological Functions In Vivo

In animal models, systemic administration of α_1-AR agonists increases IUP, while α-AR antagonists reduce IUP (Michel and Vrydag 2006). IUP studies in rats with and without prostates (due to surgical removal, castration, or by comparison of female vs. male rats) revealed that ~20% of responses are attributable to urethral muscle contraction (Michel and Vrydag 2006). The remaining component was explained by prostate smooth muscle contraction, an effect that may compress the urethra to an even greater extent in male primates than in rats, owing to the differences in anatomy. Possible contributions of the trigone to IUP responses to α_1-AR ligands are uncertain and have not been specifically addressed. Since the prostate makes the major contribution to IUP, effects of α-AR antagonists on IUP are summarized above.

Data supporting a participation of α_{1A}-AR in neuronal urethral contractions are available from a urodynamic study in healthy women, where urethral contractions were induced by single pulse stimulation of sacral roots and assessed as increases in urethral pressure at different sites before and 6 h after intake of tamsulosin (0.4 mg) (Reitz et al. 2004). Contractions were reduced by tamsulosin, in the proximal, middle, and distal third of the urethra, although not by more than one third (Reitz et al. 2004). Similar findings from in vivo studies in cats showed nerve-induced urethral contraction was α_1-AR-mediated (Michel and Vrydag 2006). Studies examined effects of α-AR antagonists on female human LUTS are less conclusive regarding the contribution of urethral muscle. Thus, α-AR antagonists may improve voiding dysfunction (resulting from bladder underactivity, urethral obstruction, or other etiologies) in female patients, while improvements in storage (due to OAB or in neurogenic bladder) appear limited or lacking (Boyd and Hilas 2014; Costantini et al. 2009; Meyer and Brown 2012; Robinson et al. 2007; Zhang et al. 2017). Again, however, separate contributions of the urethra and the trigone to improvements of voiding symptoms by α-AR antagonists cannot be distinguished on the basis of these findings. Finally, $\alpha_{1A/D}$-AR are involved via urethral contraction in the micturition reflex, which may contribute to effects of α-AR antagonists in female and male urethral resistance (Michel and Vrydag 2006; Yanase et al. 2008).

6.2 α_2-Adrenoceptors

6.2.1 Expression

Urethral α_2-AR have been detected by radioligand binding in rabbits, whereas mRNA expression is not available for any species (Michel and Vrydag 2006). Rabbit

urethral α_2-AR are of the α_{2A} subtype (Michel and Vrydag 2006), density does not differ with gender or age, and levels decrease from the distal to the proximal parts of the urethra (Michel and Vrydag 2006).

6.2.2 Function

α_2-AR activation induces urethral contraction (c.f. bladder and prostate). At least in rabbits and horses, α_2-AR-mediated urethral contractions are of similar magnitude as those to α_1-AR agonists (Michel and Vrydag 2006). In line with the expression gradient within the rabbit urethra, α_2-AR-mediated contractions are stronger in the distal than the proximal urethra (Michel and Vrydag 2006). Data for human urethra are available from one study examining contractile responses of urethra from the penile section of individuals undergoing surgery for gender reassignment (Kedia et al. 2013). Noradrenaline-induced contractions were substantially inhibited by the α_2-AR antagonist delquamine, suggesting a pro-contractile function of α_2-AR in the human urethra (Kedia et al. 2013). An earlier study failed to observe α_2-AR-mediated contractions of the human urethra (Michel and Vrydag 2006).

6.2.3 Physiological Functions In Vivo

In vivo findings are limited to animal models. The effects of the α_2-AR agonist dexmedetomidine and the α_2-AR antagonists atipamezole and MK-467 on voiding and contractions of the external sphincter were examined in rats. Dexmedetomidine decreased urinary flow rate (Streng et al. 2010), even resulting in overflow incontinence, that was paralleled by reduced external sphincter contractions but probably mediated by central actions (Aro et al. 2015). Atipamezole increased urinary flow rates and external sphincter contractions, again dependent on central effects (Streng et al. 2010; Aro et al. 2015). Another in vivo study in rats confirmed that reflex contractions of both the striated and smooth urethral muscle are decreased by α_2-AR stimulation (Furuta et al. 2015). In dogs, intravenous application of clonidine increased the IUP via α_2-AR (Michel and Vrydag 2006). However, urethral α_2-AR are not considered a potential target for treatment of LUTS.

6.3 β-Adrenoceptors

6.3.1 Expression

No reports of β-AR mRNA in the urethra are currently available. Four studies reported β_3-AR protein detection, all in non-human tissues, and that allowed only limited conclusions (Michel and Vrydag 2006). In rabbit urethra, and possibly female pig urethra, β_2-AR contribute substantially or even exclusively, while β_1-AR are minimal (Michel and Vrydag 2006). The specificity of ligands utilized limited the possibility of estimation of β_3-AR (Michel and Vrydag 2006). Immunoreactivity using a polyclonal rabbit antibody against human β_3-AR has been reported in female human urethra, in epithelial layers, and in striated muscle (Kummeling et al. 2020). Notably, radioligand binding to β-AR was lower in the urethra compared to the bladder, that was examined in rabbits (Michel and Vrydag 2006).

6.3.2 Function

The lower density of β-AR in the urethra than in the bladder, translated to maximum relaxations of urethra from rabbits and dogs to β-AR agonists being half that observed in detrusor or trigone in the same study (Michel and Vrydag 2006). In dog urethra, relaxations were larger in proximal than distal sections (Michel and Vrydag 2006). Rank orders for potencies of subtype-selective ligands differ between species but all point to a lack of relevance of β_1-AR (Michel and Vrydag 2006). Available findings suggest rank orders of $\beta_2 \geq \beta_3 > \beta_1$ in rat, $\beta_2 > \beta_3 = \beta_1$ in dog, and $\beta_3 >/\geq \beta_2 >> \beta_1$-AR in pig urethra (Michel and Vrydag 2006). In equine urethra, an involvement of β_2-AR has been reported (Michel and Vrydag 2006). Neuronal relaxation is mostly mediated by nitric oxide, while contributions from β-AR components appear to be minor (Michel and Vrydag 2006). In contrast to urethral smooth muscle, striated urethral muscle has been reported to contract in response to β-adrenoceptor stimulation, possibly via β_2-AR, an effect that may contribute to increases of urethral resistance following adrenergic stimulation in vivo (Michel and Vrydag 2006). Effects of β-AR agonists, including mirabegron on mouse urethra have been reported. Isoprenaline caused biphasic relaxations that were changed to monophasic, concentration-dependent relaxations by the β_3-AR antagonist L-748,337 (Alexandre et al. 2016). Mirabegron caused concentration-dependent relaxations of phenylephrine-induced, but not KCl-, vasopressin-, or endothelin-1-induced contractions, again somewhat biphasic and with corresponding pEC_{50} values of 7.14 and 5.4, that were right-shifted by L-748,337 (Alexandre et al. 2016). Mirabegron (1–30 μM) also right-shifted concentration-response curves for phenylephrine-induced contractions, but not at 0.1 μM (Alexandre et al. 2016). Together, this points to β_3-AR-mediated relaxations, but also to antagonism of α_1-AR by high concentrations of mirabegron, in line with findings in other tissues.

6.3.3 Physiological Functions In Vivo

Few studies have addressed effects of β-AR agonists on urethral resistance in vivo. Again, the conclusions that can be drawn are limited by possible contributions of LUT tissues other than the urethra. Systemic administration of the β_2-AR agonist terbutaline reduced the resting urethral pressure in healthy women, while the antagonist propranolol had no effect (Michel and Vrydag 2006). In rats, the β_2-AR agonist procaterol decreased urethral pressure, that was sensitive to the β_2-AR antagonist ICI 118,551 but not observed with the β_3-AR agonist CL 316,243 (Michel and Vrydag 2006). In cats, urethral relaxation following hypogastric nerve stimulation was propranolol-sensitive, and further enhanced by noradrenaline uptake inhibitors (Michel and Vrydag 2006).

6.4 Drugs and Targets

Since the urethra has a role determining bladder outlet resistance, and urethral muscle displays α_1-AR-mediated contractions, α_1-AR have been considered as a

potential target for drug treatment of stress incontinence. Consequently, α_{1A}-AR selective agonists were developed for this indication, including NS-49, A-61603, and Ro 115-1240 (Michel and Vrydag 2006). In a proof-of-principle trial, the latter improved stress incontinence in female patients, but its development was nevertheless discontinued (Musselman et al. 2004).

Clinical application for treatment of LUTS, based on α_2- or β-AR-mediated regulation of urethral muscle tone, has not been considered. The overall response of intraurethral pressure to endogenous catecholamines is clearly an increase, so that β-AR-mediated relaxations of urethral muscle most probably lack relevance under physiological conditions. Considering that effects of β_3-AR agonists on the urethra are less than on the bladder, it appears unlikely targeting β-AR in the urethra will produce novel drugs for treatment of LUTS. However, since β-AR agonists have little effect on urethral tone, adverse events to β_3-AR agonists used to treat OAB are unlikely.

7 Outlook and Conclusions

AR plays a key role in the physiological regulation of LUT function, and AR ligands have become standard medications for the treatment of LUTD, in particular α_1-AR antagonists for male LUTS attributed to BPH and β_3-AR agonists for OAB. While α_1-AR agonists have been considered for the treatment of stress urinary incontinence, no representative of this drug class has been developed beyond phase II proof-of-concept studies. Despite the widespread clinical use of α_1-AR antagonists and β_3-AR agonists in the treatment of LUTD, major gaps in our knowledge remain regarding the specific cell types targeted by these drugs and the roles they play in the therapeutic benefits. Moreover, it remains unclear which of the various signaling pathways activated by these receptors mediate the clinical effects. While α_1-AR antagonists and β_3-AR agonists bring about important symptom improvement to many patients with LUTD, LUTS are not sufficiently addressed in a significant fraction of patients, possibly because endogenous transmitters and non-neuronal mediators other than catecholamines also play a role. A better knowledge of the signal transduction pathways mediating the clinically beneficial effects may allow development of novel drugs targeting these pathways and, by interfering with the signal transduction pathways utilized by non-adrenergic receptors, may have greater efficacy. However, even with greater efficacy this must be accompanied by tolerability.

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References

Akiyama K et al (1999) KMD-3213, a uroselective and long-acting alpha(1a)-adrenoceptor antagonist, tested in a novel rat model. J Pharmacol Exp Ther 291(1):81–91

Akiyama K et al (2001) Effect of KMD-3213, an alpha1A-adrenoceptor antagonist, on the prostatic urethral pressure and blood pressure in male decerebrate dogs. Int J Urol 8(4):177–183

Alexandre EC et al (2016) Mirabegron relaxes urethral smooth muscle by a dual mechanism involving beta3 -adrenoceptor activation and alpha1 -adrenoceptor blockade. Br J Pharmacol 173(3):415–428

Andersson K-E, Michel MC (2011) Urinary tract. In: Hofmann FB (ed) Handbook of experimental pharmacology, vol 202. Springer Verlag, Heidelberg, p 577

Aro E et al (2015) Is there a peripheral site of action contributing to the voiding effects of alpha(2)-adrenoceptor agonists and antagonists? World J Urol 33(3):433–440

Azuma H et al (1989) Alpha 1-adrenoceptor antagonist activity of novel pyrimidine derivatives (SHI437 and IK29) in rabbit aorta and trigone of the bladder. Br J Pharmacol 96(4):1000–1006

Barendrecht MM et al (2005) Treatment of lower urinary tract symptoms suggestive of benign prostatic hyperplasia: the cardiovascular system. BJU Int 95(Suppl 4):19–28

Barendrecht MM et al (2008) Do alpha1-adrenoceptor antagonists improve lower urinary tract symptoms by reducing bladder outlet resistance? Neurourol Urodyn 27(3):226–230

Barendrecht MM et al (2009) The effect of bladder outlet obstruction on α_1- and ß-adrenoceptor expression and function. Neurourol Urodyn 28(4):349–355

Benson GS et al (1976) Adrenergic and cholinergic stimulation and blockade of the human bladder base. J Urol 116(2):174–175

Boyd K, Hilas O (2014) alpha-adrenergic blockers for the treatment of lower-urinary-tract symptoms and dysfunction in women. Ann Pharmacother 48(6):711–722

Boyle P et al (2001) Meta-analysis of randomized trials of terazosin in the treatment of benign prostatic hyperplasia. Urology 58(5):717–722

Brahmadevara N, Shaw AM, MacDonald A (2004) ALpha1-adrenoceptor antagonist properties of CGP 12177A and other beta-adrenoceptor ligands: evidence against beta(3)- or atypical beta-adrenoceptors in rat aorta. Br J Pharmacol 142(4):781–787

Buono R et al (2014) Silodosin and tadalafil have synergistic inhibitory effects on nerve-mediated contractions of human and rat isolated prostates. Eur J Pharmacol 744:42–51

Calmasini FB et al (2015) The beta-3 adrenoceptor agonist, mirabegron relaxes isolated prostate from human and rabbit: new therapeutic indication? Prostate 75(4):440–447

Campschroer T et al (2018) α-blockers as medical expulsive therapy for ureteric stones: a Cochrane systematic review. BJU Int 122(6):932–945

Canda AE et al (2007) Physiology and pharmacology of the human ureter: basis for current and future treatments. Urol Int 78:289–298

Cernecka H, Sand C, Michel MC (2014) The odd sibling: features of $ß_3$-adrenoceptor pharmacology. Mol Pharmacol 86(5):479–484

Chapple C (2011) Overview on the lower urinary tract. Handb Exp Pharmacol 202:1–14

Chapple CR et al (2011) Silodosin therapy for lower urinary tract symptoms in men with suspected benign prostatic hyperplasia: results of an international, randomized, double-blind, placebo- and active-controlled clinical trial performed in Europe. Eur Urol 59(3):342–352

Chapple CR et al (2014) Mirabegron in overactive bladder: a review of efficacy, safety, and tolerability. Neurourol Urodyn 33(1):17–30

Costantini E et al (2009) Open-label, longitudinal study of tamsulosin for functional bladder outlet obstruction in women. Urol Int 83(3):311–315

Cui Y et al (2021) Trigonal-sparing vs. trigonal-involved onabotulinumtoxinA injection for the treatment of overactive bladder: a systematic review and meta-analysis. Front Neurol 12:651635

Dale PR et al (2014) The pharmacological rationale for combining muscarinic receptor antagonists and ß-adrenoceptor agonists in the treatment of airway and bladder disease. Curr Opin Pharmacol 16C:31–42

Dallanoce C et al (2007) Novel chiral isoxazole derivatives: synthesis and pharmacological characterization at human beta-adrenergic receptor subtypes. Bioorg Med Chem 15(7): 2533–2543

Danuser H et al (2001) Systemic and topical drug administration in the pig ureter: effect of phosphodiesterase inhibitors α1, ß and ß2-adrenergic receptor agonists and antagonists on the frequency and amplitude of ureteral contractions. J Urol 166(2):714–720

Deplanne V, Galzin AM (1996) Functional characterization of alpha-1-adrenoceptor subtypes in the prostatic urethra and trigone of male rabbit. J Pharmacol Exp Ther 278(2):527–534

Djavan B et al (2004) Longitudinal study of men with mild symptoms of bladder outlet obstruction treated with watchful waiting for four years. Urology 64(6):1144–1148

Erdogan BR et al (2022a) Validation of fenoterol to study β_2-adrenoceptor function in the rat urinary bladder. Pharmacology 107(1–2):116–121

Erdogan BR et al (2022b) Established and emerging treatments for diabetes-associated lower urinary tract dysfunction. Naunyn Schmiedeberg's Arch Pharmacol 395(8):887–906

Eredics K, Madersbacher S, Schauer I (2017) A relevant midterm (12 months) placebo effect on lower urinary tract symptoms and maximum flow rate in male lower urinary tract symptom and benign prostatic hyperplasia – a meta-analysis. Urology 106:160–166

Feve B et al (1991) Atypical beta-adrenergic receptor in 3T3-F442A adipocytes. Pharmacological and molecular relationship with the human beta 3-adrenergic receptor. J Biol Chem 266(30): 20329–20336

Fry CH et al (2010a) Animal models and their use in understanding lower urinary tract dysfunction. Neurourol Urodyn 29(4):603–608

Fry CH, Meng E, Young JS (2010b) The physiological function of lower urinary tract smooth muscle. Auton Neurosci 154(1–2):3–13

Furuta A et al (2015) Noradrenergic mechanisms controlling urethral smooth and striated muscle function in urethral continence reflex in rats. Low Urin Tract Symptoms 7(3):155–161

Fusco F et al (2016) a_1-blockers improve benign prostatic obstruction in men with lower urinary tract symptoms: a systematic review and meta-analysis of urodynamic studies. Eur Urol 69(6): 1091–1101

Gacci M et al (2014) Impact of medical treatments for male lower urinary tract symptoms due to benign prostatic hyperplasia on ejaculatory function: a systematic review and meta-analysis. J Sex Med 11(6):1554–1566

Goepel M et al (1997) Comparison of adrenoceptor subtype expression in porcine and human bladder and prostate. Urol Res 25:199–206

Haynes JM (2007) beta(2) and beta(3)-adrenoceptor inhibition of alpha(1)-adrenoceptor-stimulated Ca(2+) elevation in human cultured prostatic stromal cells. Eur J Pharmacol 570(1–3):18–26

Hennenberg M (2022) Pharmacology of the prostate in non-infectious diseases. In: Kenakin T (ed) Comprehensive pharmacology, 1st edn. Elsevier, pp 708–744

Hennenberg M et al (2011a) beta-arrestin-2 is expressed in human prostate smooth muscle and a binding partner of alpha1A-adrenoceptors. World J Urol 29(2):157–163

Hennenberg M et al (2011b) alpha1-adrenoceptor activation induces phosphorylation of beta2-adrenoceptors in human prostate tissue. BJU Int 108(6):922–928

Hennenberg M et al (2013a) The receptor antagonist picotamide inhibits adrenergic and thromboxane-induced contraction of hyperplastic human prostate smooth muscle. Am J Physiol Renal Physiol 305(10):F1383–F1390

Hennenberg M et al (2013b) Noradrenaline induces binding of clathrin light chain A to alpha1-adrenoceptors in the human prostate. Prostate 73(7):715–723

Hennenberg M, Stief CG, Gratzke C (2014) Prostatic alpha1-adrenoceptors: new concepts of function, regulation, and intracellular signaling. Neurourol Urodyn 33(7):1074–1085

Hennenberg M et al (2016) Inhibition of adrenergic and non-adrenergic smooth muscle contraction in the human prostate by the phosphodiesterase 10-selective inhibitor TC-E 5005. Prostate 76(15):1364–1374

Hennenberg M et al (2017a) Inhibition of agonist-induced smooth muscle contraction by picotamide in the male human lower urinary tract outflow region. Eur J Pharmacol 803:39–47
Hennenberg M et al (2017b) Non-adrenergic, tamsulosin-insensitive smooth muscle contraction is sufficient to replace alpha1 -adrenergic tension in the human prostate. Prostate 77(7):697–707
Herlemann A et al (2018) Inhibition of smooth muscle contraction and ARF6 activity by the inhibitor for cytohesin GEFs, secinH3, in the human prostate. Am J Physiol Renal Physiol 314(1):F47–F57
Herschorn S et al (2017) Efficacy and safety of combinations of mirabegron and solifenacin compared with monotherapy and placebo in patients with overactive bladder (SYNERGY study). BJU Int 120(4):562–575
Hoffstedt J et al (1996) Effects of several putative beta 3-adrenoceptor agonists on lipolysis in human omental adipocytes. Int J Obes Relat Metab Disord 20(5):428–434
Honda K, Nakagawa C (1986) Alpha-1 adrenoceptor antagonist effects of the optical isomers of YM-12617 in rabbit lower urinary tract and prostate. J Pharmacol Exp Ther 239(2):512–516
Honda K, Miyata-Osawa A, Takenaka T (1985) alpha 1-adrenoceptor subtype mediating contraction of the smooth muscle in the lower urinary tract and prostate of rabbits. Naunyn Schmiedeberg's Arch Pharmacol 330(1):16–21
Huang R et al (2021) Concentration-dependent alpha1-adrenoceptor antagonism and inhibition of neurogenic smooth muscle contraction by mirabegron in the human prostate. Front Pharmacol 12:666047
Huang R et al (2022) Inhibition of human prostate smooth muscle contraction by the inhibitors of protein kinase C, GF109203X, and Go6983. Prostate 82(1):59–77
Igawa Y, Aizawa N, Michel MC (2019) β_3-adrenoceptors in the normal and diseased urinary bladder – what are the open questions? Br J Pharmacol 176(14):2525–2538
Kalodimos PJ, Ventura S (2001) Beta2-adrenoceptor-mediated inhibition of field stimulation induced contractile responses of the smooth muscle of the rat prostate gland. Eur J Pharmacol 431(1):81–89
Kanie S et al (2012) Pharmacological effect of TRK-380, a novel selective human beta3-adrenoceptor agonist, on mammalian detrusor strips. Urology 79(3):744 e1–7
Kedia GT et al (2013) Pharmacologic characterization of human male urethral smooth muscle: an in vitro approach. Urology 82(6):1451 e13–9
Kitazawa T (2013) Contractile signaling pathways in mouse prostate smooth muscle. Prostate 73(9):996–1006
Kobayashi S et al (2009) Gene expression and mechanical functions of α_1-adrenoceptor subtypes in mouse ureter. World J Urol 27:775–780
Krauwinkel W et al (2012) Pharmacokinetic properties of mirabegron, a beta3-adrenoceptor agonist: results from two phase I, randomized, multiple-dose studies in healthy young and elderly men and women. Clin Ther 34(10):2144–2160
Kummeling MT et al (2020) Initial report on distribution of beta3-adrenoceptor in the human female urethra. Neurourol Urodyn 39(1):125–132
Kurizaki Y et al (2013) Relationship between expression of ß$_3$-adrenoceptor mRNA in bladder mucosa and urodynamic findings in men with lower urinary tract symptoms. Neurourol Urodyn 32(1):88–91
Kwon SY et al (2020) Efficacy of adding mirabegron to alpha-adrenoreceptor blocker in patients with benign prostatic hyperplasia with persistent overactive bladder symptoms: a prospective study. Investig Clin Urol 61(4):419–424
Kyprianou N, Vaughan TB, Michel MC (2009) Apoptosis induction by doxazosin and other quinazoline α_1-adrenoceptor antagonists: a new mechanism for cancer treatment? Naunyn Schmiedeberg's Arch Pharmacol 380(6):473–477
Labadia A et al (1987) Alpha- and beta-adrenergic receptors in the horse ureter. Rev Esp Fisiol 43(4):421–425

Leblais V et al (2004) Role of alpha-adrenergic receptors in the effect of the beta-adrenergic receptor ligands, CGP 12177, bupranolol, and SR 59230A, on the contraction of rat intrapulmonary artery. J Pharmacol Exp Ther 309(1):137–145
Lefevre-Borg F et al (1993) Alfuzosin, a selective alpha 1-adrenoceptor antagonist in the lower urinary tract. Br J Pharmacol 109(4):1282–1289
Lepor H (2004) Pathophysiology, epidemiology, and natural history of benign prostatic hyperplasia. Rev Urol 6(Suppl 9):S3–S10
Lepor H, Kazzazi A, Djavan B (2012) alpha-blockers for benign prostatic hyperplasia: the new era. Curr Opin Urol 22(1):7–15
Li B et al (2020a) Inhibition of neurogenic and thromboxane A2 -induced human prostate smooth muscle contraction by the integrin alpha2beta1 inhibitor BTT-3033 and the integrin-linked kinase inhibitor Cpd22. Prostate 80(11):831–849
Li B et al (2020b) Regulation of smooth muscle contraction by monomeric non-RhoA GTPases. Br J Pharmacol 177(17):3865–3877
Liao CH, Kuo HC (2018) Mirabegron 25 mg monotherapy is safe but less effective in male patients with overactive bladder and bladder outlet obstruction. Urology 117:115–119
Lightner DJ et al (2019) Diagnosis and treatment of overactive bladder (non-neurogenic) in adults: AUA/SUFU Guideline Amendment 2019. J Urol 202(3):558–563
Lim I, Chess-Williams R (2022) Mirabegron attenuates porcine ureteral contractility via α1-adrenoceptor antagonism. Naunyn Schmiedeberg's Arch Pharmacol 395(7):839–847
Markiewicz W et al (2014) The influence of doxazosin, an alpha1-adrenergic receptor antagonist on the urinary bladder contractility in pigs. Pol J Vet Sci 17(3):527–529
Markiewicz W et al (2017) The influence of doxazosin on the contractility of the urinary bladder in female pigs with experimentally induced cystitis. Pol J Vet Sci 20(3):485–490
Mastrangelo D, Iselin CE (2007) Urothelium dependent inhibition of rat ureter contractile activity. J Urol 178(2):702–709
Matsumoto R et al (2013) Expression and functional role of ß$_3$-adrenoceptors in the human ureter. Int J Urol 20(10):1007–1014
McConnell JD et al (2003) The long-term effect of doxazosin, finasteride, and combination therapy on the clinical progression of benign prostatic hyperplasia. N Engl J Med 349(25):2387–2398
McLeod DG, Reynolds DG, Swan KG (1973) Adrenergic mechanisms in the canine ureter. Am J Phys 224(5):1054–1058
Mehta RC et al (2000) Biochemical and functional characterization of 1-benzyl substituted trimetoquinol affinity analogs on rat and human beta-adrenoceptors. Biochem Pharmacol 59(5):517–529
Meyer LE, Brown JN (2012) Tamsulosin for voiding dysfunction in women. Int Urol Nephrol 44(6):1649–1656
Michel MC (2011) beta-adrenergic receptor subtypes in the urinary tract. Handb Exp Pharmacol 202:307–318
Michel MC (2014) Do ß-adrenoceptor agonists induce homologous or heterologous desensitization in rat urinary bladder? Naunyn Schmiedeberg's Arch Pharmacol 387(3):215–224
Michel MC (2020) α_1-adrenoceptor activity of β-adrenoceptor ligands – an expected drug property with limited clinical relevance. Eur J Pharmacol 889:173632
Michel MC (2023) Are β3-adrenoceptor gene polymorphisms relevant for urology? Neurourol Urodyn 42(1):33–39
Michel MC, de la Rosette JJMCH (2004) Efficacy and safety of tamsulosin in the treatment of urological diseases. Expert Opin Pharmacother 5(1):151–160
Michel MC, de la Rosette JJMCH (2006) α-Blocker treatment of urolithiasis. Eur Urol 50(2): 213–214
Michel MC, Gravas S (2016) Safety and tolerability of ß$_3$-adrenoceptor agonists in the treatment of overactive bladder syndrome – insight from transcriptosome and experimental studies. Expert Opin Drug Saf 15(5):647–657

Michel MC, Staskin D (2022) Study designs for evaluation of combination treatment: focus on individual patient benefit. Biomedicine 10(2):270
Michel MC, Vrydag W (2006) α_1-, α_2- and ß-adrenoceptors in the urinary bladder, urethra and prostate. Br J Pharmacol 147(Suppl. 2):S88–S119
Michel MC et al (1998) Comparison of tamsulosin efficacy in subgroups of patients with lower urinary tract symptoms. Prostate Cancer Prostatic Dis 1(6):332–335
Michel MC, Oelke M, Peters SLM (2005) The neuro-urological connection. Eur Urol Suppl 4(1): 18–28
Michel MC et al (2023) Current and emerging pharmacological targets and treatments of urinary incontinence and related disorders. Pharmacol Rev. In press
Miyatake R et al (2001) Effects of isoproterenol and butylscopolamine on the friction between an artificial stone and the intraureteral wall in anaesthetized rabbits. J Urol 166(3):1083–1087
Mo W et al (2017) The β_3-adrenoceptor agonist mirabegron increases human atrial force through β_1-adrenoceptors: an indirect mechanism? Br J Pharmacol 174(16):2706–2715
Monneron MC et al (2000) In vitro alpha-adrenoceptor autoradiography of the urethra and urinary bladder of the female pig, cat, Guinea-pig and rat. Scand J Urol Nephrol 34(4):233–238
Musselman DM et al (2004) A randomized crossover study to evaluate Ro 115-1240, a selective $\alpha_{1A/L}$-adrenoceptor partial agonist in women with stress urinary incontinence. BJU Int 93(1): 78–83
Nergardh A, Boreus LO, Naglo AS (1977) Characterization of the adrenergic beta-receptor in the urinary bladder of man and cat. Acta Pharmacol Toxicol 40(1):14–21
Nickel JC, Sander S, Moon TD (2008) A meta-analysis of the vascular-related safety profile and efficacy of alpha-adrenergic blockers for symptoms related to benign prostatic hyperplasia. Int J Clin Pract 62(10):1547–1559
Nishimatsu H et al (1999) Contractile responses to alpha1-adrenoceptor agonists in isolated human male and female urethra. BJU Int 84(4):515–520
Nitti V et al (2013a) Results of a randomized phase III trial of mirabegron in patients with overactive bladder. J Urol 189(4):1388–1395
Nitti VW et al (2013b) Urodynamics and safety of the beta(3)-adrenoceptor agonist mirabegron in males with lower urinary tract symptoms and bladder outlet obstruction. J Urol 190(4): 1320–1327
Noda K et al (2002) Functional role of inhibitory and excitatory nerves in the porcine lower urinary tract. Eur J Pharmacol 456(1–3):81–90
Oelke M et al (2013) EAU guidelines on the treatment and follow-up of non-neurogenic male lower urinary tract symptoms including benign prostatic obstruction. Eur Urol 64(1):118–140
Oger S et al (2009) Combination of doxazosin and sildenafil exerts an additive relaxing effect compared with each compound alone on human cavernosal and prostatic tissue. J Sex Med 6(3): 836–847
Oger S et al (2010) Combination of alfuzosin and tadalafil exerts an additive relaxant effect on human detrusor and prostatic tissues in vitro. Eur Urol 57(4):699–707
Ohlstein EH, von Keitz A, Michel MC (2012) A multicenter, double-blind, randomized, placebo controlled trial of the ß 3 -adrenoceptor agonist solabegron for overactive bladder. Eur Urol 62(5):834–840
Oostendorp J et al (2000) Contribution of ß-adrenoceptor subtypes to relaxation of colon and oesophagus and pacemaker activity of ureter in wild-type and ß$_3$-adrenoceptor knockout mice. Br J Pharmacol 130(4):747–758
Park YC et al (2000) Existence of a ß3-adrenoceptor and its functional role in the human ureter. J Urol 164(4):1364–1370
Reitz A et al (2004) The effect of tamsulosin on the resting tone and the contractile behaviour of the female urethra: a functional urodynamic study in healthy women. Eur Urol 46(2):235–240. discussion 240

Robinson D et al (2007) A randomized double-blind placebo-controlled multicentre study to explore the efficacy and safety of tamsulosin and tolterodine in women with overactive bladder syndrome. BJU Int 100(4):840–845

Roehrborn CG (2006) Three months' treatment with the alpha1-blocker alfuzosin does not affect total or transition zone volume of the prostate. Prostate Cancer Prostatic Dis 9(2):121–125

Roehrborn CG et al (2008) The effects of dutasteride, tamsulosin and combination therapy on lower urinary tract symptoms in men with benign prostatic hyperplasia and prostatic enlargement: 2-year results from the CombAT study. J Urol 179(2):616–621. discussion 621

Roehrborn CG et al (2010) The effects of combination therapy with dutasteride and tamsulosin on clinical outcomes in men with symptomatic benign prostatic hyperplasia: 4-year results from the CombAT study. Eur Urol 57(1):123–131

Roosen A et al (2008) Synergistic effects in neuromuscular activation and calcium-sensitization in the bladder trigone. BJU Int 101(5):610–614

Roosen A et al (2009a) Adreno-muscarinic synergy in the bladder trigone: calcium-dependent and -independent mechanisms. Cell Calcium 45(1):11–17

Roosen A et al (2009b) Clinical and experimental aspects of adreno-muscarinic synergy in the bladder base and prostate. Neurourol Urodyn 28(8):938–943

Saban MR et al (2002) Gene expression profiling of mouse bladder inflammatory responses to LPS, substance P, and antigen stimulation. Am J Pathol 160(6):2095–2110

Sakalis V et al (2021) The effect of pharmacotherapy on prostate volume, prostate perfusion and prostate-specific antigen (prostate morphometric parameters) in patients with lower urinary tract symptoms and benign prostatic obstruction. A systematic review and meta-analysis. Cent European J Urol 74(3):388–421

Schneider T, Michel MC (2010) Can [^{125}I]-iodocyanopindolol label ß$_3$-adrenoceptors in rat urinary bladder? Front Pharmacol 1:128

Schwinn DA, Roehrborn CG (2008) Alpha1-adrenoceptor subtypes and lower urinary tract symptoms. Int J Urol 15(3):193–199

Scofield MA et al (1995) Quantification of steady state expression of mRNA for alpha-1 adrenergic receptor subtypes using reverse transcription and a competitive polymerase chain reaction. J Pharmacol Exp Ther 275:1035–1042

Sellers D, Chess-Williams R, Michel MC (2018) Modulation of lower urinary tract smooth muscle contraction and relaxation by the urothelium. Naunyn Schmiedeberg's Arch Pharmacol 391(7): 675–694

Shapiro E, Tsitlik JE, Lepor H (1987) Alpha 2 adrenergic receptors in canine prostate: biochemical and functional correlations. J Urol 137(3):565–570

Sigala S et al (2004) Alpha1 adrenoceptor subtypes in human urinary bladder: sex and regional comparison. Life Sci 76(4):417–427

Speakman MJ, Walmsley D, Brading AF (1988) An in vitro pharmacological study of the human trigone–a site of non-adrenergic, non-cholinergic neurotransmission. Br J Urol 61(4):304–309

Spek A et al (2021) Purinergic smooth muscle contractions in the human prostate: estimation of relevance and characterization of different agonists. Naunyn Schmiedeberg's Arch Pharmacol 394(6):1113–1131

Staskin D et al (2020) International phase III, randomized, double-blind, placebo- and active-controlled study to evaluate the safety and efficacy of vibegron in patients with symptoms of overactive bladder: EMPOWUR. J Urol 204(2):316–324

Streng T, Santti R, Andersson KE (2010) Voiding effects mediated by alpha2-adrenoceptors in the anaesthetized male rat. BJU Int 106(10):1546–1549

Strittmatter F et al (2011) Thromboxane A2 induces contraction of human prostate smooth muscle by Rho kinase- and calmodulin-dependent mechanisms. Eur J Pharmacol 650(2–3):650–655

Suzuki T et al (2016) The expression of beta3-adrenoceptors and their function in the human prostate. Prostate 76(2):163–171

Takahashi R et al (2007) RhoA/Rho kinase-mediated Ca2+ sensitization in the contraction of human prostate. Neurourol Urodyn 26(4):547–551

Takeda H et al (2003) Functional characterization of beta-adrenoceptor subtypes in the canine and rat lower urinary tract. J Urol 170(2 Pt 1):654–658
Tamalunas A et al (2021a) Lenalidomide and pomalidomide inhibit growth of prostate stromal cells and human prostate smooth muscle contraction. Life Sci 281:119771
Tamalunas A et al (2021b) Inhibition of human prostate stromal cell growth and smooth muscle contraction by thalidomide: a novel remedy in LUTS? Prostate 81(7):377–389
Tasler S et al (2012) An aryloxypropanolamine hbeta3-adrenoceptor agonist as bladder smooth muscle relaxant. Eur J Pharm Sci 46(5):381–387
Tatemichi S et al (2006) Uroselectivity in male dogs of silodosin (KMD-3213), a novel drug for the obstructive component of benign prostatic hyperplasia. Neurourol Urodyn 25(7):792–799. discussion 800–801
Tatemichi S et al (2012) Comparison of the effects of four alpha1-adrenoceptor antagonists on ejaculatory function in rats. Urology 80(2):486 e9–16
Teixeira CE et al (2007) Comparative pharmacological analysis of Rho-kinase inhibitors and identification of molecular components of Ca2+ sensitization in the rat lower urinary tract. Biochem Pharmacol 74(4):647–658
Thiagamoorthy G, Cardozo L, Robinson D (2016) Current and future pharmacotherapy for treating overactive bladder. Expert Opin Pharmacother 17(10):1317–1325
Tomiyama Y et al (2003a) Pharmacological profile of KUL-7211, a selective ß-adrenoceptor agonist, in isolated ureteral smooth muscle. J Pharmacol Sci 92(4):411–419
Tomiyama Y et al (2003b) Comparison between CL-316243- and CGP-12177A-induced relaxations in isolated canine ureter. Pharmacology 68(3):140–146
Tzortzis V et al (2009) Medical expulsive therapy for distal ureteral stones. Drugs 69(6):677–692
Ueda S, Satake N, Shibata S (1984) Alpha 1- and alpha 2-adrenoceptors in the smooth muscle of isolated rabbit urinary bladder and urethra. Eur J Pharmacol 103(3–4):249–254
Uhlen M et al (2015) Tissue-based map of the human proteome. Science 347(6220):1260419
Van der Graaf PH et al (1997) Analysis of alpha1-adrenoceptors in rabbit lower urinary tract and mesenteric artery. Eur J Pharmacol 327(1):25–32
van Dijk MM, de la Rosette JJ, Michel MC (2006) Effects of alpha(1)-adrenoceptor antagonists on male sexual function. Drugs 66(3):287–301
Villa L et al (2013) Effects of silodosin on the partially obstructed rat ureter in vivo and on human and rat isolated ureters. Br J Pharmacol 169(1):230–238
Walden PD et al (1997) Localization of mRNA and receptor binding sites for the alpha 1a-adrenoceptor subtype in the rat, monkey and human urinary bladder and prostate. J Urol 157(3):1032–1038
Walther S et al (2018) Adreno-muscarinic synergy in the male human urinary outflow tract. Neurourol Urodyn 37(7):2128–2134
Wanajo I et al (2004) Pharmacological characterization of ß-adrenoceptor subtypes mediating relaxation in porcine isolated ureteral smooth muscle. J Urol 172(3):1155–1159
Wang X et al (2020) Onvansertib, a polo-like kinase 1 inhibitor, inhibits prostate stromal cell growth and prostate smooth muscle contraction, which is additive to inhibition by alpha1-blockers. Eur J Pharmacol 873:172985
Weiss RM, Basset AL, Hoffman BF (1978) Adrenergic innervation of the ureter. Investig Urol 16(2):123–127
White CW, Short JL, Ventura S (2013) Rho kinase activation mediates adrenergic and cholinergic smooth muscle contractile responses in the mouse prostate gland. Eur J Pharmacol 721(1–3): 313–321
White CW et al (2019) What makes the α_{1A}-adrenoceptor gene product assume a α_{1L}-adrenoceptor phenotype? Br J Pharmacol 176(14):2358–2365
Wuest M et al (2009) Catecholamines relax detrusor through $ß_2$-adrenoceptors in mouse and $ß_3$-adrenoceptors in man. J Pharmacol Exp Ther 328(1):213–222
Wuest M et al (2011) The muscarinic receptor antagonist propiverine exhibits alpha(1)-adrenoceptor antagonism in human prostate and porcine trigonum. World J Urol 29(2):149–155

Yamanishi T et al (2003) Role of beta-adrenoceptor subtypes in mediating relaxation of the pig bladder trigonal muscle in vitro. Neurourol Urodyn 22(4):338–342

Yanagisawa T et al (2000) Selectivity and potency of agonists for the three subtypes of cloned human beta-adrenoceptors expressed in Chinese hamster ovary cells. Tohoku J Exp Med 192(3):181–193

Yanase H et al (2008) The involvement of urothelial alpha1A adrenergic receptor in controlling the micturition reflex. Biomed Res 29(5):239–244

Yoshida M et al (2018) Vibegron, a novel potent and selective b_3-adrenoreceptor agonist, for the treatment of patients with overactive bladder: a randomized, double-blind, placebo-controlled phase 3 study. Eur Urol 73(5):783–790

Zhang HL et al (2017) Tamsulosin for treatment of lower urinary tract symptoms in women: a systematic review and meta-analysis. Int J Impot Res 29(4):148–156

Asthma and COPD: A Focus on β-Agonists – Past, Present and Future

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Contents

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Abstract

Asthma has been recognised as a respiratory disorder for millennia and the focus of targeted drug development for the last 120 years. Asthma is one of the most common chronic non-communicable diseases worldwide. Chronic obstructive pulmonary disease (COPD), a leading cause of morbidity and mortality worldwide, is caused by exposure to tobacco smoke and other noxious particles and exerts a substantial economic and social burden. This chapter reviews the development of the treatments of asthma and COPD particularly focussing on the β-agonists, from the isolation of adrenaline, through the development of generations of short- and long-acting β-agonists. It reviews asthma death epidemics, considers the intrinsic efficacy of clinical compounds, and charts the improvement in selectivity and duration of action that has led to our current medications. Important β2-agonist compounds no longer used are considered, including some with additional properties, and how the different pharmacological properties of current β2-agonists underpin their different places in treatment guidelines. Finally, it concludes with a look forward to future developments that could improve the β-agonists still further, including extending their availability to areas of the world with less readily accessible healthcare.

Abbreviations

AHR	Airway hyperresponsiveness
AR	Adrenoceptor
ASM	Airway smooth muscle cells
BDP	Beclomethasone dipropionate
cAMP	Cyclic adenosine 3′,5′-monophosphate
COMT	Catecholamine o-methyltransferase
COPD	Chronic obstructive airways disease
FeNO	Fraction of exhaled nitric oxide
FEV_1	Forced expiratory volume in 1 second
GINA	Global Initiative for Asthma
ICS	Inhaled corticosteroids
IL	Interleukin
LABA	Long-acting β2-agonist
LAMA	Long-acting muscarinic antagonist
LTRA	Leukotriene receptor antagonist

PDE	Phosphodiesterase
PKA	Protein kinase A
SABA	Short-acting β2-agonist
SAMA	Short-acting muscarinic antagonist
uLABA	Ultra-long-acting β2-agonist

1 Asthma: Epidemiology, Costs, Pathophysiology

Asthma is derived from the Greek word for panting (aazein) and was recognised as a respiratory problem in ancient Egypt and by the ancient Greek, Hebrew, Roman and Chinese cultures. The Greek physician Hippocrates (460-377 BC) described panting or gasping: asthmaino (Diamant et al. 2007; Arthur 2015). Asthma is an airways disease, defined by intermittent bronchospasm leading to symptoms of wheeze and dyspnoea, and is characterised airway hyperresponsiveness (AHR), mucus hypersecretion and heterogeneous underlying inflammatory mechanisms, that all contribute to variable airflow obstruction.

Epidemiology

Globally asthma affects around 300 million individuals (Stern et al. 2020). The prevalence of asthma now appears to be plateauing, although there is marked regional variation with increases in some low-income countries. Current estimates suggest a 10% global prevalence of asthma symptoms in children and adolescents (García-Marcos et al. 2022) and 6-7% in adults – ranging from 2 to 3% in low-income countries to 10% in high-income countries (Mortimer et al. 2022).

Preschool wheeze is a hallmark of asthma in young children. Approximately 40% of pre-schoolers with wheeze go on to have respiratory symptoms later in childhood. Factors associated with progression from pre-school wheeze to asthma include wheeze severity, atopy/IgE-sensitisation (especially poly-sensitisation), high fractional exhaled nitric oxide (FeNO), allergic comorbidity, prematurity, parental asthma and season of the first reported wheeze event (Kaiser et al. 2016; Bloom et al. 2021).

Other factors associated with asthma prevalence include tobacco smoke exposure, viral exposure, air pollution, obesity, genetic risk factors, sex (males – child onset, females – adult onset), stress, certain allergen exposures (e.g. dust mites), ethnicity, urbanisation, lack of beneficial microbial exposures and socio-economic status (Stern et al. 2020) and occupational exposures in adults (Cullinan et al. 2020).

Factors associated with persistence of asthma from childhood to adulthood overlap to a great extent with the general asthma risk factors (heredity, poly-sensitisation, obesity, etc.) but also include repeated airway infections, impaired lung function, comorbidities and eosinophilia. Asthma in childhood may impair airway development and reduce maximally attained lung function, which may

persist into adulthood; severe asthma during childhood has been associated with a 30-fold increased risk for fixed airway obstruction (in some studies classified as "COPD", Tai et al. 2014).

Asthma-Related Costs

Expenditure related to asthma has continued to increase: In the USA healthcare costs rose from USD 53 billion in 2007, to USD 56 billion in 2009, to USD 82 billion in 2013 (Nurmagambetov et al. 2018). Asthma is thought to account for over 1% of the total global disability-adjusted life years lost (GBD 2015 Chronic Respiratory Disease Collaborators 2017). Most of the costs of asthma are due to emergency care and severe persistent disease (Enilari and Sinha 2019).

Pathophysiological Mechanisms

Dyspnoea and wheezing are caused by variable airflow obstruction, reflecting airway smooth muscle (ASM) hypercontractility with the central and defining pathophysiological feature of asthma being airway hyperresponsiveness. Reduced lung function may be caused by airway smooth muscle hypertrophy, mucosal oedema and mucus hypersecretion all contributing to airway narrowing and are potentially reversible with anti-inflammatory treatment (such as inhaled corticosteroids, ICS). In addition, basal membrane thickening with subepithelial fibrosis can potentially result in irreversible, "fixed" airflow obstruction. Cough with phlegm reflects mucus hypersecretion, but irritative cough may also reflect increased cough hypersensitivity due to sensory nerve dysfunction.

Airway inflammation may differ between patients (e.g. eosinophilic or neutrophilic inflammation). In classic allergic asthma, T-helper-2 (Th2) cells are activated during allergen exposure, inducing an inflammatory cascade resulting in eosinophilic airway inflammation (induced by cytokines including IL-4, IL-5, IL-13, IL-33). As the role of innate lymphoid type 2 cells has become more apparent, there has been a shift in denomination from eosinophilic versus non-eosinophilic asthma to "T2" and "T2-low" (originally referred to as "T" based on the fact T helper cells are involved in the inflammatory pathway, but now also recognising the role played by innate lymphoid cells).

T2 inflammation describes an inflammatory pathway involving Th2 cells and is characterised by high IgE antibody titres and eosinophilia. Biomarkers of T2 (Type 2) inflammation, e.g. FeNO and blood eosinophils, predict response to anti-inflammatory treatments and asthma exacerbations. As T2 biomarkers are suppressed by anti-inflammatory treatment, low levels do not preclude underlying T2 inflammation in a patient on ICS. The original terminology of type 1 and type 2 asthma which was used by some authors to describe types of "brittle" asthma is no longer in routine use.

T2-low asthma is poorly understood and the role of neutrophils, with involvement of T-helper-1 and T-helper-17 cells is debated. Neutrophilic airway inflammation may be more of a non-specific marker of severe asthma, relating to low lung function, older age, altered airway microbiome and high doses of ICS, rather than a causative factor (Nair et al. 2021).

Other factors involved in asthma pathogenesis include (1) mast cells, that release bronchoconstrictive mediators, such as histamine and leukotrienes and infiltrate airway smooth muscle; (2) changes in the airway epithelium with disrupted barrier function and an exacerbated inflammatory response to both specific triggers such as allergens and non-specific triggers such as virus or smoke, with increased release of other cytokines, e.g. thymic stromal lymphopoietin, IL-33 and IL-25 (Lambrecht et al. 2019; Lambrecht and Hammad 2012); (3) aberrant innate immune memory may contribute to increased inflammatory responses observed in asthma; and (4) trained immunity which refers to epigenetic and metabolic reprogramming of innate immune cells, resulting in an augmented secondary response to different immune triggers (Netea et al. 2020). Lastly, (5) airway smooth muscle is hypertrophic, and loss of homeostatic control causes hypercontractility with tendency to bronchospasm (Camoretti-Mercado and Lockey 2021). Airway hyperresponsiveness results from hypercontractile ASM, with an increased sensitivity to the bronchoconstrictive mediators released mainly from mast cells and eosinophils in relation to airway inflammation (Hallstrand et al. 2018). Airway remodelling contributes to airflow obstruction through a combination of subepithelial fibrosis, basal membrane thickening, mucosal oedema and ASM hypertrophy that in turn contribute to more airway narrowing and hyperresponsiveness (Tliba and Panettieri 2019; Banno et al. 2020).

Asthma may also be caused by a person's occupation, but more often is aggravated by factors in the working environment. Occupational asthma may be caused by inhalation of a wide range of allergens (related to, e.g., bakeries and seafood processing) but also irritants such as chlorine, ammonium or isocyanates and have an acute but also a delayed onset (Vandenplas et al. 2014).

1.1 Asthma: Long-Term Management Regimes and Treatments

Non-pharmacological management of asthma includes advice on avoidance of exposures such as allergens (when relevant and feasible), smoking and occupational exposures, and appropriate management of comorbidities (obesity, inducible laryngeal obstruction) contributing to poor asthma control. Providing adequate patient education and information to patients and families on disease management is key to achieving positive long-term outcomes.

Pharmacological Treatment Regimes

For decades, asthma has been treated in a step-wise manner beginning with short-acting β2-agonists (SABA) to control symptoms followed by inhaled corticosteroids (ICS) to improve inflammation. If not sufficient, the dose of ICS was increased, or second controllers (long-acting β2-agonists – LABA, long-acting muscarinic antagonists – LAMA, or leukotriene receptor antagonists – LTRA) added, until asthma control was achieved, and subsequently adjusted according to the level of asthma control.

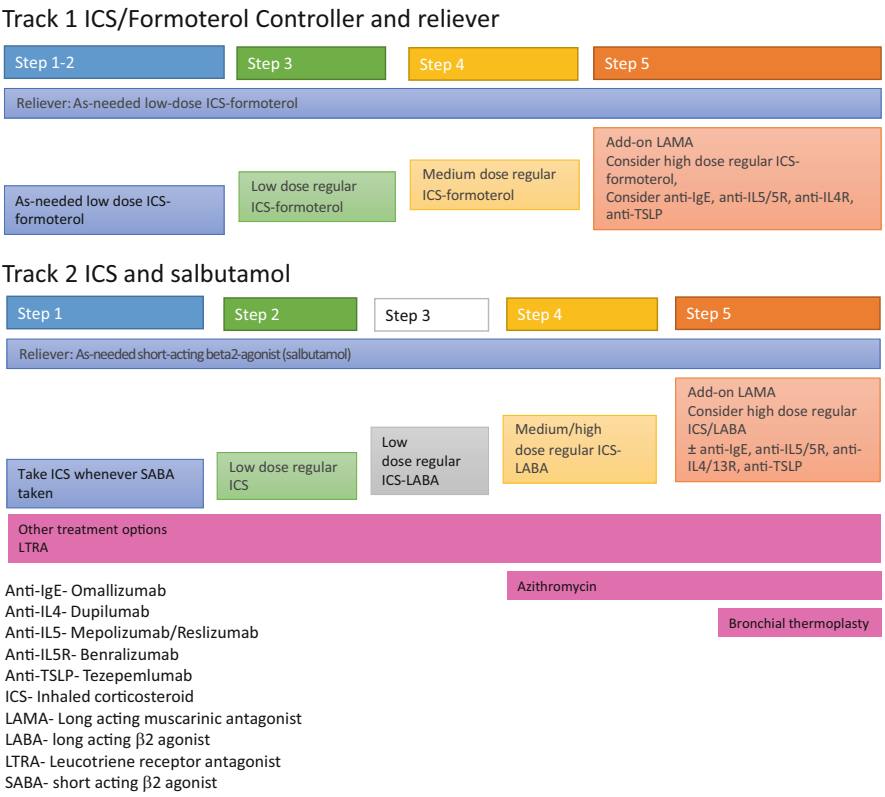

Fig. 1 Current (2022) step-wise progression of asthma treatments. Patients have both the regular medication for the step they are on and a "reliever" inhaler. If patient symptoms are not controlled on current step, they move to the next step in treatment and move on up until their asthma is controlled. After an exacerbation, patients are normally started on higher dose therapy, and if they remain symptom and exacerbation controlled, they can be stepped back down the cascade

GINA (the Global Initiative for Asthma 2022) now recommends two different "Tracks" for adults and adolescents (≥12 years; for 6–11 years a similar regimen; Fig. 1): Track 1, based on a combination of inhaled corticosteroid (ICS) and formoterol (a fast onset but long-acting bronchodilator – LABA) in a single inhaler used as both reliever and/or preventer, and Track 2, where a SABA is prescribed as a reliever, alongside intermittent or regular ICS.

This approach is based on data suggesting that SABA overuse is associated with a worse outcome (Suissa et al. 1994). However, it is difficult to determine from studies whether SABA overuse *per se* or relative ICS under use is the main driver for the poorer outcomes, or a marker of more severe disease, as the same association is observed in patients on high-dose ICS with a high use of "as-needed" SABA (Gonem et al. 2019; Suissa et al. 2000). Importantly, the use of monotherapy with a single ICS/formoterol inhaler in mild asthma is not currently licensed/approved in

all countries, or by the FDA or EMA, although it is becoming more widespread globally. More recently a salbutamol and ICS combination has been shown to be superior when compared to maintained ICS therapy with SABA reliever (Papi et al. 2022).

A step-wise approach based on increasing the dose of ICS at each step still forms the backbone of many national and international asthma guidelines. Of note, 80–90% of the maximum obtainable benefit in the long-term treatment of asthma in adults occurs below 500 mcg beclomethasone dipropionate (BDP) equivalent ICS daily. The dose administered depends not only on the formulation but also on the particle size and inhaler type. A useful source of information containing comparisons of many of the available asthma drugs and inhalers, as well as clips on inhalation technique is found at www.rightbreathe.com.

If patients are still symptomatic despite low-dose ICS/LABA combination (step 2 and 3) or medium/high-dose ICS/LABA (steps 3 and 4), GINA currently advises addition of a LAMA or LTRA. The combination of an ICS, LABA and LAMA is referred to as triple therapy and studies of "closed" triple therapy (where all three drugs are combined in one inhaler) in moderate to severe asthma have shown a reduction in severe asthma exacerbations and modest improvements in asthma control, without significant differences in quality of life or mortality, when compared to ICS/LABA therapy. Triple therapy is associated with an increased incidence of dry mouth and dysphonia (Kim et al. 2021).

Aside from ICS dose increases, other options are available to improve asthma control. In the UK, the addition of a leukotriene receptor antagonist (LTRA), e.g. Montelukast, is currently recommended as the next addition after the initial use of low-dose ICS, rather than adding a LABA. GINA suggests LTRA can be given as a treatment trial at any step to improve symptom control. Most guidelines suggest stepping down ICS dose by 25–50% or removing any additional controller medication if the patient is controlled for at least three months.

Multiple inhaler types and devices are available and teaching inhalation technique by physical demonstration of the device is important to prevent treatment failure due to poor inhalation technique, and in patients with known asthma, inhalation technique and adherence should be assessed at every clinical control. Finally, treatment regimens are based on data from randomised controlled trials, but it is recognised that people respond differently to treatments (which reflects asthma heterogeneity); in one study, both LTRA montelukast and ICS beclomethasone were associated in some patients with a reduction in FEV_1, whereas overall there was a mean improvement with both drugs (Malmstrom et al. 1999).

β2-Agonists

These are an essential part of all asthma treatment regimes in those with asthma. A detailed account of their discovery, evaluation and discussion of the individual drugs, past and present, actions and side effects, future challenges and potential pharmacological improvements are given below in Sects. 3–10.

Corticosteroids

Intramuscular, intravascular and oral steroids (cortisone and prednisolone) were used in the management of asthma in the 1950s, but due to their significant side effects were initially reserved for those with severe asthma only. Inhalers were developed in the 1950s, but it was the development of beclomethasone (BPD) in 1972 that inhaled corticosteroids (ICS) became widely used (Brown et al. 1972; Cockcroft 1999). Steroids are highly effective anti-inflammatory drugs. Via the intracellular glucocorticoid receptor, steroids alter cellular gene transcription (and therefore cell production of proteins). They reduce the transcription of pro-inflammatory genes and increase that of anti-inflammatory genes (Diamant et al. 2007). Inhaled corticosteroids are an essential part of asthma treatment, although systemic absorption does cause side effects (Chu and Drazen 2005; Chalitsios et al. 2021).

Prescribed ICS dose varies from 100 mcg BDP up to 2,000 mcg BDP; however, clinical studies have shown either marginal or no differences in clinical anti-asthmatic efficacy when comparing microgram equivalent doses of fluticasone, beclomethasone or budesonide given via the same inhaler device, particularly above 1,000 mcg/day in adults on the flatter part of the dose-response curve (Lipworth 1996; Raissy et al. 2013), and 2,000 mcg BDP is probably equivalent to an oral dose of 5-8 mg prednisolone (Lipworth 1996).

The addition of a LABA provides better symptom control than steroid alone (e.g. Larsson et al. 2020) and guidelines recommend that regular β2-agonists are only given alongside ICS (GINA 2022) as combination dual inhalers (LABA + ICS); however, there are differential effects of ICS and LABA on clinical outcomes; in general, increasing the ICS dose in people with airway inflammation reduces exacerbations, whereas adding a LABA improves symptom control and lung function (Lee et al. 2020a; Pauwels et al. 1997). ICS do not prevent lung function decline or airway remodelling.

Leukotriene Antagonists

In 1940, Kellaway and Trethewle discovered "slow reacting substance of anaphylaxis", one of the inflammatory components of asthma that was the leukotrienes (Diamant et al. 2007). Leukotrienes are released by mast cells and are potent bronchodilators (Chu and Drazen 2005). In the 1990s, a leukotriene synthesis inhibitor was developed (zileuton) and leukotriene receptor antagonists (pranlukast, zafirlukast, montelukast) as oral therapies. They have anti-inflammatory as well as bronchodilatory effects but are not as effective as ICSs or β2-agonists (Chu and Drazen 2005; Diamant et al. 2007) and are often considered in those who have concomitant allergic rhinitis. The leukotriene antagonists are now off patent and relatively inexpensive; they also have the advantage of being taken as a once daily pill, potentially aiding adherence, so some physicians have used them before the addition of a LABA to ICS if a patient is still poorly controlled. The use of leukotriene antagonists does not alter the smooth-muscle response to β2-agonists (Green and Pavord 2001).

Chromones

Disodium cromoglycate was discovered by Roger Altounyan, who had a clear asthmatic response to guinea pig dander. With colleagues at Fison Pharmaceuticals, UK, he extracted active ingredients from natural products, testing them on himself and his asthmatic reaction (Chu and Drazen 2005). From khella (bishop's weed, a member of the carrot family) he extracted khellin, and from this developed sodium cromoglycate in 1967 (FPL670) and later nedocromil (Cockcroft 1999). This is a mast cell stabiliser that reduces the release of inflammatory mediators from mast cells. Although used more in the 1970s, and useful in those with hay fever and rhinitis, its use in asthma was more limited to children (Chu and Drazen 2005). Global manufacture is now stopping, and recommendations for use no longer appear in asthma guidance.

Xanthines: Phosphodiesterase Inhibitors

Strong tea and coffee were recommended for the relief of breathlessness (bronchospasm) in asthma for centuries. The active xanthines were named after their sources – caffeine in coffee and theophylline in tea (Cockcroft 1999). It was not until the development of theophylline in 1922, followed by aminophylline in 1937 that they were regularly used as anti-asthma treatments (Diamant et al. 2007). These drugs inhibit phosphodiesterase and therefore reduce the breakdown of cAMP. This increases intracellular cAMP assisting in bronchodilation. There are also phosphodiesterase anti-inflammatory effects (Barnes 2013). From the 1920s to the 1960s, theophylline and aminophylline were the most prescribed medication for asthma, until the development of β-agonists. They have a narrow therapeutic window and significant side effects (nausea, vomiting, headaches, cardiac arrhythmias) which limits their use (Diamant et al. 2007; Barnes 2013). Although theophylline, added to low-dose ICS, has been shown to improve some clinical outcomes, data are lacking in patients on higher dose ICS or more severe asthma, and its use has declined as other inhaled drugs and injectable biologics have come to market. Consequently, theophylline use is no longer routinely recommended in GINA guidance (2022).

Muscarinic Antagonists

Muscarinic antagonists (or anticholinergics) have been used in asthma for several thousand years in the form of the Datura stramonium (thorn-apple) and Belladonna plant (deadly nightshade). The plants (leaves, flowers or fruit/leaves, stems and roots) were dried then smoked (either as fumes, cigars, pipes or asthma cigarettes). The active ingredient is the muscarinic antagonist atropine (Cockcroft 1999; Jackson 2010). Ipratropium (a short acting muscarinic antagonist, SAMA) was developed in the 1970s followed by longer acting tiotropium, glycopyrronium, aclidinium and umeclidinium. They act by blocking the Gq-coupled muscarinic M3 acetylcholine receptor on airway smooth muscle cells. Blocking this receptor stops acetylcholine activation, lowering calcium and causing cell relaxation (bronchodilation). However, actions are complicated as M1 and M2 receptors are also present in lung (Barnes 1993). Ipratropium is still used in asthma, usually nebulised along with the β2-agonist salbutamol, during severe asthma exacerbations. The first long

acting muscarinic antagonist (LAMA) to be licensed for asthma was Tiotropium, in respimat "soft mist" form. Other LAMAs are now licensed and are often combined into the so-called closed triple inhalers containing ICS, LABA and LAMA in a single inhaler, e.g. vilanterol/umeclidinium/fluticasone, indacaterol/glycopyrronium/mometasone, formoterol/glycopyrronium/beclomethasone (FitzGerald and Sadatsafavi 2019; Lee et al. 2020a; Virchow et al. 2019; Brittain et al. 2022).

Antibiotics: Azithromycin

Azithromycin is a macrolide antibiotic that has shown benefit in several respiratory diseases, including asthma (Gibson et al. 2017) and COPD (Albert et al. 2011). It is often used in patients with a chronic cough or recurrent exacerbator phenotype of asthma and is administered as 250 mg, or 500 mg, three times a week. Concerns remain about long-term safety data, especially with regard to provoking antibiotic resistance, cardiac side effects (long QT interval) and reversible hearing loss. Azithromycin is often used in preference to erythromycin as it appears to have anti-inflammatory (Slater et al. 2016) as well as antibiotic effects (Slater et al. 2014) but long-term data on its effectiveness in any airway disease are still lacking.

Allergen Immunotherapy

Allergen immunotherapy is a process of exposing an allergic individual to controlled amount of allergen in order to build up tolerance and therefore reduce the allergic response. It was first reported by Leonard Noon in 1911 with prophylactic inoculation of grass pollen to reduce hay fever and further developed by John Freeman into immunotherapy protocols in 1928 (Durham and Leung 2010). Allergic asthma is often associated with allergic rhinitis. Allergen immunotherapy is widely used and recommended to treat allergic rhinitis, and although its role in treating allergic asthma is less established, it has the potential of being disease modifying. Subcutaneous immunotherapy with a variety of allergens has been found to reduce asthma symptoms, need for asthma medication, and airway hyperresponsiveness, but there is no consistent effect on exacerbations (Abramson et al. 2010). However, there is evidence suggesting that sublingual immunotherapy with house-dust-mite allergen may decrease exacerbations and need for asthma medication (Mosbech et al. 2014; Virchow et al. 2016).

Long-Term Management of Severe Asthma

Severe asthma is defined as "asthma that is uncontrolled, despite adherence with maximal optimised high dose ICS-LABA treatment and management of contributory factors, or that requires high dose treatment to maintain good symptom control and reduce the risk of exacerbations" (Chung et al. 2014; GINA 2022). Whereas GINA guidelines define high-dose ICS as >800 mcg BDP, the American Thoracic Society and European Respiratory Society guidelines set the cut-off of >1,600 mcg BDP, which is the most widely used definition in setting the indication for biological therapies. About 5–10% of asthmatics have severe asthma, often with frequent

exacerbations, and many requiring maintenance oral corticosteroid, and severe asthma drives the majority of costs associated with health care of asthma.

A significant number of people referred to specialist care due to uncontrolled asthma despite high-dose treatment do not suffer from severe asthma, but have other causes of poor asthma control, such as treatment barriers, asthma triggers such as comorbidities or exposures to allergens or smoking. Hence, in order to make a diagnosis of severe asthma, a systematic assessment is mandated, to identify and manage these factors, prior to considering treatment escalation.

One part of this systematic assessment includes measuring airway inflammation as an improved understanding of the role of mechanisms involved in inflammation has led to effective targeted treatment options based on the use of biomarkers and the identification of "treatable traits".

This approach is based on the recognition that asthma is a heterogeneous condition composed of both different phenotypes (a set of observable characteristics of an individual resulting from the interaction of its genotype with the environment) and endotypes (subtype of a condition, which is defined by a distinct functional or pathobiological mechanism). The use of biomarkers of airway inflammation has allowed some of these endotypes to be identified and targeted (Heaney et al. 2021; Shaw et al. 2021). Better targeting and understanding of airway inhalation and its pathogenesis have led to a change in both asthma diagnosis and treatment regimens and helped the development of new therapies called monoclonal antibodies (also known as biologics) which specifically target cytokines involved in asthma inflammation. The advent of biologics has been a step change in asthma therapeutics.

Biologics (Monoclonal Antibodies)

Most current biologic therapies are directed against T2 inflammation. The first biological therapy was the humanised monoclonal antibody to IgE, Omalizumab in 2003. The stimulus for mast cells (and other inflammatory cells) to release their inflammatory mediators is IgE binding to surface IgE receptors. Omalizumab binds to IgE in the blood stream (circulating IgE), lowering blood IgE levels and preventing it from binding to IgE receptors. It is effective in those with marked allergic asthma and improves asthma control and reduces exacerbations (Dragonieri and Carpagnano 2021; Salvati et al. 2022).

With the further recognition that T2 inflammation (particularly eosinophilic inflammation) was associated with exacerbation risk, Mepolizumab, an anti-IL5 targeting monoclonal antibody was developed. Initial studies were disappointing, probably as the wrong end points of airway hyperresponsiveness and symptoms, which are not specifically related to airway inflammation, were assessed (Flood-Page et al. 2007). Later studies were positive (Ortega et al. 2014) and Mepolizumab was the first licensed anti-IL5 drug, followed by Reslizumab (Bjermer et al. 2016) and Benralizumab (FitzGerald et al. 2018). More recently, Dupilumab, an anti-IL-13/4R blocker, has shown to reduce exacerbations and oral steroid use in moderate to severe asthma and has a wider range of indication based on a blood eosinophil count of 0.15×10^9/L (compared to 0.3×10^9/L for anti-IL-5 agents) and/or an elevated FeNO (Castro et al. 2018; Rabe et al. 2018).

Latterly, Tezepelumab, a monoclonal antibody targeting thymic stromal lymphopoietin (Salvati et al. 2022), an epithelial Alarmin, has been shown to improve clinical outcomes in moderate to severe asthma, with a suggestion it also works in people without T2 inflammation (Corren et al. 2017, 2022; Menzies-Gow et al. 2021). All these agents require regular injection and are relatively expensive so are often reserved for those with severe asthma, and in the UK are only available through recognised centres of asthma expertise.

In general, the main effects of biologics are a reduction in exacerbations and in the need for maintenance oral corticosteroid, and these two traits of severe asthma set the indication for starting a biologic therapy in most countries. Patients may also experience significant improvements in symptom control and lung function. Subsequent real-life studies show more consistent effects on symptoms and lung function, which may reflect a better selection of patients through systematic assessment in the severe asthma clinics.

Bronchial Thermoplasty

Bronchial thermoplasty is an interventional bronchoscopic procedure for the treatment of severe, uncontrolled asthma patients which applies heat to the bronchial wall to disrupt smooth muscle activity and reduce airway smooth muscle mass, helping prevent bronchoconstriction. It has been shown to be safe and effective (Chaudhuri et al. 2021; Menzella et al. 2021) but since the advice of monoclonal antibodies its use has declined as the intervention requires three separate bronchoscopic procedures and requires careful sedation and after care.

1.2 Management of Emergency Exacerbation Asthma

Asthma Exacerbations

Asthma exacerbations are episodes of worsening symptoms that do not respond to rescue β2-agonist treatment (Reddel et al. 1999) and require a change in therapy, often including a short course of oral corticosteroids, to resolve. Asthma exacerbations can be life threatening and are not always preceded by poor symptom control, either because of differences in perception of dyspnoea (Magadle et al. 2002) or because day-to-day symptoms reflect more than just airway inflammation (Haldar et al. 2008). Attempts to predict asthma exacerbations using risk stratification have yet to be adopted into mainstream clinical care (Loymans et al. 2016), although as better data are collected risk factors are becoming easier to identify (Couillard et al. 2021).

Asthma exacerbations are most prevalent in young children before school-age and are associated with several factors, including seasonal change (probably representing increased exposure to viruses or allergens), previous exacerbations, and comorbid diseases including allergic rhinitis or chronic rhinosinusitis, gastro-oesophageal reflux, obesity, and lower socio-economic status.

Several treatable traits are associated with an increased risk of asthma exacerbation and need to be assessed in clinic: Overreliance on short-acting beta agonists

(SABA, for example, salbutamol) has long been known to be associated with asthma exacerbations (Patel et al. 2013; Suissa et al. 1994). Elevated blood eosinophil counts are also associated with an increased risk of asthma exacerbation (Kraft et al. 2021; Ortega et al. 2014) and are used as one parameter to identify patients who may benefit from monoclonal antibody therapy. Poor adherence is often overlooked but an important risk factor for exacerbations; the highest reduction in the odds of exacerbation is found in patients achieving 80% or more adherence with their inhaled corticosteroids (Chongmelaxme et al. 2020).

Clinical Assessment and Management of Exacerbations

Asthma exacerbations cause acute shortness of breath and wheeze. Most exacerbations can be managed in the community with bronchodilators (β2-agonists) and often a short course of oral prednisolone. More severe exacerbations require hospital admission. Asthma exacerbations can deteriorate rapidly – regular assessment via peak flow and patient examination is required. Oxygen saturations should be monitored by pulse oximetry. Salbutamol (short-acting β2-agonist, SABA) and ipratropium bromide (short-acting muscarinic antagonist; via spacer or nebulised) can be both co- and alternatively administered. If symptoms worsen, intravenous magnesium can also be given, although the evidence for treatment effect is limited (Goodacre et al. 2013).

Guidelines suggest that intravenous aminophylline should not be used because of limited evidence and the risk of life-threatening side effects (specifically tachycardia and arrhythmia), however it is still occasionally used in near-fatal asthma. Intravenous salbutamol may be considered too, but equally has a limited effect with risk of similar systemic side effects (Munro and Jacobs 2004). The use of non-invasive ventilation is not currently recommended generally but may be tried in hospital settings. It should be noted that nebulised salbutamol can cause a significant reversible increase of lactate, typically without acidosis (see Sect. 9 – clinical side effects of β2-agonists, Mountain et al. 1990). For all medications, children should be given age-adjusted (or weight-adjusted) treatment doses.

Care Following an Exacerbation

Before discharge from hospital, patients should not have had any troublesome asthma symptoms for at least 24 h, ideally have a PEF rate >75% predicted (or best) at 1-h post-treatment, had their inhaler technique checked and asthma triggers identified. A written personalised asthma action plan explaining when and how medications should be changed, when to seek help, and future on-going management, along with a peak expiratory flow meter, should be administered. Follow-up should be arranged with a health care professional in the near future.

2 COPD: Definition, Epidemiology, Treatments

Definition

Chronic obstructive pulmonary disease (COPD) is a lung condition predominantly caused by cigarette (tobacco) smoking. The disease is characterised by chronic symptoms of cough, breathlessness and sputum production caused by airway abnormalities (bronchitis and bronchiolitis) and damage to the alveoli (emphysema) that can lead to persistent, often progressive airflow obstruction (Venkatesan 2023). This airflow obstruction causes limited exercise ability and increases the risk of COPD exacerbations – acute respiratory events characterised by increased symptoms necessitating an increase in therapy. The disease is defined by this obstruction, with a postbronchodilator FEV_1/FVC ratio of <0.7 indicative of COPD. Additional lung function abnormalities include gas trapping, hyperinflation and reduced gas transfer.

Other noxious stimuli can also cause COPD, including indoor (cooking/heating fumes from burning biomass/wood) and outdoor (exhaust fumes, smoke, particulate matter) air pollution. Rarely, a genetic condition due to a mutation in the SERPINA1 gene, leading to alpha one antitrypsin deficiency can cause COPD especially in smokers.

People with COPD have increased numbers of macrophages in their peripheral airways, lung parenchyma and pulmonary vessels as well as increased activated neutrophils and lymphocytes. Chemotactic factors which attract inflammatory cells from the circulation amplify this inflammatory process and induce structural changes. This inflammation leads to airflow obstruction and some authors contend that systemic inflammation also causes comorbid conditions including muscle mass loss (Tkacova 2010).

Epidemiology

COPD is one of the top three leading causes of death worldwide. More than 3 million people died from their COPD in 2012. The current GOLD 2023 guidance (Venkatesan 2023) estimates that the current global prevalence of COPD is around 10% and is set to rise as the prevalence of cigarette smoking in lower- and middle-income countries increases. COPD exerts a heavy financial burden on society, both in terms of direct care costs and lost productivity and is second only to ischaemic heart disease as the leading cause of disability adjusted life years lost globally.

Importantly many people with COPD are also at risk of other cigarette smoking-induced disease, especially cardiovascular (myocardial infarction, hypertension, stroke) as well as other comorbidities, including metabolic syndrome, osteoporosis, obesity, depression and anxiety. COPD is also associated with other extra-pulmonary conditions including sarcopenia, weight loss and nutritional imbalance. Compared to people with asthma, COPD sufferers are often older, have more comorbidities and are consequently more likely to have polypharmacy to cover both COPD and other comorbid or co-existing conditions that are more prevalent with age (type 2 diabetes, hypertension).

The latest GOLD guidelines (2023 update) suggest that once a diagnosis is confirmed by spirometry, COPD should be assessed based on symptoms (measured by the Modified Medical Research Council (mMRC) Dyspnoea Scale or the COPD Assessment Test (CAT)), the severity of airflow limitation (FEV_1), and the occurrence of previous exacerbations. Taken together, these three factors decide what group (A, B or E) a COPD sufferer fits into. Groups A and B are separated by symptom burden but have only had one exacerbation in the previous year, whilst group E have experienced two or more moderate exacerbations or at least one causing a hospital admission, with any symptom burden. These groups then determine which treatment a person with COPD is initiated on (see below).

Treatments

The single most effective therapy in COPD is smoking cessation. Other supportive therapies such as vaccination (against SARS Co-V2, influenza and pneumococcus), and pulmonary rehabilitation (a form of exercise training with disease-specific advice), should also be considered.

The pathophysiological changes in COPD cause loss of elastic recoil and limit lung emptying during forced expiration, decreasing the FEV1 and the FEV1/FVC ratio and leading to gas trapping and lung hyperinflation. This "static" lung hyperinflation commonly becomes "dynamic" during exercise and causes exertional breathlessness and reduced exercise ability. Lung hyperinflation contributes to the impaired contractility of the respiratory muscles, especially in the diaphragm which flattens and becomes less efficient at generating negative pleural pressures and generating airflow. This inability to generate airflow increases the work of breathing and people with COPD can tire and fall into respiratory failure (Macklem 2010). Although COPD is defined by its irreversibility to SABAs, bronchodilators work on the peripheral airways and help reduce this gas trapping, which then leads to reduced hyperinflation, allowing the diaphragm to work more efficiently and improving symptoms (Belman et al. 1996). β-agonists may also have a role in skeletal muscle hypertrophy (see Sect. 4 clenbuterol where β2-agonist restrictions in elite athletes are discussed) and mucociliary clearance (see Sect. 8).

Overall pharmacological therapy in COPD is prescribed for symptomatic improvement (reduced breathlessness/increased exercise tolerance) and to reduce both the frequency and severity of acute exacerbations. The ability of drug therapy to slow lung function decline and improve mortality is debated; recent papers suggest that "triple" therapy inhalers (containing an ICS, LABA and LAMA) may reduce mortality in a sub-group of patients (Lipson et al. 2020; Martinez et al. 2021); however, there is debate as to the appropriate study design to show mortality benefit (Suissa 2021). Exacerbations of COPD are mostly due to common viral infections (rhinovirus, respiratory syncytial virus, influenza, etc.) or exposure to further noxious stimuli. During exacerbations airway inflammation increases causing gas trapping and worsening ventilation/perfusion abnormalities. ICS containing preparations reduce this inflammation and reduce the chance and severity of exacerbations.

Current inhaled therapies for COPD include SABAs, SAMAs, combined SABA/SAMA, combined LABA/LAMA, combined LABA/ICS, and closed triple devices (i.e. 3 drugs in one inhaler) containing LABA/LAMA/ICS. Oral medications including methylxanthines (theophylline/aminophylline) and Roflumilast (a phosphodiesterase inhibitor) are used less frequently. Mucolytic agents (erdosteine, carbocysteine) are utilised in cases of problematic cough or tenacious sputum.

SABAs and SAMAs have been shown to improve FEV_1 and symptoms, whereas LABAs and LAMAs have shown to also reduce exacerbation rates, with LAMAs superior to LABAs. Combined ICS and LABA therapy has also been shown to improve symptoms, FEV_1 and reduce exacerbations, but the use of ICS is associated with higher rates of pneumonia (Lipson et al. 2018), especially in people with lower blood eosinophil counts and current guidance suggests that if there is an indication for an ICS containing therapy (frequent exacerbations or concomitant asthma) triple therapy (ICS/LABA/LAMA) is preferred over LABA/ICS combination. Interestingly a similar increased risk of pneumonia with higher dose ICS has also been observed in asthma (McKeever et al. 2013; Qian et al. 2017). The current 2023 GOLD guidance states that below an eosinophil count of 100 cells/μl, ICS therapies have little effect (and may cause pneumonia) whereas above 300 cells/μl people with COPD are more likely to respond to ICS therapy, and although the risk of pneumonia remains, the risk/benefit balance tips towards using an ICS therapy. Other adverse effects of ICS include oral candidiasis and skin bruising.

The overall approach based on GOLD guidance, using Group allocation determined by symptoms and exacerbations, is to start with a SABA/SAMA in Group A (minimal symptoms, no exacerbations) and increase to a dual LABA/LAMA (preferably in a single inhaler) for symptomatic patients (Group B). For those who are in Group E (two or more exacerbations in the previous year, with/without symptoms), a LABA/LAMA combination should be prescribed, followed by the addition of an ICS to form a triple therapy in patients with blood eosinophils of greater than 300 cells/μl. Guidance on pharmacological therapy changes regularly based on large randomised controlled trials and it is worth checking current guidance when making treatment decisions.

For specific treatment of exacerbations, SABAs or SAMAs (nebulised or inhaled) are recommended and if the exacerbation is severe, systemic corticosteroids should be prescribed with a 5-day course of prednisolone normally recommended. This has been shown to improve FEV_1, oxygenation and shorten recovery time.

3 β2-Agonists and Asthma: Sites of Action

The β2-adrenoceptor (β2-AR) on airway smooth muscle cells is the main site of action for the beneficial effects of β-agonists and relief of bronchospasm, both for the short-term relief during an exacerbation and for the long-term management in asthma and COPD. The β2-AR is a G-protein-coupled receptor that sits in the cell membrane, transmitting chemical messages from hormones (adrenaline) and drugs

(e.g. salbutamol) into cellular actions (e.g. muscle relaxation). β2-ARs are present in several cell types that are important in asthma, but the most important are the bronchial smooth muscle cells.

Today most β2-agonists are taken by inhaler (including the well-known "blue" inhaler salbutamol recognised throughout the world from children to adults as a reliever of wheeze and shortness of breath in those with asthma). Inhaled drugs first reach the mucosal lining that is covered in a film of fluid. The drugs must first dissolve in this fluid layer before diffusing through the epithelial layer to reach the bronchial smooth muscle (Lötvall 2001). There is evidence that for some drugs, such as salbutamol, the drug is actively transported from the luminal surface of the epithelial cells to the basal surface (and thus underlying smooth muscle cells (Starkey et al. 2014; Unwalla et al. 2012)) rather than just relying on diffusion. When the agonist binds to β2-ARs on the smooth muscle cells, it stabilises the active conformation of the receptor. In this active state, the G-protein dissociates into a Gαs-subunit and a βγ-subunit. Gαs-proteins stimulate an increase in adenylyl cyclase activity and thus an increase in intracellular cAMP. cAMP activates protein kinase A (PKA) that phosphorylates several intracellular proteins involved in muscle tone (e.g. myosin-regulatory light chain kinase and calcium-dependent potassium channels) with the net result of relaxation of smooth muscle and dilation of the airway (bronchodilation; Ellis et al. 1995; Lötvall 2001; Johnson 2001; Anderson 2006; Billington et al. 2017).

In addition, there are protective mechanisms in place to prevent overstimulation. PKA can also phosphorylate the β2-AR, reducing the coupling to adenylyl cyclase. Sufficiently efficacious agonists can also stimulate receptor phosphorylation via G-protein receptor kinases (GRKs) that causes further decoupling from adenylyl cyclase and promotes removal of the receptors from the cell surface (internalisation) for subsequent recycling or degradation (Anderson 2006). This loss of decoupling, desensitisation and receptor loss has the potential effect of decreasing the clinical response to a drug over time – a process known as tachyphylaxis. There are some suggestions that ICS may reduce tachyphylaxis; however, the evidence for this is mixed (e.g. Booth et al. 1996; Kalra et al. 1996; Tan et al. 1997, 1998; Seco et al. 2000).

Although β2-ARs on airway smooth muscle cells are the most important target for β-agonists in the treatment of asthma and COPD, β2-ARs also exist on other cell types that may have a role in asthma. These include airway epithelial cells, goblet cells (with their role in mucus production and transport), type II pneumocytes (cells in the alveolus that produce surfactant and regenerate alveolar cells after injury) and inflammatory cells, e.g. mast cells and eosinophils.

4 The Development of β-Agonists

β2-agonists play a pivotal role in the treatment of asthma. Their development, a global achievement, spans 120 years making them one of the oldest classes of drugs, but they are also one of the most widely prescribed classes of drugs in the world.

However, the development and clinical usage has been far from straightforward, with certain earlier treatments resulting in epidemics of asthma-related deaths. The real importance of β2-AR agonists is that they treat the end problem, bronchoconstriction, by causing a relaxation of airway smooth muscle, regardless of the contractile stimulus that caused it. Thus, regardless of initial stimulus or the inflammatory mediator or pathways that are involved, β2-agonists reverse the end response of bronchoconstriction (Waldeck 2002).

The chemical structures of compounds discussed below (sections 4-7) are shown in Fig. 2 and their properties summarised in Table 1.

4.1 Adrenaline: The First β-Agonist

The Discovery of Adrenaline

One of the first suggestions of the endogenous catecholamine adrenaline being useful in asthma was a comment by the English doctor Henry Salter in 1859 – "asthma is cured in situations of either sudden alarm or violent fleeting excitements" (Arthur 2015). However, the journey of β2-agonist development began in experiments with adrenal extract.

George Oliver, a GP from Yorkshire, UK, was experimenting with extracts from many different organs prepared for him by a local chemist (and potentially using his son as subject). He found that ingestion of sheep adrenal gland caused constriction of the radial artery (Oliver and Schäfer 1894; Tattersfield 2006). Together with Edward Schafer in London, laboratory animal experiments (using handmade apparatus) proved that adrenal medulla extract caused vasoconstriction and thus increased blood pressure and enhanced ventricular contraction (Oliver and Schäfer 1895; Barcroft and Talbot 1968; Goldstein 2006; Arthur 2015; Ball and Featherstone 2017). Their findings were confirmed independently and almost simultaneously in Poland (Szymonowicz and Cybulski 1895; Millar 1955). Efforts then concentrated on purification of the active substance.

Although Oliver and Schafer had established that an active compound from the adrenal medulla caused vasoconstriction, they did not give the specific entity a name (Oliver and Schäfer 1895).

A German chemist, S Frankel, isolated a substance from the adrenal extract and called it "syphgmogenin" (Fränkel 1897). An Austrian physician Otto von Furth also isolated a similar substance and called it "suprarenin" (Von Fürth 1900). The American biochemist and pharmacologist John Jacob Abel first named it "epinephrine" in 1897 (Abel and Crawford 1897), although the extraction method was not yet perfected and the chemical entity was yet to be established (Tansey 1995). Abel did isolate a substance, referred to in his publications as "epinephrine", that was actually the inactive benzoylated derivative (Goldstein 2006; Aronson 2000). Neither "suprarenin" nor "epinephrine" had the same activity as the crude extract from the adrenal medulla.

After a visit to Abel's laboratory, the Japanese chemist Jokchi Takamine overcame the problems of purification and contamination (Goldstein 2006) and

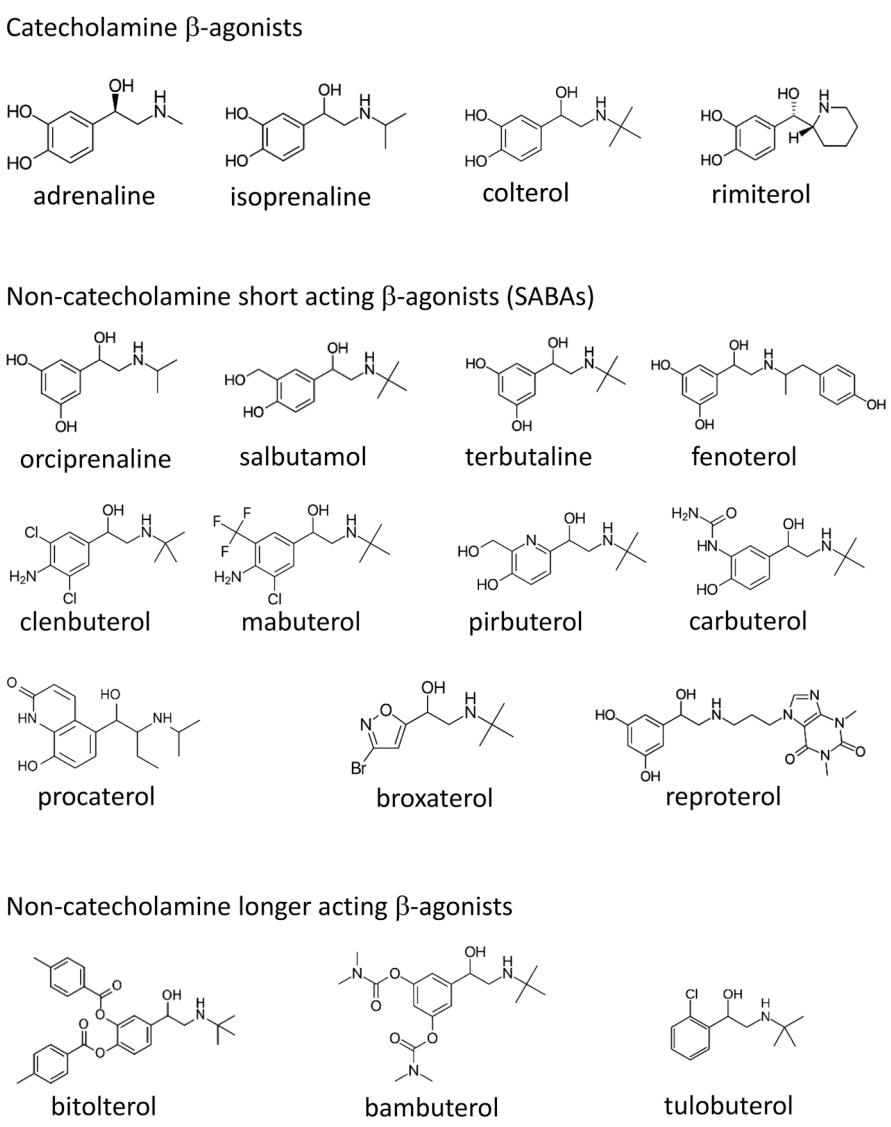

Fig. 2 The chemical structures of the β-agonists discussed in sections 4-7 developed for asthma and COPD

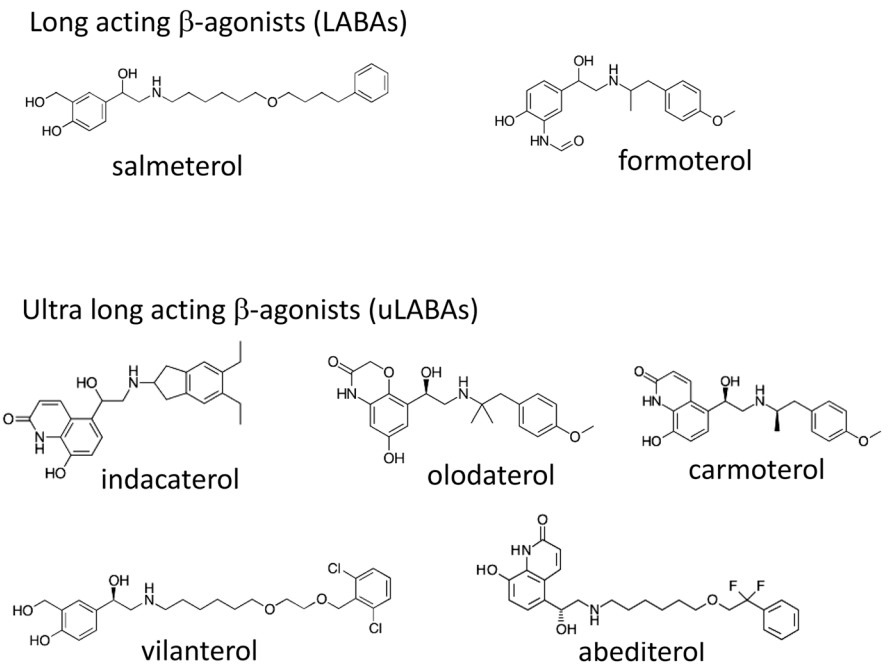

Fig. 2 (continued)

isolated a pure crystalline substance from the adrenal medulla in 1901 (Fig. 2). It was 2,000 times more potent than suprarenin or epinephrine, and he called it "adrenaline" (Yamashima 2003; Ball and Featherstone 2017). Aldrich determined its formula (Aldrich 1901) and it was patented by American company Parke-Davis and Company (Arthur 2015). He patented the techniques and arranged for the America company Parke-Davis and Company to market it commercially in the USA and Japan under the trade name "Adrenalin".

A dispute about names then arose when Henry Dale, a British pharmacologist wished to publish his findings using the name adrenaline. He argued that epinephrine (Abel's non-pure inactive extract) and adrenaline (the commonly accepted term for the isolated active compound) were not the same chemical. After a lengthy scientific and personal debate with his employer, Henry Wellcome and Wellcome Physiological Research Laboratories, that involved legal issues surrounding a compound's chemical name vs a registered trademark (Tansey 1995), Dale used the term adrenaline in his publications (Dale 1906). Today, adrenaline and epinephrine are largely used interchangeably, although it has remained "adrenaline" in most countries, but "epinephrine" in the USA, Canada and Japan, to differentiate it from commercialised "Adrenalin" (Aronson 2000).

Recently, as part of an effort to move to international non-proprietary names, there was a move to replace adrenaline with the term epinephrine worldwide.

Table 1 β2-adrenoceptor (AR) agonists developed for use in asthma and COPD

Name	Other names	First made/ published/patent	In clinical use	Pharmacological properties	Used today
Short-acting β-AR agonists (SABAs)					
Adrenaline	Epinephrine Suprarenin syphgmogenin	1901	1903	Endogenous hormone Non-selective α and β-AR agonist High efficacy full agonist COMT sensitive	[a]
Isoprenaline	Isoproterenol	1940	1948	Non-selective β-AR agonist High efficacy full agonist COMT sensitive	[b]
Orciprenaline	Metaproterenol	1961	1961	Non-selective β-AR agonist High efficacy full agonist	
Rimiterol	WG253 R789	1971		Moderately β2-AR selective agonist High efficacy full agonist COMT sensitive	
Salbutamol	Albuterol AH3365 SCH13949	1966	1969	Moderately β2-AR selective agonist Partial agonist Remains main rescue β2-AR agonist in inhalers and nebulisers	Asthma COPD
Terbutaline	KWD2019	1966	1970	Moderately β2-AR selective agonist Partial agonist	Asthma COPD
Fenoterol	Th1165a	1962	1971	Moderately β2-AR selective agonist High efficacy full agonist	
Reproterol	D-1959	1965	1977	Orciprenaline-theophylline fusion monomolecule	
Clenbuterol	NAB 365	1967	1977	Moderately β2-AR selective agonist Partial agonist Anabolic effects (increases muscle mass)	
Pirbuterol	SC-10049	1961 (patent 1971)	1983	Moderately β2-AR selective agonist Partial agonist	

(continued)

Table 1 (continued)

Name	Other names	First made/ published/patent	In clinical use	Pharmacological properties	Used today
Carbuterol	SK&F 40383-A	1970		β-AR agonist partial agonist	
Procaterol	OPC-2009	1974	1980	Highly β2-AR selective agonist Partial agonist Longer duration of action, but multiple dosing still required	
Broxaterol	Z1170	1980s		β-AR agonist, helpful in studies in asthma. Never widely used	
Mabuterol		1980s		Derivative of clenbuterol Moderately β2-AR selective agonist	
Longer acting ligands					
Bitolterol	WIN32784	1976	1984	Inactive molecule – prodrug of colterol Esterase activity needed to remove toluate groups to release active colterol Colterol = moderately β_2-AR selective full agonist catecholamine, sensitive to COMT	
Bambuterol		1984		Inactive molecule – prodrug of terbutaline Requires esterase activity to become active terbutaline, giving longer duration of action	Asthma COPD
Tulobuterol	C-78	1975		Moderately selective β2-AR agonist Partial agonist Skin patch (launched 1998) gives long duration of action	Asthma COPD
Long-acting β-AR agonists (LABAs)					
Salmeterol	SN-408	1983	1990	Highly β2-AR selective agonist Low efficacy partial agonist Salbutamol head group with hydrocarbon chain Long duration of action due to binding to unique exosite on β2-AR and high lipophilicity with membrane deposition (microkinetic diffusion theory)	Asthma COPD

Formoterol	BD 40A	1972	1986	Highly β2-AR selective agonist High efficacy agonism (below that of catecholamines) Long duration of action due to high lipophilicity with deposition in membrane (microkinetic diffusion theory)	Asthma COPD
Ultra-long-acting β-AR agonists (uLABAs)					
Indacaterol	QAB149	2000s	2009	Moderately β2-AR selective agonist High efficacy agonist Highly lipophilic with membrane diffusion (microkinetic theory)	COPD
Vilanterol	GW642444M	2000s	2013	Highly β2-AR selective agonist Partial agonist Structurally similar to salmeterol with salbutamol head group and hydrocarbon chain	COPD
Olodaterol	BI-1744 CL	2000s	2013	Moderate to highly β2-AR selective agonist High efficacy agonist – similar to formoterol	COPD
Abediterol	LAS100977 AZD-0548	2000s		Moderately β2-AR selective agonist	
Carmoterol	TA-2005 CHF-4226	1990s		Moderately β2-AR selective agonist High efficacy full agonist Not clinically developed	

[a] Adrenaline is still used today in anaphylaxis (intramuscular injection), during resuscitation from cardiac arrest (intravenous injection), and as an intravenous infusion in shock (e.g. septic shock) in the intensive care setting

[b] Isoprenaline is still used as an intravenous infusion for short-term relief of bradycardia or heart block

However, Jeffery Aronson's argument to keep the term adrenaline (from many perspectives – historical, etymological, general usage and clinic safety risk) is compelling (Aronson 2000).

Adrenaline for Asthma

At the time, the breathlessness of asthma was thought to be caused by turbid mucus blocking airways and swelling of the bronchial mucosa. Adrenal extract was useful for allergic rhinitis, hives, and decreasing bleeding during eye and nasal surgery, and so it was thought that a vasoconstrictor would reduce mucus production and swelling of the bronchial mucosa thus relieving the breathlessness in asthma (Arthur 2015; Ball and Featherstone 2017). Solomon Solis-Cohen tested adrenal extracts on patients with hay fever (including himself: Solis-Cohen 1898) and in those with asthma, and found that desiccated adrenal glands relieved symptoms (Solis-Cohen 1900; Waldeck 2002; Tattersfield 2006; Arthur 2015). In 1903, there are 2 reports of adrenaline being useful in asthma. James Burnett (Edinburgh, UK) reported the benefit of adrenaline as a "bronchodilator" therapy in asthma (Burnett 1903; Crompton 2006; Youssef et al. 2016). Jesse Bullowa and David Kaplan (New York, USA) reported the administration of subcutaneous injection of adrenaline to a patient with acute severe asthma, who felt dramatic relief within minutes (Bullowa and Kaplan 1903). But it was not until 1907 when Khan demonstrated that adrenaline caused relaxation of the smooth muscle in bronchi, hence causing bronchodilation and thus relieving the breathless with asthma, that understanding of the role of bronchial smooth muscle in asthma started to emerge (Kahn 1907).

The German chemist Fredrich Stolz and British chemist Henry Dakin independently synthesised adrenaline in 1904 (Stolz 1904; Dakin 1905). Large-scale production of adrenaline, and its subsequent distribution as a drug, was made possible following scale-up synthetic methods further developed by Stolz in 1906, and the development of glass ampoules by Parke-Davis and Company in 1909 (Arthur 2015). Adrenaline was then used in severe asthma, and following exploratory oral administration (which was ineffective), subcutaneous, intramuscular and intravenous injections, hypodermic/subcutaneous injection became the main route of administration (Arthur 2015), alongside the other asthma treatments of the day that included asthma cigarettes (containing stramonium or belladonna), cocaine and incense (Anderson 2005; Jackson 2010). Subcutaneous injections of adrenaline provided relief from asthma symptoms within minutes and were recommended by Brian Melland (Lancet 1910) and in James Adam's 1913 textbook "Asthma and its Radical Treatment".

Adrenaline however had significant drawbacks – it caused hypertension, tachycardia and tremor. It was short-acting, metabolically unstable, was rapidly metabolised by the enzyme catecholamine o-methyltransferase (COMT) and had to be given by injection as it was inactive by mouth (Waldeck 2002). Although Barger and Dale first used adrenaline as an aerosol in 1910 (Barger and Dale 1910), it was not until the evaluation and recommendation of adrenaline inhalation (Camps 1929) and the development of commercially available nebulisers (e.g. Pneumovac and DeVilbiss No.40) that adrenaline was regularly administered as an aerosol (as a

1:50 solution; Rau 2005; Arthur 2015), both in the doctor's surgery and by patients at home. Adrenaline is still used in the treatment of acute anaphylaxis (which can cause bronchospasm) and in (cardio-)respiratory arrest that may result from acute asthma, but is no longer a first-line therapy in acute asthma (Baggott et al. 2022).

4.2 Isoprenaline (Including Affinity, Intrinsic Efficacy and Selectivity)

The Discovery of Isoprenaline

The next pharmacological step towards β-agonists was isoprenaline (Konzett 1940a, b; Fig. 2). Although adrenaline analogues existed (e.g. phenylephrine), they, like adrenaline, had significant pressor effects (increased blood pressure). Ephedrine is the active ingredient of an old Chinese anti-asthma drug, from the herb *ma huang*, structurally related to adrenaline, that was used in the West for the treatment of asthma in the 1920s. It has a very poor bronchodilator effect because of limited affinity for the β-ARs (Waldeck 2002; Baker 2010). A group of Austrian pharmacologists noted that large substitutions on the amino group of ephedrine reduced effects on blood pressure but left the inhibitory effect on intestinal muscles unchanged (Konzett 1981). They surmised that similar amino group substituents on adrenaline (active in asthma) might have similar effects (Konzett 1981). Heribert Konzett and Richard Rossler developed a method for measuring pharmacological effects on bronchial smooth muscle (Konzett and Rössler 1940) and in collaboration with chemists from CH Boehringer Sohn in Ingelheim, Germany, demonstrated derivatives of adrenaline that retained bronchodilator activity but with less effect on blood pressure, in particular the compound with the amino isobutyl substitution, that became known as isoprenaline (Konzett 1981). It was developed as an aerosol for use in asthma and delivered using the Aerohaler dry powder inhaler first commercialised in 1948 (Stein and Thiel 2017). Isoprenaline was effective when given orally, sublingually, subcutaneously or via a handheld nebuliser, although palpitations were common (Gay and Long 1949; Tattersfield 2006).

The nebulisers of the day were made of fragile glass, powered by a rubber squeeze bulb. In April 1955, a 13-year-old girl called Suzie asked her father (the then President of Riker Laboratories) why she couldn't have her asthma medication in a spray-can like hairspray. Within 2 months, Riker Laboratories began testing metered dose inhalers (MDIs). In March 1956, MDIs containing adrenaline and isoprenaline were launched. By December 1956, the first clinical trial of the MDI was published, demonstrating relief of symptoms including in those who had failed to response to other treatments (Freedman 1956; Stein and Thiel 2017). The modern inhaler was born.

Affinity, Intrinsic Efficacy, Selectivity and the Pharmacological Importance of Isoprenaline

Pharmacologically, agonist molecules have two important properties – affinity (ability to bind to a receptor) and efficacy (ability to stimulate an agonist response).

The ability of a molecule to induce a response is known as intrinsic efficacy (Furchgott 1966; Clarke and Bond 1998; Kenakin 1999a, b, Strange 2008; Baker 2010). Antagonists (molecules with affinity but no intrinsic efficacy) can be selective if they have higher affinity for one receptor type over another. Although, theoretically, agonist ligands could have selectivity either from selective affinity or from selective intrinsic efficacy, most β-AR agonists to date (including the endogenous hormones adrenaline and noradrenaline) have selectivity because of selective affinity for one receptor subtype over another (Baker 2010).

Isoprenaline, similar to adrenaline and noradrenaline, has relatively low affinity for the β-ARs, but stimulates highly potent agonist responses. It needs to occupy very few receptors in order to stimulate a maximum response and is therefore a low affinity but high intrinsic efficacy agonist (Baker 2010). Clinically, however, given its high efficacy and lack of β-AR selectivity, isoprenaline also readily stimulates β1-ARs in the heart, causing tachycardia. As it is also a catecholamine, it is short acting due to rapid metabolism by COMT (Plummer 1978).

Isoprenaline was an important development being a bronchodilator without increasing blood pressure. At this time (1940), nothing about AR subtypes was known, beyond the existence of a generic "adrenoceptor". Adrenaline (and noradrenaline) activate all 9 AR subtypes (α1A, α1B, α1D, α2A, α2B, α2C, β1, β2 and β3). Although isoprenaline can still activate α-ARs with a high intrinsic efficacy, isoprenaline preferentially activates β-ARs because it has higher β than α-AR affinity (Baker 2010; Proudman et al. 2022).

Clinically this was important – the increased β2-affinity resulted in a bronchodilation but with reduced β1-mediated cardiovascular effects. Pharmacologically, isoprenaline was an important turning point. The development of ligands that stimulated some physiological effects more than others paved the way for the separation of the α and β-AR by the American pharmacist, Raymond Ahlquist (1948), but it was not until studies in the 1960s, looking at adrenaline, isoprenaline and noradrenaline, that Lands and colleagues in New York proposed the existence of two types of β-AR – the β1 in heart and β2 in bronchioles (Lands and Brown 1964; Lands et al. 1967a, b).

4.3 The First Epidemic of Asthma Deaths

The rapid symptomatic relief provided by adrenaline and isoprenaline MDIs was much appreciated by patients with asthma and use rocketed. They were introduced into England and Wales in 1960, gaining wide acceptance by 1961 (Speizer et al. 1968a). For a while, adrenaline and isoprenaline inhalers were available over-the-counter in the UK (as well as being on prescription) and from 1959 to 1965 usage of isoprenaline inhalers rose 600% (Crompton 2006). However, there was a dark side to this success.

In 1948, Benson and Perlman recorded the fatality rates in their 2236 patients with asthma. They reported a 7.4% fatality rate (48/648) in those who used "adrenaline oral spray" vs 1.4% fatality rate (22/1588) in those who did not (Benson and

Perlman 1948; Rau 2005). Gay and Long (1949) had reported 90% of patients had palpitations with isoprenaline and that oral and subcutaneous administration was associated with signs of coronary ischaemia (Gay and Long 1949; Tattersfield 2006). In the mid-1960s, letters started to appear in medical journals. McManis reported three asthma deaths in Australia that appeared to be due to cardiac arrest after adrenaline (in Kerr 1967). Greenberg and Pines (1967) described four patients who were found unexpectedly dead at home or work with an empty inhaler in hand and eight hospital deaths following asthma treatment when tachycardia (and even ventricular tachycardia with multiple ectopic beats) was recorded before death. Exon described repeated atrial flutter that was linked to excessive adrenaline inhaler use (Exon 1967). Graham (1968) reported two unexplained asthma deaths, both found with inhaler in hand and reported previous overuse. An increase in asthma deaths was starting to be noticed and a link between this and β-agonists use was suggested in England, Wales and Scotland (Greenberg 1965; Smith 1966; Kerr 1967).

In the 1960s there was a surge in asthma deaths. In England and Wales, between 1959 and 1965 there was 3.5-fold increase in asthma deaths in the 5–34 age group (from 0.66 to 2.18 per 100,000 population). This increase was highest in the 10–14 age group (sevenfold; 0.33 to 2.46 per 100,000 population; Speizer et al. 1968a; Inman and Adelstein 1969). Various hypotheses were suggested – a recent change in diagnostic criteria for asthma, environmental hazards (including pollution), use of corticosteroids, but the temporal relationship with a surge in the use of inhaled isoprenaline was clear. Increases in death rates were also being reported elsewhere in the world especially Scotland, Ireland, Norway, Australia and New Zealand, all of which (including England and Wales) had access to the stronger "isoprenaline forte" inhaler (Pearce 2007), although small increases were seen elsewhere (Speizer et al. 1968a, b; Speizer and Doll 1968). If the adrenaline and isoprenaline inhalers were to blame, several contributing factors were considered: a delay in seeking medical attention due to a belief that the inhaler would bring relief; excessive inhaler use (two canisters a day, causing desensitisation/tachyphylaxis); isoprenaline (or adrenaline) toxicity (systemic absorption causing "ventricular irritability" and fatal arrhythmias, likely made worse by the hypoxia of severe asthma); propellant allergy; an underestimation of asthma severity (with the "patient may have been living closer to their limit of ventilatory reserve") and bronchodilation exposing the patient to more allergen (Speizer et al. 1968a, b; Conolly et al. 1971; Waldeck 2002; Tattersfield 2006; Pearce 2007).

In 1967, the UK Committee on the Safety of Medicines warned of the potential danger of excessive use of inhaled isoprenaline. This had several effects. The sales of inhalers decreased dramatically (and non-prescription sale was banned; Inman and Adelstein 1969; Esdaile et al. 1987), but it also reminded clinicians and patients of the importance of better long-term asthma management, the need to seek medical help during an exacerbation and that diminishing response to the inhaler should be interpreted as heralding an asthma attack (Crompton 2006). There was a 100% increase in the number of people admitted to hospital for asthma the following year and increased use of corticosteroids (Crompton 2006). After March 1967, there

was a profound fall in the number of UK asthma deaths. It is estimated that there were 3,500 excessive deaths from asthma in England and Wales in the 1960s (Inman and Adelstein 1969). However it has never been proved whether it was the actual drugs that caused the deaths (via adrenaline or isoprenaline "toxicity" causing arrhythmia or desensitisation and tachyphylaxis), or the indirect problems of over-use/over reliance of the new inhalers combined with a lack of urgency of seeking medical help, and/or underuse of steroids (Crompton 2006; Inman and Adelstein 1969; Esdaile et al. 1987).

4.4 Orciprenaline and Rimiterol

Orciprenaline (metaproterenol, Fig. 2) was another β-agonist developed by Boehringer Ingelheim, launched in 1961. It has a slower onset of action, but as it is not metabolised by COMT, it has greater stability and thus a longer duration of action than isoprenaline (Engelhardt et al. 1961; Kennedy and Jackson 1963; Chahl and O'Donnell 1968). It is a full, high efficacy agonist similar to isoprenaline and adrenaline, although less potent due to lower receptor affinity (Engelhardt et al. 1961; Chahl and O'Donnell 1968; Baker 2010). It was also available as an inhaler at the same time as isoprenaline, and although sales were much lower, it too was linked with the asthma death epidemic (Inman and Adelstein 1969). It was briefly available in the USA again as a non-prescription drug for 2 months in 1983 before safety issues overcame the marketing and the FDA again banned non-prescription sales (Pearce 2007). Palpitations and tachycardia (including arrhythmias; Beumer 1983) were the biggest side effects and it was formally withdrawn from the UK market by the MHRA in 2010 (MHRA Public Assessment Report 2009).

Rimiterol (R789/WG253; Riker; Fig. 2) is another catecholamine (Griffin and Turner 1971). It is a fast onset bronchodilator, similar to isoprenaline, when administered intravenously or by inhaler and is not active orally. It appeared more β2-selective than isoprenaline, having less cardiac effects, although it is rapidly degraded by COMT giving it a shorter duration of action than other moderately β2-selective agents developed around the same time (e.g. salbutamol and terbutaline; Phillips et al. 1972; Bianco et al. 1975; Marlin and Turner 1975; Paterson et al. 1975; Eriksson and Lindgren 1978; Tarala et al. 1981).

4.5 Salbutamol and Terbutaline

Drug development then concentrated on generating drugs that retained significant bronchodilator effects, but with less effects on the heart. Two groups independently developed related compounds simultaneously – salbutamol, by a British group (Brittain et al. 1968; Cullum et al. 1969 at Allen and Hanbury, patented in 1966 and entered clinical use in 1969; Fischer and Ganellin 2006) and terbutaline, by a Swedish group (Wetterlin and Svensson 1968; Bergman et al. 1969; Persson and Olsson 1970 at AstraZeneca, patented in 1966, entered medical use in 1970; Fischer

and Ganellin 2006; Fig. 2). They are both non-catechol derivatives of adrenaline, with similar pharmacological actions. Because they are resistant to COMT, they are metabolically more stable than adrenaline or isoprenaline promoting both oral bioavailability and a longer duration of action (Waldeck 2002). Both are highly water soluble and have a fast onset of bronchodilator activity (Lötvall 2001).

Clinically, salbutamol and terbutaline caused bronchodilation (in tissues, and whole animals when given orally, subcutaneously or by aerosol) that was more sustained than isoprenaline or orciprenaline, with less effect on blood pressure and heart rate (Brittain et al. 1968; Cullum et al. 1969; Bergman et al. 1969; Persson and Olsson 1970). Studies in people with asthma also noted a longer duration of action for salbutamol than isoprenaline, with less increase in heart rate (Choo-Kang et al. 1969; Kelman et al. 1969; Mattila and Muittari 1969; Palmer and Diament 1969; Tattersfield and McNicol 1969; Arner 1970; Arner et al. 1970; Warrell et al. 1970; Legge et al. 1971), although tremor and tachycardia remained the biggest side effects.

Pharmacologically, salbutamol and terbutaline are similar: both have moderate (20–40-fold) higher affinity for the β2 over the β1-AR (Baker 2010). They are both partial agonists with respect to isoprenaline. This means their signal coupling is not as good as adrenaline and isoprenaline and they need to bind to more receptors to stimulate the same level of response, i.e. they have a lower intrinsic efficacy. In many tissues, there are not enough β2-ARs for salbutamol or terbutaline to generate a full response, even with full β2-AR occupancy. Thus, the response is lower, or partial, in relation to adrenaline and isoprenaline. This partial agonist response means that salbutamol and terbutaline have less potential for receptor internalisation and desensitisation (Lipworth and Grove 1997; January et al. 1997, 1998; Baker et al. 2003) and are therefore less likely to cause tachyphylaxis.

Today, salbutamol, being a fast onset and effective bronchodilator, is still the main rescue medication used during exacerbations of asthma, either as the well-known "blue inhaler" used by millions every day or as a nebuliser in more severe exacerbations in the emergency department. It is on the World Health Organisation's list of essential medicines (WHO 2019) and was the 7th most commonly prescribed medication in the USA in 2020 (Drug Usage Statistics. ClinCalc. https://clincalc.com/DrugStats/Drugs/Albuterol). Whilst inhalation minimises side effects compared to all other methods of delivery, salbutamol is directly absorbed through the alveolar epithelium into the pulmonary vasculature, and thus into the systemic circulation. It has a plasma half-life of 4–6 h (Starkey et al. 2014; Unwalla et al. 2012). The most common side effects from this systemic exposure are tremor and tachycardia.

4.6 Fenoterol

Fenoterol (Th1165a; Fig. 2), a derivative of orciprenaline, was patented in 1962 (Boehringer Ingelheim, Fischer and Gannelin 2006), evaluated in people with asthma a little later (Mattila et al. 1967; Minette 1970; Waldeck 2002) and licenced

for medical use in 1971. It had three potential improvements over isoprenaline. Firstly, fenoterol has a longer duration of action than isoprenaline because it is not metabolised by COMT (Bäcklund and Fagerberg 1968; Beardshaw et al. 1974; Pennock et al. 1977; Steen et al. 1977). Secondly, although it is an equally efficacious bronchodilator stimulating maximal tracheal relaxation (compared with isoprenaline and orciprenaline), it was more potent (i.e. needed less drug dosing to achieve the same effect). Thirdly, it had some tracheal vs atrial selectivity (i.e. β2-selectivity; O'Donnell 1970, 1972). In those with asthma, fenoterol, given by inhalation, increased heart rate but this was less than that for isoprenaline (relative to bronchodilation) and thus fenoterol was thought to have less cardiovascular side effects (Cohen 1978). Interestingly, a decline in the fenoterol-induced improvement in FEV_1 and duration of action over 3 months was also noted suggesting possible tachyphylaxis (Plummer 1978). Others noted worse asthma control longer term, when fenoterol was given regularly compared with placebo (Sears et al. 1990). Plummer's (1978) reassessment of salbutamol and terbutaline trials points out that a degree of tachyphylaxis was seen in some of these too, although the clinical significance of the modest reductions was not clear.

However, significant tachycardia remained a problem in some subjects (Beardshaw et al. 1974). Fenoterol demonstrated more tachycardia than terbutaline (a partial agonist) or adrenaline (maybe as adrenaline is metabolised quickly by COMT) for the same degree of bronchodilation (Da Costa and Goh 1973). Compared with tulobuterol (see below), fenoterol was associated with more changes in heart rate and blood pressure (including severe tremor, tachycardia and sweating; Werdermann 1990) and more frequent rescue salbutamol usage (possibly due to tachyphylaxis, Sanchez et al. 1988). Plummer (1978) reported nervousness and tachycardia were common side effects of fenoterol (occurring in 10 out of 14 of subjects). Later studies confirmed more tachycardia and systemic effects with fenoterol compared to salbutamol (e.g. Bremner et al. 1992a, 1993; Scheinin et al. 1987; Crane et al. 1989b; Wong et al. 1990). The detrimental cardiovascular effects of fenoterol appeared significantly worse during hypoxia, which may occur as part of the asthma or COPD exacerbation when β-agonists are used at higher concentrations. The combined detrimental effect of β-agonists in hypoxia was shown in dogs – high doses of isoprenaline when breathing air were tolerated, but under hypoxic conditions fatal cardiac depression occurred with considerably lower isoprenaline doses (Collins et al. 1969; McDevitt et al. 1974). Studies in man also suggest fenoterol had more cardiovascular effects under conditions of hypoxia (Bremner et al. 1992b) with concerns raised about the cardiac safety of fenoterol use in the context of asthma-induced hypoxia (Kiely et al. 1995). In a cross-over study of 15 patients, inhaled fenoterol induced more tachycardia than salbutamol for similar bronchodilation. Four patients receiving fenoterol had ventricular dysrhythmias requiring suspension from the study, and 13/15 had side effects of tremor, palpitations, headaches and sweating compared to 5/15 after salbutamol prompting the author to conclude "salbutamol may be a safer drug than fenoterol" (Tandon 1980). Similar findings were reported by others (Bremner et al. 1992a).

Pharmacologically, fenoterol is a high efficacy agonist, similar to adrenaline, noradrenaline, isoprenaline and orciprenaline. It has substantially higher ability to induce responses (intrinsic efficacy) than salbutamol or terbutaline (O'Donnell and Wanstall 1978; Delhaye et al. 1983; Crane et al. 1989b; Bremner et al. 1996; January et al. 1997; Baker 2010). It does cause potent β2-responses because it has 100-fold higher affinity for the β2-AR than the β1 (it is an affinity selective, highly efficacious β2-agonist, Baker 2010). It also causes more receptor phosphorylation, desensitisation and internalisation than salbutamol (January et al. 1997). Its high efficacy at the β1, β2 and β3-AR is the same as that for adrenaline, making it one of the most efficacious non-catecholamine β-agonists available (Baker 2010).

4.7 The Second Epidemic of Asthma Deaths

In the 1970s, there was an increase in sudden asthma deaths in New Zealand. The epidemic started in 1976 and by 1979 the asthma mortality had increased from 1.4 to 4.1 per 100,000 in the 5–34 age group (Pearce 2007). In a report of 22 cases, the 16 who died suddenly (walking or talking until a few minutes before death) ten had been using fenoterol, three salbutamol and three no or unknown β-agonist (Wilson et al. 1981). Initially it was thought this may be due to an additive effect between β-agonists and theophylline (both of which increase cAMP) causing cardiac arrest (Wilson et al. 1981). In 1985 an asthma researcher in New Zealand, Julian Crane, took fenoterol after developing wheeze from a cold. He started shaking and his heart raced. He had already noted that there appeared an excess of fenoterol users in Wilson et al.'s study and became interested in the difference in side effects between different β-agonists (Pearce 2007). In 1989, a case–control study of 5–45 year olds concluded that the epidemic of asthma death happening in New Zealand was due to unsupervised self-administration of fenoterol in people with severe asthma (Crane et al. 1989a). This study was confirmed by two more New Zealand studies (Pearce et al. 1990; Grainger et al. 1991). New Zealand was unusual in that fenoterol was more widely used with, by 1979, about 30% of β-agonist inhalers being fenoterol compared with <5% in most other countries and it was not licenced in the USA (Pearce 2007). In December 1989, the New Zealand Department of Health issued a warning about fenoterol and advised withdrawal. This was followed by a fall in the asthma mortality rate to pre-fenoterol levels (Pearce et al. 1995). Later, fenoterol was also linked to an increase in asthma deaths in Canada (Spitzer et al. 1992). Just as with the first outbreak of asthma deaths and isoprenaline in the 1960s, the cause of the New Zealand outbreak and its link with fenoterol remains contested (Garrett et al. 1996; Rea et al. 1996; Lanes et al. 1997; Suissa and Ernst 1997).

4.8 Other Short-Acting β2-Agonists: Reproterol, Clenbuterol, Mabuterol, Pirbuterol, Carbuterol, Procaterol, Broxaterol

Reproterol

Reproterol (D-1959; Degussa, Fig. 2) was patented in 1965 and came into medical use in 1977. It is a fusion between orciprenaline and theophylline, a phosphodiesterase (PDE) inhibitor that reduces cAMP breakdown and so further increases cAMP levels in cells (Klingler 1977; Habersang et al. 1977). In theory, this dual action could improve the therapeutic effects of orciprenaline. In mastocytoma cells, reproterol was more potent than theophylline at inhibiting PDE (Alvarez-Guerra et al. 2004). In monocytes, reproterol caused more cAMP production than orciprenaline or theophylline alone and this cAMP response was only partially inhibited by the β-blocker propranolol, suggesting a synergistic mode of action of β2-agonism and PDE inhibition. Reproterol and theophylline (but not orciprenaline) caused inhibition of the inflammatory mediator leukotriene B4 suggesting that this was through PDE inhibition, rather than β2-stimulation (Juergens et al. 1999; Virchow 1999). In clinical studies, reproterol was an effective bronchodilator when given, both orally and inhaled, with an overall effect greater response than orciprenaline. An alternative explanation suggested that the large xanthine side chain impaired the metabolism of reproterol rather than acting as a PDE inhibitor as the xanthine concentration is likely too low for clinical effect (Patchett et al. 1985; Alvarez-Guerra et al. 2004). Some studies suggested it has few or no cardiovascular effects, although others actively used it to increase heart rate in those with bradycardia (Diewitz 1977; Mándi et al. 1977a, b; Tabori et al. 1977; Patchett et al. 1985). With regard to its β2-agonist action, fenoterol stimulated a greater overall cAMP response than reproterol or salbutamol (Juergens et al. 2004). Weight for weight after nebulisation, reproterol was 12 times less potent than salbutamol (Foster et al. 1991). It was used as a combination treatment with cromoglycate as a combined bronchodilatory and anti-inflammatory "disease-modifying" medication in asthma (Virchow 1999).

Clenbuterol

Clenbuterol (NAB 365, Boehringer Ingelheim, Fig. 2) was patented in 1967 and came into medical use in 1977. It is also resistant to COMT activity so has a longer duration of action. Similar to salbutamol, it has a fast onset of bronchodilator action when given orally or by inhalation, but a longer duration of action (Engelhardt 1972, 1976; Salorinne et al. 1975; Anderson and Wilkins 1977; Kamburoff et al. 1977; Pasotti et al. 1979). It has 20-fold β2 vs β1 selective affinity and is of lower efficacy than isoprenaline (O'Donnell 1976; Baker 2010). However, clenbuterol also causes skeletal muscle hypertrophy, preventing protein breakdown and decreasing fat deposition. This increase in lean muscle mass is popular amongst bodybuilders, athletes and in the farming industry (including cows, sheep, chickens and horses: Baker et al. 1984; Ricks et al. 1984; Prather et al. 1995; Spann and Winter 1995; Kearns and McKeever 2009). It is still used illicitly, either intentionally for muscle gain or more recently inadvertently with adulterated heroin and can result in

tachycardia, arrhythmias, myocardial infarction, agitation, tremor and electrolyte disturbances (Hoffman et al. 2008; Barry and Graham 2013; Hieger et al. 2016). Harm has also been reported from people consuming liver and meat from clenbuterol-treated cattle (e.g. 125 individuals from 43 families who had eaten contaminated meat (97% of those who ate the meat) had symptoms of tremor, palpitations, tachycardia, nervousness, headache and myalgia that lasted for an average of 40 h; Martínez-Navarro 1990). Veterinary use of β-agonists (including clenbuterol) is not permitted (except for treating COPD in horses) and meat monitoring programmes are in place (Kuiper et al. 1998). Clenbuterol is on the International Olympic Committee and World Anti-Doping Agency (WADA) prohibited list (Barry and Graham 2013; Geyer et al. 2014). The use of β2-agonists remains restricted in elite athletes, with only inhaled salbutamol, salmeterol and formoterol being permitted (Fitch 2016).

Mabuterol

Mabuterol (Fig. 2) is a derivative of clenbuterol. In animals, mabuterol was a bronchodilator and by comparing bronchodilation and cardiac stimulation, appeared to have more β2-selectivity than clenbuterol and salbutamol (Engelhardt 1984; Krüger et al. 1984; Murai et al. 1984). Initial studies suggested it was also an effective bronchodilator in people with asthma (Ulmer et al. 1984; Kawakami 1984).

Pirbuterol

Pirbuterol (SC-10049; Pfizer, Fig. 2) is a non-catecholamine β2-agonist, structurally similar to salbutamol (with pyridine ring rather than benzene ring). It was patented in 1971 and came into clinical use in 1983 (Fischer and Ganellin 2006). It is an effective bronchodilator when given orally, intravenously or by inhalation in animals and man and seemed well tolerated over months. It has a similar pharmacological profile to salbutamol – a moderately β2-selective compound with lower efficacy and less cardiovascular effects than isoprenaline, and longer duration of action than isoprenaline or orciprenaline (Van Arman et al. 1961; Steen et al. 1974; Willey et al. 1976; Burki and Diamond 1978; Moore et al. 1978; Ence et al. 1979; Beumer 1979a, b, 1983; Dyson and MacKay 1980; Kenakin and Beek 1984; Richards and Brogden 1985). However, nervousness, tremor and increased heart rate were common side effects. It was briefly investigated for its positive β-agonist inotropic effects in people with heart failure before being found ineffective (and potential detrimental; Packer 1989). It is now discontinued from clinical use in some countries (including US).

Carbuterol

Carbuterol (SK&F 40383-A, SmithKline and French, US, Fig. 2) was another non-catecholamine β-agonist developed for asthma. Like salbutamol, it was active given orally and by inhalation and was effective in adults and children. It had more bronchodilation relative to cardiovascular stimulation, suggesting β2-selectivity, and a longer duration of action than isoprenaline (Wardell et al. 1974; Saleeby and Ziskind 1975; Rhoades et al. 1976; Colella et al. 1977). Similar to salbutamol,

most reports suggest that carbuterol is a partial agonist in relation to isoprenaline, causing similar metabolic and heart rate changes to salbutamol (Minette et al. 1976; Drachler et al. 1977; Sanders et al. 1977; Beumer et al. 1978; Potter et al. 1980).

Procaterol

Procaterol (OPC-2009, Otsuka, Japan, Fig. 2) is a β-agonist developed in the 1970s (Yoshizaki et al. 1976; Yabuuchi et al. 1977). It was patented in 1974 and entered clinical use in 1980 (Fischer and Ganellin 2006). Animal studies suggested some β2-selectivity with more bronchodilation relative to cardiac stimulation. Bronchodilation with procaterol was of longer duration and the drug was more potent than salbutamol, with similar increases in heart rate, left ventricular contractile force, myocardial oxygen consumption and coronary blood flow (Himori and Taira 1977; Yabuuchi et al. 1977). Furthermore, although the β2-selectivity of procaterol and salbutamol appeared similar in cats (Yamashita et al. 1978), procaterol selectivity was six times greater than salbutamol in guinea pig (Yabuuchi 1977) and 60 times greater in dogs; Himori and Taira 1977), with an intrinsic efficacy less than adrenaline and isoprenaline (Kusayama et al. 1994). In pre-clinical studies, procaterol appeared to have a longer duration of action, but also potentially more β2-selectivity than the other β-agonists developed at the time.

Clinical studies showed that procaterol was an effective bronchodilator (Zanetti et al. 1982) when given orally and by inhalation, be that as an inhaler for regular use, or as a nebuliser in the emergency department. Although more potent, the bronchodilation induced by procaterol was similar to that obtained with salbutamol and terbutaline (Crowe et al. 1985; Dahl et al. 1985; Siegel et al. 1985; De Candussio et al. 1986; Ioli et al. 1986; Liippo et al. 1991; Mangunnegoro et al. 2011). Although some studies suggested a longer duration of action than salbutamol, procaterol still required three-times daily dosing (whether taken orally or by inhalation, Tukiainen et al. 1988; Mazza et al. 1992).

Later studies, after the cloning of receptors and development of recombinant techniques and direct measurements of receptor subtype selectivity, showed that procaterol has more β2-affinity selectivity at human receptors than salbutamol (salbutamol 20-fold β2-selective, procaterol 200-fold β2-selective) with lower efficacy than isoprenaline and adrenaline (Delhaye et al. 1983; Baker 2010). It was therefore the most β2-selective β-agonist developed at the time.

Broxaterol

Broxaterol (Z.1170; Fig. 2) was developed in Italy in the 1980s (Rampulla et al. 1985; Chiarino et al. 1986). It improved bronchoconstriction both after oral and inhaled administration in asthma, exercise-induced bronchospasm, allergen-challenge-induced bronchospasm and was effective in children (Bianco 1989; Löfdahl et al. 1989; Simone et al. 1990). Although there was a therapeutic window between bronchodilation and pulse and blood pressure changes, and metabolic changes were not reported (including up to 12 months), tremor was the most frequent side effect (Perruchoud et al. 1987; Chetta et al. 1988; Löfdahl et al. 1989; Ziment 1989; Petraglia et al. 1990). Pharmacologically it appeared to be a non-selective

partial agonist in some studies (human receptors; Hoffmann et al. 2004), but others found it to have some β2-selectivity (in rats; Sala et al. 1991).

Conclusions: Short-Acting β2-Agonists

Pharmacologically, salbutamol, terbutaline and the other β2-agonists above share very similar bronchodilator properties. They are fast onset (<4 min), short acting, with some β2 vs β1 selectivity and are less efficacious than the catecholamines (Johnson et al. 1993; Nials et al. 1993b), meaning in systems with lower receptor expression they are partial agonists relative to isoprenaline and adrenaline. Their fast onset of action makes them ideal for the emergency rescue of bronchospasm, however being short acting, they require frequent dosing to have a sustained effect. However overall, as discussed above, the other short-acting β2-agonists developed since have not led to any significant clinical gain over the original two 1960s compounds, salbutamol and terbutaline. These two, and in particular salbutamol, remain the main short-acting β2-agonist compounds in use for rescue therapy in asthma and COPD today. Furthermore, these partial agonists have not been associated with an increase in community asthma mortality (Mullen et al. 1993; Beasley et al. 1999).

5 A Step Towards Longer Acting Ligands: Bitolterol, Bambuterol, Tulobuterol

A significant problem with salbutamol and terbutaline is that they are both short acting, meaning frequent dosing in the daytime is needed to control symptoms and, unless the patient was woken for repeat dosing during the night, there is poor nocturnal control of symptoms. Three notable compounds arose from modifications of short-acting β2-agonists that gave the short-acting parent drugs a longer duration of action.

Bitolterol

Colterol (Fig. 2) is a catecholamine with similar potency to isoprenaline. It is a full agonist with marginal β2-selectivity in guinea pigs (Kusayama et al. 1994) and is degraded by COMT. However, it was noted to have less cardiac stimulation relative to bronchodilation (i.e. some β2-selectivity) and thus analogues of this were studied with attempts at prolonging its duration of action. Bitolterol (WIN 32784, Fig. 2) was developed in the 1970s (Sterling-Winthrop Institute, Tullar et al. 1976). It is a biologically inactive prodrug of colterol. The large toluate ester groups on the aromatic ring protect it from inactivation by COMT activity. Esterases, present in the blood and tissues (including lung), hydrolyse the toluate groups to hydroxyl groups thus forming the active catecholamine colterol. The requirement for hydrolysis to active colterol was expected to prolong the duration of action of colterol by off-setting its degradation by COMT, thus creating a β-agonist with a longer duration of action than catecholamines (Walker et al. 1985). Bitolterol is an effective bronchodilator when administered orally, intravenously or by inhalation. It has a fast

onset of action (3–5 min following inhalation, presumably due to hydrolysis by lung esterases) and has a 10-times longer duration of action than its parent colterol or isoprenaline. It also has some β2-selectivity as measured by less cardiac effects relative to the concentration required for bronchodilation (Tullar et al. 1976; Minatoya 1978; Kass and Mingo 1980; Petty et al. 1984; Orgel et al. 1985; Walker et al. 1985; Friedel and Brogden 1988). It was approved in the USA in 1984, however, overall, it offered little overall benefit compared to salbutamol and other β2-agonists developed around the same time and was withdrawn in 2001.

Bambuterol

Bambuterol (Fig. 2), developed in 1984, is a prodrug of terbutaline. This was specifically developed with the goal of being an orally available drug, with a longer duration of action. It survives the first pass effect, before being slowly metabolised to terbutaline. This metabolism prolongs the generation of terbutaline thus extending the duration of action of the active drug (Olsson and Svensson 1984; Sandström et al. 1988). Bambuterol was felt by some to have a unique high uptake in the lung (rather than heart or skeletal muscle) after oral intake, but also with lung metabolism it was converted to the active bronchodilator "on-site" (Svensson 1991), as well as in the liver (Sandström et al. 1988). Whereas oral terbutaline required twice daily dosing, this longer 24 h duration of action enabled bambuterol to be effective as an oral bronchodilator including relieving symptoms through the night from a once daily oral dose (Pedersen et al. 1985; Petrie et al. 1993; Gunn et al. 1995; D'Alonzo et al. 1995; Persson et al. 1995), including appearing safe in older people (Sitar et al. 1993).

Comparisons of oral once daily bambuterol with inhaled twice daily salmeterol (see later) have shown that both achieved similar bronchodilation, improved lung function and control of nocturnal symptoms and were well tolerated in both asthma (Crompton et al. 1999; Wallaert et al. 1999) and COPD (Cazzola et al. 1999).

Tulobuterol

Tulobuterol (Fig. 2), developed in Japan, was found to be an orally active bronchodilator. Although it was less potent than isoprenaline, it had a 10-fold longer duration of bronchodilator activity than isoprenaline or salbutamol aiming to achieve better nocturnal control (Kubo et al. 1975; Aldons 1990). When inhaled, the onset of action was 1–5 min, with comparative bronchodilation to salbutamol and with no or minimal changes in blood pressure or heart rate and no tachyphylaxis (Patel 1986; Charpin 1990), however, even when inhaled there was a dose-dependent tremor in some individuals (Patel 1986, 1990). However, its duration of action was further prolonged by the development of a transdermal delivery system. The tulobuterol patch, launched in Japan in 1998, is a controlled release medication, designed to deliver tulobuterol such that plasma concentration was highest in the early morning to coincide with the normal circadian "morning-dip" but to provide a continuous 24 h drug delivery. Pharmacologically, at human receptors, tulobuterol is a partial agonist with moderate (16-fold) β2-selective affinity (Baker 2010).

Several studies have compared the tulobuterol patch with twice daily inhaled salmeterol (see below) in those with asthma and COPD with mixed results. Some favoured salmeterol (Fujimoto et al. 2006; Nishiyama et al. 2006; Kobayashi et al. 2007), whilst others favoured tulobuterol (Fukuchi et al. 2005; Sugawara et al. 2009). Overall, it may be that inhaled salmeterol has a greater bronchodilator effect (efficacy), but the effect of the tulobuterol patch is more sustained (Yamagata et al. 2008; Inoue et al. 2017). However, the patch is associated with considerably greater adherence and hence is important for symptom control improvement (Tamura and Ohta 2007; Sugawara et al. 2009; Mochizuki et al. 2013). The patch avoids the peaks and troughs of oral tulobuterol, improves morning peak flow, and is associated with a reduction in rescue β-agonist medication, whilst not causing any tachyphylaxis, desensitisation or tolerance (Kume et al. 2002; Patel 1990; Tamura et al. 2012).

Conclusions

The prodrug bambuterol is a once daily oral medication and the tulobuterol patch a once daily dermal preparation, both of which provide 24-h drug delivery and control of symptoms in asthma and COPD. Given their once daily dosing, both could therefore be considered long-acting β-agonists (LABA), or indeed ultra-long-acting β-agonists (uLABA) for asthma and COPD. They are both simpler to use than some inhalers (as once daily tablet or plaster to stick on the skin). Both are still available for clinical use and the tulobuterol patch remains widely used for asthma and COPD in Japan, China and Korea (Tamura et al. 2012). The biggest issue with both is that because they are both dosed systemically, systemic side effects remain a concern (see Sect. 9), however the simplicity of their delivery means that they may have benefits elsewhere (see Sect. 10).

6 The LABAs: Salmeterol and Formoterol

In order to avoid systemic effects, the next efforts in β2-agonist development were centred on developing inhaled long-acting β2-agonists. Two different methods were employed: creating a molecule that would be long-acting at the β2-AR by binding to a separate site within the receptor anchoring it in place (the "exosite theory") and making highly lipophilic drugs that would dissolve in the cell membrane forming a reservoir of drug to slowly leach out and bind to the receptor (the "diffusion microkinetic theory").

Salmeterol

Salmeterol (SN-408, Fig. 2) was the result of a targeted design programme at GlaxoSmithKline, UK, to develop a long-acting β2-agonist that would provide more convenient and better daytime maintenance therapy (than multiple dosing with short-acting beta agonists) and also provide control of symptoms at night (Ball et al. 1991). It was patented in 1983 and licensed for clinical use in 1990 (Fischer and Ganellin 2006).

The molecule was designed using the active head group of salbutamol with a large lipophilic alkyloxyphenyl extension that would either bind to hydrophobic regions of the cell membrane or bind to non-polar amino acids away from the active binding site on the β2-AR, thus anchoring it in place on the receptor via an "exosite" (Brittain et al. 1976; Johnson et al. 1993). Early studies suggested that salmeterol was a more potent bronchodilator than salbutamol and with a duration of action far longer than isoprenaline, salbutamol or procaterol (Ball et al. 1991; Nishimura et al. 1991).

In clinical studies, salmeterol was shown to have a longer duration of bronchodilation (over 12 h) than that for salbutamol (<6 h, Ullman and Svedmyr 1988; Ullman et al. 1990; van Noord et al. 1996). The use of rescue medication was less when salmeterol was used twice a day. Despite the lower likelihood of systemic absorption, inhaled salmeterol still caused tremor, an increase in heart rate, ECG changes and metabolic changes (Ullman and Svedmyr 1988; Smyth et al. 1993; Bennett et al. 1994; Guhan et al. 2000), although salbutamol and salmeterol caused less atrial activation than isoprenaline (Ball et al. 1991). Salmeterol did not appear to cause tachyphylaxis of either airways or systemic (skeletal or cardiac) AR (Ullman et al. 1990; Lötvall et al. 1992).

Salmeterol is more than 10,000 times more lipophilic than salbutamol and is thought to rapidly partition into biological membranes, particularly the outer phospholipid monolayer, with the molecules then thought to diffuse sideways more slowly for the head group to reach the active binding pocket (diffusion microkinetic theory; Rhodes et al. 1992; Johnson et al. 1993; Anderson et al. 1994). Thus, the high lipophilicity of salmeterol does contribute to a longer duration of action although it is not the full explanation. It does not explain why salmeterol appears to have a longer duration of action at β2 rather than β1-AR (if all lipophilicity driven, duration of action should be the same at both receptors) and neither does it explain the very high (>1,000-fold) β2 vs β1-selective affinity of salmeterol.

The addition of the alkyloxyphenyl extension to the salbutamol molecule significantly changed the molecular pharmacology of the molecule, with changes to affinity, duration of action and efficacy. With regard to affinity, the alkyloxyphenyl extension increased the β2-binding affinity ~1,000-fold. The change in the affinity at the β1-AR was small. Thus, the β2 vs β1 selective affinity was increased from salbutamol's 10–30-fold to salmeterol's over 1,000-fold, making salmeterol the most selective β2-compound available (Johnson et al. 1993; Baker 2010; Baker et al. 2015).

With regard to duration of action, studies with β2-antagonists show that salmeterol can be competitively blocked by the addition of β-AR antagonists, thus competing for the active (salbutamol) head group at the active site. However, if the β-blocker is removed or washed out, the salmeterol-agonist activity returns. This cycle can be repeated several times and indicates the on-going presence and the long duration of action of salmeterol, be that because of its membrane partitioning or because it is bound to an exosite. Apparently, studies at the time involved changing the position of the oxygen atom in the hydrocarbon chain to optimise affinity and selectivity. This had little effect on lipophilicity, but a large effect on β2-affinity,

suggesting that this part of the hydrocarbon chain, a long way from the active head group was binding to a separate part of the β2-AR. This was repeated in a study of the affinity and selectivity of other β-AR head groups with various alkyloxyphenyl extensions (Baker et al. 2020a). Thus, the best explanation is that the alkyloxyphenyl extension of salmeterol binds into an exosite, that is separate, and a distance from the active binding pocket, in a non-competitive manner and with a long duration of action. The active head group then bends round, binding to the active (orthosteric) binding site where it is fully competitive, and short acting, like salbutamol (Ball et al. 1991; Johnson et al. 1993; Nials et al. 1993a; McCrea and Hill 1996; Clark et al. 1996). There has been much debate over the precise location of this exosite. A mutagenesis study however located two amino acids crucial for the high β2-selective affinity of salmeterol (but not affecting the affinity of other β2-agonists) – a histidine at the extracellular end of transmembrane six (H296) and a lysine (K305) at the junction between transmembrane seven and extracellular loop three (Baker et al. 2015). This was later confirmed with the β2-salmeterol crystal structure (Masureel et al. 2018). It is binding into this exosite that gives salmeterol its very high β2-AR affinity, selectivity and contributes to it long duration of action.

The addition of the alkyloxyphenyl extension also influenced the intrinsic efficacy. Salmeterol is a partial agonist, with lower intrinsic efficacy than salbutamol (and therefore considerably lower than the catecholamines). This is consitstent whether efficacy is measured by comparing the maximum responses obtained by the different agonists or whether a measure of intrinsic efficacy (by using an efficacy ratio) is used (Ball et al. 1991; Johnson et al. 1993; Ellis et al. 1995; Clark et al. 1996; Baker 2010). Theoretically, there is therefore even less ability to internalise the β2-AR (suggesting fewer problems with desensitisation or tachyphylaxis) than full-agonists or even than salbutamol. Indeed, this reduced ability of salmeterol to desensitise β2-AR has been demonstrated (e.g. January et al. 1998; Scola et al. 2004; Moore et al. 2007).

Formoterol

Formoterol (BD 40A, Fig. 2) was synthesised as part of a series of molecules being optimised for β2-selectivity (Moore et al. 1998) and was patented in 1972 (Ida 1976a, b; Murase et al. 1977). Early animal studies found formoterol, given by inhalation, subcutaneously and orally, caused bronchodilation and to be substantially more potent than isoprenaline and salbutamol. Although bronchodilator responses were similar, atrial responses to formoterol and salbutamol were partial agonist responses compared to isoprenaline. However, when bronchial and cardiac responses were compared, salbutamol appeared more "bronchoselective" in these initial studies (Ida 1976a, b).

However, the clinical observation of a longer duration of action following inhalation was a serendipitous observation (Moore et al. 1998). Later inhalation studies in people with asthma confirmed the increased potency of formoterol compared to salbutamol but also found a substantially longer duration of bronchodilator activity (3–5 h for salbutamol, ~12 h for formoterol; Löfdahl and Svedmyr 1986, 1989; Becker and Simons 1989; van Noord et al. 1996; Lötvall et al. 2005) and it

entered medical use in 1986 (Fischer and Ganellin 2006). Formoterol was also associated with improved symptoms, peak flow measurements, less use of rescue medications, and patient preference over salbutamol (Arvidsson et al. 1989). However, inhaled formoterol does increase heart rate (Lötvall et al. 2005; Guhan et al. 2000).

Pharmacological studies demonstrated that formoterol has a 300-fold high affinity for the human β2- than the β1-AR (Baker 2010) although, unlike salmeterol, studies have failed to locate the amino acid interactions required for the high β2-selective affinity (Baker et al. 2015). Likewise, how it achieves its longer duration of action is unclear and in some studies formoterol appears quite short acting (e.g. Nials et al. 1993b, 1994). Formoterol has intermediate lipophilicity between salbutamol/terbutaline and salmeterol. It is possible that the diffusion microkinetic theory where formoterol partially partitions into the lipid bilayer could explain its longer duration of action. Thus, the intermediate lipophilicity enables the compound to access the receptor from the aqueous phase (hence faster onset of action) as well as from the membrane for longer duration of action (Anderson 1993b; Anderson et al. 1994; Nials et al. 1994; Van Noord et al. 1996). Formoterol has higher intrinsic efficacy than salbutamol, terbutaline and salmeterol (e.g. Jeppsson et al. 1992; Lindén et al. 1993; Scola et al. 2004; Baker 2010), yet has not been associated with tachyphylaxis in long-term studies (Faulds et al. 1991).

A Comparison of Salmeterol and Formoterol

Salmeterol and formoterol are both long-acting β2-agonists widely used in asthma. For many years they were used interchangeably as LABAs to be used in conjunction with an ICS on the same step of the asthma treatment guidelines. Both show a long duration of bronchodilator activity in man following inhalation leading to better symptom control, less use of rescue medication and improvement in nocturnal symptoms. Pharmacologically, both have high (nanomolar) affinity for the β2-AR and are selective displaying a greater ability to bind to the β2 than the β1-AR (β2-selective affinity; Baker 2010). However, there are some important pharmacological differences between formoterol and salmeterol that are responsible for their different clinical actions, and hence different place in the current guidelines for asthma treatment (GINA 2022).

Isoprenaline and salbutamol have a fast onset of action of 2–3 min and are thus ideal for quick rescue during an exacerbation (Ball et al. 1991; Johnson et al. 1993; Nials et al. 1993a, b). Formoterol has an intermediate onset of action of 5–10 min whereas salmeterol approaches 30 min (Johnson et al. 1993; Nials et al. 1993a, b; Waldeck 1996). Others found a similar speed of onset of salbutamol and formoterol of 1–5 min, whilst that of salmeterol remained slower (van Noord et al. 1996). Thus, the onset of action for salmeterol is too slow for rescue in an exacerbation, hence its place in maintenance therapy for asthma and COPD. For formoterol however, the onset of action is quick enough to be used both for rescue relief and for long-term symptom maintenance (in a dual inhaler with ICS), so patients require one inhaler rather than separate maintenance and reliever inhalers. For serious exacerbations

requiring hospitalisation, nebulised salbutamol is still the main treatment, given its faster onset of action and ease of inhalation.

Many studies show that the highly soluble, fast onset compounds, e.g. isoprenaline and salbutamol are also rapidly washed out, thus giving short duration of action both at the receptor and with clinical responses. Formoterol has an intermediate duration or action and salmeterol the longest (Jeppsson et al. 1989; Nials et al. 1993b, 1994; van Noord et al. 1996; Lötvall 2001) but both require twice daily dosing.

Salmeterol and formoterol differ in intrinsic efficacy. Salmeterol is a partial agonist (at both β1 and β2-ARs) whereas formoterol is a much higher intrinsic efficacy agonist at both receptors. Thus, the β2-selectivity of both compounds is affinity related (i.e. due to better binding to the β2-AR) and not due to a substantial difference in their ability to activate one receptor subtype over another (efficacy; Baker 2010). Many studies have shown the intrinsic efficacy order of isoprenaline > fenoterol > formoterol > salbutamol > salmeterol (O'Donnell 1972; Apperley et al. 1976; O'Donnell and Wanstall 1978; Delhaye et al. 1983; Jeppsson et al. 1992; Anderson 1993a, b; Lindén et al. 1993; Naline et al. 1994; Ellis et al. 1995; Clark et al. 1996; McCrea and Hill 1996; Bremner et al. 1996; van Noord et al. 1996; Lipworth and Grove 1997; January et al. 1997, 1998; Waldeck 1996; Lötvall 2001; Hoffmann et al. 2004; Scola et al. 2004; Moore et al. 2007; Baker 2010; Slack et al. 2013). This is important as the epidemics of asthma deaths appear to be related to the use of compounds possessing high efficacy. It appears that the partial agonism of salmeterol produces sufficient bronchodilation and good symptom control. Formoterol is substantially more efficacious (Baker 2010), approaching that of fenoterol and the catecholamines that were associated with increased death rates. Despite its higher efficacy, formoterol has not been associated with tachyphylaxis in long-term studies (Faulds et al. 1991) suggesting that it has the optimal properties of β2-selectivity combined with high, but not too high efficacy, moderate lipophilicity allowing fast onset of action in the aqueous phase yet sufficient lipophilicity to partition into the lipid layer to act as a longer term reservoir, that taken together enables it to be a safe both as a rescue and a maintenance long-term β2-agonist, alongside an ICS (GINA 2022).

Safety of LABAs

The safety of salmeterol was raised after a study in 1993 involving 25,180 individuals that showed that asthma control was better with salmeterol than salbutamol, although there was a small, non-significant trend towards asthma-related deaths in the salmeterol group (Castle et al. 1993). The SMART study followed that suggested a 4.4 relative risk of an increase in asthma deaths in those receiving salmeterol vs placebo (Nelson et al. 2006). However, the numbers were small (13 deaths out of 13,176 randomised to salmeterol vs three deaths out of 13,179 randomised to placebo). Of the 13 salmeterol deaths, nine received salmeterol monotherapy and four salmeterol plus ICS, whereas the placebo group all three had ICS. Trial design (lack of in-person follow-up and participants being given seven salmeterol inhalers at the start which could have resulted in overuse) and the

use of ICSs being optional (leading to 53% participants with salmeterol monotherapy) were heavily criticised. The results and statistics were hotly contested and despite most (including the authors) believing the results were not secondary to the β-agonist (Nelson 2006a, b Ortega and Peters 2010), the FDA issued a warning about the safety of salmeterol and formoterol, whilst international guidelines continued to recommend them as combination LABA/steroid treatments (as dual salmeterol/fluticasone, formoterol/budesonide inhalers).

Several studies since (some of which include LABA monotherapy) have not found an association between the use of salmeterol and formoterol and asthma-related deaths although there were mixed results for adverse events (Beasley et al. 1999; Anderson et al. 2005; Cates and Cates 2008; Cates et al. 2008; Nelson et al. 2009; Kramer 2009; Sears et al. 2009). Although there is some systemic absorption through the lungs and GI tract that can cause an increase in heart rate and tremor (Tattersfield 1992; Faulds et al. 1991), more recent meta-analyses (where all cases are a combination of LABA plus ICS) show no increase in asthma-related deaths with formoterol or salmeterol and no increase in adverse events (Cates et al. 2018; Janjua et al. 2019; O'Shea et al. 2021). There is also no suggestion of increased cardiovascular risk with salmeterol (Martin et al. 1998). Studies have consistently shown improvement in asthma control in patients treated with LABA and ICS (Beasley et al. 1999; Ortega and Peters 2010), and international asthma guidelines have continued to recommend combination LABA and ICS therapy (GINA 2022).

7 The uLABAS: Indacaterol, Vilanterol, Olodaterol, Abediterol and Carmoterol

A significant problem with asthma treatment, and therefore ultimately the control of asthma symptoms, is patient compliance – patients actually taking their medication regularly as prescribed, even if they feel well, in order to prevent an exacerbation. The development of drugs with a longer duration of action means less frequent dosing regimens and improved convenience for patients, that should in turn mean greater patient adherence and thus improved overall asthma and COPD control and symptoms. Ultra-long-acting β2-agonists (uLABAs) are compounds that have 24 h duration of action and thus can be taken once a day (rather than twice daily salmeterol and formoterol) for the treatment of asthma and COPD (Matera and Cazzola 2007; Beeh and Beier 2009; Cazzola et al. 2019). As above, the once daily bambuterol and tulobuterol could be considered uLABAs, although their dosing is systemic, whereas those below are given by inhalation.

Indacaterol

Indacaterol (from Novartis, Fig. 2) was developed in a program that used lipophilicity (deposition into the lipid membrane) as the route to achieve a long duration of action (Murphy et al. 2014). It is an effective once daily inhaled bronchodilator in asthma and COPD with fast onset of action (<5 min) and a 24-h bronchodilator effect (Beier et al. 2007, 2009; Beeh and Beier 2009; Jones et al.

2011; Ray et al. 2012; Geake et al. 2015). Pharmacologically, indacaterol is a relatively high efficacy β2-agonist generating agonist responses *in vitro* and *in vivo* that would suggest it has slightly less efficacy than formoterol but considerably more than salbutamol or salmeterol. It also only has moderate β2 vs β1 binding selectivity of 16–28-fold (Battram et al. 2006; Sayers et al. 2009; Slack et al. 2013). Indacaterol is systemically absorbed despite being delivered by inhalation, with 75% systemic absorption occurring through the lungs (25% through GI tract) and plasma concentrations peaking 15 min post inhalation (Beeh and Beier 2009; Blair 2021). It causes increased heart rate when given by inhalation, but less than formoterol, salbutamol and salmeterol for equivalent bronchodilator effect (Battram et al. 2006). However, there appears to be no significant clinical risks from cardiovascular or metabolic side effects in man (Chuchalin et al. 2007; Beeh and Beier 2009; Ray et al. 2012), and no increased risk of cardiovascular events (Scosyrev et al. 2021).

Vilanterol

Vilanterol (from GlaxoSmithKline, Fig. 2) is structurally similar to salmeterol. It has very similar β2-affinity to salmeterol and equally high selectivity over β1 (2,400-fold; Slack et al. 2013). It has more intrinsic efficacy than salmeterol but remains more of a partial agonist than indacaterol and formoterol. It appears to have a faster onset of action than salmeterol but a longer duration of action (Slack et al. 2013). It provides 24-h bronchodilation following a single inhaled dose (in conjunction with the ICS fluticasone) in those with asthma and COPD (Lötvall et al. 2012; Hanania et al. 2012), similar to that achieved with twice daily salmeterol/fluticasone, and twice daily formoterol/budesonide (Woodcock et al. 2013; Syed 2015; Stynes et al. 2015; Svedsater et al. 2016; Furuhashi et al. 2019).

Olodaterol

Olodaterol (from Boehringer Ingelheim, Fig. 2, Bouyssou et al. 2010a) was developed from a programme designed to look for longer acting β2-agonists but with a fast onset of action. Olodaterol has a 65-fold β2-selectivity (based on affinity) so is marginally more β2-selective than indacaterol, but considerably less so than vilanterol, salmeterol and formoterol. It is an efficacious agonist with maximum responses similar to formoterol at β2-ARs. Although there is some suggestion of less intrinsic efficacy at β1-ARs (Bouyssou et al. 2010b), it is still able to increase heart rate at higher doses (be that via cardiac β1 or β2-AR, see later; Aparici et al. 2016). It produced a prolonged bronchodilation (up to 32 h from single inhaled dose; O'Byrne et al. 2009), and studies up to 48 weeks suggest no change or increase in systemic side effects, hospital admissions or mortality in those with COPD and asthma treated with olodaterol (Lee et al. 2017).

Abediterol

Abediterol (Fig. 2) is another β2-selective agonist in development by Almirall (Spain). Its binding affinity suggests that it has similar β2 vs β1 selectivity to olodaterol, less selective than salmeterol and vilanterol and more selective than indacaterol and formoterol. Its efficacy is similar to indacaterol, vilanterol and

olodaterol (i.e. less than isoprenaline but more than > salmeterol) and has a long duration of action, similar to indacaterol (Aparici et al. 2012, 2016). Phase II studies with inhaled abediterol suggested improved lung function over seven days in patients with asthma and COPD (Beier et al. 2016, 2017).

Carmoterol

Carmoterol (TA-2005, CHF-4226, Fig. 2) is a long-acting full agonist with nanomolar β2 affinity, moderate (38-fold) β2 vs β1 selective affinity (Kikkawa et al. 1991, 1994; Summerhill et al. 2008; Patel et al. 2011). It has structural similarities to formoterol and indacaterol but was withdrawn from further clinical development.

uLABA Conclusions

The mode of action for the long duration of these uLABAs is not certain. As vilanterol is structurally similar to salmeterol, it may also have exosite binding. Carmoterol, like formoterol, lacks an aliphatic side chain and does not appear to have exosite binding like salmeterol, potentially making microkinetic/lipophilic deposition more important (Patel et al. 2011). Indacaterol is very lipophilic making the microkinetic/deposition theory more likely, although exosite binding cannot be ruled out (Patel et al. 2011). More work is needed to understand the molecular basis of both uLABA selectivity and duration of action.

Currently the use of uLABAs is restricted to COPD, in part because of the 2005 FDA Black Box warning for LABAs in asthma. This warning was removed for LABA/ICS combination products on 20th December 2017 but remains in place for single LABA products. The uLABAs are often combined with muscarinic antagonists (e.g. olodaterol/tiotropium, vilanterol/umeclidinium and indacaterol/glycopyrronium) in fixed-dose combination inhalers for use in COPD. Triple inhalers in combination with ICS are also available in the hope of improving patient symptoms and adherence. To date there is no consistent evidence that one particular uLABA agent is superior to any other, when either monotherapies are compared or when the different double uLABA/LAMA or triple LABA or uLABA/LAMA/ICS combinations are compared (Ferguson et al. 2020; Lee et al. 2020a, b, 2021; Muraki et al. 2021; Cazzola et al. 2021; Hsieh et al. 2022).

8 Other Potential Benefits of β2-Agonists: Anti-inflammatory Actions and Mucociliary Clearance

In asthma and COPD, symptom relief mediated by β2-mediated bronchodilation of the smooth muscle cells of the airway is the most important effect of β2-agonists, but there are other potential beneficial effects as well.

β2-agonists have anti-inflammatory actions that may also be important in asthma (Assem and Schild 1969; Anderson 1993b; Butchers et al. 1991). Mast cells release histamine, leukotrienes and prostanoids that cause bronchoconstriction, increase vascular permeability (important for the leakage of plasma proteins and water into

tissues contributing to oedema) and stimulate inflammatory cells (Lewis and Austen 1981). Agonist activation of β2-ARs on mast cells inhibits release of pro-inflammatory mediators, reduces vascular permeability, reduces the number and activation of eosinophils, neutrophils and alveolar macrophages (Butchers et al. 1991; Lötvall et al. 1992; Tattersfield 1992; Johnson et al. 1993; Anderson 1993b). The duration of action of the β_2-AR agonist responses in these cells is in keeping with their molecular pharmacological responses and those seen in clinical bronchodilator responses (Butchers et al. 1991; Johnson et al. 1993). Furthermore, β2-agonists have anti-inflammatory effects on the smooth muscle cells themselves. They also decrease smooth muscle and goblet cell hyperplasia and the fibrotic rearrangement of the extracellular matrix and so reduce airway remodelling (Santus et al. 2015).

β2-agonists (including salbutamol and salmeterol) increase ciliary beat frequency *in vitro* (Verdugo et al. 1980; Hesse et al. 1981; Devalia et al. 1992; Kanthakumar et al. 1994; Piatti et al. 2005). Cilia are the tiny hair-like projections on the epithelial cells that line the airways that beat to move the mucus and debris out of the airways. Increasing beat frequency should increase mucociliary clearance from the airways (Braiman and Priel 2008; Santus et al. 2015) and β-agonists do appear to increase tracheal mucous velocity (Sackner et al. 1976) and formoterol increases mucus clearance in patients with COPD (Meyer et al. 2011).

9 Clinical Side Effects of β2-Agonists: Tremor, Cardiovascular and Metabolic Problems

Most clinical side effects of β2-agonists are predictable, due to activation of known systemic β2-receptors and are expected from systemic dosing (oral or intravenous). However, systemic side effects are also common following inhaled preparations. Most systemic absorption is thought to occur directly through the lung-vascular bed, more than the buccal or oropharynx mucosa, however 20–30% of systemic effects from inhaled salbutamol and salmeterol occur through the GI tract (Collier et al. 1980; Küng et al. 1987; Lipworth et al. 1989; Newnham et al. 1993; Bennett et al. 1999).

Tremor

Tremor is a side effect of β-agonists that is difficult to control. Tremor is common (up to 40% patients) and occurs in a dose-dependent manner whether the drug is administered intravenously, orally or by inhalation. It has been reported with most β-agonists including isoprenaline, orciprenaline, salbutamol, terbutaline, fenoterol, procaterol, pirbuterol, clenbuterol, bitolterol, bambuterol, tulobuterol, salmeterol and formoterol (Thiringer and Svedmyr 1976; Anderson and Wilkins 1977; Dyson and MacKay 1980; Crowe et al. 1985; Siegel et al. 1985; Walker et al. 1985; Sanchez et al. 1988; Aldons 1990; Wong et al. 1990; Tattersfield 1992; Faulds et al. 1991; D'Alonzo et al. 1995; Persson et al. 1995; Sitar et al. 1993; Gunn et al. 1995; Cazzola et al. 1999; Wallaert et al. 1999). Some studies suggest that the degree

of tremor decreases over time, without a decrease in bronchodilator effects (Svedmyr et al. 1976; Larsson et al. 1977; Patel 1990; Tinkelman et al. 1990). The issue with tremor is that this is an "on-target" side effect: i.e. it occurs from stimulation of β2-ARs on skeletal muscle cells, the very same receptors present on bronchial smooth muscle being the intended target. Inhalation minimises the systemic exposure, but it remains an issue that will be very difficult to eliminate (Bowman and Nott 1970, 1971; Apperley et al. 1976), unless systemic absorption is totally prevented (see Sect. 10).

Cardiovascular Problems

Tachycardia is also a persistent problem with β-agonists (Van Arman et al. 1961; Greenberg and Pines 1967), and as with tremor, is observed even with the LABAs formoterol and the low-efficacy partial agonist salmeterol (Ullman and Svedmyr 1988; Smyth et al. 1993; Bennett et al. 1994, 1999; Guhan et al. 2000; Lötvall et al. 2005). The β1-AR is the main AR in the heart and is the main site of action for the beneficial effects of β-blockers in those with heart disease (Cruickshank 2007; Baker and Wilcox 2017). Many different β-blockers (i.e. the opposite of β-agonists) reduce mortality in those with heart disease, both for ischaemic heart disease and heart failure, as well as being beneficial in arrhythmias (see references in Baker and Wilcox 2017). On the other hand, drugs that increase heart rate and force of contraction increase cardiac mortality (Steeds and Channer 1998). In short: long-term β-blockade is beneficial to those with heart disease whereas β-stimulation is detrimental. β2-agonists therefore pose an increased cardiovascular risk in those with known concomitant cardiovascular disorders (Cazzola et al. 2005).

A small proportion of β2-ARs are present in heart (Bristow et al. 1986; Buxton et al. 1987). Some studies suggest that activation of β1-ARs appears to be the more deleterious for existing heart disease (Lee et al. 2008). The short-acting β2-agonists salbutamol and terbutaline have only moderate β2-selectivity and so, particularly at the high doses used in acute exacerbations (e.g. by nebuliser), activation of cardiac β1-ARs will have a significant role. However, the fact that highly selective β2-agonists (e.g. salmeterol) given by inhaler cause an increase in heart rate suggests that activation of cardiac β2-ARs does play a role in heart rate. Just like tremor, unless all systemic absorption is eliminated, this will remain a potential issue.

Thus, β2-agonists can cause an increase in heart rate through three different mechanisms: (1) direct activation of the β1-AR by poorly-selective β2-agonists, (2) direct activation of the cardiac β2-AR, or (3) indirectly from β2-mediated peripheral vasodilatation (which reduces blood flow returning to the heart return resulting in sympathetic reflexes that increase heart rate; Sears 2002).

Short-term, or acute, detrimental cardiac effects from β-agonists can occur through several routes (Robin and McCauley 1992). Arrhythmias occur through direct β-AR activation and the safety of the high efficacy agonist isoprenaline was raised in the 1960s (Lockett 1965; Greenberg and Pines 1967). ECG changes and tachyarrhythmias reported with salbutamol include premature ventricular contractions, atrial fibrillation, supraventricular tachycardia, ventricular tachycardia and cardiac asystole (Higgins et al. 1987; Wong et al. 1990; Robin and McCauley

1992; Bremner et al. 1993; Cook et al. 1994; Newhouse et al. 1996; Habashy et al. 2003; Kallergis et al. 2005; Trachsel et al. 2007; Warnier et al. 2012; Reyes-Mondragon et al. 2016). Tachycardia can also lower cardiac output (no time for diastolic filling) and limit diastolic blood flow within the heart muscle itself, resulting in hypoxic myocardial injury, thus lowering cardiac output and further increasing risk of arrhythmias. Arrhythmias are common in people with COPD who are hypoxic (Kleiger and Senior 1974) and hypoxia itself is associated with an increase in arrhythmias (Hudson et al. 1973; Flick and Block 1979). The combination of hypoxia from underlying lung disease and β2-agonist induced tachycardia and arrhythmias and metabolic changes (see below, e.g., hypokalaemia, that could alter the resting membrane potential) could further exacerbate cardiovascular risk (Senior et al. 1979; Robin and McCauley 1992). Long-term detrimental β-agonist effects (most likely from oral and regular nebulised therapy) risk worsening heart failure (Steeds and Channer 1998).

Importantly, the increased risk of adverse cardiac events affects those without existing heart disease – the asthma death epidemics attributed to isoprenaline (1960s) and fenoterol (1980s) in the 5–34/45 age groups (i.e. in those without underlying cardiac disease) have been suggested to be due to cardiac arrhythmias (beginning with Greenberg and Pines 1967). It may be that the combination of overuse of highly efficacious β-agonists, in the context of hypoxia from an asthma attack, that precipitated fatal arrhythmias (Collins et al. 1969; McDevitt et al. 1974; Bremner et al. 1992b). Hypoxia in those with COPD is associated with significant ECG changes (Tirlapur and Mir 1982). The risks associated with the partial agonists salbutamol and terbutaline are far lower (Sears 2002) and no excess deaths have been demonstrated (Drazen et al. 1996). Although a measurable increase in heart rate is seen in some studies (Lötvall et al. 2005), the cardiovascular risk with LABA and uLABA again appears far less, even in the context of COPD (Iftikhar et al. 2014; Andreas et al. 2020; Rebordosa et al. 2022).

However, the greatest increased cardiovascular risk is in those with underlying heart disease. COPD and heart disease occur frequently together (40% people with COPD have heart disease; Department of Health (UK) 2011) and these people are at particular risk of adverse cardiovascular events including arrhythmias (Higgins et al. 1987) and death (Suissa et al. 1996; Boudestein et al. 2009; Feary et al. 2010). The addition of β-agonists, with their associated tachycardia and arrhythmias, poses an extra risk, particularly from oral and nebulised delivery, rather than lower-dose MDI (Suissa et al. 1996). Thus, the use of β-agonists is associated with an increase in myocardial infarction, unstable angina, heart failure, arrhythmias, stroke and sudden death (Au et al. 2000, 2002; Sears 2002; Salpeter et al. 2004; Macie et al. 2008) and arrhythmias and myocardial injury even in children (Habashy et al. 2003; Sarnaik et al. 2013; Woodward et al. 2021).

Metabolic Effects

β-agonists cause metabolic effects including changes in potassium, glucose, insulin, lactate, free fatty acids, calcium, magnesium and phosphate (Van Arman et al. 1961;

Goldberg et al. 1975; Sanders et al. 1977; Irie et al. 1979; Phillips et al. 1980; Scheinin et al. 1987; Guhan et al. 2000; Haffner and Kendall 1992).

Potassium: β2-agonists cause a dose-dependent decrease in serum potassium (first noticed with adrenaline, D'Silva 1934; Phillips et al. 1980). Stimulation of the β2-AR in skeletal muscle, that is linked to a sodium-potassium ATPase, causes an influx of potassium into cells, and thus a transient decrease in serum potassium (Wang and Clausen 1976; Haffner and Kendall 1992; Sears 2002). Like all β2-responses, it too is related to the underlying intrinsic efficacy and dose of the β2-agonist used, with fenoterol having more effect than the partial agonists salbutamol and terbutaline (Scheinin et al. 1987; Wong et al. 1990; Sears 2002). Lower potassium levels are still measured with LABAs (Smyth et al. 1993; Guhan et al. 2000). This low potassium potentially increases the risk of cardiac abnormalities, especially in those who are hypoxic from the underlying bronchoconstriction (Sears 2002). However, this side effect is beneficially exploited in those with high serum potassium levels (e.g. Wang and Clausen 1976). Importantly, life-threatening arrhythmias due to hyperkalaemia can be averted with readily available salbutamol nebulisers, whilst waiting to administer the definitive treatments to more permanently reduce the potassium level (Bushe 1983; Batterink et al. 2015; Montassier et al. 2019).

Glucose: β2-agonists also increase glycogenolysis and gluconeogenesis so increasing glucose production, as part of the stress (fight or flight) response, causing a dose-dependent increase in plasma glucose (e.g. Kuo et al. 1977; Sanders et al. 1977; Haffner and Kendall 1992), although the clinical relevance of this in those without diabetes is uncertain. LABAs also increase plasma glucose (Guhan et al. 2000). β2-agonist stimulation can increase glucose uptake into skeletal muscle and this has a plasma glucose lowering effect longer-term (Sato et al. 2014). In reality, in those with diabetes, the detrimental effect of the addition of corticosteroid on blood glucose (particularly those given orally as they are during a significant exacerbation alongside the nebulised salbutamol) is likely to outweigh any effects that the increased dose of β2-agonist might have on blood glucose levels (Sears 2002).

Lactate: β2-agonists activate β2-AR in skeletal muscle to cause the production of lactate via an exaggerated aerobic glycolysis. This results in an increase in lactate in healthy volunteers and those with asthma, in a dose-dependent manner (Sanders et al. 1977; Liedtke et al. 2019). Of those with asthma taking high-dose β2-agonist (e.g. nebulised salbutamol), 30% have hyperlactataemia. Hyperlactataemia can also occur as part of the exacerbation of asthma or COPD. Poor oxygen delivery to the respiratory muscles, that have increased work of breathing, results in over production of lactate by the respiratory muscles combined with reduced elimination caused by liver hypoperfusion. This causes a metabolic acidosis, which results in respiratory compensation (an increased respiratory rate in order to excrete more carbon dioxide). Thus, β2-agonists can exacerbate the high lactate, potentially exacerbating respiratory failure, with the shortness of breath being due to respiratory compensation as well as bronchospasm (Starkey et al. 2014; Reyes-Mondragon et al. 2016; Liedtke et al. 2019).

β-agonists can also cause a reduction in serum calcium, magnesium, phosphate, growth hormone, and an increase in renin and lipolysis (release of free fatty acids

from adipose tissue; Goldberg et al. 1975; Phillips et al. 1980; Sears 2002; Haffner and Kendall 1992). The clinical significance of increased lipolysis is uncertain (Haffner and Kendall 1992).

The Theoretical Risk of Desensitisation and Tachyphylaxis

Desensitisation is a protective cellular mechanism to prevent damage from overstimulation by agonists. Receptors on cells exposed to highly efficacy agonists undergo phosphorylation, uncoupling from the signalling cascade, reduced coupling efficiency and removal from the cell surface (internalisation; January et al. 1997, 1998; McLean and Milligan 2000; Baker et al. 2003). The exposure required for desensitisation varies significantly between tissues (Johnson 2001; O'Connor et al. 1992; Anderson 2006) and could explain why tremor improves over time without reduction in bronchodilation with long-term treatment of certain β-agonists (Svedmyr et al. 1976; Larsson et al. 1977; Patel 1990; Tinkelman et al. 1990). The degree of receptor desensitisation is related to the efficacy of the agonist and length of exposure. Thus, partial agonists are less likely to cause receptor desensitisation and internalisation than full agonists (Clark et al. 1996; January et al. 1997, 1998; Baker et al. 2003; Johnson 2001). Tachyphylaxis is the clinical observation that a clinical response to a drug decreases after successive doses such that the drug becomes less effective.

Desensitisation (internalisation and removal of β2-AR) certainly occurs in response to high efficacy agonists in model cell systems, such as human lymphocytes (Galant et al. 1978) and bronchial smooth muscle cells (Davis and Conolly 1980). Although all β-agonists improved symptoms, some such as adrenaline, isoprenaline, orciprenaline and fenoterol were associated with an increase in asthma deaths. Pharmacologically, these agonists all have high intrinsic efficacy (need to occupy very few receptors to stimulate a full agonist response) and have greater potential for receptor phosphorylation, desensitisation and tachyphylaxis. Tachyphylaxis had been proposed as a potential cause of the asthma death epidemics (Conolly et al. 1971). The potential for tachyphylaxis remains a significant concern and "resistance" or "tolerance" to β2-agonists has been reported in many studies for isoprenaline, salbutamol, terbutaline and salmeterol (Conolly et al. 1971; Svedmyr et al. 1976; Plummer 1978; Cheung et al. 1992; O'Connor et al. 1992; Cockcroft et al. 1993; Anderson 2006). However, there is very little evidence of problematic bronchodilatory desensitisation or tachyphylaxis in current clinical practice (Larsson et al. 1977; van Schayck et al. 1990; Ullman et al. 1990; Faulds et al. 1991; Lötvall et al. 1992; Beasley et al. 1999; Cates et al. 2018; Janjua et al. 2019; O'Shea et al. 2021). Thus although a theoretical concern, with current drugs (salbutamol, terbutaline, salmeterol, formoterol and the uLABAs) this does not appear to be a major clinical problem for bronchodilation and β2-agonists continue to be important in daily symptom control, and rescue in exacerbation, and recommended in national and international guidelines.

10 β2-Agonists: Potential Future Improvements in β2-Agonists – SABAs, LABAs, Challenges Around the World and Environmental Concerns

Improvement in Short-Acting β2-Agonist (SABAs)

Salbutamol (developed in 1966) remains the main rescue medication in both inhalers and in nebulisers (used in more severe attacks of asthma and COPD). Pharmacologically it is very similar to terbutaline, both being partial agonists relative to the compounds associated with the epidemics (adrenaline, isoprenaline, orciprenaline and fenoterol; Bremner et al. 1996; Beasley et al. 1999). Salbutamol and terbutaline are poorly β2-selective. They are systemically absorbed following inhalation and cause an increase in heart rate with potentially harmful consequences, especially in those with existing heart disease (e.g. fast heart rate exacerbating anginal symptoms/ischemic heart disease – see section 9 above), and especially when used at higher dose (nebulisers) during an asthma/COPD exacerbation when the risk of hypoxia is higher (Bremner et al. 1992b). Importantly however, these partial agonists, when used as in inhalers in the community, have not been associated with an increase in asthma death rates or epidemics (Mullen et al. 1993; Beasley et al. 1999).

Considering the molecular pharmacological properties required for an ideal SABA, there are several pharmacological improvements that could be made to reduce side effects and increase safety and thus generate a safer SABA. Clearly partial agonism rather than high intrinsic efficacy is important and salbutamol and terbutaline both achieve significant bronchodilation without increased deaths when given by inhalers in the community. Keeping this level of partial agonism is important. However, salbutamol/terbutaline are poorly β2-selective: high β2-selectivity (be that achieved through selective affinity or selective efficacy) would lower β1-medicated cardiac side effects. Fast onset of action is vital that is achieved by small soluble molecules but these are also readily systemically absorbed: limiting their distribution by reducing systemic uptake would improve side effects and reduce cardiovascular risk when used at high dose (nebuliser). Thus, SABAs could be improved in several different ways:

1. Highly β2-selective SABAs could be developed. These molecules would have high selective affinity for the β2 over the β1-AR (with partial agonism) and thus not bind to the cardiac β1-ARs to minimise cardiac side effects (i.e. a β2-affinity-selective partial agonist). Highly affinity-selective small molecule β1 and β2 antagonists exist (β1-antagonists, e.g. CGP20712A, NDD-825; β2-antagonists, e.g. ICI118551; Baker et al. 2017) and β1-partial agonists (e.g. LK204-545 and analogues; Louis et al. 1999; Mistry et al. 2013) making this highly achievable from a molecular pharmacological standpoint.
2. A different pharmacological approach would be to develop a non-selective β1/β2-molecule that bound to both receptors with similar affinity, but only activated the β2-AR (efficacy-selective drug; Baker 2010). This would be an antagonist of the β1-AR but a partial agonist of the β2-AR. A molecule such as this would have the added advantage of acting as a β-blocker in the heart, thus inhibiting the

activation from high level of endogenous catecholamines and any β2-agonist treatment present during an asthma or COPD exacerbation, thus actively protecting the heart from harmful tachycardia and arrhythmias, whilst treating the bronchospasm. This may be particularly beneficial in those with pre-existing cardiac disease. Most drug discovery to date has centred on achieving affinity selectivity rather than the harder to measure intrinsic efficacy selectivity so this area is relatively unexplored. However, there are hints that some exist AR compounds can have selective intrinsic efficacy (Baker 2010; Proudman et al. 2022), making this potentially achievable.

3. Whilst both methods above would improve the major β1-medicated cardiac side effects, the "on target" β2-medicated side effects of tremor, metabolic changes and the potential β2-medicated cardiovascular side effects would remain. Furthermore, there are other potential issues with long-term β2-agonism: β-blockade may be helpful (and thus β-agonism harmful) in the growth and spread of certain cancers (see Asthma and COPD: a focus on β-agonists – past, present and future). A method to stop all systemic effects would be to ensure that the compound was inactivated upon systemic absorption. Thus, the β-partial agonist delivered by inhalation would act locally (topically) but be metabolised upon entry into the systemic circulation such that it would not be able to interact with systemic AR (or potentially cancer cells; a "soft drug" approach). Studies have shown that it is possible to develop β-blockers (aimed at topical applications for glaucoma and vascular skin tumours) with esterase-sensitive properties that are inactivated immediately such that even central intravenous administration does not affect heart rate (Baker et al. 2020b). Furthermore, it is possible to develop these β-AR compounds such that they are sensitive to different esterases (e.g. serum vs liver esterase) and this differential esterase sensitivity can be used to fine-tune their systemic duration of action (Baker et al. 2020b). Thus, there is realistic potential to develop a serum-esterase sensitive inhaled β-agonist that would act in the lungs, but would be inactivated by serum esterase immediately upon systemic absorption and thus negate all β1 and β2-mediated systemic side-effects.

Improvement in Long-Acting β-Agonists (LABAs and uLABAs)

There are several different long-acting and ultra-long-acting β2-agonists now available. They have different β1 vs β2 selectivities and span a range of efficacies (very partial salmeterol to nearly full agonism indacaterol and formoterol). The LABAs, salmeterol and formoterol do cause an increase in heart rate and metabolic changes (e.g. Guhan et al. 2000), but overall LABAs and uLABAs appear to cause few clinically significant systemic side effects. For COPD, it may be that now, with the existing uLABAs, the ultimate goal of once daily treatments as the optimum in this regard has been achieved. There may be little clinical (compliance) benefit in any longer duration with medications that need to be taken on alternative days or potentially weekly. For asthma, more recent guidelines champion the use of a single inhaler for both maintenance and reliever to simplify regimens and improve patient adherence. In this case formoterol appears to have the current best balance of onset, duration of action, efficacy without desensitisation and subtype selectivity.

However, LABAs do still increase heart rate and are systemically absorbed and have on-target β2-mediated actions. Again, these would be prevented by the development of a serum-esterase sensitive inhaled LABA (asthma and COPD) or uLABA (COPD).

Different Challenges Around the World: Easy Modifications to Existing Medications

Inhaled, and when needed nebulised, salbutamol remains the cornerstone treatment of exacerbations of asthma in many areas of the world. This requires access to healthcare facilities, education in how to use inhalers and nebulisers, equipment and correct drug storage. Most asthma studies and treatments are aimed at the developed world, but there remains a significant asthma burden in remote areas or other parts of the world with poor health resource and/or where people may live several hours walk away from a medical centre. These situations may need different solutions, especially for the management of acute exacerbations of asthma.

The tulobuterol patch is easy to use, especially in children. It could be less expensive than inhalers, potentially easier to store, requires virtually no teaching on how to use and has high compliance. Using the current patch (tulobuterol alone) separates the steroid from the β-agonist, and by its very nature of application comes with significant systemic exposure. However, topical steroid applications already exist, and steroids are also systemically absorbed through the skin (e.g. well-documented systemic absorption of steroid skin creams for eczema). The development of a joint steroid/β-agonist topical treatment would appear to be a relatively straightforward step and could be used either as a rescue medication en route to medical care or in circumstances where inhalers are not an option (expense, storage, ability to use, equipment, education, compliance, etc.).

Likewise, there is a case to use oral salbutamol in remote communities or in areas of poor health resource, particularly in children. Oral salbutamol (tablet or syrup), as expected, has a slower onset but more sustained response than inhaled salbutamol, but does improve wheeze and lung function, costs a quarter of inhaled salbutamol, has a longer shelf-life, requires no education on how to use, and has double the compliance rate of inhaled salbutamol in low-income countries (O'Reilly et al. 2015). The potential of oral salbutamol, combined with oral prednisolone in either a cheap polypill or combined solution/syrup (to ensure no β-agonist monotherapy) for treatment of exacerbations in rural or low-income settings should remain a consideration.

Climate/Environmental Concerns and Inhalers

One potential issue affecting the use of metered dose inhalers (MDIs) and driving a change to dry powder inhalers (DPIs) is the environmental impact of MDI propellants. The early CFC (chlorofluorocarbon) propellant-based MDIs have been phased out and replaced by HFA (hydrofluoroalkanes) MDIs. Although HFAs do not deplete the ozone layer, they still have a significant environmental impact as they act as powerful greenhouse gases (Wilkinson and Woodcock 2022). In the UK, HFC MDIs account for approximately 13% of the NHS's carbon footprint related to the

delivery of care. Newer HFA propellants, with a lower carbon footprint, are in development but it will take some years until these are available for prescription and until then there is a push in some countries to swap to DPIs. Recycling schemes for inhalers are limited and currently, once empty, MDIs should be incinerated, as the HFC propellant is thermally degraded into by-products with less carbon footprint.

11 Summary

β2-agonists remain a mainstay of treatment of asthma and COPD. Following the recent change in global guidance it is likely that, for maintainance therapy, the use of short-acting β2-agonists as the first step in asthma management will be replaced by the use of combined inhaled corticosteroid and short- or long-acting β2-agonists (especially formoterol). LABAs and uLABAs are likely to remain in the guidelines for asthma and COPD maintenance treatment for years to come. For rescue medication, short-acting β2-agonists (especially salbutamol) are still widely used and likely to remain so for the management of acute asthma and COPD exacerbations.

Over 120 years of drug development, a worldwide effort has resulted in excellent medications used by millions, but there is still potential for improvement of the pharmacological properties, particularly for the widely used β2-agonist salbutamol, to reduce systemic side effects.

References

Abel JJ (1901) On epinephrine, the active constituent of the suprarenal capsule and its compounds. J Physiol 27:237

Abel JJ, Crawford AC (1897) On the blood-pressure raising constituent of the suprarenal capsule. Bull Johns Hopkins Hosp 8:151–157

Abramson MJ, Puy RM, Weiner JM (2010) Injection allergen immunotherapy for asthma. Cochrane Database Syst Rev 8:CD001186. https://doi.org/10.1002/14651858.CD001186.pub2

Adam J (1913) Asthma and its radical treatment. Henry Kimpton, p 27. as referenced in Melland, 1910

Ahlquist RP (1948) A study of the adrenotropic receptors. Am J Physiol 153(3):586–600. https://doi.org/10.1152/ajplegacy.1948.153.3.586

Albert RK, Connett J, Bailey WC, Casaburi R, Cooper JA Jr, Criner GJ, Curtis JL, Dransfield MT, Han MK, Lazarus SC, Make B, Marchetti N, Martinez FJ, Madinger NE, McEvoy C, Niewoehner DE, Porsasz J, Price CS, Reilly J, Scanlon PD, Sciurba FC, Scharf SM, Washko GR, Woodruff PG, Anthonisen NR, COPD Clinical Research Network (2011) Azithromycin for prevention of exacerbations of COPD. N Engl J Med 365(8):689–698. https://doi.org/10.1056/NEJMoa1104623

Aldons PM (1990) Can a new beta 2-agonist reduce the mortality of asthma? Lung 168(Suppl):186–191. https://doi.org/10.1007/BF02718131

Aldrich TB (1901) A preliminary report on the active principle of the suprarenal gland. Am J Physiol 5:457–461

Alvarez-Guerra M, Libertus H, Garay RP (2004) Inhibition by reproterol of cAMP PDE in intact mastocytoma P-815 cells. Pulm Pharmacol Ther 17(4):213–218. https://doi.org/10.1016/j.pupt.2004.04.001

Anderson GP (1993a) Long acting inhaled beta-adrenoceptor agonists the comparative pharmacology of formoterol and salmeterol. Agents Actions Suppl 43:253–269. https://doi.org/10.1007/978-3-0348-7324-6_22

Anderson GP (1993b) Formoterol: pharmacology, molecular basis of agonism, and mechanism of long duration of a highly potent and selective beta 2-adrenoceptor agonist bronchodilator. Life Sci 52(26):2145–2160. https://doi.org/10.1016/0024-3205(93)90729-m

Anderson PJ (2005) History of aerosol therapy: liquid nebulization to MDIs to DPIs. Respir Care 50 (9):1139–1150

Anderson GP (2006) Current issues with beta2-adrenoceptor agonists: pharmacology and molecular and cellular mechanisms. Clin Rev Allergy Immunol 31(2-3):119–130. https://doi.org/10.1385/CRIAI:31:2:119

Anderson G, Wilkins E (1977) A trial of clenbuterol in bronchial asthma. Thorax 32(6):717–719. https://doi.org/10.1136/thx.32.6.717

Anderson GP, Lindén A, Rabe KF (1994) Why are long-acting beta-adrenoceptor agonists long-acting? Eur Respir J 7(3):569–578. https://doi.org/10.1183/09031936.94.07030569

Anderson HR, Ayres JG, Sturdy PM, Bland JM, Butland BK, Peckitt C, Taylor JC, Victor CR (2005) Bronchodilator treatment and deaths from asthma: case-control study. BMJ 330 (7483):117. https://doi.org/10.1136/bmj.38316.729907.8F

Andreas S, Bothner U, de la Hoz A, Kloer I, Trampisch M, Alter P (2020) A post hoc holter ECG analysis of olodaterol and formoterol in moderate-to-very-severe COPD. Int J Chron Obstruct Pulmon Dis 15:1955–1965. https://doi.org/10.2147/COPD.S246353

Aparici M, Gómez-Angelats M, Vilella D, Otal R, Carcasona C, Viñals M, Ramos I, Gavaldà A, De Alba J, Gras J, Cortijo J, Morcillo E, Puig C, Ryder H, Beleta J, Miralpeix M (2012) Pharmacological characterization of abediterol, a novel inhaled β(2)-adrenoceptor agonist with long duration of action and a favorable safety profile in preclinical models. J Pharmacol Exp Ther 342(2):497–509. https://doi.org/10.1124/jpet.112.193284

Aparici M, Gavaldà A, Ramos I, Carcasona C, Otal R, Fernández-Blanco JA, Montero JL, García VM, López R, De Alba J, Doe C, Puig C, Vilella D, Miralpeix M (2016) In vitro and in vivo preclinical profile of abediterol (LAS100977), an inhaled long-acting β2-adrenoceptor agonist, compared with indacaterol, olodaterol and vilanterol. Eur J Pharmacol 770:61–69. https://doi.org/10.1016/j.ejphar.2015.11.053

Apperley GH, Daly MJ, Levy GP (1976) Selectivity of beta-adrenoceptor agonists and antagonists on bronchial, skeletal, vascular and cardiac muscle in the anaesthetized cat. Br J Pharmacol 57 (2):235–246. https://doi.org/10.1111/j.1476-5381.1976.tb07473.x

Arner B (1970) A comparative clinical trial of different subcutaneous doses of terbutaline and orciprenaline in bronchial asthma. Acta Med Scand Suppl 512:45–48. https://doi.org/10.1111/j.0954-6820.1970.tb05289.x

Arner B, Bertler A, Karlefors T, Westling H (1970) Bronchodilator effect of a new sympathomimetic beta-receptor-stimulating agent, terbutaline, given subcutaneously to asthmatic patients. Acta Med Scand Suppl 512:41–43. https://doi.org/10.1111/j.0954-6820.1970.tb05288.x

Aronson JK (2000) "Where name and image meet" – the argument for "adrenaline". BMJ 320 (7233):506–509. https://doi.org/10.1136/bmj.320.7233.506

Arthur G (2015) Epinephrine: a short history. Lancet Respir Med 3(5):350–351. https://doi.org/10.1016/S2213-2600(15)00087-9

Arvidsson P, Larsson S, Löfdahl CG, Melander B, Wåhlander L, Svedmyr N (1989) Formoterol, a new long-acting bronchodilator for inhalation. Eur Respir J 2(4):325–330

Assem ES, Schild HO (1969) Inhibition by sympathomimetic amines of histamine release by antigen in passively sensitized human lung. Nature 224(5223):1028–1029. https://doi.org/10.1038/2241028a0

Au DH, Lemaitre RN, Curtis JR, Smith NL, Psaty BM (2000) The risk of myocardial infarction associated with inhaled beta-adrenoceptor agonists. Am J Respir Crit Care Med 161(3 Pt 1):827–830

Au DH, Curtis JR, Every NR, McDonell MB, Fihn SD (2002) Association between inhaled beta-agonists and the risk of unstable angina and myocardial infarction. Chest 121(3):846–851

Bäcklund L, Fagerberg E (1968) Ventilatory capacity after bronchodilatation with a new beta-sympathomimetic substance. Scand J Respir Dis 49(4):284–290

Baggott C, Hardy JK, Sparks J, Sabbagh D, Beasley R, Weatherall M, Fingleton J (2022) Epinephrine (adrenaline) compared to selective beta-2-agonist in adults or children with acute asthma: a systematic review and meta-analysis. Thorax 77(6):563–572. https://doi.org/10.1136/thoraxjnl-2021-217124

Baker JG (2010) The selectivity of β-adrenoceptor agonists at the human β1, β2 and β3 adrenoceptors. Br J Pharmacol 160:148–161

Baker JG, Wilcox RG (2017) β-Blockers, heart disease and COPD: current controversies and uncertainties. Thorax 72:271–276

Baker JG, Hall IP, Hill SJ (2003) Influence of agonist efficacy and receptor phosphorylation on antagonist affinity measurements: differences between second messenger and reporter gene responses. Mol Pharmacol 64:679–688

Baker JG, Proudman RGW, Hill SJ (2015) Salmeterol's extreme β2-selectivity is due to residues in both extracellular loops and transmembrane domains. Mol Pharmacol 87:103–120

Baker JG, Gardiner SM, Woolard J, Fromont C, Jadhav GP, Mistry SN, Thompson KSJ, Kellam B, Hill SJ, Fischer PM (2017) Novel selective β1-adrenoceptor antagonists for concomitant cardiovascular and respiratory disease. FASEB J 31:3150–3166

Baker J, Fromont C, Proudman R, Kellam B, Fischer P (2020a) Development of long-acting β2-selective antagonists as a potential treatment to reduce tumour growth and metastasis. BJP:2645–2647. https://bpspubs.onlinelibrary.wiley.com/doi/abs/10.1111/bph.15035

Baker JG, Fromont C, Bruder M, Thompson KSJ, Kellam B, Hill SJ, Fischer PM (2020b) Using esterase selectivity to determine the in vivo duration of systemic availability and abolish systemic side-effects of topical β-blockers. ACS Pharmacol Transl Sci 3(4):737–748. https://doi.org/10.1021/acsptsci.0c00051

Baker PK, Dalrymple RH, Ingle DL, Ricks CA (1984) Use of a β-adrenergic agonist to alter muscle and fat deposition in lambs. J Anim Sci 59(2):1256–1261. https://doi.org/10.2527/jas1984.5951256x

Ball CM, Featherstone PJ (2017) The early history of adrenaline. Anaesth Intensive Care 45(3):279–281. https://doi.org/10.1177/0310057X1704500301

Ball DI, Brittain RT, Coleman RA, Denyer LH, Jack D, Johnson M, Lunts LH, Nials AT, Sheldrick KE, Skidmore IF (1991) Salmeterol, a novel, long-acting beta 2-adrenoceptor agonist: characterization of pharmacological activity in vitro and in vivo. Br J Pharmacol 104(3):665–671. https://doi.org/10.1111/j.1476-5381.1991.tb12486.x

Banno A, Reddy AT, Lakshmi SP, Reddy RC (2020) Bidirectional interaction of airway epithelial remodeling and inflammation in asthma. Clin Sci (Lond) 134(9):1063–1079. https://doi.org/10.1042/CS20191309

Barcroft H, Talbot JF (1968) Oliver and Schäfer's discovery of the cardiovascular action of suprarenal extract. Postgrad Med J 44:6–8. https://doi.org/10.1136/pgmj.44.507.6

Barger G, Dale HH (1910) Chemical structure and sympathomimetic action of amines. J Physiol 41(1–2):19–59. https://doi.org/10.1113/jphysiol.1910.sp001392

Barnes PJ (1993) Muscarinic receptor subtypes in airways. Life Sci 52(5–6):521–527. https://doi.org/10.1016/0024-3205(93)90310-y

Barnes PJ (2013) Theophylline. Am J Respir Crit Care Med 188(8):901–906. https://doi.org/10.1164/rccm.201302-0388PP

Barry AR, Graham MM (2013) Case report and review of clenbuterol cardiac toxicity. J Cardiol Cases 8(4):131–133. https://doi.org/10.1016/j.jccase.2013.07.004

Batterink J, Cessford TA, Taylor RA (2015) Pharmacological interventions for the acute management of hyperkalaemia in adults. Cochrane Database Syst Rev 10:CD010344. https://doi.org/10.1002/14651858.CD010344.pub2

Battram C, Charlton SJ, Cuenoud B, Dowling MR, Fairhurst RA, Farr D, Fozard JR, Leighton-Davies JR, Lewis CA, McEvoy L, Turner RJ, Trifilieff A (2006) In vitro and in vivo pharmacological characterization of 5-[(R)-2-(5,6-diethyl-indan-2-ylamino)-1-hydroxy-ethyl]-8-hydroxy-1H-quinolin-2-one (indacaterol), a novel inhaled beta(2) adrenoceptor agonist with a 24-h duration of action. J Pharmacol Exp Ther 317(2):762–770. https://doi.org/10.1124/jpet.105.098251

Beardshaw J, MacLean L, Chan-Yeung M (1974) Comparison of the bronchodilator and cardiac effects of hydroxyphenylorciprenaline and orciprenaline. Chest 65(5):507–511. https://doi.org/10.1378/chest.65.5.507

Beasley R, Pearce N, Crane J, Burgess C (1999) Beta-agonists: what is the evidence that their use increases the risk of asthma morbidity and mortality? J Allergy Clin Immunol 104(2 Pt 2):S18–S30. https://doi.org/10.1016/s0091-6749(99)70270-8

Becker AB, Simons FE (1989) Formoterol, a new long-acting selective beta 2-adrenergic receptor agonist: double-blind comparison with salbutamol and placebo in children with asthma. J Allergy Clin Immunol 84(6 Pt 1):891–895. https://doi.org/10.1016/0091-6749(89)90385-0

Beeh KM, Beier J (2009) Indacaterol, a novel inhaled, once-daily, long-acting beta2-agonist for the treatment of obstructive airways diseases. Adv Ther 26(7):691–699. https://doi.org/10.1007/s12325-009-0044-3

Beier J, Chanez P, Martinot JB, Schreurs AJ, Tkácová R, Bao W, Jack D, Higgins M (2007) Safety, tolerability and efficacy of indacaterol, a novel once-daily beta(2)-agonist, in patients with COPD: a 28-day randomised, placebo controlled clinical trial. Pulm Pharmacol Ther 20(6):740–749. https://doi.org/10.1016/j.pupt.2006.09.001

Beier J, Beeh KM, Brookman L, Peachey G, Hmissi A, Pascoe S (2009) Bronchodilator effects of indacaterol and formoterol in patients with COPD. Pulm Pharmacol Ther 22(6):492–496. https://doi.org/10.1016/j.pupt.2009.05.001

Beier J, Pujol H, Seoane B, Jimenez E, Astbury C, Massana E, Ruiz S, de Miquel G (2016) Abediterol, a novel long-acting β2-agonist: bronchodilation, safety, tolerability and pharmacokinetic results from a single-dose, dose-ranging, active-comparator study in patients with COPD. BMC Pulm Med 16(1):102. https://doi.org/10.1186/s12890-016-0266-5

Beier J, Fuhr R, Seoane B, Massana E, de Miquel G, Pujol H, Ruiz S (2017) Efficacy, safety, and tolerability of once-daily abediterol in patients with stable, persistent asthma: a phase II, randomized, 7-day, crossover study. Pharmacol Res Perspect 5:e00356. https://doi.org/10.1002/prp2.356

Belman MJ, Botnick WC, Shin JW (1996) Inhaled bronchodilators reduce dynamic hyperinflation during exercise in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 153(3):967–975. https://doi.org/10.1164/ajrccm.153.3.8630581

Bennett JA, Smyth ET, Pavord ID, Wilding PJ, Tattersfield AE (1994) Systemic effects of salbutamol and salmeterol in patients with asthma. Thorax 49(8):771–774. https://doi.org/10.1136/thx.49.8.771

Bennett JA, Harrison TW, Tattersfield AE (1999) The contribution of the swallowed fraction of an inhaled dose of salmeterol to it systemic effects. Eur Respir J 13(2):445–448. https://doi.org/10.1183/09031936.99.13244599

Benson RL, Perlman F (1948) Clinical effects of epinephrine by inhalation; a survey. J Allergy 19(2):129–140. https://doi.org/10.1016/0021-8707(48)90101-4

Bergman J, Persson H, Wetterlin K (1969) 2 New groups of selective stimulants of adrenergic beta-receptors. Experientia 25(9):899–901. https://doi.org/10.1007/BF01898049

Beumer HM (1979a) Pirbuterol in the treatment of bronchial asthma. Int J Clin Pharmacol Biopharm 17(1):18–25

Beumer HM (1979b) Comparative investigations on pirbuterol, salbutamol and placebo aerosols in bronchial asthma. Int J Clin Pharmacol Biopharm 17(6):237–239

Beumer HM (1983) Pirbuterol versus orciprenaline aerosols in the treatment of bronchial asthma. Int J Clin Pharmacol Ther Toxicol 21(3):147–166

Beumer HM, Mills JG, Sharpe PC (1978) Comparative study of carbuterol and salbutamol from metered aerosols in bronchial asthma. Respiration 35(4):220–223. https://doi.org/10.1159/000193882

Bianco S (1989) Role of broxaterol in bronchial hyperresponsiveness. Respiration 55(Suppl 2):20–27. https://doi.org/10.1159/000195766

Bianco S, Kamburoff PL, Prime FJ (1975) Comparison between the bronchodilator and cardiovascular effects of inhaling 0.5 mg. rimiterol ('Pulmadil') and 0.2 mg. salbutamol. Curr Med Res Opin 3(1):30–35. https://doi.org/10.1185/03007997509113642

Billington CK, Penn RB, Hall IP (2017) β2 Agonists. Handb Exp Pharmacol 237:23–40. https://doi.org/10.1007/164_2016_64

Bjermer L, Lemiere C, Maspero J, Weiss S, Zangrilli J, Germinaro M (2016) Reslizumab for inadequately controlled asthma with elevated blood eosinophil levels: a randomized phase 3 study. Chest 150(4):789–798. https://doi.org/10.1016/j.chest.2016.03.032

Blair HA (2021) Indacaterol/glycopyrronium/mometasone: a review in asthma. Drugs 81(6):709–719. https://doi.org/10.1007/s40265-021-01518-w

Bloom CI, Franklin C, Bush A, Saglani S, Quint JK (2021) Burden of preschool wheeze and progression to asthma in the UK: population-based cohort 2007 to 2017. J Allergy Clin Immunol 147(5):1949–1958. https://doi.org/10.1016/j.jaci.2020.12.643

Booth H, Bish R, Walters J, Whitehead F, Walters EH (1996) Salmeterol tachyphylaxis in steroid treated asthmatic subjects. Thorax 51(11):1100–1104. https://doi.org/10.1136/thx.51.11.1100

Boudestein LCM, Rutten FH, Cramer MJ, Lammers JWJ, Hoes AW (2009) The impact of concurrent heart failure on prognosis in patients with chronic obstructive pulmonary disease. Eur J Heart Fail 11(12):1182–1188. https://doi.org/10.1093/eurjhf/hfp148

Bouyssou T, Hoenke C, Rudolf K, Lustenberger P, Pestel S, Sieger P, Lotz R, Heine C, Büttner FH, Schnapp A, Konetzki I (2010a) Discovery of olodaterol, a novel inhaled beta2-adrenoceptor agonist with a 24 h bronchodilatory efficacy. Bioorg Med Chem Lett 20(4):1410–1414. https://doi.org/10.1016/j.bmcl.2009.12.087

Bouyssou T, Casarosa P, Naline E, Pestel S, Konetzki I, Devillier P, Schnapp A (2010b) Pharmacological characterization of olodaterol, a novel inhaled beta2-adrenoceptor agonist exerting a 24-hour-long duration of action in preclinical models. J Pharmacol Exp Ther 334(1):53–62. https://doi.org/10.1124/jpet.110.167007

Bowman WC, Nott MW (1970) Actions of some sympathomimetic bronchodilator and beta-adrenoceptor blocking drugs on contractions of the cat soleus muscle. Br J Pharmacol 38(1):37–49. https://doi.org/10.1111/j.1476-5381.1970.tb10334.x

Bowman WC, Nott MW (1971) Muscle tremor produced by sympathomimetic bronchodilators. J Pharm Pharmacol 23:225S. https://doi.org/10.1111/j.2042-7158.1971.tb08807.x

Braiman A, Priel Z (2008) Efficient mucociliary transport relies on efficient regulation of ciliary beating. Respir Physiol Neurobiol 163(1–3):202–207. https://doi.org/10.1016/j.resp.2008.05.010

Bremner P, Burgess C, Beasley R, Woodman K, Marshall S, Crane J, Pearce N (1992a) Nebulized fenoterol causes greater cardiovascular and hypokalaemic effects than equivalent bronchodilator doses of salbutamol in asthmatics. Respir Med 186(5):419–423. https://doi.org/10.1016/s0954-6111(06)80009-0

Bremner P, Burgess CD, Crane J, McHaffie D, Galletly D, Pearce N, Woodman K, Beasley R (1992b) Cardiovascular effects of fenoterol under conditions of hypoxaemia. Thorax 47(10):814–817. https://doi.org/10.1136/thx.47.10.814

Bremner P, Woodman K, Burgess C, Crane J, Purdie G, Pearce N, Beasley R (1993) A comparison of the cardiovascular and metabolic effects of formoterol, salbutamol and fenoterol. Eur Respir J 6(2):204–210

Bremner P, Siebers R, Crane J, Beasley R, Burgess C (1996) Partial vs full beta-receptor agonism. A clinical study of inhaled albuterol and fenoterol. Chest 109(4):957–962. https://doi.org/10.1378/chest.109.4.957

Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R, Zera P, Menlove R, Shah P, Jamieson S, Stinson EB (1986) β1- and β 2-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective β 1-receptor down-regulation in heart failure. Circ Res 59:297–309

Brittain RT, Farmer JB, Jack D, Martin LE, Simpson WT (1968) Alpha-[(t-butylamino)methyl]-4-hydroxy-m-xylene-alpha 1,alpha 3-diol (AH.3365): a selective beta-adrenergic stimulant. Nature 219(5156):862–863. https://doi.org/10.1038/219862a0

Brittain RT, Dean CM, Jack D (1976) Sympathomimetic bronchodilator drugs. Pharmacol Ther B 2 (3):423–462. https://doi.org/10.1016/0306-039x(76)90001-5

Brittain D, D'Andrea P, Gruen E, Hosoe M, Jain D, Jauernig J, Pethe A, Scosyrev E, Tanase AM, Tillmann HC (2022) A review of the unique drug development strategy of indacaterol acetate/glycopyrronium bromide/mometasone furoate: a first-in-class, once-daily, single-inhaler, fixed-dose combination treatment for asthma. Adv Ther 39(6):2365–2378. https://doi.org/10.1007/s12325-021-02025-w

Brown HM, Storey G, George WH (1972) Beclomethasone dipropionate: a new steroid aerosol for the treatment of allergic asthma. Br Med J 1(5800):585–590. https://doi.org/10.1136/bmj.1.5800.585

Bullowa J, Kaplan D (1903) Treatment of asthmatic attacks: on the hypodermatic use of adrenalin chloride in the treatment of asthmatic attacks. Med News 83:787–790

Burki NK, Diamond L (1978) Long-term oral bronchodilator therapy of asthma with pirbuterol. Clin Pharmacol Ther 24(1):84–89. https://doi.org/10.1002/cpt197824184

Burnett J (1903) Adrenalin: a short account of its therapeutic applications. The Medical Times and Hopsital Gazette. 20th June, pp 385–387

Bushe C (1983) Salbutamol for hyperkalaemia. Lancet 2(8353):797. https://doi.org/10.1016/s0140-6736(83)92335-8

Butchers PR, Vardey CJ, Johnson M (1991) Salmeterol: a potent and long-acting inhibitor of inflammatory mediator release from human lung. Br J Pharmacol 104(3):672–676. https://doi.org/10.1111/j.1476-5381.1991.tb12487.x

Buxton BF, Jones CR, Molenaar P, Summers RJ (1987) Characterization and autoradiographic localization of β-beta-adrenoceptor subtypes in human cardiac tissues. Br J Pharmacol 92:299–310

Camoretti-Mercado B, Lockey RF (2021) Airway smooth muscle pathophysiology in asthma. J Allergy Clin Immunol 147(6):1983–1995. https://doi.org/10.1016/j.jaci.2021.03.035

Camps PWL (1929) A note on the inhalations treatment of asthma. Guy's Hospital Rep 79:496–498

Castle W, Fuller R, Hall J, Palmer J (1993) Serevent nationwide surveillance study: comparison of salmeterol with salbutamol in asthmatic patients who require regular bronchodilator treatment. BMJ 306(6884):1034–1037. https://doi.org/10.1136/bmj.306.6884.1034

Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, Busse WW, Ford L, Sher L, FitzGerald JM, Katelaris C, Tohda Y, Zhang B, Staudinger H, Pirozzi G, Amin N, Ruddy M, Akinlade B, Khan A, Chao J, Martincova R, Graham NMH, Hamilton JD, Swanson BN, Stahl N, Yancopoulos GD, Teper A (2018) Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. N Engl J Med 378(26):2486–2496. https://doi.org/10.1056/NEJMoa1804092

Cates CJ, Cates MJ (2008) Regular treatment with salmeterol for chronic asthma: serious adverse events. Cochrane Database Syst Rev 3:CD006363. https://doi.org/10.1002/14651858.CD006363.pub2

Cates CJ, Cates MJ, Lasserson TJ (2008) Regular treatment with formoterol for chronic asthma: serious adverse events. Cochrane Database Syst Rev 8(4):CD006923. https://doi.org/10.1002/14651858.CD006923.pub2

Cates CJ, Schmidt S, Ferrer M, Sayer B, Waterson S (2018) Inhaled steroids with and without regular salmeterol for asthma: serious adverse events. Cochrane Database Syst Rev 12(12):CD006922. https://doi.org/10.1002/14651858.CD006922.pub4

Cazzola M, Calderaro F, Califano C, Di Pema F, Vinciguerra A, Donner CF, Matera MG (1999) Oral bambuterol compared to inhaled salmeterol in patients with partially reversible chronic obstructive pulmonary disease. Eur J Clin Pharmacol 54(11):829–833. https://doi.org/10.1007/s002280050561

Cazzola M, Matera MG, Donner CF (2005) Inhaled beta2-adrenoceptor agonists: cardiovascular safety in patients with obstructive lung disease. Drugs 65(12):1595–1610. https://doi.org/10.2165/00003495-200565120-00001

Cazzola M, Rogliani P, Matera MG (2019) Ultra-LABAs for the treatment of asthma. Respir Med 156:47–52. https://doi.org/10.1016/j.rmed.2019.08.005

Cazzola M, Matera MG, Rogliani P, Calzetta L (2021) Comparative studies of dual bronchodilation in COPD. Monaldi Arch Chest Dis 91(1). https://doi.org/10.4081/monaldi.2021.1625

Chahl LA, O'Donnell SR (1968) The actions of orciprenaline and protokylol on guinea-pig trachea. Br J Pharmacol Chemother 33(3):552–559. https://doi.org/10.1111/j.1476-5381.1968.tb00504.x

Chalitsios CV, Shaw DE, McKeever TM (2021) Risk of osteoporosis and fragility fractures in asthma due to oral and inhaled corticosteroids: two population-based nested case-control studies. Thorax 76(1):21–28. https://doi.org/10.1136/thoraxjnl-2020-215664

Charpin D (1990) Acute and long-term effectiveness of tulobuterol inhaler, a new beta 2-agonist, in the treatment of asthma. Lung 168(Suppl):194–201. https://doi.org/10.1007/BF02718133

Chaudhuri R, Rubin A, Sumino K, Lapa E, Silva JR, Niven R, Siddiqui S, Klooster K, McEvoy C, Shah PL, Simoff M, Khatri S, Barbers R, Mark Grubb G, McMullen EA, Olson JL, Laviolette M, BT10+ Study Group (2021) Safety and effectiveness of bronchial thermoplasty after 10 years in patients with persistent asthma (BT10+): a follow-up of three randomised controlled trials. Lancet Respir Med 9(5):457–466. https://doi.org/10.1016/S2213-2600(20)30408-2

Chetta A, Garavaldi G, Cuomo A, Gurrieri G, Olivieri D (1988) Early bronchodilating effect of a new oral beta-2-receptor agonist (broxaterol) in bronchial asthma. Respiration 53(4):220–224. https://doi.org/10.1159/000195423

Cheung D, Timmers MC, Zwinderman AH, Bel EH, Dijkman JH, Sterk PJ (1992) Long-term effects of a long-acting beta 2-adrenoceptor agonist, salmeterol, on airway hyperresponsiveness in patients with mild asthma. N Engl J Med 327(17):1198–1203. https://doi.org/10.1056/NEJM199210223271703

Chiarino D, Fantucci M, Carenzi A, Della Bella D, Frigeni V, Sala R (1986) New isoxazole derivatives with a potent and selective beta 2-adrenergic activity. Farmaco Sci 41(6):440–453

Chongmelaxme B, Chaiyakunapruk N, Dilokthornsakul P (2020) Association between adherence and severe asthma exacerbation: a systematic review and meta-analysis. J Am Pharm Assoc 60(5):669–685.e2

Choo-Kang YF, Simpson WT, Grant IW (1969) Controlled comparison of the bronchodilator effects of three beta-adrenergic stimulant drugs administered by inhalation to patients with asthma. Br Med J 2(5652):287–289. https://doi.org/10.1136/bmj.2.5652.287

Chu EK, Drazen JM (2005) Asthma: one hundred years of treatment and onward. Am J Respir Crit Care Med 171(11):1202–1208. https://doi.org/10.1164/rccm.200502-257OE

Chuchalin AG, Tsoi AN, Richter K, Krug N, Dahl R, Luursema PB, Cameron R, Bao W, Higgins M, Woessner R, van As A (2007) Safety and tolerability of indacaterol in asthma: a randomized, placebo-controlled 28-day study. Respir Med 101(10):2065–2075. https://doi.org/10.1016/j.rmed.2007.06.002. Epub 2007 Jul 20

Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, Adcock IM, Bateman ED, Bel EH, Bleecker ER, Boulet LP, Brightling C, Chanez P, Dahlen SE, Djukanovic R, Frey U, Gaga M, Gibson P, Hamid Q, Jajour NN, Mauad T, Sorkness RL, Teague WG (2014) International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J 43(2):343–373. https://doi.org/10.1183/09031936.00202013

Clark RB, Allal C, Friedman J, Johnson M, Barber R (1996) Stable activation and desensitization of beta 2-adrenergic receptor stimulation of adenylyl cyclase by salmeterol: evidence for quasi-irreversible binding to an exosite. Mol Pharmacol 49(1):182–189

Clarke WP, Bond RA (1998) The elusive nature of intrinsic efficacy. Trends Pharmacol Sci 19:270–276

Cockcroft DW (1999) Pharmacologic therapy for asthma: overview and historical perspective. J Clin Pharmacol 39(3):216–222

Cockcroft DW, McParland CP, Britto SA, Swystun VA, Rutherford BC (1993) Regular inhaled salbutamol and airway responsiveness to allergen. Lancet 342(8875):833–837. https://doi.org/10.1016/0140-6736(93)92695-p

Cohen BM (1978) Comparison of the broncholytic and cardiovascular responses of asthmatic patients to aerosol dose of isoproterenol and fenoterol. Chest 73(6 Suppl):992–993. https://doi.org/10.1378/chest.73.6_supplement.992

Colella DF, Chakrin LW, Shetzline A, Wardell JR Jr (1977) Characterization of the adrenergic activity of carbuterol (SK&F 40383-A). Eur J Pharmacol 46(3):229–241. https://doi.org/10.1016/0014-2999(77)90338-7

Collier JG, Dobbs RJ, Williams I (1980) Salbutamol aerosol causes a tachycardia due to the inhaled rather than the swallowed fraction. Br J Clin Pharmacol 9(3):273–274. https://doi.org/10.1111/j.1365-2125.1980.tb04837.x

Collins JM, McDevitt DG, Shanks RG, Swanton JG (1969) The cardio-toxicity of isoprenaline during hypoxia. Br J Pharmacol 36(1):35–45. https://doi.org/10.1111/j.1476-5381.1969.tb08301.x

Conolly ME, Davies DS, Dollery CT, George CF (1971) Resistance to β-adrenoceptor stimulants (a possible explanation for the rise in ashtma deaths). Br J Pharmacol 43(2):389–402

Cook P, Scarfone RJ, Cook RT (1994) Adenosine in the termination of albuterol-induced supraventricular tachycardia. Ann Emerg Med 24(2):316–319. https://doi.org/10.1016/s0196-0644(94)70146-6

Corren J, Parnes JR, Wang L, Mo M, Roseti SL, Griffiths JM, van der Merwe R (2017) Tezepelumab in adults with uncontrolled asthma. N Engl J Med 377(10):936–946. https://doi.org/10.1056/NEJMoa1704064

Corren J, Pham TH, Garcia Gil E, Sałapa K, Ren P, Parnes JR, Colice G, Griffiths JM (2022) Baseline type 2 biomarker levels and response to tezepelumab in severe asthma. Allergy 77(6):1786–1796. https://doi.org/10.1111/all.15197

Couillard S, Do WIH, Beasley R, Hinks TSC, Pavord ID (2021) Predicting the benefits of type-2 targeted anti-inflammatory treatment with the prototype Oxford Asthma Attack Risk Scale (ORACLE). ERJ Open Res 8(1):00570–02021. https://doi.org/10.1183/23120541.00570-2021

Crane J, Pearce N, Flatt A, Burgess C, Jackson R, Kwong T, Ball M, Beasley R (1989a) Prescribed fenoterol and death from asthma in New Zealand, 1981-83: case-control study. Lancet 1(8644):917–922. https://doi.org/10.1016/s0140-6736(89)92505-1

Crane J, Burgess C, Beasley R (1989b) Cardiovascular and hypokalaemic effects of inhaled salbutamol, fenoterol, and isoprenaline. Thorax 44(2):136–140. https://doi.org/10.1136/thx.44.2.136

Crompton G (2006) A brief history of inhaled asthma therapy over the last fifty years. Prim Care Respir J 15(6):326–331. https://doi.org/10.1016/j.pcrj.2006.09.002

Crompton GK, Ayres JG, Basran G, Schiraldi G, Brusasco V, Eivindson A, Jamieson AH, Olsson H (1999) Comparison of oral bambuterol and inhaled salmeterol in patients with symptomatic asthma and using inhaled corticosteroids. Am J Respir Crit Care Med 159(3):824–828. https://doi.org/10.1164/ajrccm.159.3.9806117

Crowe MJ, Counihan HE, O'Malley K (1985) A comparative study of a new selective beta 2-adrenoceptor agonist, procaterol and salbutamol in asthma. Br J Clin Pharmacol 19(6):787–791. https://doi.org/10.1111/j.1365-2125.1985.tb02715.x

Cruickshank JM (2007) Are we misunderstanding beta-blockers. Int J Cardiol 120:10–27. https://doi.org/10.1016/j.ijcard.2007.01.069

Cullinan P, Vandenplas O, Bernstein D (2020) Assessment and management of occupational asthma. J Allergy Clin Immunol Pract 8(10):3264–3275. https://doi.org/10.1016/j.jaip.2020.06.031

Cullum VA, Farmer JB, Jack D, Levy GP (1969) Salbutamol: a new, selective beta-adrenoceptive receptor stimulant. Br J Pharmacol 35(1):141–151. https://doi.org/10.1111/j.1476-5381.1969.tb07975.x

D'Silva JL (1934) The action of adrenaline on serum potassium. J Physiol 82(4):393–398. https://doi.org/10.1113/jphysiol.1934.sp003190

Da Costa JL, Goh BK (1973) A comparative trial of subcutaneous terbutaline, Th1165a and adrenaline in bronchial asthma. Med J Aust 2(12):588–591. https://doi.org/10.5694/j.1326-5377.1973.tb129672.x

Dahl R, Harving H, Henriksen J, Rønne K, Schøler P (1985) Procaterol and terbutaline in bronchial asthma. A double-blind, placebo-controlled, cross-over study. Allergy 40(7):501–505. https://doi.org/10.1111/j.1398-9995.1985.tb00257.x

Dakin HD (1905) The synthesis of a substance allied to adrenalin. Proc R Soc Med 76:491–497

Dale HH (1906) On some physiological actions of ergot. J Physiol 34(3):163–206. https://doi.org/10.1113/jphysiol.1906.sp001148

D'Alonzo GE, Smolensky MH, Feldman S, Gnosspelius Y, Karlsson K (1995) Bambuterol in the treatment of asthma. A placebo-controlled comparison of once-daily morning vs evening administration. Chest 107(2):406–412. https://doi.org/10.1378/chest.107.2.406

Davis C, Conolly ME (1980) Tachyphylaxis to beta-adrenoceptor agonists in human bronchial smooth muscle: studies in vitro. Br J Clin Pharmacol 10(5):417–423. https://doi.org/10.1111/j.1365-2125.1980.tb01782.x

De Candussio G, Franchi D, Manini G, Arossa W, Castello D (1986) Duration of oral procaterol protection from methacholine-induced bronchial obstruction. Int J Clin Pharmacol Res 6 (5):403–407

Delhaye M, Taton G, Camus JC, Chatelain P, Robberecht P, Waelbroeck M, Christophe J (1983) Effects of full and partial beta-adrenergic agonists and antagonists on human lung adenylate cyclase. Biochem Pharmacol 32(12):1831–1835. https://doi.org/10.1016/0006-2952(83)90046-1

Department of Health (UK) (2011) An outcomes strategy for chronic obstructive pulmonary disease (COPD) and asthma in England

Devalia JL, Sapsford RJ, Rusznak C, Toumbis MJ, Davies RJ (1992) The effects of salmeterol and salbutamol on ciliary beat frequency of cultured human bronchial epithelial cells, in vitro. Pulm Pharmacol 5(4):257–263. https://doi.org/10.1016/0952-0600(92)90068-r

Diamant Z, Boot JD, Virchow JC (2007) Summing up 100 years of asthma. Respir Med 101 (3):378–388. https://doi.org/10.1016/j.rmed.2006.12.004

Diewitz M (1977) The influence of the broncholytic reproterol in persons with bradycardic arrhythmia upon impulse and conduction as well as irritability of the human ventricle. Arzneimittelforschung 27(12):66–72

Drachler DH, Bower JS, Miller DA, Rotman HH (1977) Long-term evaluation of a new aerosol bronchodilator, carbuterol, and comparison with isoproterenol. J Clin Pharmacol 17(11–12):734–739. https://doi.org/10.1002/j.1552-4604.1977.tb01549.x

Dragonieri S, Carpagnano GE (2021) Biological therapy for severe asthma. Asthma Res Pract 7 (1):12. https://doi.org/10.1186/s40733-021-00078-w

Drazen JM, Israel E, Boushey HA, Chinchilli VM, Fahy JV, Fish JE, Lazarus SC, Lemanske RF, Martin RJ, Peters SP, Sorkness C, Szefler SJ (1996) Comparison of regularly scheduled with as-needed use of albuterol in mild asthma. Asthma Clinical Research Network. N Engl J Med 335 (12):841–847. https://doi.org/10.1056/NEJM199609193351202

Durham SR, Leung DY (2010) One hundred years of allergen immunotherapy: time to ring the changes. J Allergy Clin Immunol 127(1):3–7. https://doi.org/10.1016/j.jaci.2010.11.032

Dyson AJ, Mackay AD (1980) Two oral beta-adrenergic stimulant drugs, pirbuterol and salbutamol, in reversible airway obstruction. Br J Dis Chest 74(1):70–77

Ellis KE, Mistry R, Boyle JP, Challiss RA (1995) Correlation of cyclic AMP accumulation and relaxant actions of salmeterol and salbutamol in bovine tracheal smooth muscle. Br J Pharmacol 116(5):2510–2516. https://doi.org/10.1111/j.1476-5381.1995.tb15103.x

Ence TJ, Tashkin DP, Ho D, Child JS (1979) Acute bronchial and cardiovascular effects of oral pirbuterol and metaproterenol. Ann Allergy 43(4):229–236
Engelhardt G (1972) Structure-activity studies in a series of new amino-halogen substituted phenyl-aminoethanoles. Arzneimittel-Forsch 22:869–876
Engelhardt G (1976) Profile of pharmacological actions of NAB 365 (clenbuterol), a novel broncholytic agent with selective activity on adrenergic beta2-receptors. Arzneimittelforschung 26(7a):1404–1420
Engelhardt G (1984) Structure-activity relationships in further series of amino-halogen substituted phenyl-aminoethanols. Arzneimittelforschung 34(11A):1625–1632
Engelhardt A, Hoefke W, Wick H (1961) Zur pharmakologie des sympathomimeticums 1-(3,5-dihydroxyphenyl)-l-hydroxy-2-isopropylaminoathan. Arzneimittel-Forsch 11:521–525
Enilari O, Sinha S (2019) The global impact of asthma in adult populations. Ann Glob Health 85(1). https://doi.org/10.5334/aogh.2412
Eriksson NE, Lindgren SB (1978) The rapidity of bronchodilatation. A comparison of isoprenaline, terbutaline and rimiterol. Scand. J Respir Dis 59(1):30–36
Esdaile JM, Feinstein AR, Horwitz RI (1987) A reappraisal of the United Kingdom epidemic of fatal asthma. Can general mortality data implicate a therapeutic agent? Arch Intern Med 147 (3):543–549
Exon PD (1967) Pressurized aerosols in asthma. Br Med J 2(5545):178. https://doi.org/10.1136/bmj.2.5545.178
Faulds D, Hollingshead LM, Goa KL (1991) Formoterol. A review of its pharmacological properties and therapeutic potential in reversible obstructive airways disease. Drugs 42 (1):115–137. https://doi.org/10.2165/00003495-199142010-00007
Feary JR, Rodrigues LC, Smith CJ, Hubbard RB, Gibson JE (2010) Prevalence of major comorbidities in subjects with COPD and incidence of myocardial infarction and stroke: a comprehensive analysis using data from primary care. Thorax 65(11):956–962. https://doi.org/10.1136/thx.2009.128082
Ferguson GT, Darken P, Ballal S, Siddiqui MK, Singh B, Attri S, Holmgren U, de Nigris E (2020) Efficacy of budesonide/glycopyrronium/formoterol fumarate metered dose inhaler (BGF MDI) versus other inhaled corticosteroid/long-acting muscarinic antagonist/long-acting β2-Agonist (ICS/LAMA/LABA) triple combinations in COPD: a systematic literature review and network meta-analysis. Adv Ther 37(6):2956–2975. https://doi.org/10.1007/s12325-020-01311-3
Fischer J, Ganellin CR (2006) Analogue-based drug discovery. Wiley, pp 542–543. ISBN 9783527607495
Fitch K (2016) The world anti-doping code: can you have asthma and still be an elite athlete? Breathe 12:148–158
FitzGerald JM, Sadatsafavi M (2019) Triple therapy in a single inhaler: a new option for uncontrolled asthma. Lancet 394(10210):1690–1692. https://doi.org/10.1016/S0140-6736(19)32216-0
FitzGerald JM, Bleecker ER, Menzies-Gow A, Zangrilli JG, Hirsch I, Metcalfe P, Newbold P, Goldman M (2018) Predictors of enhanced response with benralizumab for patients with severe asthma: pooled analysis of the SIROCCO and CALIMA studies. Lancet Respir Med 6(1):51–64. https://doi.org/10.1016/S2213-2600(17)30344-2. Epub 2017 Sep 11
Flick MR, Block AJ (1979) Nocturnal vs diurnal cardiac arrhythmias in patients with chronic obstructive pulmonary disease. Chest 75(1):8–11. https://doi.org/10.1378/chest.75.1.8
Flood-Page P, Swenson C, Faiferman I, Matthews J, Williams M, Brannick L, Robinson D, Wenzel S, Busse W, Hansel TT, Barnes NC, International Mepolizumab Study Group (2007) A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. Am J Respir Crit Care Med 176(11):1062–1071. https://doi.org/10.1164/rccm.200701-085OC
Foster RW, Atanga GK, Carpenter JR, Evans DE, Rakshi K, Small RC (1991) A method for bioassay of potency and effectiveness of inhaled bronchodilators in normal subjects. Br J Clin Pharmacol 31(4):445–455. https://doi.org/10.1111/j.1365-2125.1991.tb05561.x

Fränkel S (1897) Physiological action of the suprarenal capsules. J Chem Soc 72:63–64. Abstracts. From Wien Med Blätter 1896;14, 15, 16

Freedman T (1956) Medihaler therapy for bronchial asthma. Postgrad Med 20:667–673

Friedel HA, Brogden RN (1988) Bitolterol. A preliminary review of its pharmacological properties and therapeutic efficacy in reversible obstructive airways disease. Drugs 35(1):22–41. https://doi.org/10.2165/00003495-198835010-00002

Fujimoto K, Komatsu Y, Yasuo M, Urushihata K, Kubo K (2006) Comparison of the clinical efficacy of salmeterol and sustained-release tulobuterol (patch) on inadequately controlled asthma patients on inhaled corticosteroids. J Asthma 43(7):501–507. https://doi.org/10.1080/02770900600758432

Fukuchi Y, Nagai A, Seyama K, Nishimura M, Hirata K, Kubo K, Ichinose M, Aizawa H, Research Group TB (2005) Clinical efficacy and safety of transdermal tulobuterol in the treatment of stable COPD: an open-label comparison with inhaled salmeterol. Treat Respir Med 4(6):447–455. https://doi.org/10.2165/00151829-200504060-00008

Furchgott RF (1966) In: Harper NJ, Simmonds AB (eds) Advances in drug research, vol 3. Academic Press, pp 21–55

Furuhashi K, Fujisawa T, Hashimoto D, Kamiya Y, Yasui H, Karayama M, Suzuki Y, Hozumi H, Enomoto N, Nakamura Y, Inui N, Suda T (2019) Once-daily fluticasone furoate/vilanterol combination versus twice-daily budesonide/formoterol combination in the treatment of controlled stable asthma: a randomized crossover trial. J Asthma Allergy 12:253–261. https://doi.org/10.2147/JAA.S223093

Galant SP, Duriseti L, Underwood S, Insel PA (1978) Decreased beta-adrenergic receptors on polymorphonuclear leukocytes after adrenergic therapy. N Engl J Med 299(17):933–936. https://doi.org/10.1056/NEJM197810262991707

García-Marcos L, Asher MI, Pearce N, Ellwood E, Bissell K, Chiang CY, El Sony A, Ellwood P, Marks GB, Mortimer K, Martínez-Torres AE, Morales E, Perez-Fernandez V, Robertson S, Rutter CE, Silverwood RJ, Strachan DP, Global Asthma Network Phase I Study Group (2022) The burden of asthma, hay fever and eczema in children in 25 countries: GAN phase I study. Eur Respir J 60(3):2102866. https://doi.org/10.1183/13993003.02866-2021

Garrett JE, Lanes SF, Kolbe J, Rea HH (1996) Risk of severe life threatening asthma and beta agonist type: an example of confounding by severity. Thorax 51(11):1093–1099. https://doi.org/10.1136/thx.51.11.1093

Gay LN, Long JW (1949) Clinical evaluation of isopropyl-epinephrine in management of bronchial asthma. JAMA 39(7):452–457. https://doi.org/10.1001/jama.1949.72900240003008

GBD 2015 Chronic Respiratory Disease Collaborators (2017) Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Respir Med 9:691–706. https://doi.org/10.1016/S2213-2600(17)30293-X

Geake JB, Dabscheck EJ, Wood-Baker R, Cates CJ (2015) Indacaterol, a once-daily beta2-agonist, versus twice-daily beta$_2$-agonists or placebo for chronic obstructive pulmonary disease. Cochrane Database Syst Rev 1(1):CD010139. https://doi.org/10.1002/14651858.CD010139.pub2

Geyer H, Schänzer W, Thevis M (2014) Anabolic agents: recent strategies for their detection and protection from inadvertent doping. Br J Sports Med 48(10):820–826. https://doi.org/10.1136/bjsports-2014-093526

Gibson PG, Yang IA, Upham JW, Reynolds PN, Hodge S, James AL, Jenkins C, Peters MJ, Marks GB, Baraket M, Powell H, Taylor SL, Leong LEX, Rogers GB, Simpson JL (2017) Effect of azithromycin on asthma exacerbations and quality of life in adults with persistent uncontrolled asthma (AMAZES): a randomised, double-blind, placebo-controlled trial. Lancet 390(10095):659–668. https://doi.org/10.1016/S0140-6736(17)31281-3

GINA – Global Initiative for Asthma (2022) GINA report, global strategy for asthma management and prevention. https://ginasthma.org/gina-reports/

Goldberg R, van As M, Joffe BI, Krut L, Bersohn I, Seftel HC (1975) Metabolic responses to selective beta-adrenergic stimulation in man. Postgrad Med J 51(592):53–58. https://doi.org/10.1136/pgmj.51.592.53

Goldstein DS (2006) Adrenaline and the inner world: an introduction to scientific integrative medicine. The Johns Hopkins University Press, p 60. ISBN: 9780801882883

Gonem S, Cumella A, Richardson M (2019) Asthma admission rates and patterns of salbutamol and inhaled corticosteroid prescribing in England from 2013 to 2017. Thorax. https://doi.org/10.1136/thoraxjnl-2018-212723

Goodacre S, Cohen J, Bradburn M, Gray A, Benger J, Coats T, 3Mg Research Team (2013) Intravenous or nebulised magnesium sulphate versus standard therapy for severe acute asthma (3Mg trial): a double-blind, randomised controlled trial. Lancet Respir Med 1(4):293–300. https://doi.org/10.1016/S2213-2600(13)70070-5

Graham GS (1968) Two sudden unexplained deaths in asthmatics. Scott Med J 13(8):282–283. https://doi.org/10.1177/003693306801300

Grainger J, Woodman K, Pearce N, Crane J, Burgess C, Keane A, Beasley R (1991) Prescribed fenoterol and death from asthma in New Zealand, 1981-7: a further case-control study. Thorax 46(2):105–111. https://doi.org/10.1136/thx.46.2.105

Green RH, Pavord ID (2001) Leukotriene antagonists and symptom control in chronic persistent asthma. Lancet 357(9273):1991–1992. https://doi.org/10.1016/S0140-6736(00)05132-1

Greenberg MJ (1965) Isoprenaline in myocardial failure. Lancet 286:442–443. https://doi.org/10.1016/S0140-6736(65)90796-8

Greenberg MJ, Pines A (1967) Pressurized aerosols in asthma. Br Med J 1:563. https://doi.org/10.1136/bmj.1.5539.563

Griffin JP, Turner P (1971) Preliminary studies of a new bronchodilator (WG 253) in man. J Clin Pharmacol New Drugs 11(4):280–287

Guhan AR, Cooper S, Oborne J, Lewis S, Bennett J, Tattersfield AE (2000) Systemic effects of formoterol and salmeterol: a dose-response comparison in healthy subjects. Thorax 55(8):650–656. https://doi.org/10.1136/thorax.55.8.650

Gunn SD, Ayres JG, McConchie SM (1995) Comparison of the efficacy, tolerability and patient acceptability of once-daily bambuterol tablets against twice-daily controlled release salbutamol in nocturnal asthma. ACROBATICS Research Group. Eur J Clin Pharmacol 48(1):23–28. https://doi.org/10.1007/BF00202167

Habashy D, Lam LT, Browne GJ (2003) The administration of beta2-agonists for paediatric asthma and its adverse reaction in Australian and New Zealand emergency departments: a cross-sectional survey. Eur J Emerg Med 10(3):219–224. https://doi.org/10.1097/00063110-200309000-00012

Habersang S, Leuschner F, Stroman F, Domenico A, Schlichtegroll A (1977) Compound with bronchospasmolytical activity from the chemical class of beta-phenylethyl-aminoalkyl-xanthines. Arzneimittelforschung 27(12):22–35

Haffner CA, Kendall MJ (1992) Metabolic effects of beta 2-agonists. J Clin Pharm Ther 17(3):155–164. https://doi.org/10.1111/j.1365-2710.1992.tb01285.x

Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, Wardlaw AJ, Green RH (2008) Cluster analysis and clinical asthma phenotypes. Am J Respir Crit Care Med 178(3):218–224. https://doi.org/10.1164/rccm.200711-1754OC

Hallstrand TS, Leuppi JD, Joos G, Hall GL, Carlsen KH, Kaminsky DA, Coates AL, Cockcroft DW, Culver BH, Diamant Z, Gauvreau GM, Horvath I, de Jongh FHC, Laube BL, Sterk PJ, Wanger J, American Thoracic Society (ATS)/European Respiratory Society (ERS) Bronchoprovocation Testing Task Force (2018) ERS technical standard on bronchial challenge testing: pathophysiology and methodology of indirect airway challenge testing. Eur Respir J 52(5):1801033. https://doi.org/10.1183/13993003.01033-2018

Hanania NA, Feldman G, Zachgo W, Shim JJ, Crim C, Sanford L, Lettis S, Barnhart F, Haumann B (2012) The efficacy and safety of the novel long-acting β2 agonist vilanterol in patients with

COPD: a randomized placebo-controlled trial. Chest 142(1):119–127. https://doi.org/10.1378/chest.11-2231

Heaney LG, Busby J, Hanratty CE, Djukanovic R, Woodcock A, Walker SM, Hardman TC, Arron JR, Choy DF, Bradding P, Brightling CE, Chaudhuri R, Cowan DC, Mansur AH, Fowler SJ, Niven RM, Howarth PH, Lordan JL, Menzies-Gow A, Harrison TW, Robinson DS, Holweg CTJ, Matthews JG, Pavord ID, Investigators for the MRC Refractory Asthma Stratification Programme (2021) Composite type-2 biomarker strategy versus a symptom-risk-based algorithm to adjust corticosteroid dose in patients with severe asthma: a multicentre, single-blind, parallel group, randomised controlled trial. Lancet Respir Med 9(1):57–68. https://doi.org/10.1016/S2213-2600(20)30397-0

Hesse H, Kasparek R, Mizera W, Unterholzner C, Konietzko N (1981) Influence of reproterol on ciliary beat frequency of human bronchial epithelium in vitro. Arzneimittelforschung 31 (4):716–718

Hieger MA, Emswiler MP, Maskell KF, Sentz JT, Miller KB, Wolf CE, Cumpston KL, Wills BK (2016) A case series of clenbuterol toxicity caused by adulterated heroin. J Emerg Med 51 (3):259–261. https://doi.org/10.1016/j.jemermed.2016.05.047

Higgins RM, Cookson WO, Lane DJ, John SM, McCarthy GL, McCarthy ST (1987) Cardiac arrhythmias caused by nebulised beta-agonist therapy. Lancet 2(8563):863–864. https://doi.org/10.1016/s0140-6736(87)91057-9

Himori N, Taira N (1977) Assessment of the selectivity of OPC-2009, a new beta2-adrenoceptor stimulatn, by the use of the blood-perfused trachea in situ and of the isolated blood-perfused papillary muscle of the dog. Br J Pharmacol 61(1):9–17. https://doi.org/10.1111/j.1476-5381.1977.tb09734.x

Hoffman RS, Kirrane BM, Marcus SM, Clenbuterol Study Investigators (2008) A descriptive study of an outbreak of clenbuterol-containing heroin. Ann Emerg Med 52(5):548–553. https://doi.org/10.1016/j.annemergmed.2008.04.026

Hoffmann C, Leitz MR, Oberdorf-Maass S, Lohse MJ, Klotz KN (2004) Comparative pharmacology of human beta-adrenergic receptor subtypes – characterization of stably transfected receptors in CHO cells. Naunyn Schmiedebergs Arch Pharmacol 369(2):151–159. https://doi.org/10.1007/s00210-003-0860-y

Hsieh MJ, Chen NH, Cheng SL, Tao CW, Wei YF, Wu YK, Chan MC, Liu SF, Hsu WH, Yang TM, Lin MS, Liu CL, Kuo PH, Tsai YH (2022) Comparing clinical outcomes of tiotropium/olodaterol, umeclidinium/vilanterol, and indacaterol/glycopyrronium fixed-dose combination therapy in patients with chronic obstructive pulmonary disease in taiwan: a multicenter cohort study. Int J Chron Obstruct Pulmon Dis 17:967–976. https://doi.org/10.2147/COPD.S353799

Hudson LD, Kurt TL, Petty TL, Genton E (1973) Arrhythmias associated with acute respiratory failure in patients with chronic airway obstruction. Chest 63(5):661–665. https://doi.org/10.1378/chest.63.5.661

Ida H (1976a) Cardiorespiratory activitirs of 3-formylamino-4-hydroxy-alpha-(n-1-methyl-2-p-methoxyphenethylaminomethyl)-benzylalcohol-hemifumarate(BD 40A) and some other beta-adrenoceptor stimulants in conscious guinea pigs. Arzneimittelforschung 26(7):1337–1340

Ida H (1976b) Comparison of the action of BD 40 A and some other beta-adrenoceptor stimulants on the isolated trachea and atria of the guinea pig. Arzneimittelforschung 26(5):839–842

Iftikhar IH, Imtiaz M, Brett AS, Amrol DJ (2014) Cardiovascular safety of long acting beta agonist-inhaled corticosteroid combination products in adult patients with asthma: a systematic review. Lung 192(1):47–54. https://doi.org/10.1007/s00408-013-9525-x

Inman WH, Adelstein AM (1969) Rise and fall of asthma mortality in England and Wales in relation to use of pressurised aerosols. Lancet 2(7615):279–285. https://doi.org/10.1016/s0140-6736(69)90051-8

Inoue H, Niimi A, Matsumoto H, Ito I, Oguma T, Otsuka K, Takeda T, Nakaji H, Tajiri T, Iwata T, Nagasaki T, Mishima M (2017) A 12-week, randomized, parallel-group, proof-of-concept study of tulobuterol patch and salmeterol inhaler as add-on therapy in adult-onset mild-to-moderate asthma. Clin Exp Pharmacol Physiol 44(1):21–29. https://doi.org/10.1111/1440-1681.12683

Ioli F, Donner CF, Fracchia C, Manini G, Patessio A, Spada EL, Vecchio C (1986) A new bronchodilating agent, procaterol, in preventing exercise-induced asthma. Int J Clin Pharmacol Res 6(5):389–396

Irie Y, Igawa T, Hosokawa T, Saitoh Y (1979) Alterations in blood levels of carbohydrate and lipid metabolites and of cyclic AMP mediated by beta1- and beta2-adrenoceptors in beagle dogs: effects of procaterol, a new selective beta2-adrenoceptor agonist. Eur J Pharmacol 53(4):351–358. https://doi.org/10.1016/0014-2999(79)90459-x

Jackson M (2010) "Divine stramonium": the rise and fall of smoking for asthma. Med Hist 54 (2):171–194. https://doi.org/10.1017/s0025727300000235

Janjua S, Schmidt S, Ferrer M, Cates CJ (2019) Inhaled steroids with and without regular formoterol for asthma: serious adverse events. Cochrane Database Syst Rev 9(9):CD006924. https://doi.org/10.1002/14651858.CD006924.pub4

January B, Seibold A, Whaley B, Hipkin RW, Lin D, Schonbrunn A, Barber R, Clark RB (1997) Beta2-adrenergic receptor desensitization, internalization, and phosphorylation in response to full and partial agonists. J Biol Chem 272(38):23871–23879. https://doi.org/10.1074/jbc.272.38.23871

January B, Seibold A, Allal C, Whaley BS, Knoll BJ, Moore RH, Dickey BF, Barber R, Clark RB (1998) Salmeterol-induced desensitization, internalization and phosphorylation of the human beta2-adrenoceptor. Br J Pharmacol 123(4):701–711. https://doi.org/10.1038/sj.bjp.0701658

Jeppsson AB, Löfdahl CG, Waldeck B, Widmark E (1989) On the predictive value of experiments in vitro in the evaluation of the effect duration of bronchodilator drugs for local administration. Pulm Pharmacol 2(2):81–85. https://doi.org/10.1016/0952-0600(89)90028-8

Jeppsson AB, Källström BL, Waldeck B (1992) Studies on the interaction between formoterol and salmeterol in guinea-pig trachea in vitro. Pharmacol Toxicol 71(4):272–277. https://doi.org/10.1111/j.1600-0773.1992.tb00982.x

Johnson M (2001) Beta2-adrenoceptors: mechanisms of action of beta2-agonists. Paediatr Respir Rev 2(1):57–62. https://doi.org/10.1053/prrv.2000.0102

Johnson M, Butchers PR, Coleman RA, Nials AT, Strong P, Sumner MJ, Vardey CJ, Whelan CJ (1993) The pharmacology of salmeterol. Life Sci 52(26):2131–2143. https://doi.org/10.1016/0024-3205(93)90728-l

Jones PW, Barnes N, Vogelmeier C, Lawrence D, Kramer B (2011) Efficacy of indacaterol in the treatment of patients with COPD. Prim Care Respir J 20(4):380–388. https://doi.org/10.4104/pcrj.2011.00066

Juergens UR, Stöber M, Vetter H (1999) Reproterol – a monomolecular combination of orciprenaline and theophylline: novel aspects of its mode of action in asthma. Respiration 66 (3):220–224. https://doi.org/10.1159/000029381

Juergens UR, Stöber M, Libertus H, Darlath W, Gillissen A, Vetter H (2004) Different mechanisms of action of beta2-adrenergic receptor agonists: a comparison of reproterol, fenoterol and salbutamol on monocyte cyclic-AMP and leukotriene B4 production in vitro. Eur J Med Res 9(7):365–370

Kahn RH (1907) Zur physiologie der trachea. Arch Physiol:398–426

Kaiser SV, Huynh T, Bacharier LB, Rosenthal JL, Bakel LA, Parkin PC, Cabana MD (2016) Preventing exacerbations in preschoolers with recurrent wheeze: a meta-analysis. Pediatrics 137 (6):e20154496. https://doi.org/10.1542/peds.2015-4496

Kallergis EM, Manios EG, Kanoupakis EM, Schiza SE, Mavrakis HE, Klapsinos NK, Vardas PE (2005) Acute electrophysiologic effects of inhaled salbutamol in humans. Chest 127(6):2057–2063. https://doi.org/10.1378/chest.127.6.2057

Kalra S, Swystun VA, Bhagat R, Cockcroft DW (1996) Inhaled corticosteroids do not prevent the development of tolerance to the bronchoprotective effect of salmeterol. Chest 109(4):953–956. https://doi.org/10.1378/chest.109.4.953

Kamburoff PL, Prime FJ, Schmidt OP (1977) The bronchodilator effect of NAB 365. Br J Clin Pharmacol 4(1):67–71. https://doi.org/10.1111/j.1365-2125.1977.tb00669.x

Kanthakumar K, Cundell DR, Johnson M, Wills PJ, Taylor GW, Cole PJ, Wilson R (1994) Effect of salmeterol on human nasal epithelial cell ciliary beating: inhibition of the ciliotoxin, pyocyanin. Br J Pharmacol 112(2):493–498. https://doi.org/10.1111/j.1476-5381.1994.tb13100.x

Kass I, Mingo TS (1980) Bitolterol mesylate (WIN 32784) aerosol. A new long-acting bronchodilator with reduced chronotropic effects. Chest 78(2):283–287. https://doi.org/10.1378/chest.78.2.283

Kawakami Y (1984) First clinical studies on mabuterol. A summarizing report. Arzneimittelforschung 34(11A):1699–1700

Kearns CF, McKeever KH (2009) Clenbuterol and the horse revisited. Vet J 182(3):384–391. https://doi.org/10.1016/j.tvjl.2008.08.021

Kelman GR, Palmer KN, Cross MR (1969) Cardiovascular effects of AH.3365 (salbutamol). Nature 221(5187):1251. https://doi.org/10.1038/2211251a0

Kenakin T (1999a) Efficacy in drug receptor theory: outdated concept or under-valued tool? Trends Pharmacol Sci 20:400–405

Kenakin T (1999b) The measurement of efficacy in the drug discovery agonist selection process. J Pharmacol Toxicol Methods 42:177–187

Kenakin TP, Beek D (1984) Relative efficacy of prenalterol and pirbuterol for beta-1 adrenoceptors: measurement of agonist affinity by alteration of receptor number. J Pharmacol Exp Ther 229 (2):340–345

Kennedy MC, Jackson SL (1963) Oral sympathomimetics in treatment of asthma. Br Med J 2 (5371):1506–1509. https://doi.org/10.1136/bmj.2.5371.1506

Kerr JW (1967) Deaths from asthma. Br Med J 2(5545):177–178. https://doi.org/10.1136/bmj.2.5545.177-c

Kiely DG, Cargill RI, Grove A, Struthers AD, Lipworth BJ (1995) Abnormal myocardial repolarisation in response to hypoxaemia and fenoterol. Thorax 50(10):1062–1066. https://doi.org/10.1136/thx.50.10.1062

Kikkawa H, Naito K, Ikezawa K (1991) Tracheal relaxing effects and beta 2-selectivity of TA-2005, a newly developed bronchodilating agent, in isolated guinea pig tissues. Jpn J Pharmacol 57 (2):175–185. https://doi.org/10.1254/jjp.57.175

Kikkawa H, Kanno K, Ikezawa K (1994) TA-2005, a novel, long-acting, and selective beta 2-adrenoceptor agonist: characterization of its in vivo bronchodilating action in guinea pigs and cats in comparison with other beta 2-agonists. Biol Pharm Bull 17(8):1047–1052. https://doi.org/10.1248/bpb.17.1047

Kim LHY, Saleh C, Whalen-Browne A, O'Byrne PM, Chu DK (2021) Triple vs dual inhaler therapy and asthma outcomes in moderate to severe asthma: a systematic review and meta-analysis. JAMA 325(24):2466–2479. https://doi.org/10.1001/jama.2021.7872

Kleiger RE, Senior RM (1974) Longterm electrocardiographic monitoring of ambulatory patients with chronic airway obstruction. Chest 65(5):483–487. https://doi.org/10.1378/chest.65.5.483

Klingler KH (1977) Synthesis of bronchospasmolytically effective beta phenylethyl-aminoalkyl-xanthines. Arzneimittelforschung 27(12):4–14

Kobayashi Y, Yasuba H, Kudou M, Kita H (2007) Addition of transdermal or inhaled long-acting Beta2-agonists in adult asthmatic patients treated with inhaled corticosteroids: switchover study from tulobuterol patch to salmeterol dry powder inhaler. J Asthma 44(2):77–81. https://doi.org/10.1080/02770900601180321

Konzett H (1940a) Neue broncholytisch hochwirksame Körper der Adrenalinreihe. Arch Exp Path Pharm 197:27–40. https://doi.org/10.1007/BF01936304

Konzett H (1940b) Zur Pharmakologie neuer adrenalinverwandter Körper. Arch Exp Path Pharm 197:41–56. https://doi.org/10.1007/BF01936305

Konzett H (1981) On the discovery of isoprenaline. Trends Pharmacol Sci 2:47–49

Konzett H, Rössler R (1940) Versuchsanordnung zu Untersuchungen an der Bronchialmuskulatur. Naunyn Schmiedebergs Arch Pharmakol Exp Pathol 195:71–74. https://doi.org/10.1007/BF01861842

Kraft M, Brusselle G, FitzGerald JM, Pavord ID, Keith M, Fageråas M, Garcia Gil E, Hirsch I, Goldman M, Colice G (2021) Patient characteristics, biomarkers and exacerbation risk in severe, uncontrolled asthma. Eur Respir J 58(6):2100413. https://doi.org/10.1183/13993003.00413-2021

Kramer JM (2009) Balancing the benefits and risks of inhaled long-acting beta-agonists – the influence of values. N Engl J Med 360(16):1592–1595. https://doi.org/10.1056/NEJMp0810561

Krüger G, Keck J, Noll K, Pieper H (1984) Synthesis of further amino-halogen-substituted phenylaminoethanols. Arzneimittelforschung 34(11A):1612–1624

Kubo S, Kasé Y, Miyata T, Kito G, Uesaka I (1975) Pharmacological studies of 1-(o-chlorophenyl)-2-tert.-butylaminoethanol (C-78), a new bronchodilator. Arzneimittelforschung 25(7):1028–1037

Kuiper HA, Noordam MY, van Dooren-Flipsen MM, Schilt R, Roos AH (1998) Illegal use of beta-adrenergic agonists: European Community. J Anim Sci 76(1):195–207. https://doi.org/10.2527/1998.761195x

Kume H, Kondo M, Ito Y, Suzuki R, Yamaki K, Takagi K (2002) Effects of sustained-release tulobuterol on asthma control and beta-adrenoceptor function. Clin Exp Pharmacol Physiol 29(12):1076–1083. https://doi.org/10.1046/j.1440-1681.2002.03777.x

Küng M, Croley SW, Phillips BA (1987) Systemic cardiovascular and metabolic effects associated with the inhalation of an increased dose of albuterol. Influence of mouth rinsing and gargling. Chest 91(3):382–387. https://doi.org/10.1378/chest.91.3.382

Kuo SH, Kamaka JK, Lum BK (1977) Adrenergic receptor mechanisms involved in the hyperglycemia and hyperlactic-acidemia produced by sympathomimetic amines in the cat. J Pharmacol Exp Ther 202(2):301–309

Kusayama T, Oka J, Yabana H, Adachi-Akahane S, Nagao T (1994) Binding of a catechol derivative of denopamine (T-0509) and N-tert-butylnoradrenaline (Colterol) to beta 1- and beta 2-adrenoceptors. Biol Pharm Bull 17(8):1023–1027. https://doi.org/10.1248/bpb.17.1023

Lambrecht BN, Hammad H (2012) The airway epithelium in asthma. Nat Med 18(5):684–692. https://doi.org/10.1038/nm.2737

Lambrecht BN, Hammad H, Fahy JV (2019) The cytokines of asthma. Immunity 50(4):975–991. https://doi.org/10.1016/j.immuni.2019.03.018

Lands AM, Brown TG Jr (1964) A comparison of the cardiac stimulating and bronchodilator actions of selected sympathomimetic amines. Proc Soc Exp Biol 116:331–333. https://doi.org/10.3181/00379727-116-29239

Lands AM, Arnold A, McAuliff JP, Luduena FP, Brown TG Jr (1967a) Differentiation of receptor systems activated by sympathomimetic amines. Nature 214(5088):597–598. https://doi.org/10.1038/214597a0

Lands AM, Luduena FP, Buzzo HJ (1967b) Differentiation of receptors responsive to isoproterenol. Life Sci 6(21):2241–2249. https://doi.org/10.1016/0024-3205(67)90031-8

Lanes SF, Birmann B, Raiford D, Walker AM (1997) International trends in sales of inhaled fenoterol, all inhaled beta-agonists, and asthma mortality, 1970-1992. J Clin Epidemiol 50(3):321–328. https://doi.org/10.1016/s0895-4356(96)00375-7

Larsson S, Svedmyr N, Thiringer G (1977) Lack of bronchial beta adrenoceptor resistance in asthmatics during long-term treatment with terbutaline. J Allergy Clin Immunol 59(2):93–100. https://doi.org/10.1016/0091-6749(77)90209-3

Larsson K, Kankaanranta H, Janson C, Lehtimäki L, Ställberg B, Løkke A, Høines K, Roslind K, Ulrik CS (2020) Bringing asthma care into the twenty-first century. NPJ Prim Care Respir Med 30(1):25. https://doi.org/10.1038/s41533-020-0182-2

Lee S, Schwinger RH, Brixius K (2008) Genetically changed mice with chronic deficiency or overexpression of the β-beta-adrenoceptors – what can we learn for the therapy of heart failure? Pflugers Arch 455:767–774

Lee WH, Kim H-J, Lee C-H (2017) The impact of olodaterol on the risk of mortality and serious adverse events: a systematic review and meta-analysis. Br J Clin Pharmacol 83(6):1166–1175. https://doi.org/10.1111/bcp.13210

Lee LA, Bailes Z, Barnes N, Boulet LP, Edwards D, Fowler A, Hanania NA, Kerstjens HAM, Kerwin E, Nathan R, Oppenheimer J, Papi A, Pascoe S, Brusselle G, Peachey G, Sule N, Tabberer M, Pavord ID (2020a) Efficacy and safety of once-daily single-inhaler triple therapy (FF/UMEC/VI) versus FF/VI in patients with inadequately controlled asthma (CAPTAIN): a double-blind, randomised, phase 3A trial. Lancet Respir Med 9(1):69–84. https://doi.org/10.1016/S2213-2600(20)30389-1

Lee HW, Park J, Jang EJ, Lee CH (2020b) Comparisons of exacerbations and mortality among LAMA/LABA combinations in stable chronic obstructive pulmonary disease: systematic review and Bayesian network meta-analysis. Respir Res 21(1):310. https://doi.org/10.1186/s12931-020-01540-8

Lee HW, Kim HJ, Jang EJ, Lee CH (2021) Comparisons of efficacy and safety between triple (inhaled corticosteroid/long-acting muscarinic antagonist/long-acting beta-agonist) therapies in chronic obstructive pulmonary disease: Systematic review and Bayesian network meta-analysis. Respiration 100(7):631–643. https://doi.org/10.1159/000515133

Legge JS, Gaddie J, Palmer KN (1971) Comparison of two oral selective beta2-adrenergic stimulant drugs in bronchial asthma. Br Med J 1(5750):637–639. https://doi.org/10.1136/bmj.1.5750.637

Lewis RA, Austen KF (1981) Mediation of local homeostasis and inflammation by leukotrienes and other mast cell-dependent compounds. Nature 293(5828):103–108. https://doi.org/10.1038/293103a0

Liedtke AG, Lava SAG, Milani GP, Agostoni C, Gilardi V, Bianchetti MG, Treglia G, Faré PB (2019) Selective ß2-adrenoceptor agonists and relevant hyperlactatemia: systematic review and meta-analysis. J Clin Med 9(1):71. https://doi.org/10.3390/jcm9010071

Liippo K, Silvasti M, Tukiainen H (1991) Inhaled procaterol versus salbutamol in bronchial asthma. Eur J Clin Pharmacol 40(4):417–418. https://doi.org/10.1007/BF00265855

Lindén A, Bergendal A, Ullman A, Skoogh BE, Löfdahl CG (1993) Salmeterol, formoterol, and salbutamol in the isolated guinea pig trachea: differences in maximum relaxant effect and potency but not in functional antagonism. Thorax 48(5):547–553. https://doi.org/10.1136/thx.48.5.547

Lipson DA, Barnhart F, Brealey N, Brooks J, Criner GJ, Day NC, Dransfield MT, Halpin DMG, Han MK, Jones CE, Kilbride S, Lange P, Lomas DA, Martinez FJ, Singh D, Tabberer M, Wise RA, Pascoe SJ, Investigators IMPACT (2018) Once-daily single-inhaler triple versus dual therapy in patients with COPD. N Engl J Med 378(18):1671–1680. https://doi.org/10.1056/NEJMoa1713901

Lipson DA, Crim C, Criner GJ, Day NC, Dransfield MT, Halpin DMG, Han MK, Jones CE, Kilbride S, Lange P, Lomas DA, Lettis S, Manchester P, Martin N, Midwinter D, Morris A, Pascoe SJ, Singh D, Wise RA, Martinez FJ (2020) Reduction in all-cause mortality with fluticasone furoate/umeclidinium/vilanterol in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 201(12):1508–1516. https://doi.org/10.1164/rccm.201911-2207OC

Lipworth BJ (1996) Airway and systemic effects of inhaled corticosteroids in asthma: dose response relationship. Pulm Pharmacol 9(1):19–27. https://doi.org/10.1006/pulp.1996.0002

Lipworth BJ, Grove A (1997) Evaluation of partial beta-adrenoceptor agonist activity. Br J Clin Pharmacol 43(1):9–14. https://doi.org/10.1111/j.1365-2125.1997.tb00025.x

Lipworth BJ, Clark RA, Dhillon DP, Moreland TA, Struthers AD, Clark GA, McDevitt DG (1989) Pharmacokinetics, efficacy and adverse effects of sublingual salbutamol in patients with asthma. Eur J Clin Pharmacol 37(6):567–571. https://doi.org/10.1007/BF00562546

Lockett MF (1965) Dangerous effects of isoprenaline in myocardial failure. Lancet 2(7403):104–106. https://doi.org/10.1016/s0140-6736(65)92221-x

Löfdahl CG, Svedmyr N (1986) Effect duration of inhaled formoterol, a newβ 2-adrenoceptor agonist, compared to salbutamol in asthmatic patients. Acta Pharmacol Toxicol 229(Suppl 5 (II)):A1358

Löfdahl CG, Svedmyr N (1989) Formoterol fumarate, a newβ2-adrenoceptor agonist. Acute studies of selectivity and duration of effect after inhaled and oral administration. Allergy 44:264–271

Löfdahl CG, Sigvaldasson A, Skoogh BE, Svedmyr N (1989) Broxaterol, a new beta 2-adrenoceptor agonist compared to salbutamol in asthmatics, oral and inhalation treatment. Respiration 55(Suppl 2):15–19. https://doi.org/10.1159/000195765

Lötvall J (2001) Pharmacological similarities and differences between beta2-agonists. Respir Med 95(Suppl B):S7–S11. https://doi.org/10.1053/rmed.2001.1139

Lötvall J, Lunde H, Ullman A, Törnqvist H, Svedmyr N (1992) Twelve months, treatment with inhaled salmeterol in asthmatic patients. Effects on beta 2-receptor function and inflammatory cells. Allergy 47(5):477–483. https://doi.org/10.1111/j.1398-9995.1992.tb00668.x

Lötvall J, Palmqvist M, Ankerst J, Persson G, Rosenborg J, Bengtsson T, Rott Z, Poczi M, Devai A, Waldeck B (2005) The effect of formoterol over 24 h in patients with asthma: the role of enantiomers. Pulm Pharmacol Ther 18(2):109–113. https://doi.org/10.1016/j.pupt.2004.10.007

Lötvall J, Bateman ED, Bleecker ER, Busse WW, Woodcock A, Follows R, Lim J, Stone S, Jacques L, Haumann B (2012) 24-h duration of the novel LABA vilanterol trifenatate in asthma patients treated with inhaled corticosteroids. Eur Respir J 40(3):570–579. https://doi.org/10.1183/09031936.00121411

Louis SN, Nero TL, Iakovidis D, Jackman GP, Louis WJ (1999) LK 204-545, a highly selective beta1-adrenoceptor antagonist at human beta-adrenoceptors. Eur J Pharmacol 367(2-3):431–435. https://doi.org/10.1016/s0014-2999(99)00019-9

Loymans RJ, Honkoop PJ, Termeer EH, Snoeck-Stroband JB, Assendelft WJ, Schermer TR, Chung KF, Sousa AR, Sterk PJ, Reddel HK, Sont JK, Ter Riet G (2016) Identifying patients at risk for severe exacerbations of asthma: development and external validation of a multivariable prediction model. Thorax 71(9):838–846. https://doi.org/10.1136/thoraxjnl-2015-208138

Macie C, Wooldrage K, Manfreda J, Anthonisen N (2008) Cardiovascular morbidity and the use of inhaled bronchodilators. Int J Chron Obstruct Pulmon Dis 3:163–169

Macklem PT (2010) Therapeutic implications of the pathophysiology of COPD. Eur Respir J 35 (3):676–680. https://doi.org/10.1183/09031936.00120609

Magadle R, Berar-Yanay N, Weiner P (2002) The risk of hospitalization and near-fatal and fatal asthma in relation to the perception of dyspnea. Chest 121(2):329–333. https://doi.org/10.1378/chest.121.2.329

Malmstrom K, Rodriguez-Gomez G, Guerra J, Villaran C, Piñeiro A, Wei LX, Seidenberg BC, Reiss TF (1999) Oral montelukast, inhaled beclomethasone, and placebo for chronic asthma. A randomized, controlled trial. Montelukast/Beclomethasone Study Group. Ann Intern Med 130 (6):487–495. https://doi.org/10.7326/0003-4819-130-6-199903160-00005

Mándi A, Galfóczy G, Galambos E (1977a) On the bronchodilatory effect of reproterol. Arzneimittelforschung 27(12):60–63

Mándi A, Wilde W, Galgóczy G, Aurich R, Galambos E (1977b) Studies on the onset of effect of reproterol following inhalation from a metered aerosol. Arzneimittelforschung 27(12):64–66

Mangunnegoro H, Novariska F, Wiyono WH, Setiawati A, Louisa M (2011) The efficacy of nebulized procaterol versus nebulized salbutamol for the treatment of moderate acute asthma: a randomized, double-blind, parallel group study. Int J Clin Pharmacol Ther 49(10):614–621. https://doi.org/10.5414/cp201513

Marlin GE, Turner P (1975) Intravenous treatment with rimiterol and salbutamol in asthma. Br Med J 2(5973):715–719. https://doi.org/10.1136/bmj.2.5973.715

Martin RM, Dunn NR, Freemantle SN, Mann RD (1998) Risk of non-fatal cardiac failure and ischaemic heart disease with long acting beta 2 agonists. Thorax 53(7):558–562. https://doi.org/10.1136/thx.53.7.558

Martinez FJ, Rabe KF, Ferguson GT, Wedzicha JA, Singh D, Wang C, Rossman K, St Rose E, Trivedi R, Ballal S, Darken P, Aurivillius M, Reisner C, Dorinsky P (2021) Reduced all-cause

mortality in the ETHOS trial of budesonide/glycopyrrolate/formoterol for chronic obstructive pulmonary disease. A randomized, double-blind, multicenter, parallel-group study. Am J Respir Crit Care Med 203(5):553–564. https://doi.org/10.1164/rccm.202006-2618OC

Martínez-Navarro JF (1990) Food poisoning related to consumption of illicit beta-agonist in liver. Lancet 336(8726):1311. https://doi.org/10.1016/0140-6736(90)92990-y

Masureel M, Zou Y, Picard LP, van der Westhuizen E, Mahoney JP, Rodrigues JPGLM, Mildorf TJ, Dror RO, Shaw DE, Bouvier M, Pardon E, Steyaert J, Sunahara RK, Weis WI, Zhang C, Kobilka BK (2018) Structural insights into binding specificity, efficacy and bias of a β2AR partial agonist. Nat Chem Biol 14(11):1059–1066. https://doi.org/10.1038/s41589-018-0145-x

Matera MG, Cazzola M (2007) ultra-long-acting beta2-adrenoceptor agonists: an emerging therapeutic option for asthma and COPD? Drugs 67(4):503–515. https://doi.org/10.2165/00003495-200767040-00002

Mattila MJ, Muittari A (1969) Effect of bronchodilator drugs on the peak expiratory flow rate of asthmatic patients: oral orciprenaline and terbutaline (KWD 2019). Ann Med Exp Biol Fenn 47 (4):298–302

Mattila MJ, Muittari A, Tiitinen H (1967) The effect of orciprenaline and its p-hydroxyphenyl derivative on the peak expiratory flow rate in asthmatic patients. Arzneimittelforschung 17 (3):362–364

Mazza JA, Tashkin DP, Reed CE (1992) Evaluation of procaterol and albuterol (salbutamol) aerosol in the treatment of asthma. Ann Allergy 68(3):267–273

McCrea KE, Hill SJ (1996) Comparison of duration of agonist action at beta 1- and beta 2-adrenoceptors in C6 glioma cells: evidence that the long duration of action of salmeterol is specific to the beta 2-adrenoceptor. Mol Pharmacol 49(5):927–937

McDevitt DG, Shanks RG, Swanton JG (1974) Further observations on the cardiotoxicity of isoprenaline during hypoxia. Br J Pharmacol 50(3):335–344. https://doi.org/10.1111/j.1476-5381.1974.tb09608.x

McKeever T, Harrison TW, Hubbard R, Shaw D (2013) Inhaled corticosteroids and the risk of pneumonia in people with asthma: a case-control study. Chest 144(6):1788–1794. https://doi.org/10.1378/chest.13-0871

McLean AJ, Milligan G (2000) Ligand regulation of green fluorescent protein-tagged forms of the human beta(1)- and beta(2)-adrenoceptors; comparisons with the unmodified receptors. Br J Pharmacol 130(8):1825–1832. https://doi.org/10.1038/sj.bjp.0703506

Melland B (1910) The treatment of spasmodic asthma by the hypodermic injection of adrenalin. Lancet 175:1407–1411. https://doi.org/10.1016/S0140-6736(01)14446-6

Menzella F, Fontana M, Galeone C, D'Amato M, Canonica GW, Ghidoni G, Capobelli S, Scelfo C, Simonazzi A, Catellani C, Ruggiero P, Facciolongo N (2021) A real-world evaluation of clinical outcomes of biologicals and bronchial thermoplasty for severe refractory asthma (BIOTERM). J Asthma Allergy 14:1019–1031. https://doi.org/10.2147/JAA.S324099

Menzies-Gow A, Corren J, Bourdin A, Chupp G, Israel E, Wechsler ME, Brightling CE, Griffiths JM, Hellqvist Å, Bowen K, Kaur P, Almqvist G, Ponnarambil S, Colice G (2021) Tezepelumab in adults and adolescents with severe, uncontrolled asthma. N Engl J Med 384(19):1800–1809. https://doi.org/10.1056/NEJMoa2034975

Meyer T, Reitmeir P, Brand P, Herpich C, Sommerer K, Schulze A, Scheuch G, Newman S (2011) Effects of formoterol and tiotropium bromide on mucus clearance in patients with COPD. Respir Med 105(6):900–906. https://doi.org/10.1016/j.rmed.2011.02.007

MHRA Public Assessment Report (2009) Orciprenaline sulphate (Alupent): planned withdrawal from the UK market following a risk-benefit analysis. Available at https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/852403/Orciprenaline_sulphate__Alupent__withdrawal_from_UK_market_after_a_risk-benefit_analysis.pdf

Millar RA (1955) Adrenaline and noradrenaline. Br J Anaesth 27:603–608

Minatoya H (1978) Studies on bitolterol, di-p-toluate ester of N-tert-butylarterenol: a new long-acting bronchodilator with reduced cardiovascular effects. J Pharmacol Exp Ther 206(3):515–527

Minette A (1970) Spirometric study of the bronchodilating effects of hydroxyphenylorciprenaline (Th1165a) in various forms in a group of 124 coal miners suffering from bronchospasm. Respiration 27:276–315

Minette A, Marcq M, Gepts L (1976) Carbuterol, fenoterol, orciprenaline, salbutamol and terbutaline per os in reversible obstructive chronic bronchitis. Bull Eur Physiopathol Respir 12(4):545–553

Mistry SN, Baker JG, Fischer PM, Hill SJ, Gardiner SM, Kellam B (2013) Synthesis and in vitro and in vivo characterization of highly β1-selective β-adrenoceptor partial agonists. J Med Chem 56(10):3852–3865. https://doi.org/10.1021/jm400348g

Mochizuki H, Nanjo Y, Takahashi H (2013) Better adherence to a transdermal tulobuterol patch than inhaled salmeterol in elderly chronic obstructive pulmonary disease patients. Geriatr Gerontol Int 13(2):398–404. https://doi.org/10.1111/j.1447-0594.2012.00916.x

Montassier E, Legrand M, Rossignol P, Potel G (2019) Hyperkalemia in the emergency department: consider the use of nebulized salbutamol. Am J Emerg Med 37(5):1004. https://doi.org/10.1016/j.ajem.2018.10.024

Moore PF, Constantine JW, Barth WE (1978) Pirbuterol, a selecttve beta2 adrenergic bronchodilator. J Pharmacol Exp Ther 207(2):410–418

Moore RH, Khan A, Dickey BF (1998) Long-acting inhaled beta2-agonists in asthma therapy. Chest 113(4):1095–1108. https://doi.org/10.1378/chest.113.4.1095

Moore RH, Millman EE, Godines V, Hanania NA, Tran TM, Peng H, Dickey BF, Knoll BJ, Clark RB (2007) Salmeterol stimulation dissociates beta2-adrenergic receptor phosphorylation and internalization. Am J Respir Cell Mol Biol 36(2):254–261. https://doi.org/10.1165/rcmb.2006-0158OC

Mortimer K, Lesosky M, García-Marcos L, Asher MI, Pearce N, Ellwood E, Bissell K, El Sony A, Ellwood P, Marks GB, Martínez-Torres A, Morales E, Perez-Fernandez V, Robertson S, Rutter CE, Silverwood RJ, Strachan DP, Chiang CY, Global Asthma Network Phase I Study Group (2022) The burden of asthma, hay fever and eczema in adults in 17 countries: GAN phase I study. Eur Respir J 60(3):2102865. https://doi.org/10.1183/13993003.02865-2021

Mosbech H, Deckelmann R, de Blay F, Pastorello EA, Trebas-Pietras E, Andres LP, Malcus I, Ljørring C, Canonica GW (2014) Standardized quality (SQ) house dust mite sublingual immunotherapy tablet (ALK) reduces inhaled corticosteroid use while maintaining asthma control: a randomized, double-blind, placebo-controlled trial. J Allergy Clin Immunol 134(3):568–575.e7. https://doi.org/10.1016/j.jaci.2014.03.019

Mountain RD, Heffner JE, Brackett NC Jr, Sahn SA (1990) Acid-base disturbances in acute asthma. Chest 98(3):651–655. https://doi.org/10.1378/chest.98.3.651

Mullen M, Mullen B, Carey M (1993) The association between beta-agonist use and death from asthma. A meta-analytic integration of case-control studies. JAMA 270(15):1842–1845

Munro A, Jacobs M (2004) Best evidence topic reports. Is intravenous aminophylline better than intravenous salbutamol in the treatment of moderate to severe asthma? Emerg Med J 21(1):78–80

Murai T, Maejima T, Sanai K, Osada E (1984) Pharmacological studies of mabuterol, a new selective beta 2-stimulant. I: bronchodilating effect. Arzneimittelforschung 34(11A):1633–1640

Muraki M, Kunita Y, Shirahase K, Yamazaki R, Hanada S, Sawaguchi H, Tohda Y (2021) A randomized controlled trial of long-acting muscarinic antagonist and long-acting β2 agonist fixed-dose combinations in patients with chronic obstructive pulmonary disease. BMC Pulm Med 21(1):26. https://doi.org/10.1186/s12890-021-01403-y

Murase K, Mase T, Ida H, Takahashi K, Murakami M (1977) New beta-adrenoreceptor stimulants. Studies on 3-acylamino-4-hydroxy-alpha-(N-substituted aminomethyl)benzyl alcohols. Chem Pharm Bull (Tokyo) 25(6):1368–1377. https://doi.org/10.1248/cpb.25.1368

Murphy L, Rennard S, Donohue J, Molimard M, Dahl R, Beeh KM, Dederichs J, Fülle HJ, Higgins M, Young D (2014) Turning a molecule into a medicine: the development of indacaterol as a novel once-daily bronchodilator treatment for patients with COPD. Drugs 74(14):1635–1657. https://doi.org/10.1007/s40265-014-0284-7

Nair P, Surette MG, Virchow JC (2021) Neutrophilic asthma: misconception or misnomer? Lancet Respir Med 9(5):441–443. https://doi.org/10.1016/S2213-2600(21)00023-0

Naline E, Zhang Y, Qian Y, Mairon N, Anderson GP, Grandordy B, Advenier C (1994) Relaxant effects and durations of action of formoterol and salmeterol on the isolated human bronchus. Eur Respir J 7(5):914–920

Nelson HS (2006a) Is there a problem with inhaled long-acting beta-adrenergic agonists? J Allergy Clin Immunol 117(1):3–16.; quiz 17. https://doi.org/10.1016/j.jaci.2005.10.013

Nelson HS (2006b) Long-acting beta-agonists in adult asthma: evidence that these drugs are safe. Prim Care Respir J 15(5):271–277. https://doi.org/10.1016/j.pcrj.2006.08.006

Nelson HS, Weiss ST, Bleecker ER, Yancey SW, Dorinsky PM, SMART Study Group (2006) The salmeterol multicenter asthma research trial: a comparison of usual pharmacotherapy for asthma or usual pharmacotherapy plus salmeterol. Chest 129(1):15–26. https://doi.org/10.1378/chest.129.1.15

Nelson HS, Carr W, Nathan R, Portnoy JM (2009) Update on the safety of long-acting beta-agonists in combination with inhaled corticosteroids for the treatment of asthma. Ann Allergy Asthma Immunol 102(1):11–15. https://doi.org/10.1016/S1081-1206(10)60101-9

Netea MG, Domínguez-Andrés J, Barreiro LB, Chavakis T, Divangahi M, Fuchs E, Joosten LAB, van der Meer JWM, Mhlanga MM, Mulder WJM, Riksen NP, Schlitzer A, Schultze JL, Stabell Benn C, Sun JC, Xavier RJ, Latz E (2020) Defining trained immunity and its role in health and disease. Nat Rev Immunol 20(6):375–388. https://doi.org/10.1038/s41577-020-0285-6

Newhouse MT, Chapman KR, McCallum AL, Abboud RT, Bowie DM, Hodder RV, Paré PD, Mesic-Fuchs H, Molfino NA (1996) Cardiovascular safety of high doses of inhaled fenoterol and albuterol in acute severe asthma. Chest 110(3):595–603. https://doi.org/10.1378/chest.110.3.595

Newnham DM, McDevitt DG, Lipworth BJ (1993) Comparison of the extrapulmonary beta2-adrenoceptor responses and pharmacokinetics of salbutamol given by standard metered dose-inhaler and modified actuator device. Br J Clin Pharmacol 36(5):445–450. https://doi.org/10.1111/j.1365-2125.1993.tb00393.x

Nials AT, Sumner MJ, Johnson M, Coleman RA (1993a) Investigations into factors determining the duration of action of the beta 2-adrenoceptor agonist, salmeterol. Br J Pharmacol 108(2):507–515. https://doi.org/10.1111/j.1476-5381.1993.tb12833.x

Nials AT, Coleman RA, Johnson M, Magnussen H, Rabe KF, Vardey CJ (1993b) Effects of beta-adrenoceptor agonists in human bronchial smooth muscle. Br J Pharmacol 110(3):1112–1116. https://doi.org/10.1111/j.1476-5381.1993.tb13929.x

Nials AT, Ball DI, Butchers PR, Coleman RA, Humbles AA, Johnson M, Vardey CJ (1994) Formoterol on airway smooth muscle and human lung mast cells: a comparison with salbutamol and salmeterol. Eur J Pharmacol 251(2–3):127–135. https://doi.org/10.1016/0014-2999(94)90392-1

Nishimura M, Okiyama M, Fujiwara H, Kudo M, Simizu M, Maeda M, Yamada M, Toshimitsu Y (1991) Pharmacological action of SN-408, a novel long-acting selective beta 2-adrenoceptor agonist. Nihon Yakurigaku Zasshi 98(1):7–21. https://doi.org/10.1254/fpj.98.1_7

Nishiyama O, Taniguchi H, Kondoh Y, Kimura T, Kato K, Kume H, Shimokata K (2006) Comparison of the effects of tulobuterol patch and salmeterol in moderate to severe asthma. Clin Exp Pharmacol Physiol 33(11):1016–1021. https://doi.org/10.1111/j.1440-1681.2006.04480.x

Nurmagambetov T, Kuwahara R, Garbe P (2018) The economic burden of asthma in the United States, 2008-2013. Ann Am Thorac Soc 15(3):348–356. https://doi.org/10.1513/AnnalsATS.201703-259OC

O'Byrne PM, van der Linde J, Cockcroft DW, Gauvreau GM, Brannan JD, Fitzgerald M, Watson RM, Milot J, Davis B, O'Connor M, Hart L, Korducki L, Hamilton AL, Boulet L-P (2009) Prolonged bronchoprotection against inhaled methacholine by inhaled BI 1744, a long-acting beta(2)-agonist, in patients with mild asthma. J Allergy Clin Immunol 124(6):1217–1221. https://doi.org/10.1016/j.jaci.2009.08.047

O'Connor BJ, Aikman SL, Barnes PJ (1992) Tolerance to the nonbronchodilator effects of inhaled beta 2-agonists in asthma. N Engl J Med 327(17):1204–1208. https://doi.org/10.1056/NEJM199210223271704

O'Donnell SR (1970) A selective beta-adrenoreceptor stimulant (Th1165a) related to orciprenaline. Eur J Pharmacol 2(1):35–43. https://doi.org/10.1016/0014-2999(70)90026-9

O'Donnell SR (1972) An examination of some -adrenoreceptor stimulants for selectivity using the isolated trachea and atria of the guinea pig. Eur J Pharmacol 19(3):371–379. https://doi.org/10.1016/0014-2999(72)90104-5

O'Donnell SR, Wanstall JC (1978) Evidence that the efficacy (intrinsic activity) of fenoterol is higher than that of salbutamol on beta-adrenoceptors in guinea-pig trachea. Eur J Pharmacol 47 (3):333–340. https://doi.org/10.1016/0014-2999(78)90241-8

O'Shea O, Stovold E, Cates CJ (2021) Regular treatment with formoterol and an inhaled corticosteroid versus regular treatment with salmeterol and an inhaled corticosteroid for chronic asthma: serious adverse events. Cochrane Database Syst Rev 4(4):CD007694. https://doi.org/10.1002/14651858.CD007694.pub3

O'Donnell SR (1976) Selectivity of clenbuterol (NAB 365) in guinea-pig isolated tissues containing beta-adrenoceptors. Arch Int Pharmacodyn Ther 224(2):190–198

Oliver G, Schäfer EA (1894) On the physiological action of extracts of the suprarenal capsule. J Physiol 16:l–4

Oliver G, Schäfer EA (1895) The physiological effects of extracts of the suprarenal capsules. J Physiol 18(3):230–276. https://doi.org/10.1113/jphysiol.1895.sp000564

Olsson OA, Svensson LÅ (1984) New lipophilic terbutaline ester prodrugs with long effect duration. Pharm Res 1(1):19–23. https://doi.org/10.1023/A:1016322524471

O'Reilly DA, Awale A, Cartledge P (2015) Question 2: blast from the past: is oral salbutamol useful in resource-poor settings? Arch Dis Child 100(8):806–809. https://doi.org/10.1136/archdischild-2015-309141

Orgel HA, Kemp JP, Tinkelman DG, Webb DR Jr (1985) Bitolterol and albuterol metered-dose aerosols: comparison of two long-acting beta 2-adrenergic bronchodilators for treatment of asthma. J Allergy Clin Immunol 75(1 Pt 1):55–62. https://doi.org/10.1016/0091-6749(85)90012-0

Ortega VE, Peters SP (2010) Beta-2 adrenergic agonists: focus on safety and benefits versus risks. Curr Opin Pharmacol 3:246–253. https://doi.org/10.1016/j.coph.2010.04.009

Ortega HG, Liu MC, Pavord ID, Brusselle GG, FitzGerald JM, Chetta A, Humbert M, Katz LE, Keene ON, Yancey SW, Chanez P, MENSA Investigators (2014) Mepolizumab treatment in patients with severe eosinophilic asthma. N Engl J Med 371(13):1198–1207. https://doi.org/10.1056/NEJMoa1403290

Packer M (1989) Is activation of the sympathetic nervous system beneficial or detrimental to the patient with chronic heart failure? Lessons learned from clinical trials with beta-adrenergic agonists and antagonists. J Cardiovasc Pharmacol 14(Suppl 5):S38–S43

Palmer KN, Diament ML (1969) Effect of salbutamol on spirometry and blood-gas tensions in bronchial asthma. Br Med J 1(5635):31–32. https://doi.org/10.1136/bmj.1.5635.31

Papi A, Chipps BE, Beasley R, Panettieri RA Jr, Israel E, Cooper M, Dunsire L, Jeynes-Ellis A, Johnsson E, Rees R, Cappelletti C, Albers FC (2022) Albuterol-budesonide fixed-dose combination rescue inhaler for asthma. N Engl J Med 386(22):2071–2083. https://doi.org/10.1056/NEJMoa2203163

Pasotti C, Capra A, Vibelli C (1979) NAB 365 (clenbuterol) and salbutamol in asthmatics: a double-blind clinical trial. Int J Clin Pharmacol Biopharm 17(4):176–180

Patchett P, Patchett SM, Burge PS (1985) Bronchial and cardiovascular responses to inhaled reproterol in asthmatics: a double-blind placebo controlled dose-response study. Br J Clin Pharmacol 20(4):349–353. https://doi.org/10.1111/j.1365-2125.1985.tb05076.x

Patel KR (1986) Bronchodilator activity of a new inhaled beta 2-adrenoceptor agonist, tulobuterol and its protective effect in exercise-induced asthma. Br J Clin Pharmacol 21(2):234–237. https://doi.org/10.1111/j.1365-2125.1986.tb05182.x

Patel KR (1990) Prolonged treatment with oral and inhaled tulobuterol does not induce airways tachyphlaxis. Lung 168(Suppl):210–218. https://doi.org/10.1007/BF02718135

Patel S, Summerhill S, Stanley M, Perros-Huguet C, Trevethick MA (2011) The reassertion profiles of long acting β2-adrenoceptor agonists in the guinea pig isolated trachea and human recombinant β2-adrenoceptor. Pulm Pharmacol Ther 24(2):247–255. https://doi.org/10.1016/j.pupt.2010.11.004

Patel M, Pilcher J, Munro C, Hosking A, Pritchard A, Shaw D, Black P, Weatherall M, Beasley R, SMART Study Group (2013) Short-acting β-agonist use as a marker of current asthma control. J Allergy Clin Immunol Pract 1(4):370–377. https://doi.org/10.1016/j.jaip.2013.04.008

Paterson IC, Willey RF, Shotter V, Grant IW, Crompton GK (1975) Objective and subjective comparisons of terbutaline and rimiterol bronchodilator aerosols. Br J Dis Chest 69:267–272. https://doi.org/10.1016/0007-0971(75)90095-9

Pauwels RA, Löfdahl CG, Postma DS, Tattersfield AE, O'Byrne P, Barnes PJ, Ullman A (1997) Effect of inhaled formoterol and budesonide on exacerbations of asthma. Formoterol and Corticosteroids Establishing Therapy (FACET) International Study Group. N Engl J Med 337 (20):1405–1411. https://doi.org/10.1056/NEJM199711133372001

Pearce N (2007) Adverse reactions: the fenoterol story. Auckland University Press. ISBN: 9781869403744

Pearce N, Grainger J, Atkinson M, Crane J, Burgess C, Culling C, Windom H, Beasley R (1990) Case-control study of prescribed fenoterol and death from asthma in New Zealand, 1977-81. Thorax 45(3):170–175. https://doi.org/10.1136/thx.45.3.170

Pearce N, Beasley R, Crane J, Burgess C, Jackson R (1995) End of the New Zealand asthma mortality epidemic. Lancet 345(8941):41–44. https://doi.org/10.1016/s0140-6736(95)91159-6

Pedersen BK, Laursen LC, Gnosspelius Y, Faurschou P, Weeke B (1985) Bambuterol: effects of a new anti-asthmatic drug. Eur J Clin Pharmacol 29(4):425–427. https://doi.org/10.1007/BF00613456

Pennock BE, Rogers RM, Ryan BR, Ayers LN (1977) Aerosol administration of fenoterol hydrobromide (Th 1165a) in subjects with reversible obstructive airway disease. Chest 72 (6):731–736. https://doi.org/10.1378/chest.72.6.731

Perruchoud AP, Bründler H, Godly R, Imhof E, Herzog H (1987) Broxaterol (Z.1170), a new oral beta 2-agonist compared with salbutamol. Respiration 51(2):113–118. https://doi.org/10.1159/000195177

Persson H, Olsson T (1970) Some pharmacological properties of terbutaline (INN), 1-(3,5-dihydroxyphenyl)-2-(T-butylamino)-ethanol. A new sympathomimetic beta-receptor-stimulating agent. Acta Med Scand Suppl 512:11–19. https://doi.org/10.1111/j.0954-6820.1970.tb05284.x

Persson G, Baas A, Knight A, Larsen B, Olsson H (1995) One month treatment with the once daily oral beta 2-agonist bambuterol in asthmatic patients. Eur Respir J 8(1):34–39. https://doi.org/10.1183/09031936.95.08010034

Petraglia A, Scarpitta M, Ansalone D, Gurrieri G, Galzerano V, Calvanese RC, Federico S, Zinno A (1990) Negligible metabolic effects of long-term oral treatment with a new beta 2-agonist: broxaterol. Int J Clin Pharmacol Res 10(5):299–304

Petrie GR, Chookang JY, Hassan WU, Morrison JF, O'Reilly JF, Pearson SB, Shneerson JM, Tang OT, Ning AC, Turbitt ML (1993) Bambuterol: effective in nocturnal asthma. Respir Med 87 (8):581–585. https://doi.org/10.1016/s0954-6111(05)80260-4

Petty TL, Scoggin CH, Rollins DR, Repsher LH (1984) Bitolterol compared to isoproterenol in advanced chronic obstructive pulmonary disease. Chest 86(3):404–408. https://doi.org/10.1378/chest.86.3.404

Phillips EM, Woolnough M, Marinova VM, Turner P (1972) A comparison of isoprenaline, salbutamol, and rimiterol inhalation on skin temperature, heart rate, and respiration in man. J Clin Pharmacol New Drugs 12(4):158–168. https://doi.org/10.1002/j.1552-4604.1972.tb00045.x

Phillips PJ, Vedig AE, Jones PL, Chapman MG, Collins M, Edwards JB, Smeaton TC, Duncan BM (1980) Metabolic and cardiovascular side effects of the beta 2-adrenoceptor agonists salbutamol and rimiterol. Br J Clin Pharmacol 9(5):483–491. https://doi.org/10.1111/j.1365-2125.1980.tb05844.x

Piatti G, Ambrosetti U, Santus P, Allegra L (2005) Effects of salmeterol on cilia and mucus in COPD and pneumonia patients. Pharmacol Res 51(2):165–168. https://doi.org/10.1016/j.phrs.2004.07.006

Plummer AL (1978) The development of drug tolerance to beta2 adrenergic agents. Chest 73(6 Suppl):949–957. https://doi.org/10.1378/chest.73.6.949

Potter DE, Woodson LC, Kempen RR, Ellis S (1980) Comparative metabolic and cardiovascular effects of carbuterol, isoproterenol, metaproterenol and salbutamol in the baboon. Horm Metab Res 12(7):323–327. https://doi.org/10.1055/s-2007-996280

Prather ID, Brown DE, North P, Wilson JR (1995) Clenbuterol: a substitute for anabolic steroids? Med Sci Sports Exerc 27(8):1118–1121

Proudman RGW, Akinaga J, Baker JG (2022) The signaling and selectivity of α-adrenoceptor agonists for the human α2A, α2B and α2C-adrenoceptors and comparison with human α1 and β-adrenoceptors. Pharmacol Res Perspect 10(5):e01003. https://doi.org/10.1002/prp2.1003

Qian CJ, Coulombe J, Suissa S, Ernst P (2017) Pneumonia risk in asthma patients using inhaled corticosteroids: a quasi-cohort study. Br J Clin Pharmacol 83(9):2077–2086. https://doi.org/10.1111/bcp.13295

Rabe KF, Nair P, Brusselle G, Maspero JF, Castro M, Sher L, Zhu H, Hamilton JD, Swanson BN, Khan A, Chao J, Staudinger H, Pirozzi G, Antoni C, Amin N, Ruddy M, Akinlade B, Graham NMH, Stahl N, Yancopoulos GD, Teper A (2018) Efficacy and safety of dupilumab in glucocorticoid-dependent severe asthma. N Engl J Med 378(26):2475–2485. https://doi.org/10.1056/NEJMoa1804093

Raissy HH, Kelly HW, Harkins M, Szefler SJ (2013) Inhaled corticosteroids in lung diseases. Am J Respir Crit Care Med 187(8):798–803. https://doi.org/10.1164/rccm.201210-1853PP

Rampulla C, Corsico R, Majani U, Lodola E (1985) Broxaterol: a double-blind clinical trial comparing broxaterol and salbutamol. Respiration 47(4):299–302. https://doi.org/10.1159/000194786

Rau JL (2005) The inhalation of drugs: advantages and problems. Respir Care 50(3):367–382

Ray SM, McMillen JC, Treadway SA, Helmer RS, Franks SA (2012) Indacaterol: a novel long-acting β(2)-agonist. Pharmacotherapy 32(5):456–474. https://doi.org/10.1002/j.1875-9114.2012.01025.x

Rea HH, Garrett JE, Lanes SF, Birmann BM, Kolbe J (1996) The association between asthma drugs and severe life-threatening attacks. Chest 110(6):1446–1451. https://doi.org/10.1378/chest.110.6.1446

Rebordosa C, Farkas DK, Montonen J, Laugesen K, Voss F, Aguado J, Bothner U, Rothman KJ, Zint K, Mines D, Ehrenstein V (2022) Cardiovascular events and all-cause mortality in patients with chronic obstructive pulmonary disease using olodaterol and other long-acting beta2-agonists. Pharmacoepidemiol Drug Saf 31(8):827–839. https://doi.org/10.1002/pds.5432

Reddel H, Ware S, Marks G, Salome C, Jenkins C, Woolcock A (1999) Differences between asthma exacerbations and poor asthma control. Lancet 353(9150):364–369. https://doi.org/10.1016/S0140-6736(98)06128-5

Reyes-Mondragon A, Delgado-García G, Pacheco-Cantú A, Contreras-Garza N, Galarza-Delgado DÁ, González-Aguirre J (2016) Atrial fibrillation in an asthmatic patient with albuterol-induced lactic acidosis. Pneumologia 65(3):150–151

Rhoades RB, Leifer KN, Bloom FL, Wittig HJ (1976) Spirometric comparison of carbuterol and isoproterenol aerosol therapy in bronchial asthma. A double blind, matched-pair study of 28 adults and a double blind crossover study of 18 children. Am Rev Respir Dis 114(1):79–86. https://doi.org/10.1164/arrd.1976.114.1.79

Rhodes DG, Newton R, Butler R, Herbette L (1992) Equilibrium and kinetic studies of the interactions of salmeterol with membrane bilayers. Mol Pharmacol 42(4):596–602

Richards DM, Brogden RN (1985) Pirbuterol. A preliminary review of its pharmacological properties and therapeutic efficacy in reversible bronchospastic disease. Drugs 30(1):6–21. https://doi.org/10.2165/00003495-198530010-00002

Ricks CA, Dalrymple RH, Baker PK, Ingle DL (1984) Use of a β-agonist to alter fat and muscle deposition in steers. J Anim Sci 59(5):1247–1255. https://doi.org/10.2527/jas1984.5951247x

Robin ED, McCauley R (1992) Sudden cardiac death in bronchial asthma, and inhaled beta-adrenergic agonists. Chest 101(6):1699–1702. https://doi.org/10.1378/chest.101.6.1699

Sackner MA, Epstein S, Wanner A (1976) Effect of beta-adrenergic agonists aerosolized by freon propellant on tracheal mucous velocity and cardiac output. Chest 69(5):593–598. https://doi.org/10.1378/chest.69.5.593

Sala R, Moriggi E, Della Bella D, Carenzi A (1991) The specific binding of broxaterol, a new beta 2-selective agonist, to beta-adrenoceptors. Eur J Pharmacol 203(1):17–23. https://doi.org/10.1016/0014-2999(91)90785-o

Saleeby PR, Ziskind MM (1975) Clinical study on carbuterol (SKF 40383), a new selective bronchodilator agent aerosol: double blind comparison with isoproterenol aerosol. Curr Ther Res Clin Exp 17(3):225–233

Salorinne Y, Stenius B, Tukiainen P, Poppius H (1975) Double-blind cross-over comparison of clenbuterol and salbutamol tablets in asthmatic out-patients. Eur J Clin Pharmacol 8(3–4):189–195. https://doi.org/10.1007/BF00567113

Salpeter SR, Ormiston TM, Salpeter EE (2004) Cardiovascular effects of beta-agonists in patients with asthma and COPD: a meta-analysis. Chest 125(6):2309–2321

Salvati L, Liotta F, Annunziato F, Cosmi L (2022) Therapeutical targets in allergic inflammation. Biomedicine 10(11):2874. https://doi.org/10.3390/biomedicines10112874

Sanchez J, Rivero A, Dal-Re R, Azanza JR (1988) Open cross-over comparison of tulobuterol and fenoterol in asthmatic adult patients. Int J Clin Pharmacol Res 8(4):279–285

Sanders JP, Potter DE, Ellis S, Bee DE, Grant JA (1977) Metabolic and cardiovascular effects of carbuterol and metaproterenol. J Allergy Clin Immunol 60(3):174–179. https://doi.org/10.1016/0091-6749(77)90121-x

Sandström T, Asander L, Clemmensen IH, Eklund G, Gnosspelius Y, Klarlund Pedersen B, Persson G, Ravn S, Rosenhall L, Weeke B (1988) Bambuterol: clinical effects of different doses of a long-acting bronchodilator prodrug. Respiration 53(1):31–36. https://doi.org/10.1159/000195393

Santus P, Radovanovic D, Paggiaro P, Papi A, Sanduzzi A, Scichilone N, Braido F (2015) Why use long acting bronchodilators in chronic obstructive lung diseases? An extensive review on formoterol and salmeterol. Eur J Intern Med 26(6):379–384. https://doi.org/10.1016/j.ejim.2015.05.001

Sarnaik SM, Saladino RA, Manole M, Pitetti RA, Arora G, Kuch BA, Orr RA, Felmet KA (2013) Diastolic hypotension is an unrecognized risk factor for β-agonist-associated myocardial injury in children with asthma. Pediatr Crit Care Med 14(6):e273–e279. https://doi.org/10.1097/PCC.0b013e31828a7677

Sato M, Dehvari N, Oberg AI, Dallner OS, Sandström AL, Olsen JM, Csikasz RI, Summers RJ, Hutchinson DS, Bengtsson T (2014) Improving type 2 diabetes through a distinct adrenergic signaling pathway involving mTORC2 that mediates glucose uptake in skeletal muscle. Diabetes 63(12):4115–4129. https://doi.org/10.2337/db13-1860

Sayers I, Hawley J, Stewart CE, Billington CK, Henry A, Leighton-Davies JR, Charlton SJ, Hall IP (2009) Pharmacogenetic characterization of indacaterol, a novel beta 2-adrenoceptor agonist. Br J Pharmacol 158(1):277–286. https://doi.org/10.1111/j.1476-5381.2009.00224.x

Scheinin M, Koulu M, Laurikainen E, Allonen H (1987) Hypokalaemia and other non-bronchial effects of inhaled fenoterol and salbutamol: a placebo-controlled dose-response study in healthy volunteers. Br J Clin Pharmacol 24(5):645–653. https://doi.org/10.1111/j.1365-2125.1987.tb03224.x

Scola AM, Chong LK, Chess-Williams R, Peachell PT (2004) Influence of agonist intrinsic activity on the desensitisation of beta2-adrenoceptor-mediated responses in mast cells. Br J Pharmacol 143(1):71–80. https://doi.org/10.1038/sj.bjp.0705905

Scosyrev E, van Zyl-Smit R, Kerstjens H, Gessner C, Kornmann O, Jain D, Aubrun E, D'Andrea P, Hosoe M, Pethe A, Brittain D (2021) Cardiovascular safety of mometasone/indacaterol and mometasone/indacaterol/glycopyrronium once-daily fixed-dose combinations in asthma: pooled analysis of phase 3 trials. Respir Med 180:106311. https://doi.org/10.1016/j.rmed.2021.106311

Sears MR (2002) Adverse effects of beta-agonists. J Allergy Clin Immunol 110(6 Suppl):S322–S328. https://doi.org/10.1067/mai.2002.129966

Sears MR, Taylor DR, Print CG, Lake DC, Li QQ, Flannery EM, Yates DM, Lucas MK, Herbison GP (1990) Regular inhaled beta-agonist treatment in bronchial asthma. Lancet 336(8728):1391–1396. https://doi.org/10.1016/0140-6736(90)93098-a

Sears MR, Ottosson A, Radner F, Suissa S (2009) Long-acting beta-agonists: a review of formoterol safety data from asthma clinical trials. Eur Respir J 33(1):21–32. https://doi.org/10.1183/09031936.00145006

Seco AJ, Salgueiro ME, Villanueva MA, Manso G (2000) Treatment with high doses of terbutaline induces beta-adrenergic desensitization of guinea pig trachea not prevented by the addition of dexamethasone. Respiration 67(5):559–564. https://doi.org/10.1159/000067474

Senior RM, Lefrak SS, Kleiger RE (1979) The heart in chronic obstructive pulmonary disease: arrhythmias. Chest 75(1):1–2. https://doi.org/10.1378/chest.75.1.1

Shaw DE, Heaney LG, Thomas M, Beasley R, Gibson PG, Pavord ID (2021) Balancing the needs of the many and the few: where next for adult asthma guidelines? Lancet Respir Med 9(7):786–794. https://doi.org/10.1016/S2213-2600(21)00021-7

Siegel SC, Katz RM, Rachelefsky GS, Brandon ML, Borgen LA (1985) A placebo-controlled trial of procaterol: a new long-acting oral beta 2-agonist in bronchial asthma. J Allergy Clin Immunol 75(6):698–705. https://doi.org/10.1016/0091-6749(85)90096-x

Simone P, Borgia M, Torre L, Ventresca GP (1990) Dose-response comparison of broxaterol and salbutamol pressurized aerosols. Eur J Clin Pharmacol 39(6):565–568. https://doi.org/10.1007/BF00316096

Sitar DS, Aoki FY, Warren CP, Knight A, Grossman RF, Alexander M, Soliman S (1993) A placebo-controlled dose-finding study with bambuterol in elderly patients with asthma. Chest 103(3):771–776. https://doi.org/10.1378/chest.103.3.771

Slack RJ, Barrett VJ, Morrison VS, Sturton RG, Emmons AJ, Ford AJ, Knowles RG (2013) In vitro pharmacological characterization of vilanterol, a novel long-acting β2-adrenoceptor agonist with 24-hour duration of action. J Pharmacol Exp Ther 344(1):218–230. https://doi.org/10.1124/jpet.112.198481

Slater M, Rivett DW, Williams L, Martin M, Harrison T, Sayers I, Bruce KD, Shaw D (2014) The impact of azithromycin therapy on the airway microbiota in asthma. Thorax 69(7):673–674. https://doi.org/10.1136/thoraxjnl-2013-204517

Slater M, Torr E, Harrison T, Forrester D, Knox A, Shaw D, Sayers I (2016) The differential effects of azithromycin on the airway epithelium in vitro and in vivo. Physiol Rep 4(18):e12960. https://doi.org/10.14814/phy2.12960

Smith JM (1966) Deaths from asthma. Lancet 287:1042. https://doi.org/10.1016/S0140-6736(66)90167-X

Smyth ET, Pavord ID, Wong CS, Wisniewski AF, Williams J, Tattersfield AE (1993) Interaction and dose equivalence of salbutamol and salmeterol in patients with asthma. BMJ 306 (6877):543–545. https://doi.org/10.1136/bmj.306.6877.543

Solis-Cohen S (1898) A preliminary note on the treatment of hay-fever with suprarenal substance: with a report of personal experience. Phil Med J 11:341–343

Solis-Cohen S (1900) The use of adrenal substance in the treatment of asthma. JAMA 19:1164–1166

Spann C, Winter ME (1995) Effect of clenbuterol on athletic performance. Ann Pharmacother 29 (1):75–77

Speizer FE, Doll R (1968) A century of asthma deaths in young people. Br Med J 3(5612):245–246. https://doi.org/10.1136/bmj.3.5612.245

Speizer FE, Doll R, Heaf P (1968a) Observations on recent increase in mortality from asthma. Br Med J 1(5588):335–339. https://doi.org/10.1136/bmj.1.5588.335

Speizer FE, Doll R, Heaf P, Strang LB (1968b) Investigation into use of drugs preceding death from asthma. Br Med J 1(5588):339–343. https://doi.org/10.1136/bmj.1.5588.339

Spitzer WO, Suissa S, Ernst P, Horwitz RI, Habbick B, Cockcroft D, Boivin JF, McNutt M, Buist AS, Rebuck AS (1992) The use of beta-agonists and the risk of death and near death from asthma. N Engl J Med 326(8):501–506. https://doi.org/10.1056/NEJM199202203260801

Starkey ES, Mulla H, Sammons HM, Pandya HC (2014) Intravenous salbutamol for childhood asthma: evidence-based medicine? Arch Dis Child 99(9):873–877. https://doi.org/10.1136/archdischild-2013-304467

Steeds RP, Channer KS (1998) Drug treatment in heart failure – lowering heart rate may reduce mortality. BMJ 316(7131):567–568. https://doi.org/10.1136/bmj.316.7131.567

Steen SN, Ziment I, Thomas JS (1974) Pirbuterol: a new bronchodilator. Phase I-single dose study. Curr Ther Res Clin Exp 16(10):1077–1081

Steen SN, Smith R, Kuo J, Ziment I, Beall GN (1977) Comparison of the bronchodilator effects of aerosol fenoterol and isoproterenol. Chest 72(6):724–730. https://doi.org/10.1378/chest.72.6.724

Stein SW, Thiel CG (2017) The history of therapeutic aerosols: a chronological review. J Aerosol Med Pulm Drug Deliv 30(1):20–41. https://doi.org/10.1089/jamp.2016.1297

Stern J, Pier J, Litonjua AA (2020) Asthma epidemiology and risk factors. Semin Immunopathol 42 (1):5–15. https://doi.org/10.1007/s00281-020-00785-1

Stolz F (1904) Uber adrenalin und alkylaminoacetobrenzcatechin. Ber Dtsch Chem Ges 37:4149–4154

Strange PG (2008) Agonist binding, agonist affinity and agonist efficacy at G protein-coupled receptors. Br J Pharmacol 153:1353–1363

Stynes G, Svedsater H, Wex J, Lettis S, Leather D, Castelnuovo E, Detry M, Berry S (2015) Once-daily fluticasone furoate/vilanterol 100/25 mcg versus twice daily combination therapies in COPD – mixed treatment comparisons of clinical efficacy. Respir Res 16(1):25. https://doi.org/10.1186/s12931-015-0184-8

Sugawara T, Nanjo Y, Yamazaki M, Higashihara K, Tsuda Y, Mochizuki H, Noguchi T, Takahashi H (2009) Comparison of adherence and efficacy between inhaled salmeterol and transdermal tulobuterol patch in elderly patients with chronic obstructive pulmonary disorder. J Am Geriatr Soc 57(5):919–920. https://doi.org/10.1111/j.1532-5415.2009.02230.x

Suissa S (2021) Perplexing mortality data from triple therapy trials in COPD. Lancet Respir Med 9 (7):684–685. https://doi.org/10.1016/S2213-2600(21)00238-1

Suissa S, Ernst P (1997) Optical illusions from visual data analysis: example of the New Zealand asthma mortality epidemic. J Clin Epidemiol 50(10):1079–1088. https://doi.org/10.1016/s0895-4356(97)00158-3

Suissa S, Ernst P, Boivin JF, Horwitz RI, Habbick B, Cockroft D, Blais L, McNutt M, Buist AS, Spitzer WO (1994) A cohort analysis of excess mortality in asthma and the use of inhaled beta-agonists. Am J Respir Crit Care Med 149(3 Pt 1):604–610. https://doi.org/10.1164/ajrccm.149.3.8118625

Suissa S, Hemmelgarn B, Blais L, Ernst P (1996) Bronchodilators and acute cardiac death. Am J Respir Crit Care Med 154(6 Pt 1):1598–1602. https://doi.org/10.1164/ajrccm.154.6.8970341

Suissa S, Ernst P, Benayoun S, Baltzan M, Cai B (2000) Low-dose inhaled corticosteroids and the prevention of death from asthma. N Engl J Med 343(5):332–336. https://doi.org/10.1056/NEJM200008033430504

Summerhill S, Stroud T, Nagendra R, Perros-Huguet C, Trevethick M (2008) A cell-based assay to assess the persistence of action of agonists acting at recombinant human beta(2) adrenoceptors. J Pharmacol Toxicol Methods 58(3):189–197. https://doi.org/10.1016/j.vascn.2008.06.003

Svedmyr NL, Larsson SA, Thiringer GK (1976) Development of "resistance" in beta-adrenergic receptors of asthmatic patients. Chest 69(4):479–483. https://doi.org/10.1378/chest.69.4.479

Svedsater H, Stynes G, Wex J, Frith L, Leather D, Castelnuovo E, Detry M, Berry S (2016) Once-daily fluticasone furoate/vilanterol versus twice daily combination therapies in asthma-mixed treatment comparisons of clinical efficacy. Asthma Res Pract 2:4. https://doi.org/10.1186/s40733-015-0016-0

Svensson LA (1991) Mechanism of action of bambuterol: a beta-agonist prodrug with sustained lung affinity. Agents Actions Suppl 34:71–78

Syed YY (2015) Fluticasone furoate/vilanterol: a review of its use in patients with asthma. Drugs 75 (4):407–418. https://doi.org/10.1007/s40265-015-0354-5

Szymonowicz L, Cybulski N (1895) Jahres Bericht der Thier-Chemie 25:379

Tabori D, Conkic B, Todic V, Mijatovic M, Mirkovc S, Zecević D, Camprag D (1977) The bronchdilating action of reproterol in patients with bronchial asthma and chronic obstructive bronchitis. Arzneimittelforschung 27(12):55–60

Tai A, Tran H, Roberts M, Clarke N, Wilson J, Robertson CF (2014) The association between childhood asthma and adult chronic obstructive pulmonary disease. Thorax 69(9):805–810. https://doi.org/10.1136/thoraxjnl-2013-204815

Tamura G, Ohta K (2007) Adherence to treatment by patients with asthma or COPD: comparison between inhaled drugs and transdermal patch. Respir Med 101(9):1895–1902. https://doi.org/10.1016/j.rmed.2007.05.001

Tamura G, Ichinose M, Fukuchi Y, Miyamoto T (2012) Transdermal tulobuterol patch, a long-actingβ(2)-agonist. Allergol Int 61(2):219–229. https://doi.org/10.2332/allergolint.11-RA-0358

Tan KS, Grove A, McLean A, Gnosspelius Y, Hall IP, Lipworth BJ (1997) Systemic corticosteriod rapidly reverses bronchodilator subsensitivity induced by formoterol in asthmatic patients. Am J Respir Crit Care Med 156(1):28–35. https://doi.org/10.1164/ajrccm.156.1.9610113

Tan KS, McFarlane LC, Lipworth BJ (1998) Concomitant administration of low-dose prednisolone protects against in vivo beta2-adrenoceptor subsensitivity induced by regular formoterol. Chest 113(1):34–41. https://doi.org/10.1378/chest.113.1.34

Tandon MK (1980) Cardiopulmonary effects of fenoterol and salbutamol aerosols. Chest 77 (3):429–431. https://doi.org/10.1378/chest.77.3.429

Tansey EM (1995) What's in a name? Henry Dale and adrenaline, 1906. Med Hist 39(4):459–476. https://doi.org/10.1017/s0025727300060373

Tarala RA, Martyn V, Paterson JW (1981) Effect of intravenous injection of rimiterol in asthma. Br J Clin Pharmacol 12(3):333–340. https://doi.org/10.1111/j.1365-2125.1981.tb01222.x

Tattersfield AE (1992) Long-acting beta 2-agonists. Clin Exp Allergy 22(6):600–605. https://doi.org/10.1111/j.1365-2222.1992.tb00175.x

Tattersfield AE (2006) Current issues with beta2-adrenoceptor agonists: historical background. Clin Rev Allergy Immunol 31(2–3):107–118. https://doi.org/10.1385/CRIAI:31:2:107

Tattersfield AE, McNicol MW (1969) Salbutamol and isoproterenol. A double-blind trial to compare bronchodilator and cardiovascular activity. N Engl J Med 281(24):1323–1326. https://doi.org/10.1056/NEJM196912112812402

Thiringer G, Svedmyr N (1976) Comparison of infused and inhaled terbutaline in patients with asthma. Scand J Respir Dis 57(1):17–24

Tinkelman DG, DeJong R, Lutz C, Spangler DL (1990) Evaluation of tremor and efficacy of oral procaterol in adult patients with asthma. J Allergy Clin Immunol 85(4):719–728. https://doi.org/10.1016/0091-6749(90)90190-f

Tirlapur VG, Mir MA (1982) Nocturnal hypoxemia and associated electrocardiographic changes in patients with chronic obstructive airways disease. N Engl J Med 306(3):125–130. https://doi.org/10.1056/NEJM198201213060301

Tkacova R (2010) Systemic inflammation in chronic obstructive pulmonary disease: may adipose tissue play a role? Review of the literature and future perspectives. Mediators Inflamm 2010:585989. https://doi.org/10.1155/2010/585989

Tliba O, Panettieri RA Jr (2019) Paucigranulocytic asthma: uncoupling of airway obstruction from inflammation. J Allergy Clin Immunol 143(4):1287–1294. https://doi.org/10.1016/j.jaci.2018.06.008

Trachsel D, Newth CJ, Hammer J (2007) Adenosine for salbutamol-induced supraventricular tachycardia. Intensive Care Med 33(9):1676. https://doi.org/10.1007/s00134-007-0673-4

Tukiainen H, Jaakkola J, Torkko M, Terho EO (1988) Comparison between oral procaterol and salbutamol in patients with bronchial asthma. Curr Med Res Opin 11(4):236–241. https://doi.org/10.1185/03007998809114242

Tullar BF, Minatoya H, Lorenz RR (1976) Esters of N-tert-butylarterenol. Long-acting new bronchodilators with reduced cardiac effects. J Med Chem 19(6):834–838. https://doi.org/10.1021/jm00228a020

Ullman A, Svedmyr N (1988) Salmeterol, a new long acting inhaled beta 2 adrenoceptor agonist: comparison with salbutamol in adult asthmatic patients. Thorax 43(9):674–678. https://doi.org/10.1136/thx.43.9.674

Ullman A, Hedner J, Svedmyr N (1990) Inhaled salmeterol and salbutamol in asthmatic patients. An evaluation of asthma symptoms and the possible development of tachyphylaxis. Am Rev Respir Dis 142(3):571–575. https://doi.org/10.1164/ajrccm/142.3.571

Ulmer WT, Scholz HJ, Baines A (1984) Pilot clinical trial with a new beta 2-sympathomimetic bronchodilator, mabuterol. Arzneimittelforschung 34(11A):1697–1698

Unwalla HJ, Horvath G, Roth FD, Conner GE, Salathe M (2012) Albuterol modulates its own transepithelial flux via changes in paracellular permeability. Am J Respir Cell Mol Biol 46(4):551–558. https://doi.org/10.1165/rcmb.2011-0220OC. Epub 2011 Dec 8

Van Arman CG, Miller LM, O'Malley MP (1961) SC-10049: a catecholamine bronchodilator and hyperglycemic agent. J Pharmacol Exp Ther 133:90–97

van Noord JA, Smeets JJ, Raaijmakers JA, Bommer AM, Maesen FP (1996) Salmeterol versus formoterol in patients with moderately severe asthma: onset and duration of action. Eur Respir J 8:1684–1688. https://doi.org/10.1183/09031936.96.09081684

van Schayck CP, Graafsma SJ, Visch MB, Dompeling E, van Weel C, van Herwaarden CL (1990) Increased bronchial hyperresponsiveness after inhaling salbutamol during 1 year is not caused by subsensitization to salbutamol. J Allergy Clin Immunol 86(5):793–800. https://doi.org/10.1016/s0091-6749(05)80185-x

Vandenplas O, Wiszniewska M, Raulf M, de Blay F, Gerth van Wijk R, Moscato G, Nemery B, Pala G, Quirce S, Sastre J, Schlünssen V, Sigsgaard T, Siracusa A, Tarlo SM, van Kampen V, Zock JP, Walusiak-Skorupa J, European Academy of Allergy and Clinical Immunology (2014) EAACI position paper: irritant-induced asthma. Allergy 69(9):1141–1153. https://doi.org/10.1111/all.12448

Venkatesan P (2023) GOLD COPD report: 2023 update. Lancet Respir Med 11(1):18. https://doi.org/10.1016/S2213-2600(22)00494-5

Verdugo P, Johnson NT, Tam PY (1980) beta-Adrenergic stimulation of respiratory ciliary activity. J Appl Physiol Respir Environ Exerc Physiol 48(5):868–871. https://doi.org/10.1152/jappl.1980.48.5.868

Virchow JC Jr (1999) Reproterol: beta-2-agonist, theophylline, or both? Respiration 66(3):210–211. https://doi.org/10.1159/000029379

Virchow JC, Backer V, Kuna P, Prieto L, Nolte H, Villesen HH, Ljørring C, Riis B, de Blay F (2016) Efficacy of a house dust mite sublingual allergen immunotherapy tablet in adults with allergic asthma: a randomized clinical trial. JAMA 315(16):1715–1725. https://doi.org/10.1001/jama.2016.3964

Virchow JC, Kuna P, Paggiaro P, Papi A, Singh D, Corre S, Zuccaro F, Vele A, Kots M, Georges G, Petruzzelli S, Canonica GW (2019) Single inhaler extrafine triple therapy in uncontrolled asthma (TRIMARAN and TRIGGER): two double-blind, parallel-group, randomised, controlled phase 3 trials. Lancet 394(10210):1737–1749. https://doi.org/10.1016/S0140-6736(19)32215-9

Von Fürth O (1900) Zur Kenntniss der brenzcatechinähnlichen Substanz der Nebennieren. III. Mittheilung. Z Physiol Chem 29:105–123

Waldeck B (1996) Some pharmacodynamic aspects on long-acting beta-adrenoceptor agonists. Gen Pharmacol 27(4):575–580. https://doi.org/10.1016/0306-3623(95)02052-7

Waldeck B (2002) Beta-adrenoceptor agonists and asthma – 100 years of development. Eur J Pharmacol 445(1–2):1–12. https://doi.org/10.1016/s0014-2999(02)01728-4

Walker SB, Kradjan WA, Bierman CW (1985) Bitolterol mesylate: a beta-adrenergic agent. Chemistry, pharmacokinetics, pharmacodynamics, adverse effects and clinical efficacy in asthma. Pharmacotherapy 5(3):127–137. https://doi.org/10.1002/j.1875-9114.1985.tb03410.x

Wallaert B, Brun P, Ostinelli J, Murciano D, Champel F, Blaive B, Montané F, Godard P (1999) A comparison of two long-acting beta-agonists, oral bambuterol and inhaled salmeterol, in the treatment of moderate to severe asthmatic patients with nocturnal symptoms. The French Bambuterol Study Group. Respir Med 93(1):33–38. https://doi.org/10.1016/s0954-6111(99)90074-4

Wang P, Clausen T (1976) Treatment of attacks in hyperkalaemic familial periodic paralysis by inhalation of salbutamol. Lancet 1(7953):221–223. https://doi.org/10.1016/s0140-6736(76)91340-4

Wardell JR, Colella DF, Shetzline A, Fowler PJ (1974) Studies on carbuterol (SK & F 40383-A), a new selective bronchodilator agent. J Pharmacol Exp Ther 189(1):167–184

Warnier MJ, Rutten FH, Kors JA, Lammers JW, de Boer A, Hoes AW, de Bruin ML (2012) Cardiac arrhythmias in adult patients with asthma. J Asthma 49(9):942–946. https://doi.org/10.3109/02770903.2012.724132

Warrell DA, Robertson DG, Howes JN, Conolly ME, Paterson JW, Beilin LJ, Dollery CT (1970) Comparison of cardiorespiratory effects of isoprenaline and salbutamol in patients with bronchial asthma. Br Med J 1(5688):65–70

Werdermann K (1990) Two-month comparative study of tulobuterol aerosol versus fenoterol aerosol in patients with chronic obstructive lung disease. Lung 168(Suppl):202–209. https://doi.org/10.1007/BF02718134

Wetterlin KIL, Svensson LA (1968) Belgium patent No. 704932

WHO (2019) World Health Organization model list of essential medicines: 21st list 2019. World Health Organization, Geneva

Wilkinson A, Woodcock A (2022) The environmental impact of inhalers for asthma: a green challenge and a golden opportunity. Br J Clin Pharmacol 88(7):3016–3022. https://doi.org/10.1111/bcp.15135

Willey RF, Grant IW, Pocock SJ (1976) Effects of oral salbutamol and pirbuterol on FEV1, heart rate and blood pressure in asthmatics. Br J Clin Pharmacol 3(4):595–600. https://doi.org/10.1111/j.1365-2125.1976.tb04881.x

Wilson JD, Sutherland DC, Thomas AC (1981) Has the change to beta-agonists combined with oral theophylline increased cases of fatal asthma? Lancet 1(8232):1235–1237. https://doi.org/10.1016/s0140-6736(81)92403-x

Wong CS, Pavord ID, Williams J, Britton JR, Tattersfield AE (1990) Bronchodilator, cardiovascular, and hypokalaemic effects of fenoterol, salbutamol, and terbutaline in asthma. Lancet 336(8728):1396–1399. https://doi.org/10.1016/0140-6736(90)93099-b

Woodcock A, Bleecker ER, Lötvall J, O'Byrne PM, Bateman ED, Medley H, Ellsworth A, Jacques L, Busse WW (2013) Efficacy and safety of fluticasone furoate/vilanterol compared with

fluticasone propionate/salmeterol combination in adult and adolescent patients with persistent asthma: a randomized trial. Chest 144(4):1222–1229. https://doi.org/10.1378/chest.13-0178

Woodward S, Mundorff M, Weng C, Gamboa DG, Johnson MD (2021) Incidence of supraventricular tachycardia after inhaled short-acting beta agonist treatment in children. J Asthma 58 (4):471–480. https://doi.org/10.1080/02770903.2019.1709867

Yabuuchi Y (1977) The beta-adrenoceptor stimulant properties of OPC-2009 on guinea-pig isolated tracheal, right atrial and left atrial preparations. Br J Pharmacol 61(4):513–521. https://doi.org/10.1111/j.1476-5381.1977.tb07543.x

Yabuuchi Y, Yamashita S, Tei SS (1977) Pharmacological studies of OPC-2009, a newly synthesized selective beta adrenoceptor stimulant, in the broncho-motor and cardiovascular system of the anesthetized dog. J Pharmacol Exp Ther 202(2):326–336

Yamagata T, Hirano T, Sugiura H, Yanagisawa S, Ichikawa T, Ueshima K, Akamatsu K, Nakanishi M, Matsunaga K, Minakata Y, Ichinose M (2008) Comparison of bronchodilatory properties of transdermal and inhaled long-acting beta 2-agonists. Pulm Pharmacol Ther 21(1):160–165. https://doi.org/10.1016/j.pupt.2007.05.004

Yamashima T (2003) Jokichi Takamine (1854-1922), the samurai chemist, and his work on adrenalin. J Med Biogr 11:95–102

Yamashita S, Takai M, Yabuuchi Y (1978) Actions of procaterol (OPC-2009), a new beta2-adrenoceptor stimulant, on pulmonary resistance, contractions of the soleus muscle, and cardiovascular system of the anaesthetized cat. J Pharm Pharmacol 30(5):273–279. https://doi.org/10.1111/j.2042-7158.1978.tb13228.x

Yoshizaki S, Tanimura K, Tamada S, Yabuuchi Y, Nakagawa K (1976) Sympathomimetic amines having a carbostyril nucleus. J Med Chem 19(9):1138–1142. https://doi.org/10.1021/jm00231a011

Youssef M, Kanagaratham C, Saad MI, Radzioch D (2016) Genetics of allergic asthma and current perpectivs on therapeutic management. In: Pereira C (ed) Asthma – from childhood asthma to ACOS phenotypes. ISBN 978-953-51-2441-2

Zanetti CL, Rotman HH, Dresner AJ (1982) Efficacy and duration of action of procaterol, a new bronchodilator. J Clin Pharmacol 22(5–6):250–253. https://doi.org/10.1002/j.1552-4604.1982.tb02669.x

Ziment I (1989) Broxaterol: therapeutic trials and safety profile. Respiration 55(Suppl 2):28–40. https://doi.org/10.1159/000195767

Adrenoceptors in the Eye – Physiological and Pathophysiological Relevance

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Contents

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Abstract

The autonomic nervous system plays a crucial role in the innervation of the eye. Consequently, it comes as no surprise that catecholamines and their corresponding receptors have been extensively studied and characterized in numerous ocular structures, including the cornea, conjunctiva, lacrimal gland, trabecular meshwork, uvea, and retina. These investigations have unveiled substantial clinical implications, particularly in the context of treating glaucoma, a progressive neurodegenerative disorder responsible for irreversible vision loss on a global scale. The primary therapeutic approaches for glaucoma frequently involve the modulation of α_1-, α_2-, and β-adrenoceptors, making them pivotal targets. In this chapter, we offer a comprehensive overview of the expression, distribution, and functional roles of adrenoceptors within various components of the eye and its associated structures. Additionally, we delve into the pivotal role of adrenoceptors in the pathophysiology of glaucoma. Furthermore, we provide a concise historical perspective on adrenoceptor research, examine the distinct contributions of individual adrenoceptor subtypes to the treatment of various ocular conditions, and propose potential future avenues of exploration in this field.

Keywords

Adrenoceptors · Distribution · Eye · Function · Therapy

1 Introduction

The discovery of adrenoceptors (AR) by Ahlquist more than seven decades ago unveiled the adrenergic signaling pathways as central regulators of blood pressure and of metabolic and central nervous system functions (Ahlquist 1948). ARs are members of the superfamily of guanosine triphosphate-binding protein (G protein)-coupled receptors (GPCRs) and are targeted by catecholamines, particularly adrenaline and noradrenaline (Ahlquist 1948; Alexander et al. 2013; Civantos Calzada and Aleixandre de Artinano 2001; Piascik and Perez 2001). Based on their pharmacological properties, amino acid sequences, and signaling mechanisms, the AR family is divided into three subfamilies, α_1, α_2, and β AR (Wikberg-Matsson 2001). Each AR subfamily consists of three subtypes (α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, and β_3) (Fig. 1) (Bylund et al. 1994).

Members of the α_1-AR subfamily are widely expressed throughout the cardiovascular system (McGrath 2015). They critically participate in the regulation of vascular tone and blood flow primarily by mediating the vasoconstrictive effects of catecholamines (Ciccarelli et al. 2008; Cotecchia 2010; Faber et al. 2001; Gericke et al. 2007; Guimaraes and Moura 2001; Hrometz et al. 1999; McGrath 2015; Tuttle and Falcone 2001). However, at least in rodents, they are also expressed in the heart, where they are involved in mediating hypertrophy, positive inotropy, ischemic

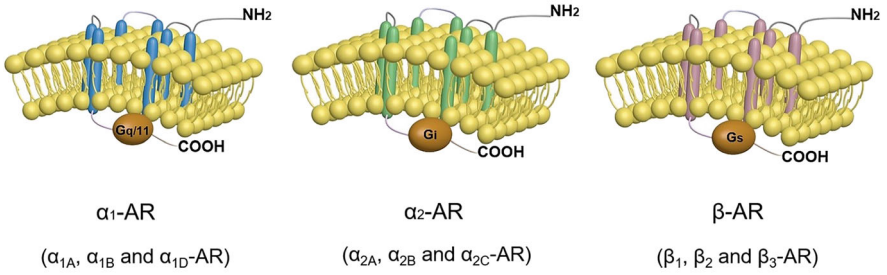

Fig. 1 The three adrenoceptor (AR) subfamilies and their subtypes. α_1-, α_2-, and β-ARs mainly couple to $G_{q/11}$, G_i, and G_s proteins, respectively

preconditioning, and protection from cell death (Zhang et al. 2021). Notably, the expression pattern of individual α_1-AR subtypes and their involvement in mediating vascular responses to catecholamines differs between individual vascular beds and between species (Graham et al. 1996; Guimaraes and Moura 2001; Hosoda et al. 2005; Marti et al. 2005; Rudner et al. 1999).

Members of the α_2-AR subfamily are expressed throughout the body including the central and the peripheral nervous systems. They are localized either pre- or postsynaptically and can mediate inhibition of neurotransmitter release (Drouin et al. 2017; Hieble 2009). Moreover, the α_{2A}-AR subtype has an unusual dual pharmacological effect by coupling to G_i proteins at low agonist concentrations and mainly coupling to Gs proteins at high concentrations (Qu et al. 2019).

β-ARs are widely distributed in both the central and peripheral nervous systems and are involved in essential functions activated by circulating catecholamines, such as heart rate regulation, vasorelaxation, bronchodilation, and facilitation of neurotransmitter release (Djurup 1981). It was also discovered that some ARs are involved in the production of reactive oxygen species (ROS) underscoring their important role as a potential target in retinal diseases (Corbi et al. 2013; Li et al. 2019; Matsuo et al. 2019). β-ARs are mainly coupled to G_s proteins, and both β_1- and β_2-ARs may switch their G protein-coupling specificity from G_s to G_i proteins (Magocsi et al. 2007).

The eye contains both sympathetic and parasympathetic nerves (McDougal and Gamlin 2015). Therefore, it is not surprising that ARs have been detected in most parts of the eye and its adnexa, including the cornea, conjunctiva, lacrimal gland, iris, trabecular meshwork, retina and blood vessels (Candia and Neufeld 1978; Chen et al. 2006; Gericke et al. 2011; Ríos et al. 2000a, b; Schwinn and Afshari 2006; Stamer et al. 1996; Suzuki et al. 2002). Activation or inhibition of ARs provides potential therapeutic strategies in various eye diseases, such as glaucoma, allergic conjunctivitis, dry eye disease, retinopathy of prematurity (ROP), and diabetic retinopathy (Dal Monte et al. 2012; Du et al. 2015; Greenfield et al. 1997; Liu et al. 2020b; Woodward and Nieves 1985). Within this chapter, we delve into the pathophysiology of glaucoma, a globally leading cause of blindness and the

prevailing form of optic neuropathy. We elucidate the pivotal role played by adrenoceptors as potential therapeutic targets in the management of this condition.

In addition, we focus on the current state of research regarding the role of α_1-, α_2-, and β-ARs in the eye. Our goal is to provide an overview of ocular expression, structural distribution, and regulation of the individual AR subfamilies and their subtypes and discuss potential therapeutic approaches of ARs in the eye.

2 Adrenoceptors and Glaucoma

2.1 General Characteristics in Glaucoma

The term "glaucoma" encompasses a group of disorders characterized by the progressive loss of retinal ganglion cells (RGCs) and subsequent atrophy of the optic nerve. This condition is often associated with elevated intraocular pressure (IOP) and is typically accompanied by optic disc cupping and thinning of the retinal nerve fiber layer (RNFL) (Burgoyne 2015; Casson et al. 2012; Waisberg and Micieli 2021; Weinreb et al. 2014). In this context, classic visual field deficits manifest as arc-shaped defects, initially sparing the horizontal equator and corresponding to the pattern of fiber nerve bundles (Anderson and Patella 1999). During the early stages, the progression of glaucoma is generally asymptomatic, thanks to binocular compensation. Patients typically experience noticeable symptoms only in advanced disease stages when significant visual field losses have already occurred (Crabb et al. 2013; Schuster et al. 2020). In advanced stages, perimetric defects develop, resulting in what is known as a Bjerrum scotoma – a distinctive feature characterized by an asymmetric ring-shaped visual field loss. This scotoma extends vertically in both upper and lower quadrants and horizontally from the blind spot temporally, sparing the macular area and delineating a central region of vision. It terminates in a sharp boundary nasally (Drance 1972; Harrington 1964). As the glaucomatous progression continues, extensive perimetric defects, advanced cupping of the optic disc, and substantial thinning of the RNFL lead to a progressive narrowing of central vision, ultimately resulting in visual impairment (Moroi et al. 2019). Glaucoma is the second most prevalent cause of blindness worldwide, following cataracts (Kingman 2004; Quigley and Broman 2006). In 2013, it affected approximately 64.3 million people aged 40-80 years globally, and it is projected that by 2040, this number will exceed 110 million (Tham et al. 2014). According to current literature, 15–20% of glaucoma patients experience blindness in at least one eye (Chen 2003; Kwon et al. 2001; Lichter 2003; Rossetti et al. 2015).

The primary risk factor for this disease is widely acknowledged to be elevated IOP (Acott et al. 2017; Cesareo et al. 2020). This is typically defined as a pressure value above the 97.5th percentile in the population under consideration, often assumed to be higher than 21 mmHg (Casson et al. 2012; Kroese and Burton 2003). However, it is important to note that glaucomatous damage can also occur in the absence of high IOP levels, as observed in normal tension glaucoma (Killer and Pircher 2018). In this context, other potential risk factors have been proposed,

including genetic factors, systemic vascular dysregulation, and endothelial dysfunction (Geyer and Levo 2020; Killer and Pircher 2018; Leung and Tham 2022; Trivli et al. 2019).

Mechanistically, elevated IOP can lead to the compression of RGC axons, which constitute the optic nerve, thereby impeding axoplasmic transport. Alternatively, elevated IOP may compress blood vessels that supply the optic nerve head, leading to hypoperfusion and ischemia in RGCs (Downs et al. 2008; McMonnies 2018). The increase in IOP is thought to result from a loss of integrity in the trabecular meshwork (TM), exacerbating pathological resistance to the flow of aqueous humor (AH) (Nita and Grzybowski 2016). AH is produced by the ciliary body and flows along the lens and iris toward the anterior chamber, reaching the iridocorneal angle where the TM is located. It then passes through the Schlemm's canal and finally drains into the episcleral veins of the conjunctiva, contributing to the normal turnover of AH (Llobet et al. 2003). The permeability of the TM to AH plays a critical role in regulating AH turnover and, consequently, modulating IOP (Llobet et al. 2003).

2.2 Expression and Role of Adrenoceptors in the Trabecular Meshwork

To gain a deeper understanding of the impacts of certain antiglaucoma medications that function by binding to adrenoceptors, we will elucidate, drawing upon the current body of literature, the specific adrenoceptor subtypes present in the TM and elucidate their respective functions. Exposure of cultured human trabecular endothelium to adrenaline was reported to block normal cytokinetic cell movements, to inhibit mitotic and phagocytic activity, and to promote cell degeneration via involvement of α- and β-ARs (Tripathi and Tripathi 1984). In cultures of the human TM, functional α_{2A}-ARs have been detected (Stamer et al. 1996). Moreover, autoradiographic studies revealed predominant expression of β_2-ARs in sections of the human TM (Jampel et al. 1987). Also, pharmacological and radioligand binding studies on human TM revealed abundant expression and functional relevance of β_2-ARs (Crider and Sharif 2002; Hudson and Kelly 2012; Wax et al. 1989). Moreover, studies in monkey and human eyes suggested that adrenaline and noradrenaline increased outflow facility through the TM via involvement of β_2-ARs (Robinson and Kaufman 1990). Functional studies on isolated TM strips revealed that α-AR agonists elicited contractions, whereas β-adrenergic agonists induced relaxations (Wiederholt et al. 1996). In summary, there is some evidence that activation of β_2-ARs increases outflow facility in the TM.

2.3 Conservative and Surgical Approaches in Glaucoma

Since the initial stages of glaucoma are often asymptomatic, early detection of the disease is paramount. Screening methods such as tonometry, analysis of the retinal

nerve fiber layer (RNFL), perimetry, and fundoscopy play a crucial role in identifying glaucoma and initiating timely intervention to halt its progression (Aspberg et al. 2021; Schuster et al. 2020). Once diagnosed, the first-line treatment typically involves the use of topical eye-drop medications. The primary objective of antiglaucomatous drugs is to lower intraocular pressure (IOP) to a target level that is individually determined to prevent the advancement of the disease (Prum et al. 2016). These antiglaucoma medications can be categorized into the following different classes based on their mechanisms of action:

1. β-blockers such as timolol, levobunolol, betaxolol, and carteolol work by inhibiting the production of aqueous humor through the blockade of β-ARs in the ciliary body (Sidjanin et al. 2008). Additionally, they may potentially enhance trabecular outflow facility (Böhm et al. 2023).
2. α_2-adrenoceptor agonists such as apraclonidine, clonidine, and brimonidine reduce the production of aqueous humor and enhance trabecular outflow by stimulating α_2-adrenergic receptors (Sidjanin et al. 2008).
3. Prostaglandin analogs such as latanoprost and bimatoprost promote increased outflow of aqueous humor through both the trabecular and uveoscleral pathways (Schuster et al. 2020).
4. Carbonic anhydrase inhibitors like dorzolamide and brinzolamide function by suppressing the production of aqueous humor through the inhibition of carbonic anhydrase in the ciliary body (Sidjanin et al. 2008).
5. Miotic agents, such as pilocarpine, widen the chamber angle via a constriction of the pupil, and further exert neuroprotective effects by activating muscarinic acetylcholine receptors (Tan et al. 2014).
6. Rho-associated coiled-coil kinase (ROCK) inhibitors, such as netarsudil, suppress the ROCK pathway, block fibrotic events in the TM, and improve AH flow (Li et al. 2021a).

Figure 2 summarizes the anatomical structures in the anterior segment of the eye, the expression of adrenoceptors in the TM, and the mechanisms of action of the main antiglaucoma drugs.

In case of conservative treatment failure and chronically unstable IOP, a variety of surgical options can be considered. These include destruction of the ciliary body by cryo- or laser coagulation, which reduces the AH production, stent implantations aimed at decreasing outflow resistance of the TM, nonfiltering procedures augmenting the outflow pathways without incising the TM, and finally filtering procedures, which create an additional drainage route for AH into the subconjunctival space (Schuster et al. 2020).

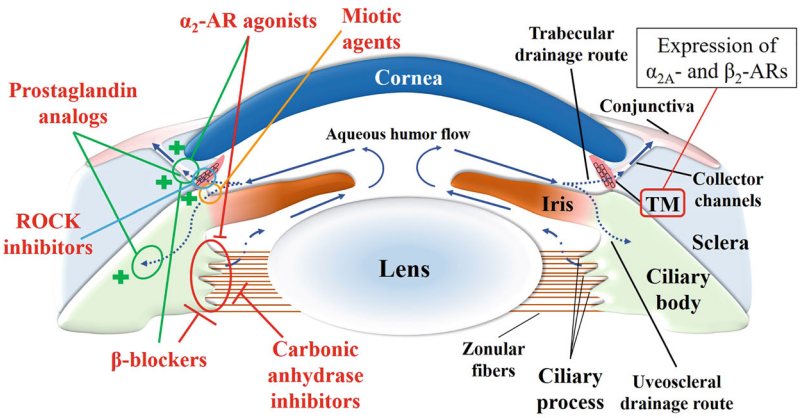

Fig. 2 Anatomical representation of the main structures containing targets of antiglaucoma agents, including ARs, and illustration of the pharmacological mechanisms of actions. ROCK: Rho-associated coiled-coil kinase; TM: trabecular meshwork

2.4 Antiglaucoma Drugs: A Short Historical Overview

The journey of antiglaucoma drugs through history has been marked by significant milestones. These therapeutic agents have evolved to effectively manage IOP and, in some cases, offer neuroprotection, transcending the reliance on IOP reduction alone.

Miotics were the first class of drugs to be introduced to fight glaucoma, as the German ophthalmologist Adolf Weber in 1876 published a study on the effect of pilocarpine in antagonizing angle-closure glaucoma through pupillary constriction, guiding to lowering IOP (Weber 1876). Mechanistically, pilocarpine is a parasympathomimetic drug acting as an agonist of muscarinic acetylcholine receptors, which are abundantly expressed in the iris sphincter muscle, which by activation can lead to miosis finally widening the anterior chamber angle (Shiroma and Costa 2015). Accommodative spasms, myopia, reduced night visual acuity, and cataract have been described as possible side effects of pilocarpine and limited its use in clinical practice (Podos and Ritch 1980). To date, its employment is valuable in emergency cases of devastating angle-closure presentations (Shiroma and Costa 2015). Moreover, very recently (2021), the FDA approved pilocarpine for treating presbyopia, in 1.25% pilocarpine hydrochloride ophthalmic solution per eye drop (Grzybowski and Ruamviboonsuk 2022).

The inception of β-blockers in clinical practice dates back to 1964 with the introduction of propranolol, originally designed to treat cardiovascular conditions (Black et al. 1964). In 1967, Phillips et al. conducted one of the pioneering studies, administering propranolol systemically, and observed a positive effect in lowering IOP (Phillips et al. 1967). Subsequently, in 1968, topical propranolol also demonstrated IOP-reducing efficacy (Bucci et al. 1968). However, its corneal anesthetic effect hindered its use in glaucoma (Vale et al. 1972). During the 1970s, other β-blockers, including practolol, were tested for their IOP-reducing

effects (Elliot et al. 1975; Vogel 1983). However, the use of practolol was abandoned in 1975 due to severe dry eye side effects and corneal scarring (Vogel 1983). In 1976, timolol maleate, a non-subtype-selective β-blocker, was tested and proved effective in lowering IOP when applied topically, which marked a significant milestone (Demailly et al. 1976). Subsequent clinical trials showed IOP-reducing effects for timolol without exhibiting serious ocular or systemic side effects (Boger et al. 1978; Demailly et al. 1978; Zimmerman and Kaufman 1977). Hence, a topical formulation of timolol became the first β-blocker approved by the FDA for glaucoma treatment in 1978, revolutionizing glaucoma management (Goldberg 2002). This paved the way for subsequent FDA approvals of other β-blockers, including levobunolol in 1985. It is worth noting that topical application of β-blockers can lead to rapid systemic absorption, potentially causing systemic side effects (Bhagey and James 2004). The tissue-specific expression of various β-AR subtypes plays a role in the diverse systemic side effects associated with nonselective β-blockers. For example, β_1-ARs are mainly found in the heart and kidney, affecting heart rate and contractility, and renin release (Brodde and Michel 1999).

β_2-ARs are prevalent in bronchial and vascular smooth muscle cells, influencing bronchodilation and vasodilation (Guimarães and Moura 2001). β_3-ARs are primarily located in adipose tissue, regulating lipolysis and thermogenesis in rodents (Coman et al. 2009).

Nonselective β-blockers like timolol were reported to cause β_2-induced pulmonary airway obstruction, making them contraindicated in asthma and chronic obstructive pulmonary diseases (Van Buskirk 1980). To address β_2-related respiratory side effects, selective β-blockers like betaxolol, which antagonize only β_1-ARs, were developed and FDA-approved in 1985 (Stewart et al. 1986). Betaxolol also demonstrated efficacy in patients with normal tension glaucoma, hinting at potential neuroprotective effects (Costagliola et al. 2009). Another noteworthy nonselective β-blocker is carteolol, characterized by partial agonist activity and no local anesthetic effects (Chrisp and Sorkin 1992). Carteolol, developed in 1972 and initially approved in Japan in 1980 (Fischer and Ganellin 2006), differed from timolol or betaxolol in its potential to improve optic nerve head perfusion (Henness et al. 2007).

Interestingly, adrenaline was discovered as a drug able to reduce IOP in open-angle glaucoma already in 1914 by Erdmann P. (1914). The compound was first abandoned due to acute episodes of IOP elevation and then retested by Hamburger C. in 1923 (Gradle 1925; Hamburger 1923, 1926; Thiel 1924; Vannas 1927). However, due to the instability in controlling IOP (Gifford 1928), adrenaline use for glaucoma treatment was limited. Later, in the 1950s its use was reinvestigated by Weekers and co-workers, who indicated a possible effect in decreasing AH production (Aasved 1964; Weekers et al. 1954, 1955). According to a mechanistic view, adrenaline, a nonselective potent agonist of β_1-ARs and moderate agonist of α_1- and β_2-ARs, is able to induce an expansion of the Schlemm's canal diameter and of the TM width, leading to an optimized AH flow (Ye et al. 2019). At present, its use is contraindicated in case of angle-closure, and strongly limited in open-angle glaucoma variants due to severe cardiovascular side effects, such as premature

ventricular contractions or other arrhythmias (Ballin et al. 1966). Another group of α-AR modulators was tested for glaucoma in the late 1980s and early 1990s: α_2-AR agonists emerged as a novel class of antiglaucoma medications. Clonidine, initially employed as a nasal decongestant in 1962 due to its vasoconstrictor properties (Schmitt 1977; Stähle 2000), was later found to effectively lower IOP (Harrison and Kaufmann 1977). However, clonidine's high permeability through the blood-brain barrier and associated systemic hypotension limited its use in glaucoma (Dikopf et al. 2017). Additionally, it was reported to worsen visual field defects in glaucoma patients (Hoyng and van Beek 2000). In an effort to create a molecule with reduced blood-brain barrier permeability, apraclonidine (also known as iopidine) was developed and tested, demonstrating IOP-lowering effectiveness after laser iridotomy (Van Buskirk and Shields 1997). The FDA approved this drug in 1987 for the treatment of transient IOP elevations after laser procedures (Dikopf et al. 2017), and a formula for short-term IOP management was designed in 1993 (Realini 2011). Brimonidine, another α_2-AR agonist introduced in 1996, exhibited greater selectivity for α_2-ARs and greater lipophilicity, enabling effective IOP reduction at lower concentrations and reducing side effects (Costagliola et al. 2009; Dikopf et al. 2017; Realini 2011). Furthermore, brimonidine demonstrated neuroprotective properties, preserving RGC function following ischemia-induced damage (Conti et al. 2021).

In the 1990s, two other relevant classes of antiglaucoma medications gained clinical approval: prostaglandin analogs and carbonic anhydrase inhibitors. Latanoprost, an $F_{2\alpha}$ prostaglandin receptor agonist developed in the 1980s, became the first prostaglandin derivative to receive FDA approval in 1996 (Realini 2011). Dorzolamide, the first carbonic anhydrase inhibitor, was approved in 1994, followed shortly by brinzolamide in 1998 (Realini 2011; Sharma et al. 2015).

The development of combination therapies or coformulations, such as the combination of timolol and latanoprost, has enhanced the efficacy of IOP-lowering in cases where monotherapies are insufficient or unsuccessful (Alm et al. 1995).

In the recent years, the possibility to treat diseases with fibrogenetic backgrounds through the employment of ROCK inhibitors has been considered in diverse pathological sets (Li et al. 2021b). Several investigations tested the effectiveness of this class of drugs for glaucoma, showing an antifibrogenic effect, and thereby diminishing the level of remodeling in the TM and facilitating AH outflow, ultimately leading to IOP reduction (Isobe et al. 2014; Kopczynski and Heah 2018; Serle et al. 2018; Tanihara et al. 2015). Worldwide, the first ROCK inhibitor to be licensed for glaucoma was ripasudil in Japan (2014) with an ophthalmic solution of 0.4% (Garnock-Jones 2014), whereas netarsudil was the first ROCK inhibitor to be authorized in the United States (2017) and then in Europe (2019), according to the formulation of a 0.02% ophthalmic topical once-daily application (Batra et al. 2021).

Currently, there is growing interest in new generations of molecules with neuroprotective activity, like brimonidine (Conti et al. 2021), which can prevent RGC loss and axonal degeneration. These agents offer new avenues for combating glaucoma that go beyond solely relying on IOP reduction. Additional candidates in

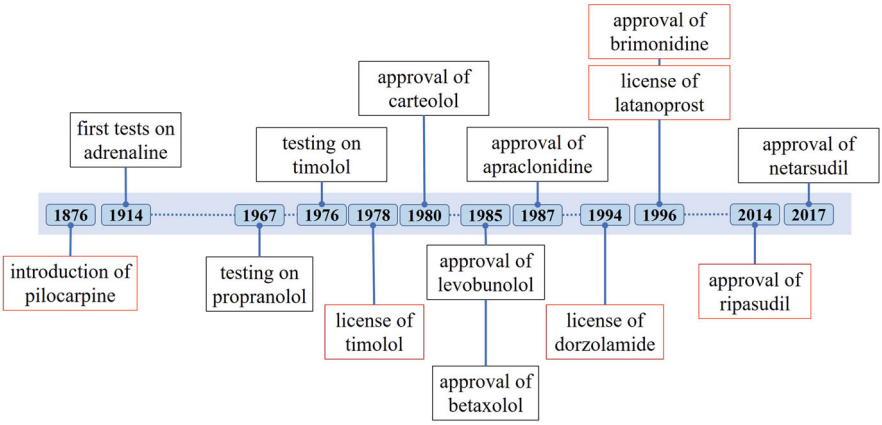

Fig. 3 Timeline on the introduction and/or approval of the most common antiglaucoma drugs

this context include immunomodulatory and antioxidant drugs (Bariş and Tezel 2019; Buonfiglio et al. 2023; Ruan et al. 2020).

In Fig. 3, a schematic timeline of the most relevant antiglaucoma drugs is presented.

3 Adrenoceptors on the Ocular Surface

3.1 Expression and Role of Adrenoceptors in the Cornea

The ocular surface comprises the cornea, conjunctiva, meibomian glands, and lacrimal glands (Gipson 2007). The cornea of mammals is supplied by adrenergic nerve fibers from the superior cervical ganglion. Adrenergic nerve fibers could be identified in adult human corneas by sodium-potassium-glyoxylic acid-induced fluorescence (Müller et al. 2003; Toivanen et al. 1987). Both α-ARs and β-ARs have been identified in corneas, and the neurotransmitter, noradrenaline, is normally found in the corneal epithelium (Candia and Neufeld 1978; Colley and Cavanagh 1982; Grayson et al. 1998; Müller et al. 2003; Musayeva et al. 2018). In mice, mRNA for all three α_1-AR subtypes was detected in the corneal epithelium, while no α_1-AR mRNA was found in the stroma and only mRNA for the α_{1B}-AR subtype was detected in the endothelium (Musayeva et al. 2018).

Previous studies in humans and other species demonstrated that α_1-ARs activate intracellular signaling pathways in corneal tissue (Akhtar 1987; Grueb et al. 2008; Musayeva et al. 2018). For example, activation of α_1-ARs was shown to stimulate inositol phosphate turnover in the rabbit cornea and human corneal epithelial cells (Akhtar 1987). Furthermore, α_1-ARs have been reported to modulate ion transport in the corneal epithelium (Bonanno 2003; Grueb et al. 2008). Other researchers reported that noradrenaline stimulates differentiation and proliferation of corneal epithelial cells (Garcia-Hirschfeld et al. 1994; Murphy et al. 1998). Later, Musayeva et al. suggested that the α_{1A}-AR subtype exerts trophic effects in the corneal

epithelium, because its lack was associated with a thinned corneal epithelial layer and reduced corneal epithelial cell size in genetically modified mice (Musayeva et al. 2018). Despite these findings, the exact physiological role of individual α_1-AR subtypes in corneal anatomy and physiology is largely unknown at present.

Little data is available on the role of α_2-ARs in the cornea. It has been reported that topical application of the α_2-AR agonist, brimonidine, induces a reversible increase in corneal thickness in humans (Grueb et al. 2011). Another α_2-AR agonist, clonidine, induced cell apoptosis both in vitro and in vivo, that was mediated by a Fas/TNFR1 death receptor-mediated signaling pathway (Fan and Fan 2017). Moreover, clonidine and the α_2-AR agonist, xylazine, were shown to induce corneal calcification in young rodents (Zhou et al. 2017). Recently, it has been suggested that α_2-ARs modulate noradrenaline release by sympathetic nerves in the cornea (Figueira et al. 2018). However, as yet, no suggestion has been made regarding the contribution of individual α_2-AR subtypes to the reported effects in the cornea.

Some more data have been gained regarding the expression and role of the β-AR subfamily in corneal tissue. Of note, high protein levels of β_2-ARs have been detected in corneal epithelial cells (Elena et al. 1990; Pullar et al. 2007; Walkenbach et al. 1984). The first published studies on that topic presented conflicting results on the role of β_2-ARs in corneal re-epithelialization (Liu et al. 1990; Nork et al. 1984; Reidy et al. 1994). Later, several researchers suggested that β_2-AR antagonists could enhance corneal epithelial cell migration and corneal wound healing by increasing extracellular signal-regulated kinase (ERK) phosphorylation (Ghoghawala et al. 2008; Pullar et al. 2007). However, a very recent study suggested that β_2-AR antagonists inhibited corneal wound healing by mediating the expression of Ki67 and phosphorylation of ERK1/2 limbal and regenerated corneal epithelium (Yuan et al. 2021). Due to these conflicting results, the role of the β_2-AR in homeostasis of the corneal epithelium and wound healing remains to be pursued further.

3.2 Expression and Role of Adrenoceptors in the Conjunctiva

Sympathetic nerves were shown to contribute to the regulation of conjunctival cell function, such as mucous secretion from goblet cells and expression of conjunctival eosinophils, which might be involved in the pathophysiology of allergic conjunctivitis and dry eye disease (Dartt et al. 1995; Diebold et al. 2001; Gabanyi et al. 2016; Gautheron and Sugrue 1987).

Pharmacological studies revealed the presence of β_2-ARs in primary human conjunctival epithelial cell cultures (Sharif et al. 1997). In developing rat conjunctival goblet cells, β_1- and β_2-ARs were detected by fluorescence microscopy (Ríos et al. 2000a, b). Another study that used receptor-specific antibodies on mouse and human conjunctival tissue displayed an expression of β_1- and β_2-ARs in both epithelial and goblet cells in murine conjunctiva but not in human conjunctiva. The expression of α_{1A}-ARs was detected in both epithelial and goblet cells of the mouse and human conjunctiva, whereas the β_3-AR was found in epithelial and goblet cells of the human conjunctiva but not in mouse conjunctival cells although the target validity of the antibodies being used has not been determined (Diebold

et al. 2001). A study in the human conjunctival epithelial cell line, IOBA-NHC, that used western blot, flow cytometry, and fluorescence microscopy to detect individual AR subtypes reported that western blot analyses showed bands for all receptors. Flow cytometry revealed constitutive expression of the α_{1A}-, α_{1B}-, α_{1D}-, α_{2A}-, α_{2B}-, α_{2C}-, β_1-, and β_3-ARs on cell membranes and intracellularly, whereas the β_2-AR was detected only intracellularly under normal culturing conditions. Using immunofluorescence microscopy, α_{1A}-, α_{2A}-, α_{2B}-, β_1-, and β_2-AR subtypes were detected in IOBA-NHC cells, but α_{1B}-, α_{1D}-, α_{2C}-, and β_3-ARs were not found (Enríquez de Salamanca et al. 2005). Interestingly, α_{1B}- and α_{2B}-AR expression was upregulated when cells were treated with the proinflammatory cytokines, interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) (Enríquez de Salamanca et al. 2005). Notably, in human conjunctival biopsy specimens, all AR subtypes except the α_{2C}-AR were detected (Enríquez de Salamanca et al. 2005). Sympathetic nerves were suggested to activate eosinophils via the α_{1A}-AR subtype in allergic conjunctivitis, suggesting that this receptor subtype may be a potential therapeutic target to treat this disease (Gautheron and Sugrue 1987; Liu et al. 2020b; Woodward and Nieves 1985).

In vernal keratoconjunctivitis, an irregular β_1-AR expression pattern was observed in all epithelial cell layers of human conjunctival biopsy specimens, indicative of autonomic nervous system involvement (Liu et al. 2020b). Moreover, the β_2-AR agonists, salbutamol and terbutaline, reduced microvascular permeability and exerted anti-inflammatory effects in allergic conjunctivitis (Gautheron and Sugrue 1987; Woodward and Nieves 1985). Based on these findings, modulation of AR may be a therapeutic approach to treat inflammatory ocular surface diseases, such as allergic conjunctivitis. Figure 4 illustrates the anterior segment of the eye is presented and the expression of ARs in the cornea and conjunctiva.

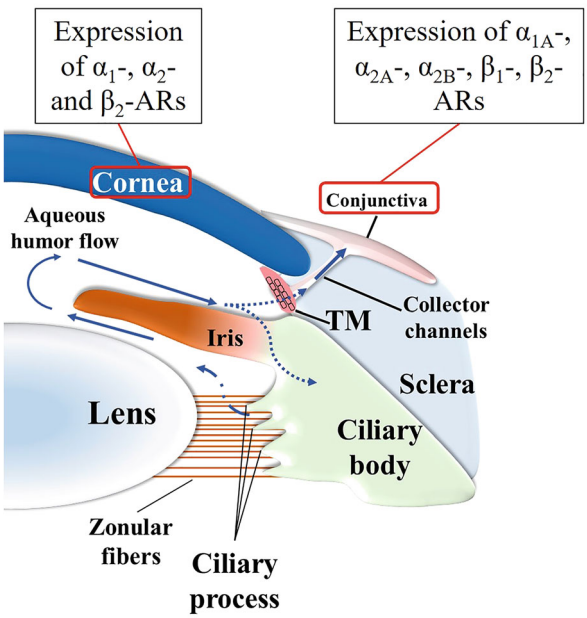

Fig. 4 Illustration of the anterior eye segment with the expression of ARs in the cornea and conjunctiva. TM: trabecular meshwork

3.3 Expression and Role of Adrenoceptors in the Lacrimal Gland

The lacrimal gland consists of acinar cells, myoepithelial cells, and ductal cells (García-Posadas et al. 2020). In dry eye disease, including Sjögren's syndrome, an altered neural control of lacrimal gland fluid regulation may play a crucial pathophysiological role (García-Posadas et al. 2020; Ikeda-Kurosawa et al. 2015). Almost 50 years ago, functional α- and β-ARs have been detected in the lacrimal gland of various species by pharmacological tools (Botelho et al. 1973). For example, in acinar cells of the rat lacrimal gland, α-AR activation increased membrane permeability to potassium (Parod and Putney 1978). Moreover, α-AR agonists were shown to induce secretion in the lacrimal gland of rats (Putney et al. 1978). Another study has shown that mRNA for all AR subtypes except the α_{2C}-, β_1-, and β_3-ARs was detected in acinar cells of the rat lacrimal gland by RT-PCR (Ikeda-Kurosawa et al. 2015).

Also, in the rat lacrimal gland, α_1-ARs were found to be the main receptors mediating sympathetic modulation of Ca^{2+}-related cell homeostasis and protein secretion (Ikeda-Kurosawa et al. 2015). Among the α_1-AR subfamily, the α_{1D}-AR subtype was suggested to stimulate protein secretion via involvement of endothelial nitric oxide synthase, NO, and cGMP (Hodges et al. 2005). Moreover, activation of the α_{1D}-AR was suggested to release ATP, which induces P2X(7) receptors to increase intracellular Ca^{2+} (Dartt and Hodges 2011). However, other studies suggested that the α_{1A}-AR may be the primary receptor mediating the sympathetic increase in intracellular calcium ion levels and mucin secretion in acinar cells of the rat lacrimal gland (Hodges et al. 1992; Ikeda-Kurosawa et al. 2015).

Apart from α_1-ARs, β-ARs were reported to contribute to protein secretion in the lacrimal gland of rats and mice (Aberg et al. 1979; Ding et al. 2007; Hodges et al. 1992; Mauduit et al. 1986). For example, β_1- and β_2-ARs were suggested to contribute to secretion in the lacrimal gland of rabbits (Aberg et al. 1979; Petounis and Akritopoulos 1989). Mauduit et al. reported that stimulation of the α_1- and β_1-ARs might have a synergistic effect in protein secretion from rat lacrimal glands, while it has also been reported that there is no synergism in protein secretion by activating the α_1- and β_1-ARs in the mouse lacrimal gland (Bromberg 1981; Ding et al. 2007; Mauduit et al. 1986; Meneray and Fields 2000).

Also, some studies examined the role of ARs in accessory lacrimal glands. For example, immunohistochemical studies in human specimens revealed that β_1-ARs were the predominant AR subtype in the glands of Wolfring (Esmaeli-Gutstein et al. 1999). Moreover, activation of β_2-ARs was reported to enhance lipid synthesis in human meibomian gland epithelial cells (Jun et al. 2022).

Taken together, there is accumulating evidence that both α- and β-ARs are involved in the regulation of tear secretion. Based on these findings, activation of adrenergic pathways may be a potential therapeutic approach to treat dry eye disease. Figure 5 illustrates the lacrimal gland and summarizes the expression of ARs in this organ.

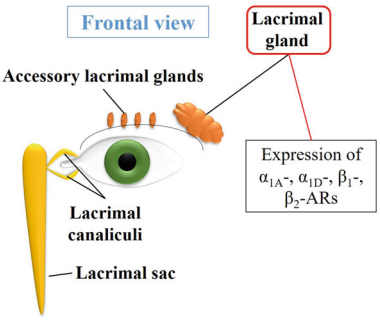

Fig. 5 Anatomical representation of the lacrimal gland frontal view, and summary of the expression of ARs

4 Adrenoceptors in the Uvea

The uvea is divided into three parts, the iris, ciliary body, and choroid. Subtypes of α_1- and α_2-ARs were detected abundantly in the iris of rabbits and rats, while high densities of β_1- and β_2-ARs were found in the human ciliary body (Nakamura et al. 1999; Suzuki et al. 2002; Wax and Molinoff 1987; Wikberg-Matsson et al. 1996; Yu and Koss 2003).

Expression studies in mice, rats, and rabbits revealed that in iris tissue the α_{1A}-AR subtype is either expressed most abundantly or equally as high as the α_{1B}-AR, whereas the α_{1D}-AR subtype is least abundantly expressed both at the mRNA and protein level (Kordasz et al. 2014; Nakamura et al. 1999; Suzuki et al. 2002; Vidovic and Hill 1995; Wikberg-Matsson et al. 2000). In addition, most functional studies in mice, rats, and rabbits suggested that the α_{1A}-AR is the main mediator of adrenergic pupil dilation (Ishikawa et al. 1996; Kordasz et al. 2014; Muramatsu et al. 2009; Nakamura et al. 1999). Moreover, it has been proposed that the low-affinity phenotype of the α_{1A}-AR mediates adrenergic pupil dilation in humans (Ishikawa et al. 1996). Clinically, pharmacological blockade of α_1-ARs appears to be relevant in intraoperative floppy iris syndrome (IFIS), a triad of billowing iris, iris prolapse, and progressive pupil constriction during cataract surgery (Chang and Campbell 2005). IFIS was first observed in patients under medication with the α_{1A}-AR–preferring antagonist tamsulosin for treating lower urinary tract symptoms but has later been observed with many other drugs having affinity for α_1-ARs (Oelke et al. 2014). Silodosin; a highly selective antagonist for the α_{1A}-AR, has also been documented to induce IFIS when prescribed for the treatment of benign prostatic hyperplasia (Christou et al. 2022).

Based on autoradiography and ligand binding studies, β-ARs were shown to be expressed in the ciliary process epithelium of rabbit eyes, indicating that β-ARs may participate in aqueous humor formation (Bromberg et al. 1980; Elena et al. 1987). Other studies in various species, including humans, demonstrated that the ciliary process epithelium was enriched in β_2-ARs coupled to adenylyl cyclase (Elena et al. 1984; Nathanson 1981b; Oelke et al. 2014). Later, it has been shown that adrenergic

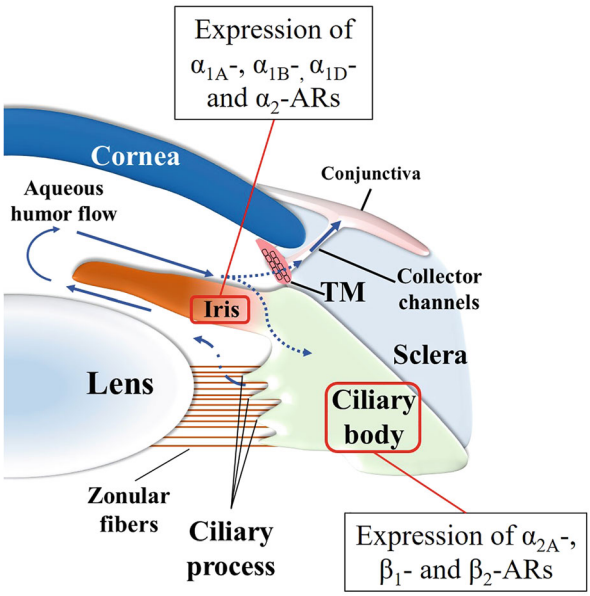

Fig. 6 Expression of ARs in iris and ciliary body. TM: trabecular meshwork

agonists, such as adrenaline, induce a desensitization of the β-AR-adenylyl cyclase complex, which may explain the delayed IOP decrease after topical adrenergic agonist application and the paradox that both β-AR agonists and antagonists lower IOP (Mittag and Tormay 1981). Another proposed mechanism for the IOP-lowering effect of β-AR blockers was a decrease of AH production by vasoconstriction of the uveal vasculature (Potter 1981). Of note, the non-subtype-selective β-AR blocker, timolol, and some β_2-AR antagonists were shown to bind potently to β-ARs on ciliary processes in radioligand binding studies and lowered IOP in various species, including humans, suggesting that β_2-ARs are a pharmacological target for glaucoma treatment (Nathanson 1981a, 1984; Neufeld 1979; Trope and Clark 1984). Apart from β-ARs, also α-ARs were shown to contribute to IOP regulation (Mittag et al. 1985). Further studies pointed toward an involvement of α_2-ARs in the modulation of AH formation because α_2-AR agonists inhibited adenylate cyclase activity in ciliary processes and reduced IOP in various species (Bausher et al. 1987; Burke et al. 1995; Cepelík and Hynie 1990; Jumblatt et al. 1987; Kintz et al. 1988). Radioligand binding studies detected abundant expression of the α_{2A}-AR in the ciliary body of various species, including humans, and suggested that activation of the α_{2A}-AR reduces adenylate cyclase activity (Bylund and Chacko 1999; Jin et al. 1994a; Jin et al. 1994b; Wikberg-Matsson et al. 1996). In Fig. 6, we summarize the expression of AR subtypes in iris and ciliary body.

In the choroid, also rich expression of β-ARs has been reported (Elena et al. 1987). In humans, the presence of β-ARs was confirmed in the choroid by showing an increased choroidal vascular tone following systemic administration of the nonselective β-AR blocker, timolol (Grajewski et al. 1991). It has been suggested

that sympathetic nerves play a role in maintaining normal choroidal vascular architecture via involvement of β-ARs (Steinle and Smith 2002). Based on experiments employing the agonist, BRL37344, it has been suggested that β_3-ARs contribute to various aspects of human choroidal endothelial cell behavior, including invasion, proliferation, and elongation in vitro. Based on these findings it has been concluded that β_3-ARs may contribute to the neovascularization processes observed in age-related macular degeneration (Steinle et al. 2005). However, these findings need to be interpreted with caution, since BRL37344 has also been shown to activate β_2-ARs (Evans et al. 2013; Mukaida et al. 2019; Ngala et al. 2009, 2013), and to reduce α_1-AR-mediated (Huang et al. 2022) and purinergic responses (Fong et al. 2019).

Other studies in experimental animals reported that β_2-ARs regulate VEGF and IL-6 expression in cells of the choroidal endothelium and other cells and suggested that blockade of these receptors may attenuate formation of choroidal neovascularization (Lavine et al. 2013, 2017).

Vasoconstrictor responses in the choroid induced by electrical stimulation of the preganglionic cervical sympathetic nerve were blunted by α_1-AR blockade (Kawarai and Koss 1998). Among the subfamily of α_1-ARs, the α_{1A}-AR subtype was found to be expressed most abundantly in the choroid (Suzuki et al. 2002). Moreover, the α_{1A}-AR antagonist, tamsulosin, was reported to increase choroidal thickness in humans, which may be a result of choroidal vasodilation in a consequence of the blockade of α_{1A}-ARs (Kanar et al. 2021; Sari et al. 2015). Also, rich expression of α_2-ARs, especially of the α_{2A}-AR subtype, has been demonstrated in the choroid (Matsuo and Cynader 1992; Wikberg-Matsson et al. 1996). Moreover, α_2-AR activation produced pronounced depression of anterior segment choroidal blood flow (Koss and Gherezghiher 1994). It has also been suggested that α_2-ARs play a key role in inducing choroidal neovascularization, and that α_2-AR agonists may represent novel therapeutic drugs for patients with neovascular age-dependent macular degeneration (Tanaka et al. 2021). Figure 7 represents the expression of ARs in the choroid.

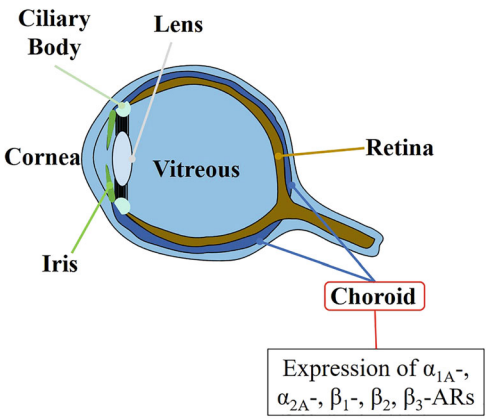

Fig. 7 Expression of ARs in the choroid

5 The Retina

5.1 Anatomy and Potential Sources of Catecholamines

The retina is a neuronal multilayer that recognizes, processes, and sends visual information to the brain (Amini et al. 2018). The neuronal lamination in the retina includes neural circuits that contain six major types of neuronal cells: RGCs, amacrine cells, bipolar cells, horizontal cells, and the cone and rod photoreceptors (Masland 2012). Despite the peripheral location of the retina, retinal neurons utilize the same types of neurotransmitters (noradrenaline, dopamine, and acetylcholine) as those of the central nervous system (Haider et al. 2014). Because visual formation highly depends on the complex neuronal structure of the retina, a variety of detrimental factors, such as ischemia and oxidative stress, may lead to deterioration of retinal cell function and consequently to retinal pathologies (Herzlich et al. 2010).

The retina is supplied with oxygen and nutrients by two distinct circulatory systems that both branch from the ophthalmic artery, the retinal circulation, and the choroidal circulation (Kur et al. 2012). Whereas choroidal blood vessels are innervated and modulated by the autonomic nerve fibers, no such nerve fiber terminals have been detected in or on the wall of human retinal blood vessels (Hogan and Feeney 1963). Retinal blood vessels have been shown to respond to local chemical factors, including oxygen (O_2), carbon dioxide (CO_2), nitric oxide (NO), and hydrogen sulfide (H_2S) (Ferrari-Dileo et al. 1989; Gericke et al. 2013b; Liu et al. 2020a). Although no evidence for sympathetic nerve fibers in the retina has been provided, catecholamines including noradrenaline, adrenaline, and dopamine have been detected (Hadjiconstantinou et al. 1983). A putative source of noradrenaline in the mammalian retina are sympathetic nerve terminals located in the choroid (Nguyen-Legros 1988). Following the removal of the superior cervical ganglion, which provides sympathetic input to the choroid, a reduction in retinal noradrenaline concentration has been observed (Hadjiconstantinou et al. 1983). This finding may suggest that noradrenaline originates from sympathetic nerve terminals in the choroid and reaches retinal ARs by paracrine diffusion (Casini et al. 2014).

Dopaminergic amacrine cells, a class of retinal neurons, synthetize and release dopamine, which is the predominant catecholamine in the retina (Haeggendal and Malmfors 1965; Hirasawa et al. 2015). In the bovine retina, noradrenaline was detected in the inner nuclear and plexiform layers, and Osborne suggested that retinal tissue can metabolize dopamine to form noradrenaline (Osborne 1981). Other amacrine cells have been detected in the retina, which differ morphologically from dopaminergic amacrine cells and can only be detected after exposure to exogenous noradrenaline (Frederick et al. 1982; Nguyen-Legros 1988). These cells have been suggested to contain low levels of endogenous catecholamines, but are equipped with high-affinity uptake properties for exogenous catecholamines (Nguyen-Legros 1988). In addition to noradrenaline, dopamine may also activate α_1-, α_2-, and β-ARs (Lei 2014). Importantly, members of all three adrenoceptor subfamilies, α_1-, α_2-, and β-ARs, have been detected in retinal tissue, including endothelial cells of retinal blood vessels (Forster et al. 1987; Gericke et al. 2013a;

Mori et al. 2011a; Steinle et al. 2003; Wikberg-Matsson et al. 2000). According to Steinle et al., β-AR density may decrease with increasing age, which could affect sympathetic neurotransmission in the retina (Smith et al. 2007). Moreover, Steinle et al. suggested that sympathetic neurotransmission might regulate expression of inducible nitric oxide synthase (iNOS), angiogenic growth factors, and the number of pericytes in the retina (Steinle 2007; Wiley et al. 2005, 2006).

5.2 α_1-ARs

5.2.1 Expression of α_1-ARs in the Retina

The retinal vasculature appears to lack autonomic, i.e., adrenergic, cholinergic, or peptidergic innervation (Ehinger 1966; Ferrari-Dileo et al. 1989; Hogan and Feeney 1963; Laties 1967; Ye et al. 1990). However, α_1-ARs have been detected in retinal tissue of various mammalian species (Suzuki et al. 2002; Wikberg-Matsson et al. 2000). Forster et al. revealed the presence of α_1-adrenergic binding sites in homogenates of isolated bovine retinal arteries and veins, which had few but high agonist affinity (Forster et al. 1987). In homogenates of isolated murine retinal arterioles our laboratory has detected mRNA for all three α_1-AR subtypes expressed at similar levels (Böhmer et al. 2014a).

The cellular localization of α_1-ARs in retinal blood vessels may be of pathophysiological and pharmacological relevance. For example, an intact blood-retinal barrier may prevent circulating hydrophilic catecholamines from reaching α_1-ARs localized in vascular smooth muscle cells (Riva et al. 2011; Yu et al. 2003). However, under pathophysiological conditions associated with an increased permeability of the blood-retinal barrier they may be reached by circulating catecholamines with potential impact on vascular tone and smooth muscle growth (Ferrari-Dileo et al. 1990; Forster et al. 1987). Since numerous enzymes involved in catecholamine synthesis have been detected in retinal cells of different species, retinal vascular α_1-ARs may also be targeted by locally released agonists (Chen et al. 1999; Hadjiconstantinou et al. 1984; Kolb et al. 1990; Nguyen-Legros et al. 1994).

Zarbin et al. localized α_1-ARs in the outer plexiform layer of the rat retina in vitro by semiquantitative autoradiography using [^{3}H] prazosin. The authors reported that α_1-adrenergic binding sites were only enriched in the outer plexiform layer (Zarbin et al. 1986). Other research groups demonstrated that α_1-ARs are expressed on retinal pigment epithelium (RPE) of rabbit and bovine retinas, where they modulate K^+ and Cl^- transport and electrical currents (Frambach et al. 1988; Joseph and Miller 1992). Unfortunately, at present, antibody-based methods for localizing α_1-AR subtypes within the tissue structure appear to lack high specificity (Böhmer et al. 2014b; Jensen et al. 2009; Michel and Seifert 2015; Pradidarcheep et al. 2009). A combined approach of ligand-receptor binding techniques and immunostainings and/or functional studies in tissues from knockout animal models lacking the respective receptor subtype may represent a more appropriate methodological alternative to determine the location and function of α_1-ARs within the retina, which remains a subject of further research (McGrath 2015).

5.2.2 α_1-ARs and Retinal Vascular Reactivity

Although some studies in experimental animals and healthy humans have investigated the impact of systemically administered α_1-adrenergic agonists on retinal vascular reactivity, the results are contradictory, making it difficult to draw unequivocal conclusions on the functional role of α_1-ARs in the regulation of retinal vascular tone and perfusion. For example, Mori et al. observed a dose-dependent constriction of rat retinal arterioles in response to intravenous administration of noradrenaline (Mori et al. 2011a). In contrast, Alm et al. observed no effect of intraarterially administered noradrenaline on retinal blood flow in cats (Alm 1972; Mori et al. 2011a). Dollery et al. reported that intravenously administered noradrenaline decreased retinal vascular diameter in healthy humans, whereas other studies detected only a negligible impact of circulating noradrenaline and of the α_1-adrenergic agonist, phenylephrine, on human retinal vessel diameter and blood flow (Dollery et al. 1963; Jandrasits et al. 2002; Polak et al. 2000).

In vivo studies testing the effects of systemically applied adrenergic agonists or antagonists on retinal vascular responses are hampered by marked changes in systemic blood pressure induced by these ligands. Due to the strong autoregulatory ability of the retinal vascular bed, the resulting changes in ocular perfusion pressure may also induce compensatory responses of the retinal vasculature, making it difficult to distinguish between direct pharmacological effects on retinal vessels and their reactive responses to systemic blood pressure changes (Ferrari-Dileo et al. 1990; Garhöfer and Schmetterer 2012; Jandrasits et al. 2002; Yu et al. 2003). This methodological dilemma may at least partially explain the contradictory results of the in vivo studies outlined above.

To reduce systemic influences of catecholamines, Ichikawa et al. and Hara et al. injected drugs intravitreally and observed constriction of retinal arteries in rabbits in response to phenylephrine, which was attenuated after application of the α_1-AR antagonist, bunazosin (Hara et al. 2005; Ichikawa et al. 2004). Moreover, in vitro studies with isolated eyes, retinal tissue grafts, and preparations of retinal arterioles revealed vasoconstriction effects of α_1-adrenergic agonists (Ferrari-Dileo et al. 1990; Hoste et al. 1989; Nielsen and Nyborg 1989; Spada et al. 2001; Yu et al. 1994).

Hoste et al. reported that contractile responses of bovine retinal arteries to α_1-adrenergic stimuli were strongly masked under resting conditions (Hoste et al. 1989). However, retinal arteries became sensitive to α_1-adrenergic agonists when activated by circumferential stretch and displayed an enhanced myogenic vasoconstrictor response to elevated perfusion pressure during α_1-adrenergic stimulation (Hoste et al. 1989). In a study by Yu et al., the extent of vasoconstriction in isolated porcine retinal arterioles with intact endothelium in response to α_1-adrenergic agonists differed between intra- and extra-luminal drug application. Adrenaline and noradrenaline elicited concentration-dependent vasoconstriction, which was stronger when applied via the lumen (Yu et al. 1994, 2003).

Although the in vitro studies mentioned above found constrictive effects of α_1-adrenergic agonists on retinal vessels with an intact endothelium, the reported vascular reactions were relatively mild and had a high threshold (Hoste et al. 1989; Nielsen and Nyborg 1989; Spada et al. 2001; Yu et al. 1994). In a study

performed on murine retinal explants, our group revealed that retinal arteriole constriction to α_1-adrenergic stimulation is largely masked by endothelial mechanisms but becomes relevant when the endothelium is damaged (Böhmer et al. 2014a). Conversely, a prior study of our own conducted on endothelium-intact mouse ophthalmic arteries under similar experimental conditions has shown strong vasoconstrictive responses to α_1-adrenergic stimulation (Gericke et al. 2011). These findings indicate that endothelial modulation of α_1-adrenergic vasoconstriction differs considerably between retinal and retrobulbar blood vessels.

Due to its characteristic properties, the retinal vascular endothelium represents a mechanical barrier that is thought to prevent most blood-borne hydrophilic compounds, including catecholamines, from reaching vascular smooth muscle cells (Delaey and Van De Voorde 2000; Ehinger 1966; Ferrari-Dileo et al. 1989; Hoste et al. 1990; Laties 1967; Riva et al. 2011; Ye et al. 1990; Yu et al. 2003). In the referred study, however, the blood-retinal barrier was circumvented, since vasoactive substances were applied extraluminally (Böhmer et al. 2014a). Therefore, the observed attenuating influence of the endothelium most likely results from endothelium-derived vascular mechanisms that functionally antagonized constrictive responses of retinal arterioles to α_1-adrenergic stimuli. Endothelium-dependent attenuation of retinal α_1-AR-mediated vasoconstriction seems physiologically plausible, since it would confer a safety net by protecting the retina from inappropriate reductions in blood flow induced by elevated catecholamine levels during exercise, hemorrhage, or stress. Based on studies in various vascular beds, including cerebral vessels, it is well documented that vascular responses to α_1-adrenergic stimuli are modulated by the vascular endothelium and are altered when endothelial function is impaired (Alosachie and Godfraind 1988; Bauknight et al. 1992; Carrier and White 1985; Egleme et al. 1984; Furchgott 1984; Godfraind et al. 1985; Lues and Schumann 1984; Miller and Vanhoutte 1989; Sercombe et al. 1985; Verrecchia et al. 1985; White and Carrier 1986). Apparently, the endothelium attenuates the vasoconstrictive impact of elevated circulating catecholamine levels particularly in organs whose uncompromised blood supply and functioning are critical during fight-and-flight responses (Guimaraes and Moura 2001; Jones et al. 1993; Rudner et al. 1999). Conversely, under conditions of endothelial impairment, e.g., in atherosclerosis and diabetes, the vascular sensitivity to α_1-adrenergic vasoconstrictors appears to be increased (Baumgart et al. 1999; Berkenboom et al. 1991; Cocks and Angus 1983; Heusch et al. 2000; Jones et al. 1993; Vita et al. 1992).

In general, endothelial compensation of α_1-AR-mediated vasoconstriction is attributed to relaxing factors, such as nitric oxide, released by endothelial cells (Ignarro et al. 1988; Koss 1999; Moncada and Higgs 2006; Palmer et al. 1987, 1988; Schmetterer and Polak 2001; Zembowicz et al. 1991). While nitric oxide release in response to increased shear stress during vascular constriction is implicated as a possible endothelial mechanism counteracting vasoconstriction, some other studies suggest that activation of α_1-ARs located on endothelial cells also promotes the endothelial release of nitric oxide, which functionally counteracts adrenergic vasoconstriction (Angus et al. 1986; de Andrade et al. 2006; Filippi et al.

2001; Jones et al. 1993; Kaneko and Sunano 1993; Sun et al. 2001; Tesfamariam and Cohen 1988; Tuttle and Falcone 2001).

Retinal pathologies, such as diabetic retinopathy, arterial occlusive disease, or glaucoma, are associated with impairment of vascular endothelial function (Cipolla et al. 1996; Gericke et al. 2019; Nakazawa et al. 2007; Resch et al. 2009; Toda and Nakanishi-Toda 2007). However, no compelling evidence has been provided to date that under retinal pathological conditions α_1-AR-mediated vasoconstriction becomes a relevant contributing factor, although an in vivo study in rabbits suggested that blockade of α_1-AR signaling may alleviate the impairment in blood flow and retinal function caused by nitric oxide synthase inhibition (Goto et al. 2003). Recently, agonistic GPCR autoantibodies (GPCR-agAAb), including agonistic α_1-AR autoantibodies (α_1-agAAb) and agonistic β_2-AR autoantibodies (β_2-agAAb), have been detected in patients following infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), suggesting a link between seropositivity of GPCR-agAAbs to impaired retinal microcirculation and prolonged symptoms after coronavirus disease (Szewczykowski et al. 2022).

In summary, various studies have revealed distinct findings concerning retinal vascular reactivity when exposed to α_1-AR stimulation. For example, in cases of endothelial dysfunction, as seen in conditions such as atherosclerosis or diabetes, an increased sensitivity to α_1-AR-mediated vasoconstriction has been observed, often leading to endothelial compensation through the release of nitric oxide. Additionally, the responsiveness of ocular blood vessels to α_1-AR stimulation varies depending on the specific vessel under consideration. Retrobulbar vessels, for example, tend to exhibit pronounced vasoconstrictive responses to α_1-AR stimulation, while this reaction is weak in retinal vessels due to endothelial regulatory mechanisms. In aggregate, the reactivity of the retinal vasculature in response to the modulation of α_1-ARs exhibits complex characteristics, and investigations in this area have reported conflicting results. This complexity may arise from the potent autoregulatory mechanisms governing retinal vasculature, which obscure and modify the direct effects of drugs and reactions to changes in systemic blood pressure.

5.2.3 Role of Individual α_1-Adrenoceptor Subtypes in the Retina

In their in vivo study, Mori et al. aimed to identify the α_1-AR subtype(s) involved in noradrenaline-induced constriction of retinal arterioles in anesthetized rats by evaluating the vascular effects of systemically administered subtype-preferring agonists and antagonists. The authors concluded from their results that vasoconstriction to noradrenaline in rat retinal arterioles is primarily mediated by the α_{1A}- and the α_{1D}-AR, and that this finding corresponds to the situation in the rat peripheral circulation (Mori et al. 2011a). From a methodological perspective, the interpretation of the results is hampered by the confounding impact of retinal vascular autoregulation in an in vivo setting and by the lack of highly selective agonists and antagonists for all α_1-AR subtypes (Chen and Minneman 2005; Cotecchia 2010; Ferrari-Dileo et al. 1990; Garhöfer and Schmetterer 2012; Jandrasits et al. 2002; McGrath 2015; Piascik and Perez 2001; Yu et al. 2003; Zhong and Minneman 1999).

Using an in vitro approach and gene-targeted mice deficient in individual α_1-AR subtypes, our group demonstrated that α_1-AR-mediated vasoconstriction in retinal arterioles with damaged endothelium is predominantly conveyed by the α_{1B}-AR subtype (Böhmer et al. 2014a). In contrast, in the murine ophthalmic artery, which is directly afferent to the retinal vasculature, vasoconstriction responses to adrenergic stimuli were shown to be mediated primarily via the α_{1A}-AR subtype (Gericke et al. 2011). These results indicate that the retrobulbar (α_{1A}-AR) and the retinal (α_{1B}-AR) vasculature are under the functional control of different α_1-AR subtypes. This finding is consistent with previous studies reporting that the distribution of individual α_1-AR subtypes and their contribution to adrenergic vasoconstriction varies considerably between circulatory beds, between different-sized vessels of a given vascular bed, and between different species (Docherty 2010; Guimaraes and Moura 2001; Han et al. 1990; Piascik and Perez 2001; Rudner et al. 1999; Shen et al. 2000).

Although mRNA for all three α_1-AR subtypes has been found to be expressed at similar levels in murine retinal arterioles, α_1-AR-mediated vasoconstriction is predominantly mediated by the α_{1B}-AR subtype (Böhmer et al. 2014a). Several studies have provided evidence that the presence of mRNA or protein of a particular α_1-AR subtype is not necessarily associated with its participation in vasoconstriction (Gericke et al. 2011; Hrometz et al. 1999; Piascik et al. 1995; Yang et al. 1997, 1998). Furthermore, each receptor subtype can activate distinct downstream signaling components in the Gq/11 signaling pathways and also couple to other independent signaling pathways (Cotecchia 2010; Gonzalez-Cabrera et al. 2003; Graham et al. 1996; Hague et al. 2003; Hein and Michel 2007; Zhong and Minneman 1999). Therefore, a particular α_1-AR subtype that does not contribute to vasoconstriction despite its expression may, nevertheless, be involved in the regulation of other physiological or pathophysiological processes of the retinal vasculature. However, whether these results derived from animal models correctly represent the expression and function of α_1-AR subtypes in the human retinal vasculature remains to be elucidated. Except for the contribution of α_1-AR subtypes to retinal vascular reactivity, α_1-ARs also exert neuroprotective effects in the retina by inhibiting oxidative stress. For example, doxazosin, a α_1-AR antagonist, may decrease oxidative stress and proinflammatory cytokines in photoreceptors in retinal detachment by systemic administration (Li et al. 2019). Another α_1-AR antagonist, nipradilol, was shown to exert neuroprotective properties in cultured RGC-5 cells via the Keap1/Nrf2 pathway by Keap1 S-nitrosylation (Koriyama et al. 2012).

5.3 α_2-ARs

5.3.1 Expression of α_2-ARs in the Retina

In 1982, Osborne detected retinal α_2-ARs in the bovine retina in binding studies (Osborne 1982). In 1986, α_2-AR binding sites were found in the inner plexiform layer of the rat retina (Zarbin et al. 1986). Most of these sites were also related to the proximal layer of cell bodies in the inner nuclear layer and with some putative displaced amacrine or ganglion cell bodies (Zarbin et al. 1986). Furthermore, α_2-

ARs were identified in calf retinal cellular membranes by binding experiments employing radiolabeled antagonists (Convents et al. 1987). Matsuo and Cynader found α_2-AR binding sites in the human retina by an in vitro ligand-binding technique and autoradiography (Matsuo and Cynader 1992).

All three subtypes of α_2-ARs have been identified by molecular and pharmacological characterization techniques (Harrison et al. 1991). The International Union of Pharmacology has identified species orthologues and termed them α_{2A}-AR in humans and α_{2D}-AR in rats, mice, and cows (Bylund et al. 1994). Venkataraman et al. indicated that the α_{2D}-*AR* gene in the bovine retina was a structural variant of the rat and mouse genes and defined the α_{2D}-AR subtype in the bovine retina (Venkataraman et al. 1996). The expression of the α_{2D}-AR subtype was detected in the bovine retina and its photoreceptors (Venkataraman et al. 1996). Messenger RNA of the α_{2D}-AR was identified in the bovine retina by reverse transcription-polymerase chain reaction (RT-PCR) (Venkataraman et al. 1996). It is now generally accepted that the α_{2D}-AR represents a species variant of the α_{2A}-AR (Bylund 1998).

Immunohistochemical studies revealed the presence of α_2-ARs (specifically α_{2A}-ARs) on rat RGCs and inner nuclear layer cells (Kalapesi et al. 2005). In the human retina, Kalapesi et al. found α_{2A}-ARs on human RGCs and cells in the inner and outer nuclear layers (Kalapesi et al. 2005). In addition, Woldemussie et al. reported that α_{2A}-ARs in the rat retina were mainly located in cell bodies located in the ganglion cell layer, the inner plexiform layer, and the outer plexiform layer (Woldemussie et al. 2007). In other immunohistochemical studies, α_{2A}-AR staining was also observed in the membrane of cells located in the inner nuclear layer, specifically amacrine and horizontal cells. In human and monkey retinas, the α_{2A}-AR staining pattern was relatively consistent with that observed in rats (Woldemussie et al. 2007). In contrast to α_{2A}-ARs, α_{2B}-AR immunoreactivity was observed in all layers of the retina, especially in the presynaptic regions of neurons. In the outer retina, α_{2B}-AR immunoreactivity has been observed in more than one cell type, such as the inner segments of photoreceptors, Müller cells, and bipolar cells (Woldemussie et al. 2007). Moreover, Woldemussie et al. observed that α_{2A}-ARs were mainly present in the cell membrane of photoreceptor cells and in their inner segments (Woldemussie et al. 2007). Notably, some studies reported that many commercially available antibodies directed against ARs, including α_2-ARs, lack sufficient specificity (Böhmer et al. 2014b; Jensen et al. 2009; Pradidarcheep et al. 2009). Based on these findings, expression studies employing commercially available AR antibodies need to be interpreted with caution (Pradidarcheep et al. 2009).

5.3.2 α_2-AR in Retinal Neuroprotection

The α_2-AR family is one of the pharmacological targets of the natural stress hormone, noradrenaline, and is involved in the modulation of cellular resistance and adaptation to stress stimuli (Wheeler et al. 2001). α_2-ARs were first described as presynaptic receptors that inhibit the release of various transmitters from neurons in the central and peripheral nervous systems (Gilsbach and Hein 2012). In vivo studies revealed that α_2-AR stimulation reduces ischemic damage in the brain (Maier et al. 1993). This effect has been largely attributed to its classic presynaptic inhibition of

signaling molecules released by blocking Ca^{2+} channels, activating K^+ channels, or reducing active release sites (Dong et al. 2008; Ma et al. 2004). Neuroprotective treatment strategies for retinal diseases whose course includes neuronal degeneration have aroused a great deal of interest. A few studies have assessed the functional role of α_2-ARs in the retina in a variety of animal models through the mechanism of α_2-AR-mediated neuroprotection. For example, Donello et al. proposed that activation of α_2-ARs could reduce ischemic retinal injury and preserve retinal function following transient ischemia by preventing extracellular glutamate and aspartate accumulation (Donello et al. 2001). An in vivo study by Manuel et al. revealed neuroprotective effects of α_2-ARs in preventing RGC death after transient retinal ischemia (Vidal-Sanz et al. 2001). In the study, pretreatment with two α_2-AR-selective agonists, AGN 191,103 and brimonidine tartrate, proved to be very effective in preventing not only rapid RGCs' loss, but also long-term RGCs' loss in a rat model (Vidal-Sanz et al. 2001).

Glaucoma with elevated IOP often continues to progress even after the IOP has been reduced to normal levels (Quigley and Broman 2006). The progressive loss of vision in eyes with glaucoma is a result of RGCs' degeneration, emphasizing the need for neuroprotective therapy (WoldeMussie et al. 2001). The α_2-AR agonist, brimonidine, and other α_2-AR agonists have been shown to lower IOP mainly by reducing AH production and by increasing uveoscleral outflow (Greenfield et al. 1997; Katsimpris et al. 2003; Reitsamer et al. 2006). In a rat model of chronically elevated IOP, pharmacological activation of α_2-ARs exerted neuroprotective effects in RGCs, irrespective of the IOP level (WoldeMussie et al. 2001). In addition, the α_2-AR agonist, brimonidine, preserved visual function in glaucoma patients with low/normal IOP and high IOP, suggesting that pharmacological α_2-AR activation may exert IOP-independent neuroprotective effects (Evans et al. 2003; Krupin et al. 2011; Shim et al. 2017). By spectral domain optical coherence tomography, a current study demonstrated that the neuroprotective effects of brimonidine could delay changes to retinal nerve fiber layer thickness, which is independent of its effect on IOP (Takahashi et al. 2021). Various mechanisms have been proposed for the neuroprotective action of α_2-AR agonists. For example, activation of α_2-ARs has been shown to decrease IOP-induced overexpression of intermediate filament glial fibrillary acidic protein (GFAP) in Müller cells, suggesting that activation of α_2-ARs may reduce stress responses in glial cells (WoldeMussie et al. 2001). Furthermore, the α_2-AR agonist, brimonidine, was reported to protect RGCs from the effects of chronic ocular hypertension through mechanisms involving α_2-AR-mediated survival signal activation and upregulation of endogenous neurotrophic factors in the rat retina (Kim et al. 2007). Another study demonstrated that α_2-adrenergic modulation of N-methyl-d-aspartate (NMDA) receptor function was an important mechanism for neuroprotection in experimental glaucoma models (Dong et al. 2008). Brimonidine may protect RGCs by preventing abnormal elevation of cytosolic-free Ca^{2+} evoked by NMDA receptors in RGCs under stress conditions (Dong et al. 2007; Han and Wu 2002). Other studies suggested that brimonidine-mediated inhibition of the cyclic adenosine 3′,5′-monophosphate/protein kinase A (cAMP/PKA) pathway could be an important mechanism to protect RGCs from

glaucomatous neurodegeneration (Shim et al. 2017). Furthermore, RGCs' protective effect of brimonidine may also be through the blockade of glutamate excitotoxicity-induced oxidative stress in the ischemic retina (Lee et al. 2012). Based on a recent study in a rat glaucoma model, Zhou et al. proposed that α_2-AR activation hyperpolarizes RGCs by enhancing the γ-aminobutyric acid (GABA) receptor response to spontaneous and elicited GABA release, thereby reducing the risk for excitotoxicity and RGC injury (Zhou et al. 2019). Activation of α_2-ARs has also been shown to exert neuroprotective effects in other retinal diseases, such as light-induced photoreceptor damage, retinal detachment, and optic nerve injury (Fujita et al. 2013; Li et al. 2019; Wen et al. 1996). For example, α_2-adrenergic agonists were shown to induce the expression of basic fibroblast growth factor in photoreceptors in vivo and to ameliorate photoinduced damage in the retina (Wen et al. 1996). In retinal detachment, stimulation of α_2-AR signaling protected photoreceptors by inhibiting oxidative stress and inflammation (Li et al. 2019). Furthermore, in a mouse optic neuritis model, topical administration of the α_2-AR agonist, brimonidine, prevented RGCs' death by increasing retinal basic fibroblast growth factor expression (Guo et al. 2015). In a mechanic optic nerve injury model, activation of α_2-AR signaling promoted optic nerve regeneration via activation of extracellular-signal-regulated kinase (ERK) phosphorylation (Fujita et al. 2013). It has been suggested that activation of α_2-ARs induces vasoconstriction in the vasculature distal to the ophthalmic artery, such as ciliary and retinal blood vessels (Kaya et al. 2011; Weigert et al. 2007; Wikberg-Matsson and Simonsen 2001). While the α_{2A}-AR subtype was proposed to mediate adrenergic vasoconstriction in porcine ciliary arteries, no suggestion regarding the contribution of individual α_2-AR subtypes has been made for retinal blood vessels (Wikberg-Matsson and Simonsen 2001). Little is known about the functional role of individual α_2-AR subtypes in the retina. A study by Harun-Or-Rashid et al. reported α_{2A}-AR expression in cells of chicken, and that pharmacological activation of the α_{2A}-AR subtype triggers a mitogen-activated protein kinase (MAPK)-dependent response with phosphorylation of ERK1/2 both in vivo and in vitro.

Taken together, most studies indicate that activation of α_2-ARs increases neuronal resistance to retinal injury suggesting that the receptor subfamily may become a potent treatment target in various retinal diseases. However, the role of individual α_2-AR subtypes remains to be better characterized in the retina.

5.4 β-ARs

5.4.1 Expression of β-ARs in the Retina

In 1986, Zarbin et al. localized β-AR binding sites in the outer nuclear layer, the outer plexiform layer, the inner nuclear layer, and the inner plexiform layer of the rat retina (Zarbin et al. 1986). Also in the human retina, β-AR binding sites were visualized in vitro by autoradiography (Elena et al. 1990).

Three distinct β-AR subtypes, β_1-AR, β_2-AR, and β_3-AR, have been identified (Granneman 2001). In 1997, the β_4-AR was initially proposed by Kaumann and

Molenaar as a potential novel state of the β_1-AR (Kaumann and Molenaar 1997). Molenaar described these "atypical" β-ARs as having distinctive features that warranted further exploration in terms of pharmacological and genetic characterization (Molenaar 2003). However, Kompa and Summers subsequently conducted research in a rat model of cardiac failure, revealing that β_4-ARs undergo processes of desensitization and resensitization in a manner similar to β_1-ARs. This discovery highlighted overlapping characteristics between β_1- and β_4-ARs, ultimately challenging and refuting the initial β_4 hypothesis (Kompa and Summers 1999). Subsequent studies on knockout mice definitively demonstrated that activities, before attributed to the novel β_4-ARs, were evidently mediated via an atypical interaction with β_1-ARs (Kaumann et al. 2001; Konkar et al. 2000). Among the β-AR subtypes, β_1-AR and β_2-AR binding sites were found in bovine retinal vessels and in the neural retina (Ferrari-Dileo 1988). The presence of functional β_3-ARs has also been verified in rat retinal blood vessels (Mori et al. 2010). By immunohistochemistry, β_3-ARs were localized in the inner capillary plexus of the mouse mid-peripheral retina (Dal Monte et al. 2012; Ristori et al. 2011). Expression of β_3-ARs has been shown for the first time on cultured human retinal endothelial cells in 2003 (Steinle et al. 2003). In that study, pharmacological activation of β_3-ARs promoted cell migration and proliferation of endothelial cells (Steinle et al. 2003). In 2014, a study has reported that β_1- and β_3-ARs were expressed in human retinal endothelial cells (Safi et al. 2014). Based on the widespread distribution of β-ARs in retinal blood vessels and in the neural retina, β-ARs are believed to play an important role in the vascular and neuronal function of the retina. The localization of individual AR subfamilies in individual retinal layers and structures is shown in Fig. 8.

5.4.2 Role of β-ARs in the Retina

Stress conditions, such as hypoxia, can cause catecholaminergic overstimulation in the cardiovascular system, which in turn may activate β-ARs (Lindgren and Altimiras 2009). A study in mice reported that the level of noradrenaline in the hypoxic retina increased by approximately 90% compared to normoxic conditions (Dal Monte et al. 2012). Activation of β-ARs is considered to upregulate the hypoxia-inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF), which plays a key role in the formation of pathogenic blood vessels in various retinal diseases, such as ROP and diabetic retinopathy (Aiello et al. 1994; Chen and Smith 2007; Dal Monte et al. 2012). The β-AR blocker, propranolol, effectively inhibited the increase of VEGF expression caused by hypoxia and the consecutive neovascular response in the retina (Casini et al. 2014). Likewise, subcutaneous administration of propranolol reduced VEGF and HIF-1α levels in a mouse model of oxygen-induced retinopathy (OIR), suggesting that β-AR blockade was protective against retinal angiogenesis and ameliorated blood-retinal barrier dysfunction (Ristori et al. 2011).

Intriguingly, a novel β-AR agonist, compound 49b, was reported to decrease VEGF levels in the diabetic rat retina (Jiang et al. 2015). The effect of compound 49b was attributed to an increase in insulin-like growth factor binding protein 3, which reduced VEGF levels via modulation of endothelial nitric oxide synthase and protein

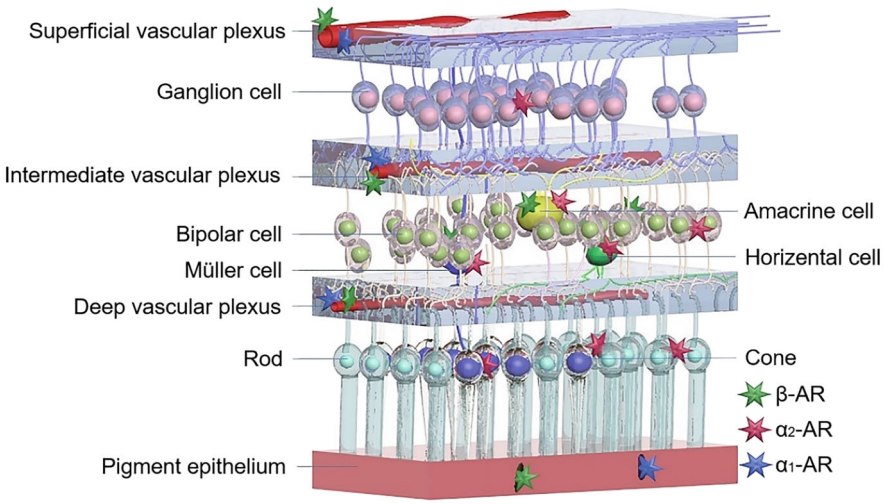

Fig. 8 The distribution of ARs in individual retinal layers and structures

kinase C pathways (Jiang et al. 2015). These seemingly contradictory findings regarding the impact of β-AR agonists and antagonists on VEGF levels suggest that the effects may be mediated through diverse regulatory mechanisms depending on the retinal disease and the experimental setting.

β-AR activation was also shown to increase human and mouse pericyte survival under diabetic conditions (Yun et al. 2018). Conversely, a significant decrease in the number of pericytes has been reported in the rat retina after surgical removal of the right superior cervical ganglion, which supplies the eye with sympathetic nerve fibers (Wiley et al. 2005). These findings suggest that proper β-AR signaling is essential for pericyte survival. An in vitro study proposed that β-ARs are involved in the regulation of inducible nitric oxide synthase (iNOS) expression (Steinle et al. 2008). Activation of β-ARs reduced levels of iNOS and other inflammatory molecules, such as interleukin (IL)-1β, TNF-α, and prostaglandin E_2 in human retinal endothelial cells and rat Müller cells in an in vitro model of hyperglycemia (Steinle 2007). The proposed mechanism for the protective effects was that the stimulatory β-ARs decrease the levels of the MAPK family members, PKA, p38 MAPK, and p42/p44 MAPK, in human retinal endothelial cells (Steinle et al. 2008).

Stimulation of β-ARs by agonists can activate members of the G protein-coupled receptor kinase family, which is a potential mechanism leading to β-AR desensitization (Wallukat 2002). A first mechanism underlying the desensitization is the phosphorylation of the GPCR (Willets et al. 2003). After coupling to activated receptors, G proteins are phosphorylated by the family of G protein-coupled receptor kinases (GRKs) (Kelly et al. 2008; Lymperopoulos and Bathgate 2012). Currently, seven GRKs (GRK1-7) have been identified (Pierce and Lefkowitz 2001). As a rhodopsin kinase, GRK1 is responsible for phosphorylating rhodopsin, which is

abundantly expressed in retinal rod and cone cells (Lorenz et al. 1991). β-ARs can be phosphorylated by a protein kinase termed GRK2 (Benovic et al. 1986). The GRK-phosphorylated receptor binds to arrestins, leading to uncoupling of the receptor from the G protein, desensitizing the agonist-induced response and subsequently mediating the internalization of receptors (Lymperopoulos 2018; Lymperopoulos and Bathgate 2012). Consequently, the GRK-arrestin pathway plays a central role in the desensitization of GPCR responses (Lymperopoulos 2018; Lymperopoulos and Bathgate 2012). Two arrestin subtypes were found to be expressed in the retina, arrestin-1 and -4. These arrestins are specialized in binding light-activated phosphorylated rhodopsin and suppressing G protein activation (Gurevich et al. 2018; Kühn et al. 1984). It has been shown that retinal and nonretinal arrestins mediate suppression of GPCRs, suggesting a common mechanism for AR desensitization (Alloway and Dolph 1999). Apart from this role, arrestins were also reported to be involved in receptor-mediated endocytosis through clathrin-coated pits (Alloway and Dolph 1999; Hanyaloglu and Zastrow 2008). It is worth noting that receptor phosphorylation and/or recruitment of β-arrestins is observed only in the case of stimulation by full agonists like adrenaline or noradrenaline. However, these events do not occur when partial agonists, such as albuterol, formoterol, or salmeterol, are involved (Littmann et al. 2015).

5.4.3 Functional Role of Individual β-ARs in the Retina

It has been reported that hypoxia triggers the release of catecholamines, which have been shown to contribute to the increase in retinal VEGF expression and to cause pathologic neovascularization (Aiello et al. 1994; Chen and Smith 2007; Dal Monte et al. 2012). In a mouse model of OIR, deletion of β_1- and β_2-ARs reduced retinal VEGF receptor-2 expression and abolished the development of vascular abnormalities in the superficial plexus of the retina (Dal Monte et al. 2015). In another study employing a mouse model of OIR, β_1- and β_2-AR blockade by propranolol was shown to reduce VEGF expression and to ameliorate retinal dysfunction (Ristori et al. 2011). In a neonatal rat intermittent hypoxia model, topical ocular propranolol failed to prevent severe OIR, but showed positive effects on preserving the astrocytes (Qadri et al. 2021). Other studies demonstrated that ICI 118,551, a selective β_2-AR blocker, decreased retinal levels of proangiogenic factors and reduced pathogenic neovascularization in a mouse OIR model, suggesting that β_2-AR blockade may be instrumental in blocking retinal angiogenesis (Martini et al. 2011).

As previously mentioned, there are conflicting results regarding the role of β-AR activation or blockade on VEGF expression and pathogenic vascularization. While most studies reported on inhibitory effects of pharmacological β-AR blockade on VEGF formation, some other studies observed blockade of VEGF formation by β-AR agonist exposure (Aiello et al. 1994; Casini et al. 2014; Chen and Smith 2007; Dal Monte et al. 2012; Lindgren and Altimiras 2009; Ristori et al. 2011). An explanation for these seemingly contradictory results is provided by a study by Dal Monte et al., which suggests that the nonselective β-ARs agonist isoproterenol can cause agonist-induced β_2-AR desensitization that downregulates the expression

of β_2-ARs in the retina, which in turn exerts a downregulatory effect on VEGF expression in OIR (Dal Monte et al. 2012). Jiang et al. proposed that β_2-AR knockout mice exhibited certain features similar to diabetic retinopathy, resulting in retinal cell death (Jiang et al. 2013). A study in β_2-AR knockout mice has shown a functional connection between β-ARs and insulin receptor signaling pathways in the retina (Jiang et al. 2013). Furthermore, β_2-ARs can maintain insulin receptor signaling in retinal Müller cells, which potentially supports neuroprotective effects promoted by β-AR stimulation in diabetic retinopathy models (Jiang et al. 2013). Xamoterol, a β_1-AR agonist, attenuated iNOS expression in human retinal endothelial cells grown in high glucose medium (Steinle 2007). Studies by Mori et al. have demonstrated that stimulation of β_1-, β_2-, and β_3-ARs in rat models can cause dilation of retinal arterioles and increase retinal blood flow (Mori et al. 2011b, 2017). Moreover, the latest study by Mori et al. reported that retinal vasodilation by β_2-AR stimulation is mediated via a Gi protein through the activation of large-conductance Ca^{2+}-activated K^+ channels (Mori et al. 2020).

β_3-ARs were shown to be involved in the neovascularization processes of various retinal vascular diseases (Ristori et al. 2011). For example, β_3-ARs were upregulated in response to hypoxia in an OIR mouse model with dense β_3-ARs immunoreactivity in engorged retinal tufts, suggesting that activation of β_3-ARs is likely to constitute an important part in pathologic angiogenesis (Ristori et al. 2011). Furthermore, the newest study published in 2022 has shown that the hypoxia-inducible factor-1 (HIF-1) could enhance the expression of the β_3-AR gene in the hypoxic retina of mouse, supporting that β_3-ARs may participate in the angiogenic response to hypoxia (Amato et al. 2022). However, the systemic administration of HIF-1 inhibitors might cause severe side effects including thromboembolism or hyperkalemia (Filippi et al. 2022; Hirota 2021). It has been demonstrated that the β_3-AR antagonists, L-748,337 and SR59230A, downregulated retinal VEGF release in hypoxia via modulation of the nitric NO signaling pathway (Dal Monte et al. 2013). In addition, the β_3-AR agonist, CL316243, was shown to reduce retinal damage following intravitreal injection of N-methyl-D-aspartate (NMDA) in rats (Oikawa et al. 2012). Furthermore, β_3-ARs, which differ from β_2-ARs with regard to a lack of consensus phosphorylation sites required for interaction with G protein receptor kinase, are resistant to agonist-induced desensitization (Jean-Luc 2013; Wallukat 2002). Taken together, based on the studies performed so far, the β_3-AR appears to be an attractive therapeutic target for the treatment of ischemic retinal diseases.

Remarkably, β-AR genes exhibit genetic polymorphisms caused by mutations in the gene promoter, leading to changes in the expression of receptors and the regulation of signal transduction (Leineweber et al. 2004; Lymperopoulos and Bathgate 2012; Zalewska et al. 2014). The mutations of ARs mainly affect receptor responses and are associated with some diseases (Zalewska et al. 2014). An in vivo study tested the effects of two polymorphisms (codon 16 and codon 27) of the β_2-AR on agonist-mediated vascular desensitization, suggesting that the arginine at position 16 (Arg16) polymorphism (the substitution of glycine for arginine) of the β_2-AR is associated with enhanced agonist-mediated desensitization in the vasculature (Dishy

et al. 2001). However, to the best of our knowledge, no association between genetic polymorphisms of β-AR genes and retinal diseases has been described, at present.

6 Future Directions and Clinical Implications

There is some evidence that ARs participate in the regulation of cell differentiation and proliferation in the corneal epithelium and also contribute to wound healing. However, due to the partially conflicting findings, the role of individual ARs in the cornea needs to be pursued further. In the conjunctiva, modulation of AR signaling may become a therapeutic approach to treat inflammatory ocular surface diseases, such as allergic conjunctivitis. Since both α- and β-ARs are involved in the regulation of tear secretion, activation of adrenergic pathways may be a potential therapeutic approach to treat dry eye disease. The role of individual AR subtypes in these physiological processes as well as under pathophysiological conditions remains to be identified. In the iris, blockade of the α_{1A}-AR appears to be a risk factor for IFIS. This syndrome derives from the inhibition of iris dilator muscle contraction and establishes in men and women, even after cessation of α-blocker administration. A careful drug history, postponing of α-blocker treatment for patients with scheduled cataract surgery, and careful counseling of patients taking α-blockers may reduce the risk of IFIS (Oelke et al. 2014). In the ciliary body epithelium, blockade of β_2-ARs and activation of α_2-ARs induces a reduction in AH formation and thus IOP. Although β-AR antagonists and α_2-AR agonists are established therapeutic approaches in glaucoma therapy, selective modulation of individual AR subtypes may help to reduce systemic side effects in the future. Since autoantibodies to the β_2-AR may be involved in the pathogenesis of glaucoma, neutralization or removal of these antibodies, e.g., by extracorporeal immunoadsorption, might become a potential strategy for glaucoma treatment (Jünemann et al. 2018).

Retinal α_1-adrenergic vasoconstriction is masked by endothelial mechanisms under physiological conditions and becomes more relevant when the endothelium is damaged. Therefore, α_1-AR signaling pathways may represent therapeutic targets primarily in the context of retinal pathologies associated with impaired endothelial function. However, it remains to be determined which α_1-AR subtype mediates vascular responses in the human retina. The dearth of highly selective pharmacological ligands and antibodies for individual α_1-AR subtypes has so far delayed the progress in this research area. From a clinical point of view, subtype-selective agonists and antagonists would constitute a therapeutic approach to specifically influence retinal perfusion. Various animal experiments and cell culture studies revealed neuroprotective effects of the selective α_2-AR agonist, brimonidine, in the retina (Kalapesi et al. 2005; Prokosch et al. 2010; Wheeler et al. 2001). In 2011, a randomized clinical trial reported on the neuroprotective effects of topically applied brimonidine tartrate 0.2% in preventing visual field loss progression in patients with low-pressure glaucoma, which supports the concept of direct activation of retinal α_2-ARs (Krupin et al. 2011). In contrast, another trial showed no neuroprotective effects of 0.2% brimonidine tartrate in patients with nonarteritic

anterior ischemic optic neuropathy (Wilhelm et al. 2006). In a pilot study on patients with retinal dystrophies, topical treatment with brimonidine indicated a trend toward reduced disease progression (Merin et al. 2008). However, the number of patients ($n = 26$) was relatively small and the mean follow-up period (29 months) relatively short, which does not allow to draw unequivocal conclusions. Overall, the retinal neuroprotective effects of brimonidine obtained in various experimental disease models remain to be confirmed in large human trials. Apart from the above-mentioned topical treatments, an intravitreal brimonidine implant, Brimonidine Posterior Segment Drug Delivery System (brimonidine DDS), has been studied for posterior segment pharmacokinetics in monkey eyes (Tamhane et al. 2021). Simulated tissue concentration-time profiles indicated maintenance of pharmacologically effective brimonidine concentrations for about 3 months in the human retina, suggesting that brimonidine DDS could be a potential clinical treatment approach (Tamhane et al. 2021). Propranolol, which has a high affinity to β_1- and β_2-AR subtypes, is the only β-AR antagonist that has been tested in clinical trials to date (Casini et al. 2014). Propranolol 0.1% eye micro-drops have been developed and administered in a multicenter pilot clinical trial to analyze the safety and efficacy in the treatment of preterm newborns with stage 2 ROP (Filippi et al. 2017). However, the second stage of this study was discontinued, since one of the 19 newborns showed a progression to stage 2 or 3 with additional disease (Filippi et al. 2017). Based on animal studies, β_3-ARs appear to be involved in pathogenic vessel formation in the ischemic retina. Hence, future studies are needed to explore β_3-AR ligands in human ischemic retinal diseases.

7 Conclusions

In summary, the comprehensive examination of studies in this chapter underscores the presence of α_1-, α_2-, and β-ARs in various components of the eye and its adnexa, including the lacrimal gland, cornea, conjunctiva, TM, uvea, and retina, spanning across multiple species, including humans. While β-AR blockers and α_2-AR agonists have played a pivotal role in glaucoma therapy for years, effectively reducing IOP and preventing blindness in millions of patients, the therapeutic application of AR agonists or antagonists for other eye conditions remains largely experimental. The introduction of β-blockers and α_2-agonists in glaucoma treatment represented a groundbreaking advancement in ophthalmology. However, the full potential of adrenoceptor modulators in addressing other ocular diseases is yet to be fully explored. In the retina and choroid, α_1-ARs primarily function as stimulatory receptors, playing a pivotal role in vascular smooth muscle activation, resulting in vasoconstriction and pupil dilation. In contrast, α_2-ARs predominantly serve as inhibitory receptors, with their pharmacological activation demonstrating efficacy in lowering IOP and offering neuroprotective effects in the retina. Furthermore, β-ARs are prominently expressed in critical ocular structures, including the corneal epithelium, ciliary body, and retinal blood vessels, neurons, and glial cells. While β-AR antagonists have firmly established their place in glaucoma therapy, ligands

targeting this particular receptor subgroup have yet to find clinical application in the treatment of other ocular disorders. In the retina, β-ARs have been implicated in the regulation of vascular diameter and responses to hypoxia, suggesting the potential of β-AR ligands as prospective therapeutic agents for managing ischemic retinal diseases.

References

Aasved H (1964) The effect of adrenalin in glaucoma simplex. Acta Ophthalmol 42:378–386. https://doi.org/10.1111/j.1755-3768.1964.tb03625.x

Aberg G, Adler G, Wikberg J (1979) Inhibition and facilitation of lacrimal flow by beta-adrenergic drugs. Acta Ophthalmol 57:225–235. https://doi.org/10.1111/j.1755-3768.1979.tb00486.x

Acott TS, Keller KE, Kelley MJ (2017) Role of proteoglycans in the trabecular meshwork. Reference module in neuroscience and biobehavioral psychology. Elsevier

Ahlquist RP (1948) A study of the adrenotropic receptors. Am J Physiol 153:586–600. https://doi.org/10.1152/ajplegacy.1948.153.3.586

Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, Pasquale LR, Thieme H, Iwamoto MA, Park JE et al (1994) Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 331:1480–1487. https://doi.org/10.1056/nejm199412013312203

Akhtar RA (1987) Effects of norepinephrine and 5-hydroxytryptamine on phosphoinositide-PO4 turnover in rabbit cornea. Exp Eye Res 44:849–862. https://doi.org/10.1016/s0014-4835(87)80047-7

Alexander SP, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M, Peters JA, Harmar AJ, Collaborators C (2013) The concise guide to PHARMACOLOGY 2013/14: G protein-coupled receptors. Br J Pharmacol 170:1459–1581. https://doi.org/10.1111/bph.12445

Alloway PG, Dolph PJ (1999) A role for the light-dependent phosphorylation of visual arrestin. Proc Natl Acad Sci 96:6072. https://doi.org/10.1073/pnas.96.11.6072

Alm A (1972) Effects of norepinephrine, angiotensin, dihydroergotamine, papaverine, isoproterenol, histamine, nicotinic acid, and xanthinol nicotinate on retinal oxygen tension in cats. Acta Ophthalmol 50:707–719

Alm A, Widengård I, Kjellgren D, Söderström M, Friström B, Heijl A, Stjerschantz J (1995) Latanoprost administered once daily caused a maintained reduction of intraocular pressure in glaucoma patients treated concomitantly with timolol. Br J Ophthalmol 79:12–16. https://doi.org/10.1136/bjo.79.1.12

Alosachie I, Godfraind T (1988) The modulatory role of vascular endothelium in the interaction of agonists and antagonists with alpha-adrenoceptors in the rat aorta. Br J Pharmacol 95:619–629

Amato R, Pisani F, Laudadio E, Cammalleri M, Lucchesi M, Marracci S, Filippi L, Galeazzi R, Svelto M, Dal Monte M, Bagnoli P (2022) HIF-1-dependent induction of β3 adrenoceptor: evidence from the mouse retina. Cell 11. https://doi.org/10.3390/cells11081271

Amini R, Rocha-Martins M, Norden C (2018) Neuronal migration and lamination in the vertebrate retina. Front Neurosci 11. https://doi.org/10.3389/fnins.2017.00742

Anderson DR, Patella VM (1999) Automated static perimetry, 2nd edn. Mosby, Saint Louis

Angus JA, Cocks TM, Satoh K (1986) The alpha adrenoceptors on endothelial cells. Fed Proc 45:2355–2359

Aspberg J, Heijl A, Bengtsson B (2021) Screening for open-angle glaucoma and its effect on blindness. Am J Ophthalmol 228:106–116. https://doi.org/10.1016/j.ajo.2021.03.030

Ballin N, Becker B, Goldman ML (1966) Systemic effects of epinephrine applied topically to the eye. Invest Ophthalmol Vis Sci 5:125–129

Bariş M, Tezel G (2019) Immunomodulation as a neuroprotective strategy for glaucoma treatment. Curr Ophthalmol Rep 7:160–169

Batra M, Gupta S, Nair AB, Dhanawat M, Sandal S, Morsy MA (2021) Netarsudil: a new ophthalmic drug in the treatment of chronic primary open angle glaucoma and ocular hypertension. Eur J Ophthalmol 31:2237–2244. https://doi.org/10.1177/11206721211008783

Bauknight GC Jr, Faraci FM, Heistad DD (1992) Endothelium-derived relaxing factor modulates noradrenergic constriction of cerebral arterioles in rabbits. Stroke 23:1522–1525. discussion 1525–6

Baumgart D, Haude M, Gorge G, Liu F, Ge J, Grosse-Eggebrecht C, Erbel R, Heusch G (1999) Augmented alpha-adrenergic constriction of atherosclerotic human coronary arteries. Circulation 99:2090–2097

Bausher LP, Gregory DS, Sears ML (1987) Interaction between alpha 2- and beta 2-adrenergic receptors in rabbit ciliary processes. Curr Eye Res 6:497–505. https://doi.org/10.3109/02713688709025206

Benovic JL, Strasser RH, Caron MG, Lefkowitz RJ (1986) Beta-adrenergic receptor kinase: identification of a novel protein kinase that phosphorylates the agonist-occupied form of the receptor. Proc Natl Acad Sci U S A 83:2797–2801. https://doi.org/10.1073/pnas.83.9.2797

Berkenboom G, Unger P, Fang Z, Fontaine J (1991) Endothelium-derived relaxing factor and protection against contraction to norepinephrine in isolated canine and human coronary arteries. J Cardiovasc Pharmacol 17(Suppl 3):S127–S132. https://doi.org/10.1097/00005344-199100001-00023

Bhagey J, James B (2004) Topical timolol prevented migraine attacks. Eye 18:751–751. https://doi.org/10.1038/sj.eye.6701303

Black JW, Crowther AF, Shanks RG, Smith LH, Dornhorst AC (1964) A new adrenergic betareceptor antagonist. Lancet 1:1080–1081. https://doi.org/10.1016/s0140-6736(64)91275-9

Boger WP 3rd, Puliafito CA, Steinert RF, Langston DP (1978) Long-term experience with timolol ophthalmic solution in patients with open-angle glaucoma. Ophthalmology 85:259–267. https://doi.org/10.1016/s0161-6420(78)35673-6

Böhm EW, Stoffelns B, Gericke A (2023) β-Adrenoreceptors as therapeutic targets for ocular tumors and other eye diseases – historical aspects and nowadays understanding. Int J Mol Sci 24:4698

Böhmer T, Manicam C, Steege A, Michel MC, Pfeiffer N, Gericke A (2014a) The α_1B-adrenoceptor subtype mediates adrenergic vasoconstriction in mouse retinal arterioles with damaged endothelium. Br J Pharmacol 171:3858–3867. https://doi.org/10.1111/bph.12743

Böhmer T, Pfeiffer N, Gericke A (2014b) Three commercial antibodies against α1-adrenergic receptor subtypes lack specificity in paraffin-embedded sections of murine tissues. Naunyn Schmiedebergs Arch Pharmacol 387:703–706. https://doi.org/10.1007/s00210-014-0992-2

Bonanno JA (2003) Identity and regulation of ion transport mechanisms in the corneal endothelium. Prog Retin Eye Res 22:69–94. https://doi.org/10.1016/s1350-9462(02)00059-9

Botelho SY, Goldstein AM, Martinez EV (1973) Norepinephrine-responsive beta-adrenergic receptors in rabbit lacrimal gland. Am J Physiol 224:1119–1122. https://doi.org/10.1152/ajplegacy.1973.224.5.1119

Brodde O-E, Michel MC (1999) Adrenergic and muscarinic receptors in the human heart. Pharmacol Rev 51:651–690

Bromberg BB (1981) Autonomic control of lacrimal protein secretion. Invest Ophthalmol Vis Sci 20:110–116

Bromberg BB, Gregory DS, Sears ML (1980) Beta-adrenergic receptors in ciliary processes of the rabbit. Invest Ophthalmol Vis Sci 19:203–207

Bucci MG, Missiroli A, Pecori Giraldi J, Virno M (1968) The local administration of propranolo in the therapy of glaucoma. Boll Ocul 47:51–60

Buonfiglio F, Pfeiffer N, Gericke A (2023) Immunomodulatory and antioxidant drugs in glaucoma treatment. Pharmaceuticals 16:1193

Burgoyne C (2015) The morphological difference between glaucoma and other optic neuropathies. J Neuroophthalmol 35(Suppl 1):S8–S21. https://doi.org/10.1097/wno.0000000000000289

Burke J, Kharlamb A, Shan T, Runde E, Padillo E, Manlapaz C, Wheeler L (1995) Adrenergic and imidazoline receptor-mediated responses to UK-14,304-18 (brimonidine) in rabbits and monkeys. A species difference. Ann N Y Acad Sci 763:78–95. https://doi.org/10.1111/j.1749-6632.1995.tb32392.x

Bylund DB (1998) Adrenoceptors. IUPHAR Media, Londen

Bylund DB, Chacko DM (1999) Characterization of alpha2 adrenergic receptor subtypes in human ocular tissue homogenates. Invest Ophthalmol Vis Sci 40:2299–2306

Bylund DB, Eikenberg DC, Hieble JP, Langer S, Lefkowitz R, Minneman KP, Molinoff P, Ruffolo R, Trendelenburg UJPR (1994) International union of pharmacology nomenclature of adrenoceptors. Pharmacol Rev 46(2):121–136

Candia OA, Neufeld AH (1978) Topical epinephrine causes a decrease in density of β-adrenergic receptors and catecholamine-stimulated chloride transport in the rabbit cornea. Biochim Biophys Acta 543:403–408

Carrier GO, White RE (1985) Enhancement of alpha-1 and alpha-2 adrenergic agonist-induced vasoconstriction by removal of endothelium in rat aorta. J Pharmacol Exp Ther 232:682–687

Casini G, Dal Monte M, Fornaciari I, Filippi L, Bagnoli P (2014) The β-adrenergic system as a possible new target for pharmacologic treatment of neovascular retinal diseases. Prog Retin Eye Res 42:103–129. https://doi.org/10.1016/j.preteyeres.2014.06.001

Casson RJ, Chidlow G, Wood JP, Crowston JG, Goldberg I (2012) Definition of glaucoma: clinical and experimental concepts. Clin Exp Ophthalmol 40:341–349. https://doi.org/10.1111/j.1442-9071.2012.02773.x

Cepelík J, Hynie S (1990) Inhibitory effects of clonidine and dopamine on adenylate cyclase of rabbit ciliary processes. Curr Eye Res 9:111–120. https://doi.org/10.3109/02713689008995197

Cesareo M, Giannini C, Martucci A, Di Marino M, Pocobelli G, Aiello F, Mancino R, Nucci C (2020) Chapter 2 – links between obstructive sleep apnea and glaucoma neurodegeneration. In: Bagetta G, Nucci C (eds) Progress in brain research, vol 257. Elsevier, pp 19–36

Chang DF, Campbell JR (2005) Intraoperative floppy iris syndrome associated with tamsulosin. J Cataract Refract Surg 31:664–673. https://doi.org/10.1016/j.jcrs.2005.02.027

Chen PP (2003) Blindness in patients with treated open-angle glaucoma. Ophthalmology 110:726–733. https://doi.org/10.1016/s0161-6420(02)01974-7

Chen ZJ, Minneman KP (2005) Recent progress in alpha1-adrenergic receptor research. Acta Pharmacol Sin 26:1281–1287. https://doi.org/10.1111/j.1745-7254.2005.00224.x

Chen J, Smith LEH (2007) Retinopathy of prematurity. Angiogenesis 10:133–140. https://doi.org/10.1007/s10456-007-9066-0

Chen Z, Jia W, Kaufman PL, Cynader M (1999) Immunohistochemical localization of dopamine-beta-hydroxylase in human and monkey eyes. Curr Eye Res 18:39–48

Chen L, Hodges RR, Funaki C, Zoukhri D, Gaivin RJ, Perez DM, Dartt DA (2006) Effects of alpha1D-adrenergic receptors on shedding of biologically active EGF in freshly isolated lacrimal gland epithelial cells. Am J Physiol Cell Physiol 291:C946–C956. https://doi.org/10.1152/ajpcell.00014.2006

Chrisp P, Sorkin EM (1992) Ocular carteolol. Drugs Aging 2:58–77. https://doi.org/10.2165/00002512-199202010-00007

Christou CD, Kourouklidou M, Mataftsi A, Oustoglou E, Ziakas N, Tzamalis A (2022) Silodosin as a predisposing factor of intraoperative floppy iris syndrome (IFIS): an observational propensity score-matching cohort study. Int Ophthalmol 42:393–399. https://doi.org/10.1007/s10792-021-02054-y

Ciccarelli M, Santulli G, Campanile A, Galasso G, Cervero P, Altobelli GG, Cimini V, Pastore L, Piscione F, Trimarco B, Iaccarino G (2008) Endothelial alpha1-adrenoceptors regulate neo-angiogenesis. Br J Pharmacol 153:936–946. https://doi.org/10.1038/sj.bjp.0707637

Cipolla MJ, Harker CT, Porter JM (1996) Endothelial function and adrenergic reactivity in human type-II diabetic resistance arteries. J Vasc Surg 23:940–949

Civantos Calzada B, Aleixandre de Artinano A (2001) Alpha-adrenoceptor subtypes. Pharmacol Res 44:195–208. https://doi.org/10.1006/phrs.2001.0857

Cocks TM, Angus JA (1983) Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. Nature 305:627–630

Colley A, Cavanagh HD (1982) Binding of [3H] dihydroalprenolol and [3H] quinuclidinyl benzilate to intact cells of cultured corneal epithelium. Metab Pediatr Syst Ophthalmol 6:75–86

Coman OA, Păunescu H, Ghiță I, Coman L, Bădărău A, Fulga I (2009) Beta 3 adrenergic receptors: molecular, histological, functional and pharmacological approaches. Rom J Morphol Embryol 50:169–179

Conti F, Romano GL, Eandi CM, Toro MD, Rejdak R, Di Benedetto G, Lazzara F, Bernardini R, Drago F, Cantarella G, Bucolo C (2021) Brimonidine is neuroprotective in animal paradigm of retinal ganglion cell damage. Front Pharmacol 12:705405. https://doi.org/10.3389/fphar.2021.705405

Convents A, De Backer JP, Vauquelin G (1987) Characterization of alpha 2-adrenergic receptors of calf retina membranes by [3H]-rauwolscine and [3H]-RX 781094 binding. Biochem Pharmacol 36:2497–2503. https://doi.org/10.1016/0006-2952(87)90522-3

Corbi G, Conti V, Russomanno G, Longobardi G, Furgi G, Filippelli A, Ferrara N (2013) Adrenergic signaling and oxidative stress: a role for sirtuins? Front Physiol 4:324. https://doi.org/10.3389/fphys.2013.00324

Costagliola C, dell'Omo R, Romano MR, Rinaldi M, Zeppa L, Parmeggiani F (2009) Pharmacotherapy of intraocular pressure: part I. Parasympathomimetic, sympathomimetic and sympatholytics. Expert Opin Pharmacother 10:2663–2677. https://doi.org/10.1517/14656560903300103

Cotecchia S (2010) The alpha1-adrenergic receptors: diversity of signaling networks and regulation. J Recept Signal Transduct Res 30:410–419. https://doi.org/10.3109/10799893.2010.518152

Crabb DP, Smith ND, Glen FC, Burton R, Garway-Heath DF (2013) How does glaucoma look?: patient perception of visual field loss. Ophthalmology 120:1120–1126. https://doi.org/10.1016/j.ophtha.2012.11.043

Crider JY, Sharif NA (2002) Adenylyl cyclase activity mediated by beta-adrenoceptors in immortalized human trabecular meshwork and non-pigmented ciliary epithelial cells. J Ocul Pharmacol Ther 18:221–230. https://doi.org/10.1089/108076802760116142

Dal Monte M, Martini D, Latina V, Pavan B, Filippi L, Bagnoli P (2012) Beta-adrenoreceptor agonism influences retinal responses to hypoxia in a model of retinopathy of prematurity. Invest Ophthalmol Vis Sci 53:2181–2192. https://doi.org/10.1167/iovs.11-9408

Dal Monte M, Filippi L, Bagnoli P (2013) Beta3-adrenergic receptors modulate vascular endothelial growth factor release in response to hypoxia through the nitric oxide pathway in mouse retinal explants. Naunyn Schmiedebergs Arch Pharmacol 386:269–278. https://doi.org/10.1007/s00210-012-0828-x

Dal Monte M, Cammalleri M, Mattei E, Filippi L, Bagnoli P (2015) Protective effects of β1/2 adrenergic receptor deletion in a model of oxygen-induced retinopathy. Invest Ophthalmol Vis Sci 56:59–73. https://doi.org/10.1167/iovs.14-15263

Dartt DA, Hodges RR (2011) Interaction of alpha1D-adrenergic and P2X(7) receptors in the rat lacrimal gland and the effect on intracellular [Ca2+] and protein secretion. Invest Ophthalmol Vis Sci 52:5720–5729. https://doi.org/10.1167/iovs.11-7358

Dartt DA, McCarthy DM, Mercer HJ, Kessler TL, Chung EH, Zieske JD (1995) Localization of nerves adjacent to goblet cells in rat conjunctiva. Curr Eye Res 14:993–1000. https://doi.org/10.3109/02713689508998520

de Andrade CR, Fukada SY, Olivon VC, de Godoy MA, Haddad R, Eberlin MN, Cunha FQ, de Souza HP, Laurindo FR, de Oliveira AM (2006) Alpha1D-adrenoceptor-induced relaxation on rat carotid artery is impaired during the endothelial dysfunction evoked in the early stages of hyperhomocysteinemia. Eur J Pharmacol 543:83–91. https://doi.org/10.1016/j.ejphar.2006.06.003

Delaey C, Van De Voorde J (2000) Regulatory mechanisms in the retinal and choroidal circulation. Ophthalmic Res 32:249–256. https://doi.org/10.1159/000055622

Demailly P, Lehner MA, Duperré J (1976) A new beta-blocking agent in the treatment of chronic glaucoma: timolol maleate. Bull Soc Ophtalmol Fr 76:801–802

Demailly P, Lehner MA, Etienne R, Trepsat C, Haut J, Raynaud G, Massin M, Tatry C (1978) Results of a double-blind medium-term study comparing effects of timolol maleate and epinephrine in 120 patients with chronic open-angle glaucoma. J Fr Ophtalmol 1:727–732

Diebold Y, Ríos JD, Hodges RR, Rawe I, Dartt DA (2001) Presence of nerves and their receptors in mouse and human conjunctival goblet cells. Invest Ophthalmol Vis Sci 42:2270–2282

Dikopf MS, Vajaranant TS, Edward DP (2017) Topical treatment of glaucoma: established and emerging pharmacology. Expert Opin Pharmacother 18:885–898. https://doi.org/10.1080/14656566.2017.1328498

Ding C, Walcott B, Keyser KT (2007) The alpha1- and beta1-adrenergic modulation of lacrimal gland function in the mouse. Invest Ophthalmol Vis Sci 48:1504–1510. https://doi.org/10.1167/iovs.05-1634

Dishy V, Sofowora GG, Xie H-G, Kim RB, Byrne DW, Stein CM, Wood AJJ (2001) The effect of common polymorphisms of the β2-adrenergic receptor on agonist-mediated vascular desensitization. N Engl J Med 345:1030–1035. https://doi.org/10.1056/NEJMoa010819

Djurup R (1981) Adrenoceptors: molecular nature and role in atopic diseases. Allergy 36:289–307. https://doi.org/10.1111/j.1398-9995.1981.tb01581.x

Docherty JR (2010) Subtypes of functional alpha1-adrenoceptor. Cell Mol Life Sci 67:405–417. https://doi.org/10.1007/s00018-009-0174-4

Dollery CT, Hill DW, Hodge JV (1963) The response of normal retinal blood vessels to angiotensin and noradrenaline. J Physiol 165:500–507

Donello JE, Padillo EU, Webster ML, Wheeler LA, Gil DW (2001) Alpha(2)-adrenoceptor agonists inhibit vitreal glutamate and aspartate accumulation and preserve retinal function after transient ischemia. J Pharmacol Exp Ther 296:216–223

Dong CJ, Guo Y, Wheeler L, Hare WA (2007) Alpha2 adrenergic receptor-mediated modulation of cytosolic Ca++ signals at the inner plexiform layer of the rat retina. Invest Ophthalmol Vis Sci 48:1410–1415. https://doi.org/10.1167/iovs.06-0890

Dong C-J, Guo Y, Agey P, Wheeler L, Hare WA (2008) α2 adrenergic modulation of NMDA receptor function as a major mechanism of RGC protection in experimental glaucoma and retinal excitotoxicity. Invest Ophthalmol Vis Sci 49:4515–4522. https://doi.org/10.1167/iovs.08-2078

Downs JC, Roberts MD, Burgoyne CF (2008) Mechanical environment of the optic nerve head in glaucoma. Optom Vis Sci 85:425–435. https://doi.org/10.1097/OPX.0b013e31817841cb

Drance SM (1972) The glaucomatous visual field. Br J Ophthalmol 56:186–200. https://doi.org/10.1136/bjo.56.3.186

Drouin C, Bobadilla A-C, Tassin J-P (2017) Norepinephrine. Reference module in neuroscience and biobehavioral psychology. Elsevier

Du Y, Cramer M, Lee CA, Tang J, Muthusamy A, Antonetti DA, Jin H, Palczewski K, Kern TS (2015) Adrenergic and serotonin receptors affect retinal superoxide generation in diabetic mice: relationship to capillary degeneration and permeability. FASEB J 29:2194–2204. https://doi.org/10.1096/fj.14-269431

Egleme C, Godfraind T, Miller RC (1984) Enhanced responsiveness of rat isolated aorta to clonidine after removal of the endothelial cells. Br J Pharmacol 81:16–18

Ehinger B (1966) Distribution of adrenergic nerves in the eye and some related structures in the cat. Acta Physiol Scand 66:123–128. https://doi.org/10.1111/j.1748-1716.1966.tb03176.x

Elena PP, Fredj-Reygrobellet D, Moulin G, Lapalus P (1984) Pharmacological characteristics of beta-adrenergic-sensitive adenylate cyclase in non pigmented and in pigmented cells of bovine ciliary process. Curr Eye Res 3:1383–1389. https://doi.org/10.3109/02713688409000833

Elena PP, Kosina-Boix M, Moulin G, Lapalus P (1987) Autoradiographic localization of beta-adrenergic receptors in rabbit eye. Invest Ophthalmol Vis Sci 28:1436–1441

Elena P-P, Denis P, Kosina-Boix M, Saraux H, Lapalus P (1990) Beta adrenergic binding sites in the human eye: an autoradiographic study. J Ocul Pharmacol Ther 6:143–149. https://doi.org/10.1089/jop.1990.6.143

Elliot MJ, Cullen PM, Phillips CI (1975) Ocular hypotensive effect of atenolol (Tenormin, I.C.I.). A new beta-adrenergic blocker. Br J Ophthalmol 59:296–300. https://doi.org/10.1136/bjo.59.6.296

Enríquez de Salamanca A, Siemasko KF, Diebold Y, Calonge M, Gao J, Juárez-Campo M, Stern ME (2005) Expression of muscarinic and adrenergic receptors in normal human conjunctival epithelium. Invest Ophthalmol Vis Sci 46:504–513. https://doi.org/10.1167/iovs.04-0665

Erdmann P (1914) Subconjunctivale Injectionen von Nebennierenpräparaten. Zeitschrift für Augenheilkunde 32:216–225

Esmaeli-Gutstein B, Hewlett BR, Harvey JT (1999) Characterization of adrenergic receptors in the accessory lacrimal glands of the upper eyelid. Ophthal Plast Reconstr Surg 15:245–251. https://doi.org/10.1097/00002341-199907000-00005

Evans DW, Hosking SL, Gherghel D, Bartlett JD (2003) Contrast sensitivity improves after brimonidine therapy in primary open angle glaucoma: a case for neuroprotection. Br J Ophthalmol 87:1463–1465. https://doi.org/10.1136/bjo.87.12.1463

Evans BA, Hutchinson DS, Summers RJ (2013) β2-Adrenoceptor-mediated regulation of glucose uptake in skeletal muscle – ligand-directed signalling or a reflection of system complexity? Naunyn Schmiedebergs Arch Pharmacol 386:757–760. https://doi.org/10.1007/s00210-013-0879-7

Faber JE, Yang N, Xin X (2001) Expression of alpha-adrenoceptor subtypes by smooth muscle cells and adventitial fibroblasts in rat aorta and in cell culture. J Pharmacol Exp Ther 298:441–452

Fan D, Fan TJ (2017) Clonidine induces apoptosis of human corneal epithelial cells through death receptors-mediated, mitochondria-dependent signaling pathway. Toxicol Sci 156:252–260. https://doi.org/10.1093/toxsci/kfw249

Ferrari-Dileo G (1988) Beta 1 and beta 2 adrenergic binding sites in bovine retina and retinal blood vessels. Invest Ophthalmol Vis Sci 29:695–699

Ferrari-Dileo G, Davis EB, Anderson DR (1989) Biochemical evidence for cholinergic activity in retinal blood vessels. Invest Ophthalmol Vis Sci 30:473–477

Ferrari-Dileo G, Davis EB, Anderson DR (1990) Response of retinal vasculature to phenylephrine. Invest Ophthalmol Vis Sci 31:1181–1182

Figueira L, Janeiro C, Ferreirinha F, Serrao P, Perestrelo S, Falcao-Reis F, Correia-de-Sa P, Moura D (2018) Regulation of corneal noradrenaline release and topography of sympathetic innervation: functional implications for adrenergic mechanisms in the human cornea. Exp Eye Res 174:121–132. https://doi.org/10.1016/j.exer.2018.05.023

Filippi S, Parenti A, Donnini S, Granger HJ, Fazzini A, Ledda F (2001) Alpha(1D)-adrenoceptors cause endothelium-dependent vasodilatation in the rat mesenteric vascular bed. J Pharmacol Exp Ther 296:869–875

Filippi L, Cavallaro G, Bagnoli P, Dal Monte M, Fiorini P, Berti E, Padrini L, Donzelli G, Araimo G, Cristofori G, Fumagalli M, la Marca G, Della Bona ML, Pasqualetti R, Fortunato P, Osnaghi S, Tomasini B, Vanni M, Calvani AM, Milani S, Cortinovis I, Pugi A, Agosti M, Mosca F (2017) Propranolol 0.1% eye micro-drops in newborns with retinopathy of prematurity: a pilot clinical trial. Pediatr Res 81:307–314. https://doi.org/10.1038/pr.2016.230

Filippi L, Cammalleri M, Amato R, Ciantelli M, Pini A, Bagnoli P, Dal Monte M (2022) Decoupling oxygen tension from retinal vascularization as a new perspective for management of retinopathy of prematurity. New opportunities from β-adrenoceptors. Front Pharmacol 13:835771. https://doi.org/10.3389/fphar.2022.835771

Fischer J, Ganellin CR (2006) Analogue-based drug discovery

Fong Z, Griffin CS, Hollywood MA, Thornbury KD, Sergeant GP (2019) β(3)-adrenoceptor agonists inhibit purinergic receptor-mediated contractions of the murine detrusor. Am J Physiol Cell Physiol 317:C131–C142. https://doi.org/10.1152/ajpcell.00488.2018

Forster BA, Ferrari-Dileo G, Anderson DR (1987) Adrenergic alpha 1 and alpha 2 binding sites are present in bovine retinal blood vessels. Invest Ophthalmol Vis Sci 28:1741–1746
Frambach DA, Valentine JL, Weiter JJ (1988) Alpha-1 adrenergic receptors on rabbit retinal pigment epithelium. Invest Ophthalmol Vis Sci 29:737–741
Frederick JM, Rayborn ME, Laties AM, Lam DMK, Hollyfield JG (1982) Dopaminergic neurons in the human retina. J Comp Neurol 210:65–79. https://doi.org/10.1002/cne.902100108
Fujita Y, Sato A, Yamashita T (2013) Brimonidine promotes axon growth after optic nerve injury through Erk phosphorylation. Cell Death Dis 4:e763–e763. https://doi.org/10.1038/cddis.2013.298
Furchgott RF (1984) The role of endothelium in the responses of vascular smooth muscle to drugs. Annu Rev Pharmacol Toxicol 24:175–197. https://doi.org/10.1146/annurev.pa.24.040184.001135
Gabanyi I, Muller PA, Feighery L, Oliveira TY, Costa-Pinto FA, Mucida DJC (2016) Neuro-immune interactions drive tissue programming in intestinal macrophages. Cell 164:378–391
Garcia-Hirschfeld J, Lopez-Briones LG, Belmonte C (1994) Neurotrophic influences on corneal epithelial cells. Exp Eye Res 59:597–605. https://doi.org/10.1006/exer.1994.1145
García-Posadas L, Hodges RR, Utheim TP, Olstad OK, Delcroix V, Makarenkova HP, Dartt DA (2020) Lacrimal gland myoepithelial cells are altered in a mouse model of dry eye disease. Am J Pathol 190:2067–2079. https://doi.org/10.1016/j.ajpath.2020.06.013
Garhöfer G, Schmetterer L (2012) Endothelial and adrenergic control. In: Schmetterer L, Kiel J (eds) Ocular blood flow. Springer, Berlin, pp 311–346
Garnock-Jones KP (2014) Ripasudil: first global approval. Drugs 74:2211–2215
Gautheron P, Sugrue MF (1987) The ability of salbutamol and theophylline to suppress immediate allergic conjunctivitis in the Guinea pig. Graefes Arch Clin Exp Ophthalmol 225:331–334. https://doi.org/10.1007/bf02153399
Gericke A, Martinka P, Nazarenko I, Persson PB, Patzak A (2007) Impact of alpha1-adrenoceptor expression on contractile properties of vascular smooth muscle cells. Am J Physiol Regul Integr Comp Physiol 293:R1215–R1221. https://doi.org/10.1152/ajpregu.00076.2007
Gericke A, Kordasz ML, Steege A, Sanbe A, Goloborodko E, Vetter JM, Patzak A, Pfeiffer N (2011) Functional role of alpha1-adrenoceptor subtypes in murine ophthalmic arteries. Invest Ophthalmol Vis Sci 52:4795–4799. https://doi.org/10.1167/iovs.11-7516
Gericke A, Böhmer T, Michel MC (2013a) β3-adrenoceptors: a drug target in ophthalmology? Naunyn Schmiedebergs Arch Pharmacol 386:265–267. https://doi.org/10.1007/s00210-013-0835-6
Gericke A, Goloborodko E, Sniatecki JJ, Steege A, Wojnowski L, Pfeiffer N (2013b) Contribution of nitric oxide synthase isoforms to cholinergic vasodilation in murine retinal arterioles. Exp Eye Res 109:60–66. https://doi.org/10.1016/j.exer.2013.01.012
Gericke A, Mann C, Zadeh JK, Musayeva A, Wolff I, Wang M, Pfeiffer N, Daiber A, Li H, Xia N, Prokosch V (2019) Elevated intraocular pressure causes abnormal reactivity of mouse retinal arterioles. Oxid Med Cell Longev 2019:9736047. https://doi.org/10.1155/2019/9736047
Geyer O, Levo Y (2020) Glaucoma is an autoimmune disease. Autoimmun Rev 19:102535. https://doi.org/10.1016/j.autrev.2020.102535
Ghoghawala SY, Mannis MJ, Pullar CE, Rosenblatt MI, Isseroff RR (2008) Beta2-adrenergic receptor signaling mediates corneal epithelial wound repair. Invest Ophthalmol Vis Sci 49:1857–1863. https://doi.org/10.1167/iovs.07-0925
Gifford S (1928) Acute rise of tension following the use of adrenalin in glaucoma. Am J Ophthalmol 11:628–631
Gilsbach R, Hein L (2012) Are the pharmacology and physiology of α2adrenoceptors determined by α2-heteroreceptors and autoreceptors respectively? Br J Pharmacol 165:90–102. https://doi.org/10.1111/j.1476-5381.2011.01533.x
Gipson IK (2007) The ocular surface: the challenge to enable and protect vision: the Friedenwald lecture. Invest Ophthalmol Vis Sci 48(4390):4391–4398. https://doi.org/10.1167/iovs.07-0770

Godfraind T, Egleme C, Al Osachie I (1985) Role of endothelium in the contractile response of rat aorta to alpha-adrenoceptor agonists. Clin Sci (Lond) 68(Suppl 10):65s–71s
Goldberg I (2002) Should beta blockers be abandoned as initial monotherapy in chronic open angle glaucoma? The controversy. Br J Ophthalmol 86:691–692. https://doi.org/10.1136/bjo.86.6.691
Gonzalez-Cabrera PJ, Gaivin RJ, Yun J, Ross SA, Papay RS, McCune DF, Rorabaugh BR, Perez DM (2003) Genetic profiling of alpha 1-adrenergic receptor subtypes by oligonucleotide microarrays: coupling to interleukin-6 secretion but differences in STAT3 phosphorylation and gp-130. Mol Pharmacol 63:1104–1116
Goto W, Oku H, Okuno T, Sugiyama T, Ikeda T (2003) Amelioration by topical bunazosin hydrochloride of the impairment in ocular blood flow caused by nitric oxide synthase inhibition in rabbits. J Ocul Pharmacol Ther 19:63–73. https://doi.org/10.1089/108076803762718123
Gradle HS (1925) The use of EPINEPHRIN in ocular hypertension. JAMA 84:675–675. https://doi.org/10.1001/jama.1925.26620350002011c
Graham RM, Perez DM, Hwa J, Piascik MT (1996) Alpha 1-adrenergic receptor subtypes. Molecular structure, function, and signaling. Circ Res 78:737–749
Grajewski AL, Ferrari-Dileo G, Feuer WJ, Anderson DR (1991) Beta-adrenergic responsiveness of choroidal vasculature. Ophthalmology 98:989–995. https://doi.org/10.1016/s0161-6420(91)32216-4
Granneman JG (2001) The putative beta4-adrenergic receptor is a novel state of the beta1-adrenergic receptor. Am J Physiol Endocrinol Metab 280:E199–E202. https://doi.org/10.1152/ajpendo.2001.280.2.E199
Grayson TH, Ellis JM, Chen S, Graham R, Dale Brown R, Hill CJC (1998) Immunohistochemical localisation of α1B-adrenergic receptors in the rat iris. Cell Tissue Res 293:435–444
Greenfield DS, Liebmann JM, Ritch R (1997) Brimonidine: a new alpha2-adrenoreceptor agonist for glaucoma treatment. J Glaucoma 6:250–258
Grueb M, Bartz-Schmidt K, Rohrbach J (2008) Adrenergic regulation of cAMP/protein kinase a pathway in corneal epithelium and endothelium. Ophthalmic Res 40:322–328. https://doi.org/10.1159/000150446
Grueb M, Mielke J, Rohrbach JM, Schlote T (2011) Effect of brimonidine on corneal thickness. J Ocul Pharmacol Ther 27:503–509. https://doi.org/10.1089/jop.2010.0198
Grzybowski A, Ruamviboonsuk V (2022) Pharmacological treatment in presbyopia. J Clin Med 11:1385
Guimaraes S, Moura D (2001) Vascular adrenoceptors: an update. Pharmacol Rev 53:319–356
Guimarães S, Moura D (2001) Vascular adrenoceptors: an update. Pharmacol Rev 53:319–356
Guo X, Namekata K, Kimura A, Noro T, Azuchi Y, Semba K, Harada C, Yoshida H, Mitamura Y, Harada T (2015) Brimonidine suppresses loss of retinal neurons and visual function in a murine model of optic neuritis. Neurosci Lett 592:27–31. https://doi.org/10.1016/j.neulet.2015.02.059
Gurevich VV, Chen Q, Gurevich EV (2018) Arrestins: introducing signaling bias into multifunctional proteins. Prog Mol Biol Transl Sci 160:47–61. https://doi.org/10.1016/bs.pmbts.2018.07.007
Hadjiconstantinou M, Cohen J, Neff NH (1983) Epinephrine: a potential neurotransmitter in retina. J Neurochem 41:1440–1444. https://doi.org/10.1111/j.1471-4159.1983.tb00843.x
Hadjiconstantinou M, Mariani AP, Panula P, Joh TH, Neff NH (1984) Immunohistochemical evidence for epinephrine-containing retinal amacrine cells. Neuroscience 13:547–551
Haeggendal J, Malmfors T (1965) Identification and cellular localization of the CATECHOLAMINES in the retina and the choroid of the rabbit. Acta Physiol Scand 64:58–66. https://doi.org/10.1111/j.1748-1716.1965.tb04153.x
Hague C, Chen Z, Uberti M, Minneman KP (2003) Alpha(1)-adrenergic receptor subtypes: non-identical triplets with different dancing partners? Life Sci 74:411–418
Haider NB, Cruz NM, Allocca M, Yuan J (2014) Pathobiology of the outer retina: genetic and nongenetic causes of disease. In: McManus LM, Mitchell RN (eds) Pathobiology of human disease. Academic Press, San Diego, pp 2084–2114
Hamburger C (1923) Experimentelle Glaukomtherapie. Med Klin 19:21

Hamburger C (1926) Treatment of glaucoma with Glaucosan, Glaucosan drops, and amino-Glaucosan. Arch Ophthalmol 55:533–544

Han Y, Wu SM (2002) NMDA-evoked [Ca2+]i increase in salamander retinal ganglion cells: modulation by PKA and adrenergic receptors. Vis Neurosci 19:249–256. https://doi.org/10.1017/s0952523802192029

Han C, Li J, Minneman KP (1990) Subtypes of alpha 1-adrenoceptors in rat blood vessels. Eur J Pharmacol 190:97–104

Hanyaloglu AC, Zastrow MV (2008) Regulation of GPCRs by endocytic membrane trafficking and its potential implications. Annu Rev Pharmacol Toxicol 48:537–568. https://doi.org/10.1146/annurev.pharmtox.48.113006.094830

Hara H, Ichikawa M, Oku H, Shimazawa M, Araie M (2005) Bunazosin, a selective alpha1-adrenoceptor antagonist, as an anti-glaucoma drug: effects on ocular circulation and retinal neuronal damage. Cardiovasc Drug Rev 23:43–56

Harrington DO (1964) The BJERRUM scotoma. Trans Am Ophthalmol Soc 62:324–348

Harrison R, Kaufmann CS (1977) Clonidine. Effects of a topically administered solution on intraocular pressure and blood pressure in open-angle glaucoma. Arch Ophthalmol 95:1368–1373. https://doi.org/10.1001/archopht.1977.04450080078007

Harrison JK, Angelo DD, Zeng DW, Lynch KR (1991) Pharmacological characterization of rat alpha 2-adrenergic receptors. Mol Pharmacol 40:407

Hein P, Michel MC (2007) Signal transduction and regulation: are all alpha1-adrenergic receptor subtypes created equal? Biochem Pharmacol 73:1097–1106. https://doi.org/10.1016/j.bcp.2006.11.001

Henness S, Harrison TS, Keating GM (2007) Ocular carteolol. Drugs Aging 24:509–528. https://doi.org/10.2165/00002512-200724060-00007

Herzlich AA, Patel M, Charles Sauer T, Chan C-C (2010) Chapter 2 – retinal anatomy and pathology. In: Nguyen QD, Rodrigues EB, Farah ME, Mieler WF (eds) Retinal pharmacotherapy. W.B. Saunders, Edinburgh, pp 5–14

Heusch G, Baumgart D, Camici P, Chilian W, Gregorini L, Hess O, Indolfi C, Rimoldi O (2000) Alpha-adrenergic coronary vasoconstriction and myocardial ischemia in humans. Circulation 101:689–694

Hieble JP (2009) Adrenergic receptors. In: Squire LR (ed) Encyclopedia of neuroscience. Academic Press, Oxford, pp 135–139

Hirasawa H, Contini M, Raviola E (2015) Extrasynaptic release of GABA and dopamine by retinal dopaminergic neurons. Philos Trans R Soc Lond B Biol Sci 370:20140186. https://doi.org/10.1098/rstb.2014.0186

Hirota K (2021) HIF-α prolyl hydroxylase inhibitors and their implications for biomedicine: a comprehensive review. Biomedicine 9. https://doi.org/10.3390/biomedicines9050468

Hodges RR, Dicker DM, Rose PE, Dartt DA (1992) Alpha 1-adrenergic and cholinergic agonists use separate signal transduction pathways in lacrimal gland. Am J Physiol 262:G1087–G1096. https://doi.org/10.1152/ajpgi.1992.262.6.G1087

Hodges RR, Shatos MA, Tarko RS, Vrouvlianis J, Gu J, Dartt DA (2005) Nitric oxide and cGMP mediate alpha1D-adrenergic receptor-stimulated protein secretion and p42/p44 MAPK activation in rat lacrimal gland. Invest Ophthalmol Vis Sci 46:2781–2789. https://doi.org/10.1167/iovs.05-0022

Hogan MJ, Feeney L (1963) The ultrastructure of the retinal blood vessels: I. The large vessels. J Ultrastruct Res 9:10–28. https://doi.org/10.1016/S0022-5320(63)80033-7

Hosoda C, Tanoue A, Shibano M, Tanaka Y, Hiroyama M, Koshimizu TA, Cotecchia S, Kitamura T, Tsujimoto G, Koike K (2005) Correlation between vasoconstrictor roles and mRNA expression of alpha1-adrenoceptor subtypes in blood vessels of genetically engineered mice. Br J Pharmacol 146:456–466. https://doi.org/10.1038/sj.bjp.0706325

Hoste AM, Boels PJ, Brutsaert DL, De Laey JJ (1989) Effect of alpha-1 and beta agonists on contraction of bovine retinal resistance arteries in vitro. Invest Ophthalmol Vis Sci 30:44–50

Hoste AM, Boels PJ, Andries LJ, Brutsaert DL, De Laey JJ (1990) Effects of beta-antagonists on contraction of bovine retinal microarteries in vitro. Invest Ophthalmol Vis Sci 31:1231–1237

Hoyng PFJ, van Beek LM (2000) Pharmacological therapy for glaucoma. Drugs 59:411–434. https://doi.org/10.2165/00003495-200059030-00003

Hrometz SL, Edelmann SE, McCune DF, Olges JR, Hadley RW, Perez DM, Piascik MT (1999) Expression of multiple alpha1-adrenoceptors on vascular smooth muscle: correlation with the regulation of contraction. J Pharmacol Exp Ther 290:452–463

Huang R, Tamalunas A, Waidelich R, Strittmatter F, Stief CG, Hennenberg M (2022) Antagonism of α(1)-adrenoceptors by β(3)-adrenergic agonists: structure-function relations of different agonists in prostate smooth muscle contraction. Biochem Pharmacol 202:115148. https://doi.org/10.1016/j.bcp.2022.115148

Hudson BD, Kelly ME (2012) Identification of novel competing β2AR phospho-extracellular signal regulated kinase 1/2 signaling pathways in human trabecular meshwork cells. J Ocul Pharmacol Ther 28:17–25. https://doi.org/10.1089/jop.2011.0016

Ichikawa M, Okada Y, Asai Y, Hara H, Ishii K, Araie M (2004) Effects of topically instilled bunazosin, an alpha1-adrenoceptor antagonist, on constrictions induced by phenylephrine and ET-1 in rabbit retinal arteries. Invest Ophthalmol Vis Sci 45:4041–4048. https://doi.org/10.1167/iovs.03-1395

Ignarro LJ, Byrns RE, Buga GM, Wood KS, Chaudhuri G (1988) Pharmacological evidence that endothelium-derived relaxing factor is nitric oxide: use of pyrogallol and superoxide dismutase to study endothelium-dependent and nitric oxide-elicited vascular smooth muscle relaxation. J Pharmacol Exp Ther 244:181–189

Ikeda-Kurosawa C, Higashio H, Nakano M, Okubo M, Satoh Y, Kurosaka D, Saino T (2015) α1-adrenoceptors relate ca(2+) modulation and protein secretions in rat lacrimal gland. Biomed Res 36:357–369. https://doi.org/10.2220/biomedres.36.357

Ishikawa H, Miller DD, Patil PN (1996) Comparison of post-junctional alpha-adrenoceptors in iris dilator muscle of humans, and albino and pigmented rabbits. Naunyn Schmiedebergs Arch Pharmacol 354:765–772. https://doi.org/10.1007/bf00166903

Isobe T, Mizuno K, Kaneko Y, Ohta M, Koide T, Tanabe S (2014) Effects of K-115, a rho-kinase inhibitor, on aqueous humor dynamics in rabbits. Curr Eye Res 39:813–822

Jampel HD, Lynch MG, Brown RH, Kuhar MJ, De Souza EB (1987) Beta-adrenergic receptors in human trabecular meshwork. Identification and autoradiographic localization. Invest Ophthalmol Vis Sci 28:772–779

Jandrasits K, Luksch A, Soregi G, Dorner GT, Polak K, Schmetterer L (2002) Effect of noradrenaline on retinal blood flow in healthy subjects. Ophthalmology 109:291–295

Jean-Luc B (2013) Beta3-adrenoreceptors in cardiovasular diseases: new roles for an "old" receptor. Curr Drug Deliv 10:64–66. https://doi.org/10.2174/1567201811310010011

Jensen BC, Swigart PM, Simpson PC (2009) Ten commercial antibodies for alpha-1-adrenergic receptor subtypes are nonspecific. Naunyn Schmiedebergs Arch Pharmacol 379:409–412. https://doi.org/10.1007/s00210-008-0368-6

Jiang Y, Zhang Q, Liu L, Tang J, Kern TS, Steinle JJ (2013) β2-adrenergic receptor knockout mice exhibit a diabetic retinopathy phenotype. PloS One 8:e70555. https://doi.org/10.1371/journal.pone.0070555

Jiang Y, Zhang Q, Steinle J (2015) Beta-adrenergic receptor agonist decreases VEGF levels through altered eNOS and PKC signaling in diabetic retina. Growth Factors 33:1–8. https://doi.org/10.3109/08977194.2015.1054990

Jin Y, Gooding JR, Yorio T (1994a) Ocular alpha 2-receptor subclasses and antiglaucoma efficacy. J Ocul Pharmacol 10:359–369. https://doi.org/10.1089/jop.1994.10.359

Jin Y, Verstappen A, Yorio T (1994b) Characterization of alpha 2-adrenoceptor binding sites in rabbit ciliary body membranes. Invest Ophthalmol Vis Sci 35:2500–2508

Jones CJ, DeFily DV, Patterson JL, Chilian WM (1993) Endothelium-dependent relaxation competes with alpha 1- and alpha 2-adrenergic constriction in the canine epicardial coronary microcirculation. Circulation 87:1264–1274

Joseph DP, Miller SS (1992) Alpha-1-adrenergic modulation of K and cl transport in bovine retinal pigment epithelium. J Gen Physiol 99:263–290. https://doi.org/10.1085/jgp.99.2.263

Jumblatt JE, Liu JG, North GT (1987) Alpha-2 adrenergic modulation of norepinephrine secretion in the perfused rabbit iris-ciliary body. Curr Eye Res 6:767–777. https://doi.org/10.3109/02713688709034843

Jun I, Choi YJ, Kim BR, Seo KY, Kim TI (2022) Activation of ADRB2/PKA signaling pathway facilitates lipid synthesis in meibocytes, and beta-blocker glaucoma drug impedes PKA-induced lipid synthesis by inhibiting ADRB2. Int J Mol Sci 23. https://doi.org/10.3390/ijms23169478

Jünemann A, Hohberger B, Rech J, Sheriff A, Fu Q, Schlötzer-Schrehardt U, Voll RE, Bartel S, Kalbacher H, Hoebeke J, Rejdak R, Horn F, Wallukat G, Kunze R, Herrmann M (2018) Agonistic autoantibodies to the β2-adrenergic receptor involved in the pathogenesis of open-angle glaucoma. Front Immunol 9:145. https://doi.org/10.3389/fimmu.2018.00145

Kalapesi FB, Coroneo MT, Hill MA (2005) Human ganglion cells express the alpha-2 adrenergic receptor: relevance to neuroprotection. Br J Ophthalmol 89:758–763. https://doi.org/10.1136/bjo.2004.053025

Kanar HS, Olcucu MT, Ozdemir I (2021) Comparison of effects of tamsulosin and silodosin on subfoveal choroidal thickness and pupil size diameters in patients with prostatic hyperplasia. Int Ophthalmol 41:3921–3927. https://doi.org/10.1007/s10792-021-01961-4

Kaneko K, Sunano S (1993) Involvement of alpha-adrenoceptors in the endothelium-dependent depression of noradrenaline-induced contraction in rat aorta. Eur J Pharmacol 240:195–200

Katsimpris JM, Siganos D, Konstas AG, Kozobolis V, Georgiadis N (2003) Efficacy of brimonidine 0.2% in controlling acute postoperative intraocular pressure elevation after phacoemulsification. J Cataract Refract Surg 29:2288–2294. https://doi.org/10.1016/j.jcrs.2003.08.029

Kaumann AJ, Molenaar P (1997) Modulation of human cardiac function through 4 β-adrenoceptor populations. Naunyn Schmiedebergs Arch Pharmacol 355:667–681. https://doi.org/10.1007/PL00004999

Kaumann AJ, Engelhardt S, Hein L, Molenaar P, Lohse M (2001) Abolition of (−)-CGP 12177-evoked cardiostimulation in double beta1/beta2-adrenoceptor knockout mice. Obligatory role of beta1-adrenoceptors for putative beta4-adrenoceptor pharmacology. Naunyn Schmiedebergs Arch Pharmacol 363:87–93. https://doi.org/10.1007/s002100000336

Kawarai M, Koss MC (1998) Sympathetic vasoconstriction in the rat anterior choroid is mediated by alpha1-adrenoceptors. Eur J Pharmacol 363:35–40. https://doi.org/10.1016/s0014-2999(98)00790-0

Kaya S, Kolodjaschna J, Berisha F, Polska E, Pemp B, Garhöfer G, Schmetterer L (2011) Effect of the α2-adrenoceptor antagonist yohimbine on vascular regulation of the middle cerebral artery and the ophthalmic artery in healthy subjects. Microvasc Res 81:117–122. https://doi.org/10.1016/j.mvr.2010.10.001

Kelly E, Bailey CP, Henderson G (2008) Agonist-selective mechanisms of GPCR desensitization. Br J Pharmacol 153:S379–S388. https://doi.org/10.1038/sj.bjp.0707604

Killer HE, Pircher A (2018) Normal tension glaucoma: review of current understanding and mechanisms of the pathogenesis. Eye (Lond) 32:924–930. https://doi.org/10.1038/s41433-018-0042-2

Kim HS, Chang YI, Kim JH, Park CK (2007) Alteration of retinal intrinsic survival signal and effect of alpha2-adrenergic receptor agonist in the retina of the chronic ocular hypertension rat. Vis Neurosci 24:127–139. https://doi.org/10.1017/s0952523807070150

Kingman S (2004) Glaucoma is second leading cause of blindness globally. Bull World Health Organ 82:887–888

Kintz P, Himber J, de Burlet G, Andermann G (1988) Characterization of alpha 2-adrenergic receptors, negatively coupled to adenylate cyclase, in rabbit ciliary processes. Curr Eye Res 7:287–292. https://doi.org/10.3109/02713688809047034

Kolb H, Cuenca N, Wang HH, Dekorver L (1990) The synaptic organization of the dopaminergic amacrine cell in the cat retina. J Neurocytol 19:343–366

Kompa AR, Summers RJ (1999) Desensitization and resensitization of beta 1- and putative beta 4-adrenoceptor mediated responses occur in parallel in a rat model of cardiac failure. Br J Pharmacol 128:1399–1406. https://doi.org/10.1038/sj.bjp.0702920

Konkar AA, Zhai Y, Granneman JG (2000) beta1-adrenergic receptors mediate beta3-adrenergic-independent effects of CGP 12177 in brown adipose tissue. Mol Pharmacol 57:252–258

Kopczynski C, Heah T (2018) Netarsudil ophthalmic solution 0.02% for the treatment of patients with open-angle glaucoma or ocular hypertension. Drugs Today 54:467–478

Kordasz ML, Manicam C, Steege A, Goloborodko E, Amato C, Laspas P, Brochhausen C, Pfeiffer N, Gericke A (2014) Role of α_1-adrenoceptor subtypes in pupil dilation studied with gene-targeted mice. Invest Ophthalmol Vis Sci 55:8295–8301. https://doi.org/10.1167/iovs.14-15706

Koriyama Y, Kamiya M, Takadera T, Arai K, Sugitani K, Ogai K, Kato S (2012) Protective action of nipradilol mediated through S-nitrosylation of Keap1 and HO-1 induction in retinal ganglion cells. Neurochem Int 61:1242–1253. https://doi.org/10.1016/j.neuint.2012.09.004

Koss MC (1999) Functional role of nitric oxide in regulation of ocular blood flow. Eur J Pharmacol 374:161–174

Koss MC, Gherezghiher T (1994) Ocular effects of alpha 2-adrenoceptor activation in anesthetized cats. J Ocul Pharmacol 10:149–156. https://doi.org/10.1089/jop.1994.10.149

Kroese M, Burton H (2003) Primary open angle glaucoma. The need for a consensus case definition. J Epidemiol Community Health 57:752–754. https://doi.org/10.1136/jech.57.9.752

Krupin T, Liebmann JM, Greenfield DS, Ritch R, Gardiner S (2011) A randomized trial of brimonidine versus timolol in preserving visual function: results from the low-pressure glaucoma treatment study. Am J Ophthalmol 151:671–681. https://doi.org/10.1016/j.ajo.2010.09.026

Kühn H, Hall SW, Wilden U (1984) Light-induced binding of 48-kDa protein to photoreceptor membranes is highly enhanced by phosphorylation of rhodopsin. FEBS Lett 176:473–478. https://doi.org/10.1016/0014-5793(84)81221-1

Kur J, Newman EA, Chan-Ling T (2012) Cellular and physiological mechanisms underlying blood flow regulation in the retina and choroid in health and disease. Prog Retin Eye Res 31:377–406. https://doi.org/10.1016/j.preteyeres.2012.04.004

Kwon YH, Kim CS, Zimmerman MB, Alward WL, Hayreh SS (2001) Rate of visual field loss and long-term visual outcome in primary open-angle glaucoma. Am J Ophthalmol 132:47–56. https://doi.org/10.1016/s0002-9394(01)00912-6

Laties AM (1967) Central retinal artery innervation. Absence of adrenergic innervation to the intraocular branches. Arch Ophthalmol 77:405–409. https://doi.org/10.1001/archopht.1967.00980020407021

Lavine JA, Sang Y, Wang S, Ip MS, Sheibani N (2013) Attenuation of choroidal neovascularization by β(2)-adrenoreceptor antagonism. JAMA Ophthalmol 131:376–382. https://doi.org/10.1001/jamaophthalmol.2013.1476

Lavine JA, Farnoodian M, Wang S, Darjatmoko SR, Wright LS, Gamm DM, Ip MS, Sorenson CM, Sheibani N (2017) β2-adrenergic receptor antagonism attenuates CNV through inhibition of VEGF and IL-6 expression. Invest Ophthalmol Vis Sci 58:299–308. https://doi.org/10.1167/iovs.16-20204

Lee D, Kim KY, Noh YH, Chai S, Lindsey JD, Ellisman MH, Weinreb RN, Ju WK (2012) Brimonidine blocks glutamate excitotoxicity-induced oxidative stress and preserves mitochondrial transcription factor a in ischemic retinal injury. PloS One 7:e47098. https://doi.org/10.1371/journal.pone.0047098

Lei S (2014) Cross interaction of dopaminergic and adrenergic systems in neural modulation. Int J Physiol Pathophysiol Pharmacol 6:137–142

Leineweber K, Büscher R, Bruck H, Brodde OE (2004) β-Adrenoceptor polymorphisms. Naunyn Schmiedebergs Arch Pharmacol 369:1–22. https://doi.org/10.1007/s00210-003-0824-2

Leung DYL, Tham CC (2022) Normal-tension glaucoma: current concepts and approaches-a review. Clin Exp Ophthalmol 50:247–259. https://doi.org/10.1111/ceo.14043

Li T, Yang S, She X, Yan Q, Zhang P, Zhu H, Wang F, Luo X, Sun X (2019) Modulation of α-adrenoceptor signalling protects photoreceptors after retinal detachment by inhibiting oxidative stress and inflammation. Br J Pharmacol 176:801–813. https://doi.org/10.1111/bph.14565

Li G, Lee C, Read AT, Wang K, Ha J, Kuhn M, Navarro I, Cui J, Young K, Gorijavolu R, Sulchek T, Kopczynski C, Farsiu S, Samples J, Challa P, Ethier CR, Stamer WD (2021a) Anti-fibrotic activity of a rho-kinase inhibitor restores outflow function and intraocular pressure homeostasis. Elife 10. https://doi.org/10.7554/eLife.60831

Li G, Lee C, Read AT, Wang K, Ha J, Kuhn M, Navarro I, Cui J, Young K, Gorijavolu R, Sulchek T, Kopczynski C, Farsiu S, Samples J, Challa P, Ethier CR, Stamer WD (2021b) Anti-fibrotic activity of a rho-kinase inhibitor restores outflow function and intraocular pressure homeostasis. Elife 10:e60831. https://doi.org/10.7554/eLife.60831

Lichter PR (2003) Glaucoma clinical trials and what they mean for our patients. Am J Ophthalmol 136:136–145. https://doi.org/10.1016/s0002-9394(03)00143-0

Lindgren I, Altimiras J (2009) Chronic prenatal hypoxia sensitizes beta-adrenoceptors in the embryonic heart but causes postnatal desensitization. Am J Physiol Regul Integr Comp Physiol 297:R258–R264. https://doi.org/10.1152/ajpregu.00167.2009

Littmann T, Göttle M, Reinartz MT, Kälble S, Wainer IW, Ozawa T, Seifert R (2015) Recruitment of β-arrestin 1 and 2 to the β2-adrenoceptor: analysis of 65 ligands. J Pharmacol Exp Ther 355: 183–190. https://doi.org/10.1124/jpet.115.227959

Liu GS, Trope GE, Basu PK (1990) Beta adrenoceptors and regenerating corneal epithelium. J Ocul Pharmacol Ther 6:101–112. https://doi.org/10.1089/jop.1990.6.101

Liu H, Mercieca K, Anders F, Prokosch V (2020a) Hydrogen sulfide and β-Synuclein are involved and interlinked in the aging glaucomatous retina. J Ophthalmol:8642135–8642135. https://doi.org/10.1155/2020/8642135

Liu J, Huang S, Li F, Wu M, He J, Xue Y, Fu T, Yu R, Chen X, Wang Y, Li Z (2020b) Sympathetic nerves positively regulate eosinophil-driven allergic conjunctivitis via α1-adrenergic receptor signaling. Am J Pathol 190:1298–1308. https://doi.org/10.1016/j.ajpath.2020.02.004

Llobet A, Gasull X, Gual A (2003) Understanding trabecular meshwork physiology: a key to the control of intraocular pressure? News Physiol Sci 18:205–209. https://doi.org/10.1152/nips.01443.2003

Lorenz W, Inglese J, Palczewski K, Onorato JJ, Caron MG, Lefkowitz RJ (1991) The receptor kinase family: primary structure of rhodopsin kinase reveals similarities to the beta-adrenergic receptor kinase. Proc Natl Acad Sci U S A 88:8715–8719

Lues I, Schumann HJ (1984) Effect of removing the endothelial cells on the reactivity of rat aortic segments to different alpha-adrenoceptor agonists. Naunyn Schmiedebergs Arch Pharmacol 328:160–163

Lymperopoulos A (2018) Arrestins in the cardiovascular system: an update. Prog Mol Biol Transl Sci 159:27–57. https://doi.org/10.1016/bs.pmbts.2018.07.003

Lymperopoulos A, Bathgate A (2012) Pharmacogenomics of the heptahelical receptor regulators G-protein-coupled receptor kinases and arrestins: the known and the unknown. Pharmacogenomics 13:323–341. https://doi.org/10.2217/pgs.11.178

Ma D, Rajakumaraswamy N, Maze M (2004) alpha2-adrenoceptor agonists: shedding light on neuroprotection? Br Med Bull 71:77–92. https://doi.org/10.1093/bmb/ldh036

Magocsi M, Vizi ES, Selmeczy Z, Brózik A, Szelenyi J (2007) Multiple G-protein-coupling specificity of beta-adrenoceptor in macrophages. Immunology 122:503–513. https://doi.org/10.1111/j.1365-2567.2007.02658.x

Maier C, Steinberg GK, Sun GH, Zhi GT, Maze M (1993) Neuroprotection by the alpha 2-adrenoreceptor agonist dexmedetomidine in a focal model of cerebral ischemia. Anesthesiology 79:306–312. https://doi.org/10.1097/00000542-199308000-00016

Marti D, Miquel R, Ziani K, Gisbert R, Ivorra MD, Anselmi E, Moreno L, Villagrasa V, Barettino D, D'Ocon P (2005) Correlation between mRNA levels and functional role of alpha1-adrenoceptor subtypes in arteries: evidence of alpha1L as a functional isoform of the

alpha1A-adrenoceptor. Am J Physiol Heart Circ Physiol 289:H1923–H1932. https://doi.org/10.1152/ajpheart.00288.2005

Martini D, Monte MD, Ristori C, Cupisti E, Mei S, Fiorini P, Filippi L, Bagnoli P (2011) Antiangiogenic effects of β2 -adrenergic receptor blockade in a mouse model of oxygen-induced retinopathy. J Neurochem 119:1317–1329. https://doi.org/10.1111/j.1471-4159.2011.07530.x

Masland RH (2012) The neuronal organization of the retina. Neuron 76:266–280. https://doi.org/10.1016/j.neuron.2012.10.002

Matsuo T, Cynader MS (1992) Localization of Alpha-2 adrenergic receptors in the human eye. Ophthalmic Res 24:213–219. https://doi.org/10.1159/000267170

Matsuo M, Kuse Y, Takahashi K, Kuwahara K, Tanito M, Kaidzu S, Shimazawa M, Hara H, Ohira A (2019) Carteolol hydrochloride reduces visible light-induced retinal damage in vivo and BSO/glutamate-induced oxidative stress in vitro. J Pharmacol Sci 139:84–90. https://doi.org/10.1016/j.jphs.2018.11.010

Mauduit P, Herman G, Rossignol B (1986) Protein secretion in lacrimal gland: alpha 1-beta-adrenergic synergism. Am J Physiol 250:C704–C712. https://doi.org/10.1152/ajpcell.1986.250.5.C704

McDougal DH, Gamlin PD (2015) Autonomic control of the eye. Compr Physiol 5:439–473. https://doi.org/10.1002/cphy.c140014

McGrath JC (2015) Localization of α-adrenoceptors: JR Vane medal lecture. Br J Pharmacol 172:1179–1194. https://doi.org/10.1111/bph.13008

McMonnies C (2018) Reactive oxygen species, oxidative stress, glaucoma and hyperbaric oxygen therapy. J Optom 11:3–9. https://doi.org/10.1016/j.optom.2017.06.002

Meneray MA, Fields TY (2000) Adrenergic stimulation of lacrimal protein secretion is mediated by G(q/11)alpha and G(s)alpha. Curr Eye Res 21:602–607

Merin S, Obolensky A, Farber MD, Chowers I (2008) A pilot study of topical treatment with an alpha2-agonist in patients with retinal dystrophies. J Ocul Pharmacol Ther 24:80–86. https://doi.org/10.1089/jop.2007.0022

Michel MC, Seifert R (2015) Selectivity of pharmacologic tools: implications for use in cell physiology. A review in the theme: cell signaling: proteins, pathways and mechanisms. Am J Physiol Cell Physiol:ajpcell 00389 2014. https://doi.org/10.1152/ajpcell.00389.2014

Miller VM, Vanhoutte PM (1989) Role of the endothelium in modulating vascular adrenergic receptor actions. Prog Clin Biol Res 286:33–39

Mittag T, Tormay A (1981) Desensitization of the beta-adrenergic receptor-adenylate cyclase complex in rabbit iris-ciliary body induced by topical epinephrine. Exp Eye Res 33:497–503. https://doi.org/10.1016/s0014-4835(81)80124-8

Mittag TW, Tormay A, Severin C, Podos SM (1985) Alpha-adrenergic antagonists: correlation of the effect on intraocular pressure and on alpha 2-adrenergic receptor binding specificity in the rabbit eye. Exp Eye Res 40:591–599. https://doi.org/10.1016/0014-4835(85)90081-8

Molenaar P (2003) The, 'state' of beta-adrenoceptors. Br J Pharmacol 140:1–2. https://doi.org/10.1038/sj.bjp.0705420

Moncada S, Higgs EA (2006) Nitric oxide and the vascular endothelium. Handb Exp Pharmacol:213–254

Mori A, Miwa T, Sakamoto K, Nakahara T, Ishii K (2010) Pharmacological evidence for the presence of functional β3-adrenoceptors in rat retinal blood vessels. Naunyn Schmiedebergs Arch Pharmacol 382:119–126. https://doi.org/10.1007/s00210-010-0526-5

Mori A, Hanada M, Sakamoto K, Nakahara T, Ishii K (2011a) Noradrenaline contracts rat retinal arterioles via stimulation of alpha(1A)- and alpha(1D)-adrenoceptors. Eur J Pharmacol 673:65–69. https://doi.org/10.1016/j.ejphar.2011.10.012

Mori A, Nakahara T, Sakamoto K, Ishii K (2011b) Role of β3-adrenoceptors in regulation of retinal vascular tone in rats. Naunyn Schmiedebergs Arch Pharmacol 384:603–608. https://doi.org/10.1007/s00210-011-0682-2

Mori A, Sekito A, Sakamoto K, Ishii K, Nakahara T (2017) Stimulation of β1- and β2-adrenoceptors dilates retinal blood vessels in rats. Naunyn Schmiedebergs Arch Pharmacol 390:527–533. https://doi.org/10.1007/s00210-017-1349-4

Mori A, Taniai A, Hasegawa M, Sakamoto K, Nakahara T (2020) Involvement of Gi protein–dependent BKCa channel activation in β2-adrenoceptor-mediated dilation of retinal arterioles in rats. Naunyn Schmiedebergs Arch Pharmacol 393:2043–2052. https://doi.org/10.1007/s00210-020-01895-1

Moroi SE, Reed DM, Sanders DS, Almazroa A, Kagemann L, Shah N, Shekhawat N, Richards JE (2019) Precision medicine to prevent glaucoma-related blindness. Curr Opin Ophthalmol 30: 187–198. https://doi.org/10.1097/icu.0000000000000564

Mukaida S, Sato M, Öberg AI, Dehvari N, Olsen JM, Kocan M, Halls ML, Merlin J, Sandström AL, Csikasz RI, Evans BA, Summers RJ, Hutchinson DS, Bengtsson T (2019) BRL37344 stimulates GLUT4 translocation and glucose uptake in skeletal muscle via β(2)-adrenoceptors without causing classical receptor desensitization. Am J Physiol Regul Integr Comp Physiol 316:R666–r677. https://doi.org/10.1152/ajpregu.00285.2018

Müller LJ, Marfurt CF, Kruse F, Tervo TMT (2003) Corneal nerves: structure, contents and function. Exp Eye Res 76:521–542. https://doi.org/10.1016/S0014-4835(03)00050-2

Muramatsu I, Suzuki F, Nishimune A, Anisuzzaman AS, Yoshiki H, Su TH, Chang CK, Morishima S (2009) Expression of distinct alpha 1-adrenoceptor phenotypes in the iris of pigmented and albino rabbits. Br J Pharmacol 158:354–360. https://doi.org/10.1111/j.1476-5381.2009.00254.x

Murphy CJ, Campbell S, Araki-Sasaki K, Marfurt CF (1998) Effect of norepinephrine on proliferation, migration, and adhesion of SV-40 transformed human corneal epithelial cells. Cornea 17: 529–536. https://doi.org/10.1097/00003226-199809000-00011

Musayeva A, Manicam C, Steege A, Brochhausen C, Straub BK, Bell K, Pfeiffer N, Gericke A (2018) Role of α(1)-adrenoceptor subtypes on corneal epithelial thickness and cell proliferation in mice. Am J Physiol Cell Physiol 315:C757–c765. https://doi.org/10.1152/ajpcell.00314.2018

Nakamura S, Taniguchi T, Suzuki F, Akagi Y, Muramatsu I (1999) Evaluation of alpha1-adrenoceptors in the rabbit iris: pharmacological characterization and expression of mRNA. Br J Pharmacol 127:1367–1374. https://doi.org/10.1038/sj.bjp.0702675

Nakazawa T, Kaneko Y, Mori A, Saito M, Sakamoto K, Nakahara T, Ishii K (2007) Attenuation of nitric oxide- and prostaglandin-independent vasodilation of retinal arterioles induced by acetylcholine in streptozotocin-treated rats. Vascul Pharmacol 46:153–159. https://doi.org/10.1016/j.vph.2006.09.002

Nathanson JA (1981a) Effects of a potent and specific beta 2-adrenoceptor antagonist on intraocular pressure. Br J Pharmacol 73:97–100. https://doi.org/10.1111/j.1476-5381.1981.tb16776.x

Nathanson JA (1981b) Human ciliary process adrenergic receptor: pharmacological characterization. Invest Ophthalmol Vis Sci 21:798–804

Nathanson JA (1984) ICI 118,551: an effective ocular hypotensive agent with selectivity for the ciliary process beta 2-adrenoceptor and with minimal cardiac side effects. Br J Pharmacol 83: 821–829. https://doi.org/10.1111/j.1476-5381.1984.tb16238.x

Neufeld AH (1979) Experimental studies on the mechanism of action of timolol. Surv Ophthalmol 23:363–370. https://doi.org/10.1016/0039-6257(79)90229-7

Ngala RA, O'Dowd J, Wang SJ, Stocker C, Cawthorne MA, Arch JR (2009) Beta2-adrenoceptors and non-beta-adrenoceptors mediate effects of BRL37344 and clenbuterol on glucose uptake in soleus muscle: studies using knockout mice. Br J Pharmacol 158:1676–1682. https://doi.org/10.1111/j.1476-5381.2009.00472.x

Ngala RA, O'Dowd JF, Stocker CJ, Cawthorne MA, Arch JR (2013) β2-adrenoceptor agonists can both stimulate and inhibit glucose uptake in mouse soleus muscle through ligand-directed signalling. Naunyn Schmiedebergs Arch Pharmacol 386:761–773. https://doi.org/10.1007/s00210-013-0860-5

Nguyen-Legros J (1988) Chapter 5 morphology and distribution of catecholamine-neurons in mammalian retina. Prog Retin Res 7:113–147. https://doi.org/10.1016/0278-4327(88)90007-7

Nguyen-Legros J, Krieger M, Simon A (1994) Immunohistochemical localization of L-dopa and aromatic L-amino acid-decarboxylase in the rat retina. Invest Ophthalmol Vis Sci 35:2906–2915

Nielsen PJ, Nyborg NC (1989) Adrenergic responses in isolated bovine retinal resistance arteries. Int Ophthalmol 13:103–107

Nita M, Grzybowski A (2016) The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxid Med Cell Longev 2016:3164734. https://doi.org/10.1155/2016/3164734

Nork TM, Holly FJ, Hayes J, Wentlandt T, Lamberts DW (1984) Timolol inhibits corneal epithelial wound healing in rabbits and monkeys. Arch Ophthalmol 102:1224–1228. https://doi.org/10.1001/archopht.1984.01040030994034

Oelke M, Gericke A, Michel MC (2014) Cardiovascular and ocular safety of α1-adrenoceptor antagonists in the treatment of male lower urinary tract symptoms. Expert Opin Drug Saf 13:1187–1197. https://doi.org/10.1517/14740338.2014.936376

Oikawa F, Nakahara T, Akanuma K, Ueda K, Mori A, Sakamoto K, Ishii K (2012) Protective effects of the β3-adrenoceptor agonist CL316243 against N-methyl-D-aspartate-induced retinal neurotoxicity. Naunyn Schmiedebergs Arch Pharmacol 385:1077–1081. https://doi.org/10.1007/s00210-012-0796-1

Osborne NN (1981) Noradrenaline, a transmitter candidate in the retina. J Neurochem 36:17–27. https://doi.org/10.1111/j.1471-4159.1981.tb02372.x

Osborne NN (1982) Binding of (−)[3H]noradrenaline to bovine membrane of the retina. Evidence for the existence of alpha 2-receptors. Vision Res 22:1401–1407. https://doi.org/10.1016/0042-6989(82)90230-9

Palmer RM, Ferrige AG, Moncada S (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327:524–526. https://doi.org/10.1038/327524a0

Palmer RM, Ashton DS, Moncada S (1988) Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 333:664–666. https://doi.org/10.1038/333664a0

Parod RJ, Putney JW Jr (1978) An alpha-adrenergic receptor mechanism controlling potassium permeability in the rat lacrimal gland acinar cell. J Physiol 281:359–369. https://doi.org/10.1113/jphysiol.1978.sp012427

Petounis AD, Akritopoulos P (1989) Influence of topical and systemic beta-blockers on tear production. Int Ophthalmol 13:75–80. https://doi.org/10.1007/bf02028642

Phillips CI, Howitt G, Rowlands DJ (1967) Propranolol as ocular hypotensive agent. Br J Ophthalmol 51:222–226. https://doi.org/10.1136/bjo.51.4.222

Piascik MT, Perez DM (2001) Alpha1-adrenergic receptors: new insights and directions. J Pharmacol Exp Ther 298:403–410

Piascik MT, Guarino RD, Smith MS, Soltis EE, Saussy DL Jr, Perez DM (1995) The specific contribution of the novel alpha-1D adrenoceptor to the contraction of vascular smooth muscle. J Pharmacol Exp Ther 275:1583–1589

Pierce KL, Lefkowitz RJ (2001) Classical and new roles of β-arrestins in the regulation of G-PROTEIN-COUPLED receptors. Nat Rev Neurosci 2:727–733. https://doi.org/10.1038/35094577

Podos SM, Ritch R (1980) Epinephrine as the initial therapy in selected cases of ocular hypertension. Surv Ophthalmol 25:188–194. https://doi.org/10.1016/0039-6257(80)90097-1

Polak K, Dorner G, Kiss B, Polska E, Findl O, Rainer G, Eichler HG, Schmetterer L (2000) Evaluation of the Zeiss retinal vessel analyser. Br J Ophthalmol 84:1285–1290

Potter DE (1981) Adrenergic pharmacology of aqueous humor dynamics. Pharmacol Rev 33:133–153

Pradidarcheep W, Stallen J, Labruyère WT, Dabhoiwala NF, Michel MC, Lamers WH (2009) Lack of specificity of commercially available antisera against muscarinergic and adrenergic receptors. Naunyn Schmiedebergs Arch Pharmacol 379:397–402. https://doi.org/10.1007/s00210-009-0393-0

Prokosch V, Panagis L, Volk GF, Dermon C, Thanos S (2010) α2-adrenergic receptors and their core involvement in the process of axonal growth in retinal explants. Invest Ophthalmol Vis Sci 51:6688–6699. https://doi.org/10.1167/iovs.09-4835

Prum BE Jr, Rosenberg LF, Gedde SJ, Mansberger SL, Stein JD, Moroi SE, Herndon LW Jr, Lim MC, Williams RD (2016) Primary open-angle glaucoma preferred practice pattern(®) guidelines. Ophthalmology 123:P41–P111. https://doi.org/10.1016/j.ophtha.2015.10.053

Pullar CE, Zhao M, Song B, Pu J, Reid B, Ghoghawala S, McCaig C, Isseroff RR (2007) Beta-adrenergic receptor agonists delay while antagonists accelerate epithelial wound healing: evidence of an endogenous adrenergic network within the corneal epithelium. J Cell Physiol 211:261–272. https://doi.org/10.1002/jcp.20934

Putney JW Jr, VandeWalle CM, Leslie BA (1978) Stimulus-secretion coupling in the rat lacrimal gland. Am J Physiol 235:C188–C198. https://doi.org/10.1152/ajpcell.1978.235.5.C188

Qadri A, Cai CL, Deslouches K, Siddiqui F, Aranda JV, Beharry KD (2021) Ocular versus oral propranolol for prevention and/or treatment of oxygen-induced retinopathy in a rat model. J Ocul Pharmacol Ther 37:112–130. https://doi.org/10.1089/jop.2020.0092

Qu L, Zhou Q, Xu Y, Guo Y, Chen X, Yao D, Han GW, Liu Z-J, Stevens RC, Zhong G, Wu D, Zhao S (2019) Structural basis of the diversity of adrenergic receptors. Cell Rep 29:2929–2935. e4. https://doi.org/10.1016/j.celrep.2019.10.088

Quigley HA, Broman AT (2006) The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 90:262–267. https://doi.org/10.1136/bjo.2005.081224

Realini T (2011) A history of glaucoma pharmacology. Optom Vis Sci 88:36–38. https://doi.org/10.1097/OPX.0b013e3182058ead

Reidy JJ, Zarzour J, Thompson HW, Beuerman RW (1994) Effect of topical beta blockers on corneal epithelial wound healing in the rabbit. Br J Ophthalmol 78:377. https://doi.org/10.1136/bjo.78.5.377

Reitsamer HA, Posey M, Kiel JW (2006) Effects of a topical alpha2 adrenergic agonist on ciliary blood flow and aqueous production in rabbits. Exp Eye Res 82:405–415. https://doi.org/10.1016/j.exer.2005.07.015

Resch H, Garhofer G, Fuchsjager-Mayrl G, Hommer A, Schmetterer L (2009) Endothelial dysfunction in glaucoma. Acta Ophthalmol 87:4–12. https://doi.org/10.1111/j.1755-3768.2007.01167.x

Ríos JD, Forde K, Diebold Y, Lightman J, Zieske JD, Dartt DA (2000a) Development of conjunctival goblet cells and their neuroreceptor subtype expression. Invest Ophthalmol Vis Sci 41:2127–2137

Ríos JD, Forde K, Diebold Y, Lightman J, Zieske JD, Dartt DA (2000b) Development of conjunctival goblet cells and their neuroreceptor subtype expression. Invest Ophthalmol Vis Sci 41:2127–2137

Ristori C, Filippi L, Dal Monte M, Martini D, Cammalleri M, Fortunato P, la Marca G, Fiorini P, Bagnoli P (2011) Role of the adrenergic system in a mouse model of oxygen-induced retinopathy: antiangiogenic effects of beta-adrenoreceptor blockade. Invest Ophthalmol Vis Sci 52:155–170. https://doi.org/10.1167/iovs.10-5536

Riva C, Alm A, Pouraras C (2011) Ocular circulation. In: Kaufman P, Alm A, Levin L, Nilsson S, ver Hoeve J, Wu S (eds) Adler's physiology of the eye. Elsevier, Mosby, pp. 243–273

Robinson JC, Kaufman PL (1990) Effects and interactions of epinephrine, norepinephrine, timolol, and betaxolol on outflow facility in the cynomolgus monkey. Am J Ophthalmol 109:189–194. https://doi.org/10.1016/s0002-9394(14)75985-9

Rossetti L, Digiuni M, Montesano G, Centofanti M, Fea AM, Iester M, Frezzotti P, Figus M, Ferreras A, Oddone F, Tanga L, Rolle T, Battaglino V, Posarelli C, Motolese I, Mittica P, Bagaglia SA, Menicacci C, De Cilla S, Autelitano A, Fogagnolo P (2015) Blindness and glaucoma: a multicenter data review from 7 academic eye clinics. PloS One 10:e0136632. https://doi.org/10.1371/journal.pone.0136632

Ruan Y, Jiang S, Musayeva A, Gericke A (2020) Oxidative stress and vascular dysfunction in the retina: therapeutic strategies. Antioxidants 9. https://doi.org/10.3390/antiox9080761

Rudner XL, Berkowitz DE, Booth JV, Funk BL, Cozart KL, D'Amico EB, El-Moalem H, Page SO, Richardson CD, Winters B, Marucci L, Schwinn DA (1999) Subtype specific regulation of human vascular alpha(1)-adrenergic receptors by vessel bed and age. Circulation 100:2336–2343

Safi SZ, Qvist R, Yan GO, Ismail IS (2014) Differential expression and role of hyperglycemia induced oxidative stress in epigenetic regulation of β1, β2 and β3-adrenergic receptors in retinal endothelial cells. BMC Med Genet 7:29. https://doi.org/10.1186/1755-8794-7-29

Sari E, Sari ES, Yazici A, Koç A, Bulbul E, Koytak A, Ermis SS, Erol MK (2015) The effect of systemic tamsulosin hydrochloride on choroidal thickness measured by enhanced depth imaging spectral domain optical coherence tomography. Curr Eye Res 40:1068–1072. https://doi.org/10.3109/02713683.2014.971935

Schmetterer L, Polak K (2001) Role of nitric oxide in the control of ocular blood flow. Prog Retin Eye Res 20:823–847

Schmitt H (1977) The pharmacology of clonidine and related products. In: Gross F (ed) Antihypertensive agents. Springer, Berlin, pp 299–396

Schuster AK, Erb C, Hoffmann EM, Dietlein T, Pfeiffer N (2020) The diagnosis and treatment of glaucoma. Dtsch Arztebl Int 117:225–234. https://doi.org/10.3238/arztebl.2020.0225

Schwinn DA, Afshari NA (2006) α1-adrenergic receptor antagonists and the iris: new mechanistic insights into floppy iris syndrome. Surv Ophthalmol 51:501–512. https://doi.org/10.1016/j.survophthal.2006.06.011

Sercombe R, Verrecchia C, Oudart N, Dimitriadou V, Seylaz J (1985) Pial artery responses to norepinephrine potentiated by endothelium removal. J Cereb Blood Flow Metab 5:312–317. https://doi.org/10.1038/jcbfm.1985.40

Serle JB, Katz LJ, McLaurin E, Heah T, Ramirez-Davis N, Usner DW, Novack GD, Kopczynski CC (2018) Two phase 3 clinical trials comparing the safety and efficacy of netarsudil to timolol in patients with elevated intraocular pressure: Rho kinase elevated IOP treatment trial 1 and 2 (ROCKET-1 and ROCKET-2). Am J Ophthalmol 186:116–127

Sharif NA, Crider JY, Griffin BW, Davis TL, Howe WE (1997) Pharmacological analysis of mast cell mediator and neurotransmitter receptors coupled to adenylate cyclase and phospholipase C on immunocytochemically-defined human conjunctival epithelial cells. J Ocul Pharmacol Ther 13:321–336. https://doi.org/10.1089/jop.1997.13.321

Sharma S, Trikha S, Perera SA, Aung T (2015) Clinical effectiveness of brinzolamide 1%-brimonidine 0.2% fixed combination for primary open-angle glaucoma and ocular hypertension. Clin Ophthalmol 9:2201–2207. https://doi.org/10.2147/opth.S72380

Shen H, Peri KG, Deng XF, Chemtob S, Varma DR (2000) Distribution of alpha1-adrenoceptor subtype proteins in different tissues of neonatal and adult rats. Can J Physiol Pharmacol 78:237–243

Shim MS, Kim KY, Ju WK (2017) Role of cyclic AMP in the eye with glaucoma. BMB Rep 50 (2):60–70. https://doi.org/10.5483/bmbrep.2017.50.2.200

Shiroma LO, Costa VP (2015) 56 – Parasympathomimetics. In: Shaarawy TM, Sherwood MB, Hitchings RA, Crowston JG (eds) Glaucoma 2nd edn. W.B. Saunders, pp. 577–582

Sidjanin DJ, McCarty CA, Patchett R, Smith E, Wilke RA (2008) Pharmacogenetics of ophthalmic topical beta-blockers. Pers Med 5:377–385. https://doi.org/10.2217/17410541.5.4.377

Smith CP, Sharma S, Steinle JJ (2007) Age-related changes in sympathetic neurotransmission in rat retina and choroid. Exp Eye Res 84:75–81. https://doi.org/10.1016/j.exer.2006.08.018

Spada CS, Nieves AL, Burke JA, Wheeler LA, Woodward DF (2001) Differential effects of alpha-adrenoceptor agonists on human retinal microvessel diameter. J Ocul Pharmacol Ther 17:255–277. https://doi.org/10.1089/108076801750295290

Stähle H (2000) A historical perspective: development of clonidine. Best Pract Res Clin Anaesthesiol 14:237–246. https://doi.org/10.1053/bean.2000.0079

Stamer WD, Huang Y, Seftor RE, Svensson SS, Snyder RW, Regan JW (1996) Cultured human trabecular meshwork cells express functional alpha 2A adrenergic receptors. Invest Ophthalmol Vis Sci 37:2426–2433

Steinle JJ (2007) Sympathetic neurotransmission modulates expression of inflammatory markers in the rat retina. Exp Eye Res 84:118–125. https://doi.org/10.1016/j.exer.2006.09.006
Steinle JJ, Smith PG (2002) Role of adrenergic receptors in vascular remodelling of the rat choroid. Br J Pharmacol 136:730–734. https://doi.org/10.1038/sj.bjp.0704771
Steinle JJ, Booz GW, Meininger CJ, Day JN, Granger HJ (2003) β3-adrenergic receptors regulate retinal endothelial cell migration and proliferation. J Biol Chem 278:20681–20686
Steinle JJ, Zamora DO, Rosenbaum JT, Granger HJ (2005) Beta 3-adrenergic receptors mediate choroidal endothelial cell invasion, proliferation, and cell elongation. Exp Eye Res 80:83–91. https://doi.org/10.1016/j.exer.2004.08.015
Steinle JJ, Chin VC, Williams KP, Panjala SR (2008) Beta-adrenergic receptor stimulation modulates iNOS protein levels through p38 and ERK1/2 signaling in human retinal endothelial cells. Exp Eye Res 87:30–34. https://doi.org/10.1016/j.exer.2008.04.008
Stewart RH, Kimbrough RL, Ward RL (1986) Betaxolol vs timolol. A six-month double-blind comparison. Arch Ophthalmol 104:46–48. https://doi.org/10.1001/archopht.1986.01050130056019
Sun D, Huang A, Recchia FA, Cui Y, Messina EJ, Koller A, Kaley G (2001) Nitric oxide-mediated arteriolar dilation after endothelial deformation. Am J Physiol Heart Circ Physiol 280:H714–H721
Suzuki F, Taniguchi T, Nakamura S, Akagi Y, Kubota C, Satoh M, Muramatsu I (2002) Distribution of alpha-1 adrenoceptor subtypes in RNA and protein in rabbit eyes. Br J Pharmacol 135:600–608. https://doi.org/10.1038/sj.bjp.0704503
Szewczykowski C, Mardin C, Lucio M, Wallukat G, Hoffmanns J, Schröder T, Raith F, Rogge L, Heltmann F, Moritz M, Beitlich L, Schottenhamml J, Herrmann M, Harrer T, Ganslmayer M, Kruse FE, Kräter M, Guck J, Lämmer R, Zenkel M, Gießl A, Hohberger B (2022) Long COVID: association of functional autoantibodies against G-protein-coupled receptors with an impaired retinal microcirculation. Int J Mol Sci:23. https://doi.org/10.3390/ijms23137209
Takahashi N, Matsunaga N, Natsume T, Kitazawa C, Itani Y, Hama A, Hayashi I, Shimazawa M, Hara H, Takamatsu H (2021) A longitudinal comparison in cynomolgus macaques of the effect of brimonidine on optic nerve neuropathy using diffusion tensor imaging magnetic resonance imaging and spectral domain optical coherence tomography. Heliyon 7:e06701. https://doi.org/10.1016/j.heliyon.2021.e06701
Tamhane M, Luu KT, Attar M (2021) Ocular pharmacokinetics of brimonidine drug delivery system in monkeys and translational modeling for selection of dose and frequency in clinical trials. J Pharmacol Exp Ther 378:207–214. https://doi.org/10.1124/jpet.120.000483
Tan PP, Yuan HH, Zhu X, Cui YY, Li H, Feng XM, Qiu Y, Chen HZ, Zhou W (2014) Activation of muscarinic receptors protects against retinal neurons damage and optic nerve degeneration in vitro and in vivo models. CNS Neurosci Ther 20:227–236. https://doi.org/10.1111/cns.12187
Tanaka M, Inoue Y, Imai T, Tanida N, Takahashi K, Hara H (2021) Guanabenz and clonidine, α2-adrenergic receptor agonists, inhibit choroidal neovascularization. Curr Neurovasc Res 18:85–92. https://doi.org/10.2174/1567202618666210518133634
Tanihara H, Inoue T, Yamamoto T, Kuwayama Y, Abe H, Suganami H, Araie M, K-115 Clinical Study Group (2015) Intra-ocular pressure-lowering effects of a Rho kinase inhibitor, ripasudil (K-115), over 24 hours in primary open-angle glaucoma and ocular hypertension: a randomized, open-label, crossover study. Acta Ophthalmol 93:e254–e260
Tesfamariam B, Cohen RA (1988) Inhibition of adrenergic vasoconstriction by endothelial cell shear stress. Circ Res 63:720–725
Tham YC, Li X, Wong TY, Quigley HA, Aung T, Cheng CY (2014) Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. Ophthalmology 121:2081–2090. https://doi.org/10.1016/j.ophtha.2014.05.013
Thiel R (1924) Zur Wirkung des Adrenalins auf den Binnendruck des Glaucomauges. Klin Monatsbl f Augenh 72:534
Toda N, Nakanishi-Toda M (2007) Nitric oxide: ocular blood flow, glaucoma, and diabetic retinopathy. Prog Retin Eye Res 26:205–238. https://doi.org/10.1016/j.preteyeres.2007.01.004

Toivanen M, Tervo T, Partanen M, Vannas A, Hervonen A (1987) Histochemical demonstration of adrenergic nerves in the stroma of human cornea. Invest Ophthalmol Vis Sci 28:398–400

Tripathi BJ, Tripathi RC (1984) Effect of epinephrine in vitro on the morphology, phagocytosis, and mitotic activity of human trabecular endothelium. Exp Eye Res 39:731–744. https://doi.org/10.1016/0014-4835(84)90072-1

Trivli A, Koliarakis I, Terzidou C, Goulielmos GN, Siganos CS, Spandidos DA, Dalianis G, Detorakis ET (2019) Normal-tension glaucoma: pathogenesis and genetics. Exp Ther Med 17:563–574. https://doi.org/10.3892/etm.2018.7011

Trope GE, Clark B (1984) Binding potencies of 3 new beta 2 specific blockers to beta receptors in the ciliary processes and the possible relevance of these drugs to intraocular pressure control. Br J Ophthalmol 68:245–247. https://doi.org/10.1136/bjo.68.4.245

Tuttle JL, Falcone JC (2001) Nitric oxide release during alpha1-adrenoceptor-mediated constriction of arterioles. Am J Physiol Heart Circ Physiol 281:H873–H881

Vale J, Gibbs AC, Phillips CI (1972) Topical propranolol and ocular tension in the human. Br J Ophthalmol 56:770–775. https://doi.org/10.1136/bjo.56.10.770

Van Buskirk EM (1980) Adverse reactions from timolol administration. Ophthalmology 87:447–450. https://doi.org/10.1016/s0161-6420(80)35215-9

Van Buskirk EM, Shields MB (1997) 100 years of progress in glaucoma

Vannas M (1927) Klinische Untersuchungen über die Einwirkung des Adrenalins bei Glaukom: mit 8 Texttabellen und 1 graphischen Tabelle im Anhang: akademische Abhandlung, Levin & Munksgaard Publishers

Venkataraman V, Duda T, Galoian K, Sharma RK (1996) Molecular and pharmacological identity of the α2D-adrenergic receptor subtype in bovine retina and its photoreceptors. Mol Cell Biochem 159:129–138. https://doi.org/10.1007/BF00420915

Verrecchia C, Sercombe R, Seylaz J (1985) Influence of endothelium on noradrenaline-induced vasoconstriction in rabbit central ear artery. Clin Exp Pharmacol Physiol 12:169–179

Vidal-Sanz M, Lafuente MAP, Mayor S, de Imperial JM, Villegas-Pérez MAP (2001) Retinal ganglion cell death induced by retinal ischemia: neuroprotective effects of two alpha-2 agonists. Surv Ophthalmol 45:S261–S267. https://doi.org/10.1016/S0039-6257(01)00205-3

Vidovic M, Hill CE (1995) Alpha adrenoceptor gene expression in the rat iris during development and maturity. Brain Res Dev Brain Res 89:309–313. https://doi.org/10.1016/0165-3806(95)00118-w

Vita JA, Treasure CB, Yeung AC, Vekshtein VI, Fantasia GM, Fish RD, Ganz P, Selwyn AP (1992) Patients with evidence of coronary endothelial dysfunction as assessed by acetylcholine infusion demonstrate marked increase in sensitivity to constrictor effects of catecholamines. Circulation 85:1390–1397

Vogel R (1983) Beta-adrenoceptor blocking drugs and the eye. J Clin Pharm Ther 8:209–218. https://doi.org/10.1111/j.1365-2710.1983.tb01052.x

Waisberg E, Micieli JA (2021) Neuro-ophthalmological optic nerve cupping: an overview. Eye Brain 13:255–268. https://doi.org/10.2147/eb.S272343

Walkenbach RJ, Gibbs SR, Bylund DB, Chao W-T (1984) Characteristics of β-adrenergic receptors in bovine corneal epithelium: comparison of fresh tissue and cultured cells. Biochem Biophys Res Commun 121:664–672

Wallukat G (2002) The beta-adrenergic receptors. Herz 27:683–690. https://doi.org/10.1007/s00059-002-2434-z

Wax MB, Molinoff PB (1987) Distribution and properties of beta-adrenergic receptors in human iris-ciliary body. Invest Ophthalmol Vis Sci 28:420–430

Wax MB, Molinoff PB, Alvarado J, Polansky J (1989) Characterization of beta-adrenergic receptors in cultured human trabecular cells and in human trabecular meshwork. Invest Ophthalmol Vis Sci 30:51–57

Weber A (1876) Über die Wirkung des Pilocarpin muriaticum. Centralblatt für die medicinischen Wissenschaften 44:769–772

Weekers R, Prijot E, Gustin J (1954) Recent advances and future prospects in the medical treatment of ocular hypertension. Br J Ophthalmol 38:742

Weekers R, Dflmarcelle Y, Gustin J (1955) Treatment of ocular hypertension by adrenalin and diverse sympathomimetic amines. Am J Ophthalmol 40:666–672

Weigert G, Resch H, Luksch A, Reitsamer HA, Fuchsjager-Mayrl G, Schmetterer L, Garhofer G (2007) Intravenous administration of clonidine reduces intraocular pressure and alters ocular blood flow. Br J Ophthalmol 91:1354–1358. https://doi.org/10.1136/bjo.2007.116574

Weinreb RN, Aung T, Medeiros FA (2014) The pathophysiology and treatment of glaucoma: a review. JAMA 311:1901–1911. https://doi.org/10.1001/jama.2014.3192

Wen R, Cheng T, Li Y, Cao W, Steinberg RH (1996) α_2-adrenergic agonists induce basic fibroblast growth factor expression in photoreceptors – in vivo – and ameliorate light damage. J Neurosci 16:5986–5992. https://doi.org/10.1523/JNEUROSCI.16-19-05986.1996

Wheeler LA, Gil DW, WoldeMussie E (2001) Role of alpha-2 adrenergic receptors in neuroprotection and glaucoma. Surv Ophthalmol 45:S290–S294. https://doi.org/10.1016/S0039-6257(01)00206-5

White RE, Carrier GO (1986) Alpha 1- and alpha 2-adrenoceptor agonist-induced contraction in rat mesenteric artery upon removal of endothelium. Eur J Pharmacol 122:349–352

Wiederholt M, Schäfer R, Wagner U, Lepple-Wienhues A (1996) Contractile response of the isolated trabecular meshwork and ciliary muscle to cholinergic and adrenergic agents. Ger J Ophthalmol 5:146–153

Wikberg-Matsson A (2001) α1- and α2-adrenoceptors in the eye : pharmacological and functional characterization. Doctoral thesis, comprehensive summary, Acta Universitatis Upsaliensis

Wikberg-Matsson A, Simonsen U (2001) Potent α2A-adrenoceptor–mediated vasoconstriction by brimonidine in porcine ciliary arteries. Invest Ophthalmol Vis Sci 42:2049–2055

Wikberg-Matsson A, Wikberg JE, Uhlén S (1996) Characterization of alpha 2-adrenoceptor subtypes in the porcine eye: identification of alpha 2A-adrenoceptors in the choroid, ciliary body and iris, and alpha 2A- and alpha 2C-adrenoceptors in the retina. Exp Eye Res 63:57–66. https://doi.org/10.1006/exer.1996.0091

Wikberg-Matsson A, Uhlén S, Wikberg JE (2000) Characterization of alpha(1)-adrenoceptor subtypes in the eye. Exp Eye Res 70:51–60. https://doi.org/10.1006/exer.1999.0753

Wiley LA, Rupp GR, Steinle JJ (2005) Sympathetic innervation regulates basement membrane thickening and pericyte number in rat retina. Invest Ophthalmol Vis Sci 46:744–748. https://doi.org/10.1167/iovs.04-1023

Wiley LA, Berkowitz BA, Steinle JJ (2006) Superior cervical ganglionectomy induces changes in growth factor expression in the rat retina. Invest Ophthalmol Vis Sci 47:439–443. https://doi.org/10.1167/iovs.05-0656

Wilhelm B, Lüdtke H, Wilhelm H (2006) Efficacy and tolerability of 0.2% brimonidine tartrate for the treatment of acute non-arteritic anterior ischemic optic neuropathy (NAION): a 3-month, double-masked, randomised, placebo-controlled trial

Willets JM, Challiss RAJ, Nahorski SR (2003) Non-visual GRKs: are we seeing the whole picture? Trends Pharmacol Sci 24:626–633. https://doi.org/10.1016/j.tips.2003.10.003

WoldeMussie E, Ruiz G, Wijono M, Wheeler LA (2001) Neuroprotection of retinal ganglion cells by brimonidine in rats with laser-induced chronic ocular hypertension. Invest Ophthalmol Vis Sci 42:2849–2855

Woldemussie E, Wijono M, Pow D (2007) Localization of alpha 2 receptors in ocular tissues. Vis Neurosci 24:745–756. https://doi.org/10.1017/S0952523807070605

Woodward DF, Nieves AL (1985) Topical anti-inflammatory activity of beta 2-adrenoceptor agonists in the conjunctiva. J Ocul Pharmacol 1:391–396. https://doi.org/10.1089/jop.1985.1.391

Yang M, Verfurth F, Buscher R, Michel MC (1997) Is alpha1D-adrenoceptor protein detectable in rat tissues? Naunyn Schmiedebergs Arch Pharmacol 355:438–446

Yang M, Reese J, Cotecchia S, Michel MC (1998) Murine alpha1-adrenoceptor subtypes. I. Radioligand binding studies. J Pharmacol Exp Ther 286:841–847

Ye XD, Laties AM, Stone RA (1990) Peptidergic innervation of the retinal vasculature and optic nerve head. Invest Ophthalmol Vis Sci 31:1731–1737

Ye M, Chen Z, Li M, Chen W, Zhang H, Wang J (2019) Effect of topical application of adrenaline on Schlemm canal, trabecular meshwork and intraocular pressure. Medicine 98:e15558. https://doi.org/10.1097/md.0000000000015558

Yu Y, Koss MC (2003) Functional characterization of alpha-adrenoceptors mediating pupillary dilation in rats. Eur J Pharmacol 471:135–140. https://doi.org/10.1016/s0014-2999(03)01824-7

Yu DY, Alder VA, Cringle SJ, Su EN, Yu PK (1994) Vasoactivity of intraluminal and extraluminal agonists in perfused retinal arteries. Invest Ophthalmol Vis Sci 35:4087–4099

Yu DY, Su EN, Cringle SJ, Yu PK (2003) Isolated preparations of ocular vasculature and their applications in ophthalmic research. Prog Retin Eye Res 22:135–169

Yuan X, Ma X, Yang L, Zhou Q, Li Y (2021) β-Blocker eye drops affect ocular surface through β2 adrenoceptor of corneal limbal stem cells. BMC Ophthalmol 21:419. https://doi.org/10.1186/s12886-021-02186-w

Yun J-H, Jeong H-S, Kim K-J, Han MH, Lee EH, Lee K, Cho C-H (2018) β-Adrenergic receptor agonists attenuate pericyte loss in diabetic retinas through Akt activation. FASEB J 32:2324–2338. https://doi.org/10.1096/fj.201700570RR

Zalewska M, Siara M, Sajewicz W (2014) G protein-coupled receptors: abnormalities in signal transmission, disease states and pharmacotherapy. Acta Pol Pharm 71:229–243

Zarbin MA, Wamsley JK, Palacios JM, Kuhar MJ (1986) Autoradiographic localization of high affinity GABA, benzodiazepine, dopaminergic, adrenergic and muscarinic cholinergic receptors in the rat, monkey and human retina. Brain Res 374:75–92. https://doi.org/10.1016/0006-8993(86)90396-3

Zembowicz A, Hecker M, Macarthur H, Sessa WC, Vane JR (1991) Nitric oxide and another potent vasodilator are formed from NG-hydroxy-L-arginine by cultured endothelial cells. Proc Natl Acad Sci U S A 88:11172–11176

Zhang J, Simpson PC, Jensen BC (2021) Cardiac α1A-adrenergic receptors: emerging protective roles in cardiovascular diseases. Am J Physiol Heart Circ Physiol 320:H725–h733. https://doi.org/10.1152/ajpheart.00621.2020

Zhong H, Minneman KP (1999) Alpha1-adrenoceptor subtypes. Eur J Pharmacol 375:261–276

Zhou TE, Sayah DN, Noueihed B, Mazzaferri J, Costantino S, Brunette I, Chemtob S (2017) Preventing corneal calcification associated with xylazine for longitudinal optical coherence tomography in Young Rodents. Invest Ophthalmol Vis Sci 58:461–469. https://doi.org/10.1167/iovs.16-20526

Zhou X, Zhang T, Wu J (2019) Brimonidine enhances inhibitory postsynaptic activity of OFF- and ON-type retinal ganglion cells in a Wistar rat chronic glaucoma model. Exp Eye Res 189:107833. https://doi.org/10.1016/j.exer.2019.107833

Zimmerman TJ, Kaufman HE (1977) Timolol. A beta-adrenergic blocking agent for the treatment of glaucoma. Arch Ophthalmol 95:601–604. https://doi.org/10.1001/archopht.1977.04450040067008

Adrenoceptors: A Focus on Psychiatric Disorders and Their Treatments

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Abstract

Research into the involvement of adrenoceptor subtypes in the cause(s) of psychiatric disorders is particularly challenging. This is partly because of difficulties in developing animal models that recapitulate the human condition but also because no evidence for any causal links has emerged from studies of patients. These, and other obstacles, are outlined in this chapter. Nevertheless, many drugs that are used to treat psychiatric disorders bind to adrenoceptors to some extent. Direct or indirect modulation of the function of specific adrenoceptor subtypes mediates all or part of the therapeutic actions of drugs in various psychiatric disorders. On the other hand, interactions with central or peripheral adrenoceptors can also explain their side effects. This chapter discusses both aspects of the field, focusing on disorders that are prevalent: depression, schizophrenia, anxiety, attention-deficit hyperactivity disorder, binge-eating disorder, and substance use disorder. In so doing, we highlight some unanswered questions that need to be resolved before it will be feasible to explain how changes in the function of any adrenoceptor subtype affect mood and behavior in humans and other animals.

Keywords

Adrenoceptor subtypes · Anxiety · Attention-deficit hyperactivity disorder · Binge-eating disorder · Cognition · Depression · Neurogenesis · Opiate/opioid withdrawal syndrome · Schizophrenia

Abbreviations

5-CSRT	5-Choice serial reaction-time (test)
ACC	Anterior cingulate cortex
ADHD	Attention-deficit hyperactivity disorder
AN	Anorexia nervosa
BED	Binge-eating disorder
BN	Bulimia nervosa
cAMP	Cyclic adenosine monophosphate
CHO	Chinese hamster ovary
CNS	Central nervous system
DAT	Dopamine reuptake transporter
DMN	Default mode network
EN	Executive network
LTP	Long-term potentiation
NARI	Norepinephrine reuptake inhibitor

NET	Norepinephrine reuptake transporter
NK1	Neurokinin-1 (receptor)
PET	Positron emission tomography
PFC	Prefrontal cortex
SN	Salience network
SNP	Single-nucleotide polymorphism
SSRI	Selective serotonin reuptake inhibitor
vPFC	Ventral prefrontal cortex

1 Introduction

The possibility that norepinephrine might influence brain function directly, rather than merely regulating the intracranial vasculature, was highly controversial until the 1960s (Vogt 1954). However, the development of fluorescence histochemistry enabled mapping of norepinephrine-releasing ("noradrenergic") neurons in the brain, which revealed clusters of their cell bodies in the brainstem and a diffuse distribution of their terminal fibers to nearly all brain regions; the majority of these terminals derive from neurons with cell bodies within the nucleus locus coeruleus (Dahlström and Fuxe 1964; Ungerstedt 1971; Szabadi 2013).

The subsequent development of radioligand binding enabled quantitation of α- and β-adrenoceptors in different regions of the brain, albeit initially using fairly non-selective ligands to study large brain regions of large animals (U'Prichard and Snyder 1977). The discovery of subtypes of α_1- (α_{1A}, α_{1B}, and α_{1D}), α_2- (α_{2A}, α_{2B}, and α_{2C}), and β- (β_1, β_2, and β_3) adrenoceptors then led to a plethora of research that has produced evidence, in progressively finer detail, that has helped to refine our knowledge of the distribution of adrenoceptors in the brain.

The rationale for the classification of these nine subtypes is described, in detail, elsewhere in this volume. Here, we shall discuss the evidence that has informed our understanding of the extent to which they are implicated in the cause(s) of, and/or treatments for, prevalent psychiatric disorders: anxiety, depression, schizophrenia, attention-deficit hyperactivity disorder (ADHD), binge-eating disorder, and opiate/opioid withdrawal syndrome.

2 Experimental Approaches and Their Limitations

As with other neurotransmitter systems, research of the role of adrenoceptors in psychiatric disorders has followed three main approaches. One is to study humans suffering from a disorder and to hunt for biomarkers that could offer clues to its cause. So far, none has come to light as a causal factor for any disorder, but there are many candidates that might increase vulnerability. However, these studies are subject to many potential confounders, including the limitations of what can be

sampled in patients, the influence of any medication, and uncertainty about whether findings relate to the primary disorder or its comorbidities. Additional problems are that the expression of symptoms and signs that are used to diagnose psychiatric disorders can vary between patients, change with time, and, for most disorders, rely on patients' self-reporting of symptoms, which cannot be evaluated objectively.

Another approach is to characterize the pharmacodynamics of drugs that are used to treat the disorder of interest. However, it has to be acknowledged that drug treatments for a disorder might not cure the cause(s) of the illness but might simply recruit different brain mechanisms that mask its consequences. Also, as will become evident below, psychotropic drugs are promiscuous in their binding to adrenoceptors (as well as receptors for other neurotransmitters) and so it has not been possible to attribute dedicated functionality to any adrenoceptor subtype.

A third approach is to develop animal models of the disorder, using experimental interventions, such as a genetic mutation, neuronal lesioning, or drug administration, and to look for parallel changes in the underlying neurobiology and behavioral phenotype. However, there is growing skepticism about the extent to which such experimental interventions can produce animal models that recapitulate the diagnostic criteria for any full-blown human psychiatric disorder. Instead, a more circumspect interpretation of behavioral abnormalities as being plausibly analogous to specific aspects (symptom domains or endophenotypes) of the human disorder is more likely (see Stanford 2017; Stanford 2020; Pratt et al. 2022).

Regarding studies of the different adrenoceptor subtypes, each successive technological development (radioligand binding, immunoblotting, in situ hybridization, etc.) has prompted a new wave of efforts to map their distribution and role in the brain. Although much has been learned from these different approaches, they all have limitations. For instance, almost none of the ligands bind exclusively to only one receptor family, still less one adrenoceptor subtype; their binding to membrane homogenates does not detect intracellular receptors under most experimental conditions; the rate of receptor internalization depends on both the activating ligand and receptor subtype (see Akinaga et al. 2019); and weak affinity and cross-reactivity of binding of antibodies to their target.

The use of in situ hybridization to quantify mRNA for each of the receptor subtypes avoids some of these problems, but mismatches between the intensity of a given mRNA signal and expression of its protein product have been problematic. A notable example is the concentration of mRNA for the β_3-adrenoceptor subtype, which is high in the cortex, hippocampus, and striatum (Summers et al. 1995) but, with the possible exception of cerebellar Purkinje cells (Lippiello et al. 2020), the expression of this subtype has not been detected in the brain (e.g., Sugama et al. 2019). A similar discrepancy has been reported for α_{1D}-adrenoceptors (Yang et al. 1997).

Mapping individual adrenoceptor subtypes in the human brain, using positron emission tomography (PET), is even more challenging on account of the need for safe, selective, high-affinity ligands that cross the blood–brain barrier: many candidates have been tested, but with limited success (Alluri et al. 2020).

All these factors need to be considered when appraising the evidence discussed in the following sections.

3 Adrenoceptor Subtypes in the Brain

3.1 The Distribution of Adrenoceptors in the Brain

Evidence suggests that about 55% of α_1-receptors are the α_{1A}-subtype, 35% are α_{1B}-adrenoceptors, and only 10% are α1D-adrenoceptors (see refs in Perez 2021). Important progress was made by studies of transgenic mice expressing human α_{1A}- or α_{1B}-adrenoceptors (Papay et al. 2004, 2006). Their findings broadly, but not invariably, confirmed those from studies using pre-existing techniques. In respect of their distribution in brain regions of particular interest in research of psychiatric disorders, both subtypes are prominent in the amygdala, but are relatively scarce in the basal ganglia and thalamus (see Table 1). α_{1A}-Adrenoceptors have a high concentration in the hippocampus and brainstem, unlike α_{1B}-adrenoceptors, and are expressed by many different types of neurons, including glutamatergic and GABAergic interneurons (Papay et al. 2006). There have been no equivalent studies for mapping α_{1D}-adrenoceptors, which appear to be almost exclusively intracellular.

No studies to date have used transgenic mice expressing human α_2-subtypes, but the majority are α_{2A}-adrenoceptors, which are ubiquitous in the brain. Between 11 and 44% are α_{2B}-adrenoceptors, most of which are in the cerebellum and thalamus. α_{2C}-Adrenoceptors are mainly in the striatum and hippocampus. The majority of α_{2A}-adrenoceptors are postsynaptic, but α_{2C}-adrenoceptors are more evenly expressed on both pre- and postsynaptic membranes (Erdozain et al. 2019) where they function as autoreceptors, on noradrenergic neurons, and heteroceptors on other neuronal phenotypes, both of which blunt neurotransmitter release (Scheibner et al. 2001).

Using radioligand binding to map the distribution of β-adrenoceptors is problematic because most ligands also bind to 5-HT_{1A}-receptors and their lipophilicity affects their binding. However, evidence suggests that the densities of β_1- and β_2-adrenoceptors are similar in many brain regions, but they differ in respect of their membrane vs. intracellular distribution (Guo and Li 2007). The majority of β_1-adrenoceptors are postsynaptic, but some are presynaptic (Gereau and Conn 1994), expressed by catecholaminergic neurons (Levin and Biegon 1984; Aoki et al. 1989). β_2-Adrenoceptors are mainly in the cerebellum and thalamus. Although no β_3-adrenoceptor binding has been detected in the brain, there is pharmacological evidence that they modulate metabolism in the frontal cortex (Mirbolooki et al. 2015) and the firing rate of noradrenergic neurons in the locus coeruleus (Claustre et al. 2008).

Adrenoceptors are also expressed by glial cells, which is interesting because this will affect neuronal signaling indirectly. There is a good deal of evidence that α_1- and α_2-adrenoceptors on astrocytes promote glutamate uptake, glycogen synthesis, and glucose metabolism (see Hertz et al. 2010; O'Donnell et al. 2012). Some

Table 1 The distribution of adrenoceptor subtypes in the brain and the behavioral abnormalities expressed by subtype-selective knockout mice

Adrenoceptor subtype	Technique	Species	Distribution in the brain	Behavioral phenotype abnormalities (wild-type versus knockout mouse)
α1A	Transgenic mice expressing humanized receptor	Mouse	*High*: amygdala, brainstem, cerebellum, hippocampus, hypothalamus *Moderate*: cerebral cortex, midbrain *Low*: basal ganglia, thalamus (Papay et al. 2006)	• Impaired cognitive performance (Barnes maze) (Doze et al. 2011)
α1B	Transgenic mice expressing humanized receptor	Mouse	*High*: amygdala, cerebral cortex *Moderate*: cerebellum, midbrain, hypothalamus *Low*: brainstem, basal ganglia, hippocampus, thalamus (Papay et al. 2004)	• Reduced locomotor and exploratory activity • Impaired passive avoidance • Diminished response and sensitization to CNS stimulants • Impaired response to reward • Exaggerated response to novel environmental stimuli • Impaired learning in spatial memory task (Spreng et al. 2001; Drouin et al. 2002; Knauber and Müller 2000)
α1D	Radioligand binding to brain membranes	Mouse	No receptor binding detected (Yang et al. 1998; Harasawa et al. 2003)	• Improved performance on rotarod • No change in locomotor activity • Impaired alternation in Y maze • Reduced auditory startle response • Normal spatial learning in the Morris Water Maze • Impaired thermal nociception (Harasawa et al. 2003; Mishima et al. 2004)
	In situ hybridization	Mouse	Amygdala, cerebral cortex, reticular formation, thalamus	
	Immunoblotting	Rat	Receptors detected in the brain (cerebral cortex), but mainly confined to cell cytosol (Shen et al. 2000; Segura et al. 2010)	
	In situ hybridization	Rat	mRNA present in amygdala, cerebral (prefrontal) cortex, hippocampus, olfactory bulb, reticular thalamic nuclei (Day et al. 1997; Santana et al. 2013)	

α2A	Autoradiography	Mouse	Expressed in most brain regions (Holmberg et al. 2003)	• Heightened Pavlovian fear conditioning • No change in contextual memory • No change in learning • Reduced working memory performance • Increased immobility in forced swim test and loss of response to imipramine (Schramm et al. 2001; Franowicz et al. 2002; Davies et al. 2003)
	In situ hybridization	Rat	*High*: brainstem, (deep) cerebellar nuclei, cerebral cortex, locus coeruleus, paraventricular nucleus (hypothalamus), pontine nuclei, reticular nucleus (thalamus) (Nicholas et al. 1993)	
α2B	Radioligand binding	Mouse	Cerebellum, cortex, olfactory bulb, striatum, thalamus (Luhrs et al. 2016)	• Impaired motor habitation in the Open Field • Sensitization of motor response to amphetamine and increased stereotypy • Increased marble-burying (Luhrs et al. 2016)
	In situ hybridization	Rat	Confined to the thalamus (Nicholas et al. 1993)	
α2C	Autoradiography	Mouse	Wide distribution but: *Moderate:* hippocampus, striatum (dorsal and ventral) *Low*: other regions investigated (Holmberg et al. 2003)	• Increased acoustic startle but normal prepulse inhibition • Hyperactivity • Impaired motor habitation in the open field • Increased locomotor response to amphetamine • Impaired performance in delayed alternation task (increased perseveration errors) (Luhrs et al. 2016; Tanila et al. 1999)
	In situ hybridization	Rat	Prominent mRNA expression in many areas including cerebellar cortex, cerebral cortex (especially pyriform cortex), hippocampus, olfactory bulb, striatum, (Nicholas et al. 1993)	
β1	Autoradiography	Mouse	*High:* cerebral cortex, cingulate cortex; hippocampus, thalamus *Moderate*: amygdala, hypothalamus, septum, striatum *Low*: cerebellum, certain thalamic nuclei (Lorton and Davis 1987)	• Impaired contextual fear response (freezing) (Murchison et al. 2011)

(continued)

Table 1 (continued)

Adrenoceptor subtype	Technique	Species	Distribution in the brain	Behavioral phenotype abnormalities (wild-type versus knockout mouse)
β2	Autoradiography	Mouse	*High:* cerebellum, certain thalamic nuclei (Lorton and Davis 1987)	• Cognition impaired in aged mice • Inducible astrocytic receptor deletion • No effect on motor performance (rotarod or swimming) (Jensen et al. 2016)
β3	In situ hybridization	Rat	*High*: cortex, hippocampus, striatum *Moderate:* midbrain, hypothalamus *Low*: brainstem, cerebellum (Summers et al. 1995)	• No effect on motor function • Impaired memory in novel object and social preference discrimination tests (Souza-Braga et al. 2018)

β-adrenoceptors are similarly expressed by glial cells (Hertz et al. 2010; Milner et al. 2000) and promote glycogenolysis (O'Donnell et al. 2012). This has profound implications for neuronal function in the light of the evidence that lactate, produced by astrocytes, influences neuronal signaling (e.g., Magistretti and Allaman 2018). A recent study of mixed cultures of neurons and astrocytes from the mouse cerebral cortex further suggests that β_3-adrenoceptors promote glutathione release from astrocytes, which could have a neuroprotective effect (Yoshioka et al. 2021).

Further interesting possibilities for the complex functional interplay between neuronal and glial adrenoceptors have been discussed elsewhere (e.g., O'Donnell et al. 2012; Wahis and Holt 2021).

3.2 Functional Implications for Psychiatric Disorders of the Regional Distribution of Adrenoceptors

Norepinephrine is only one of a family of catecholamine neurotransmitters. Dopamine, another catecholamine, is prevalent in the brain, but has negligible affinity for α_1-adrenoceptors. However, its affinity for α_{2A}- and α_{2C}-adrenoceptors is similar to that for dopamine D_2 receptors (Sánchez-Soto et al. 2018), which makes it likely that these adrenoceptor subtypes contribute to dopaminergic transmission, especially in brain regions with both a dense dopaminergic innervation and a high density of these subtypes. Such regions include the dorsal striatum (which is one of the few brain regions to lack a noradrenergic innervation) and the (medial prefrontal) cortex. Dopaminergic activation of these subtypes in these brain regions has important implications for the causes of, and treatments for, several psychiatric disorders, such as depression, schizophrenia, and attention-deficit hyperactivity disorder, which are thought to involve abnormal dopaminergic transmission.

There is also evidence for neurons in the brain that express the enzyme, phenylethanolamine-*N*-methyltransferase, especially in the amygdala, hypothalamus, and brainstem regions (Mefford 1988; Howe et al. 1980; Bochorishvili et al. 2014). Although this suggests that these neurons have an epinephrine-releasing phenotype, their release of that catecholamine has not been confirmed. Nevertheless, the affinity of epinephrine for binding to α_{1D}-adrenoceptors is similar to that of norepinephrine and is even higher for binding to the α_{1A}-subtype (Proudman and Baker 2021), which is densely expressed in the brainstem. Likewise, the affinity of epinephrine for binding to α_2-adrenoceptors is similar to that of norepinephrine (Audinot et al. 2002). The functional implications of a role for epinephrine in the brain, mediated by adrenoceptors, merit more consideration.

Another key variable is the location of different receptor subtypes within the brain matrix: those that are close to the norepinephrine release sites (the active zone) will be exposed to higher concentrations of neurotransmitter than receptors that are activated by norepinephrine that has diffused through the extracellular space to remote targets ("volume transmission"; see, for example, Fuxe et al. 2015). As a consequence, low-affinity adrenoceptor antagonists are likely to have a proportionally greater influence on extrasynaptic receptors than on receptors that lie close to the

release sites. Unfortunately, the mapping of the synaptic vs. extrasynaptic location of different adrenoceptor subtypes and the pharmacokinetic modeling of these variables are not sufficiently refined to ascertain how this affects the overall noradrenergic response. Nevertheless, this variable is highly relevant to understanding the contribution of different subtypes to the overall effects of systemic administration of drugs that affect mood and behavior (see below).

In summary, the extent to which different adrenoceptor subtypes are physiologically activated *in vivo* will depend on both their neurotransmitter environment and the extent to which the released neurotransmitter escapes neuronal reuptake to reach extrasynaptic receptors. This will be especially relevant to the actions of psychotropic drugs because the majority, if not all, modify norepinephrine release and/or its reuptake. Because these compounds also bind to adrenoceptors directly, to varying extents, it follows that their overall effects will depend not only on their receptor binding affinity (which is measured *in vitro)* but also on the location of their target receptors, i.e., synaptic or extrasynaptic.

4 Adrenoceptor Subtypes and Behavior

Owing to all the variables discussed above, it is difficult to assign specific roles to each of the adrenoceptor subtypes, on the basis of findings from studies using pharmacological tools. Studies of the effects of how gene knockout for each of the adrenoceptor subtypes affects animals' behavioral phenotype avoid many such confounders and some examples are included in Table 1. Although compensatory adaptive changes could mask the effect of the gene loss of function, that factor is also likely to be the case in humans with genetic mutations that impair the function of the receptor. However, none of the changes in Table 1 link any adrenoceptor subtype with phenotypic abnormalities that could qualify as a model of any psychiatric disorder in humans.

5 Depression and Antidepressants

5.1 Adrenoceptors and Depression

Drawing on observations of the side effects of drugs on the mood of patients being treated for hypertension or tuberculosis, it was inferred that depression was explained by a deficit in catecholamine transmission in the brain, especially that of norepinephrine (Schildkraut 1965). That proposal, which was later refined to include serotonin and rebranded as the "monoamine theory of depression," has been deprecated for decades, mainly because no consistent supporting evidence has emerged, still less a biomarker (e.g., McTavish et al. 2005; Strawbridge et al. 2022).

By contrast, the corollary of the monoamine theory (i.e., drugs that augment noradrenergic neurotransmission are effective antidepressants) is borne out by clinical experience. This is further supported by evidence that norepinephrine

makes a vital contribution to the *relief* of depression in patients who have responded to antidepressants that augment noradrenergic transmission (e.g., Booij et al. 2003).

5.2 Adrenoceptors and Antidepressants

Antidepressants can augment noradrenergic transmission in three different ways. One is to expand the vesicular neurotransmitter store, so that more norepinephrine is released when the neurons are active (as in the case for monoamine oxidase inhibitors). Another is to block neuronal uptake of norepinephrine, which blunts its clearance from the extracellular fluid (as do tricyclic antidepressants and selective norepinephrine reuptake inhibitors). Both these processes will increase activation of adrenoceptors indirectly. The third is to block presynaptic α_2-autoreceptors, which are responsible for feedback inhibition of impulse-evoked neurotransmitter release (see Starke 1977).

However, the lag of several weeks before any beneficial effects of antidepressants become apparent makes it clear that none of these mechanisms explain the therapeutic response, directly. Instead, evidence that prolonged, but not acute, administration of monoamine oxidase inhibitors (see Mobley and Sulser 1981) or tricyclic antidepressants (Banerjee et al. 1977) caused a long-latency downregulation of β-adrenoceptors in the rat brain, and a reduction in the production of their intracellular second messenger, cAMP (cyclic adenosine monophosphate: Vetulani and Sulser 1975; Mishra et al. 1983), suggested an alternative explanation for the therapeutic lag. That tranche of research turned out to be a red-herring, mainly because β-adrenoceptor downregulation was not found after treatment with antidepressants that were developed subsequently, e.g., the selective serotonin reuptake inhibitors ("SSRIs"; Maggi et al. 1980; Mobley and Sulser 1981). Despite exhaustive research on other adrenoceptor subtypes, no changes in any neurotransmitter receptors have been found that are shared by all antidepressants.

5.3 Binding of Antidepressants to Adrenoceptors

As well as increasing the activation of adrenoceptors by norepinephrine, indirectly, almost all antidepressants bind to adrenoceptors directly, albeit to different extents. As discussed below, this binding accounts for some of their side effects and could help to ameliorate certain aspects of depression.

The chance discovery in the 1950s that the dibenzazepine, imipramine, was an effective treatment for depression led to the development of the family of tricyclic antidepressants, which block neuronal reuptake of extracellular norepinephrine and serotonin. However, their binding to α_1-adrenoceptors, as antagonists, attracted attention because this causes orthostatic hypotension, which is one of the problematic side effects of this class of drugs. All tricyclic antidepressants have a K_D of less than 100 nM for binding to α_1-adrenoceptors in homogenates of human brain tissue *postmortem* (Richelson and Nelson 1984). As a consequence, drug development of antidepressants aimed to produce compounds with a lower affinity for binding to this

Table 2 Rank order of affinities for binding of antidepressant ligands to α_1- and α_2-adrenoceptor subtypes for compounds discussed in Sect. 5. Binding affinities were estimated using CHO cells expressing each of the human adrenoceptor subtypes. ">>" indicates a difference in affinity of 10-fold, at least. ">" indicates a difference in affinity of between 3- and 10-fold. [ ] indicates that that there was no measurable, or negligible, binding and so K_D was not estimated. The ranks are based on information provided in full datasets in Audinot et al. (2002), Proudman et al. (2020), Proudman and Baker (2021), Proudman et al. (2022)

Drug class	Compound	Rank	
		α1-adrenoceptors	α2-adrenoceptors
	Epinephrine	α1D > α1A >> α1B	α2C= α2A = α2B
	Norepinephrine	α1D > α1A >> α1B	α2C = α2B = α2A
	Clonidine	α1A > α1B = α1D	α2A = α2B = α2C
	Dexmedetomidine	α1D = α1A > α1B (α1B >> α1D)	α2A = α2B = α2C
Antagonists	Yohimbine Idazoxan	α1C = α1A = α1B α1A > α1D = α1B	α2C = α2A > α2B α2A> α2C= α2B
Tricyclic antidepressants	Amitriptyline	α1A >> α1D = α1B	α2B = α2C > α2A (α1A >> α1B)
	Doxepin	α1A >> α1D = α1B	α2B > α2C = α2A (α1A >> α1B)
	Imipramine	α1A >> α1D = α1B	α2B > α2C > α2A (α2B >> α2A)
Tetracyclic antidepressant	Mirtazapine	α1A = α1D > α1B (α1A >> α1B)	α2C = α2A > α2B
Norepinephrine reuptake inhibitor	Reboxetine	α1A = α1D > α1B	α2C ≥ [α2A/α2B]
Norepinephrine and serotonin reuptake inhibitor	Duloxetine Venlafaxine	α1A = α1D > α1B α1D > α1A > α1B (α1D >> α1B)	α2C = α2A = α2B α2C = α2A [α2B]
Selective serotonin reuptake inhibitors	Citalopram Fluoxetine	α1A >> α1D > α1B α1A > α1D > α1B (α1A >> α1B)	[α2] α2B = α2C = α2A
Serotonin reuptake inhibitors	Vortioxetine Trazodone	α1A > α1D = α1B α1A >α1B = α1D	α2C = α2A = α2B (α2C > α2B) α2C > α2A = α2B

receptor. With the exception of mianserin, maprotiline, and trazodone, that strategy was broadly successful (Richelson and Nelson 1984).

A recent and comprehensive study compared the binding of antidepressants from a wide range of mechanistic categories to all three subtypes of human α_1-adrenoceptors. These were expressed by transfected Chinese hamster ovary (CHO) cells, with each cell line expressing one of the human α_1-adrenoceptor subtypes (Proudman et al. 2020). Importantly, the binding assays were carried out under

conditions that should enable comparisons of the binding parameters across the entire range of compounds and different classes of antidepressants.

That study confirmed that the affinity of binding of tricyclic antidepressants to all α_1-adrenoceptor subtypes was typically considerably higher than that of more recent compounds, apart from mirtazapine, trazodone, and vortioxetine. Reboxetine and venlafaxine showed particularly low affinity for all α_1-adrenoceptor subtypes (Table 2). The full dataset is published in Proudman et al. (2020)

In a different study, Proudman et al. (2022) compared the binding of antidepressants to the three human α_2-adrenoceptor subtypes, using the same CHO expression system as before. The binding affinity of tricyclic antidepressants for all α_2-adrenoceptor subtypes was considerably higher than for the majority of antidepressants that were developed subsequently (e.g., reboxetine and venlafaxine), but mirtazapine and trazodone were again exceptions, together with vortioxetine. Apart from sertraline (K_D in the low μM range), the binding of selective serotonin reuptake inhibitors (SSRIs) to any of the α_2-adrenoceptor subtypes was either low or negligible.

It must be acknowledged that such comparisons do not take into account the possibility that active metabolites of these drugs might have a different binding profile. However, insofar as these two separate studies can be compared (*c.f.,* Proudman et al. 2020, 2022), the binding affinity of the majority of antidepressants to α_1-adrenoceptor subtypes remains considerably higher than for α_2-adrenoceptors, but mirtazapine, duloxetine, and the SSRIs are exceptions.

Despite the lower affinity of antidepressants for α_2-adrenoceptors, it should be borne in mind that antagonism of any α_1-adrenoceptors that are close to the site of release will need much higher concentrations of an antagonist than would blockade of any extrasynaptic α_2-adrenoceptors that are recruited through volume transmission. This is likely to be particularly important for antidepressants because they all increase the concentration of extracellular norepinephrine and so will amplify the activation of extrasynaptic adrenoceptors. For this reason, it should not be assumed that the lower K_D of antidepressants for α_2-adrenoceptors indicates that their interaction with these receptors makes a negligible contribution to the therapeutic response.

Finally, apart from vortioxetine, none of a wide range of antidepressants show any appreciable binding to β_1- or β_2-adrenoceptors (Proudman et al. 2022).

5.4 The Binding Profile of Antidepressants and the Therapeutic Response

No selective adrenoceptor agonist or antagonist is an effective antidepressant but, on the grounds that antagonism of α_2-adrenoceptors blocks feedback inhibition of release of norepinephrine (and other neurotransmitters) and so increases noradrenergic transmission, any antagonism of these receptors by antidepressants could be therapeutically advantageous. Also, α_2-adrenoceptor antagonism would tend to

mask any orthostatic hypotension caused by their α_1-adrenoceptor antagonism (Shibao et al. 2010; Jones et al. 2015).

It is striking that all the tricyclic antidepressants have a higher binding affinity for α_{2B}- than α_{2A}- or α_{2C}-adrenoceptors, especially amitriptyline; only mirtazapine has a lower affinity for this subtype than for other α_2-subtypes. This makes the high density of α_B-adrenoceptors in the thalamus particularly interesting in the light of emerging evidence that adjusting neurotransmission within the lateral habenula (in the epithalamus) is an effective treatment for depression (Webster et al. 2021). Given the evidence that α_{2B}-adrenoceptors have a role in negative emotional processing, which is apparent in depression (Gibbs et al. 2013), the contribution of this receptor subtype to the therapeutic response of antidepressants clearly needs further investigation.

Comparison of the *relative affinities* (selectivity) of antidepressants across all adrenoceptor subtypes, which is a variable that will determine their net effect on noradrenergic transmission, points to another interesting possibility. By preferentially blunting activation of α_1- (and α_2-adrenoceptors to some extent), antidepressants will increase the relative influence of β-adrenoceptors, for which they have negligible affinity. The possibility that such a shift in the receptor profile of noradrenergic transmission is a component of antidepression is interesting because the opposite shift, from activation of β- to α_1-adrenoceptors (i.e., activation of G_s and inhibition of $G_{q/11}$ G protein-coupled receptors), seems to happen after a bout of chronic stress (Stanford 1995). The possibility of such a shift is supported by evidence, albeit controversial, that β-adrenoceptor antagonists can exacerbate depression (Luijendijk et al. 2011; Andrade 2021) and that the newest antidepressant, vortioxetine, increases activation of β-adrenoceptors (Todorović et al. 2022).

In short, the important components of the therapeutic actions of antidepressants could depend on the relative contributions of their effects on neurotransmission mediated by synaptic vs. extrasynaptic adrenoceptors, together with a reduction in the contribution of α_1-adrenoceptor-mediated transmission, *in combination with* an increase in the relative contribution of β-adrenoceptor activation.

6 Schizophrenia and Antipsychotics

6.1 Adrenoceptors and Schizophrenia

The neurotransmitters, dopamine, serotonin, and glutamate, have been investigated extensively for their involvement in schizophrenia, especially in respect of the positive symptoms of this disorder. By contrast, there have been comparatively few studies of noradrenergic transmission in this context. Although there are isolated reports of abnormalities in adrenoceptors in schizophrenic patients, no consistent findings have come to light to suggest that dysfunctional noradrenergic transmission is a causal factor in schizophrenia (e.g., Bennett et al. 1979; Dean 2003; Clark et al. 2006; Brocos-Mosquera et al. 2021). Nevertheless, this does not rule out the

possibility that modifying noradrenergic transmission might be beneficial when treating this disorder.

6.2 Adrenoceptors and Antipsychotics

It should be noted that both the first-generation antipsychotics (phenothiazines) and the tricyclic antidepressants (dibenzazepines) derive from the same parent molecule (promazine) and so it is not surprising that they have similar receptor-binding profiles. This doubtless contributes to the cardiovascular side effects, partly mediated by α_1-adrenoceptor antagonism, which can be problematic with antipsychotics as well as antidepressants.

An early report that chronic administration of antipsychotics caused upregulation of α_1-adrenoceptors suggested that antagonism of these receptors might have a crucial role in the therapeutic response to these drugs (Cohen and Lipinski 1986). This finding was largely ignored for many years. An influential proposal for the involvement of α_1- and α_2-adrenoceptors in the actions of antipsychotics was that antagonism of α-adrenoceptors blunts dopaminergic transmission by neurons that project from the ventral tegmentum to the ventral striatum and so relieves the positive symptoms of schizophrenia. Furthermore, antagonism of α_2-adrenoceptors is thought to disinhibit noradrenergic release by neurons projecting from the locus coeruleus, which augments dopamine release in the prefrontal cortex (PFC) and so relieves the negative symptoms and cognitive deficits of schizophrenia (Svensson 2003). This explanation for the beneficial pharmacology of antipsychotics still prevails.

Interest in these receptors increased following the development of the first of a new class of (atypical) antipsychotics, clozapine. Unlike its predecessors, this compound turned out to have comparatively low D_2 dopamine receptor binding, which hitherto had been thought to explain the efficacy of antipsychotic drugs. In addition to a reduced incidence and severity of extrapyramidal side effects, another notable ("atypical") feature of clozapine was that it was the first antipsychotic to show any appreciable improvement in both Type 2 (negative) symptoms of schizophrenia, which include symptoms that resemble depression, and cognitive deficits (Hagger et al. 1993). This also turned out to be the case with atypical antipsychotics that were developed subsequently. The role of different adrenoceptors in cognition is discussed in Sect. 7.

6.3 Binding of Antipsychotics to Adrenoceptors

Studies of the binding of antipsychotics to a wide range of neurotransmitter receptors in homogenates of human brain tissue *postmortem* revealed that, for compounds that are licensed for use in the UK or USA, their K_D for binding to α_1-adrenoceptors was consistently in the low nM range. However, apart from ziprasidone and zotepine, the binding affinity of atypical antipsychotics for α_1-adrenoceptors was similar to that

Table 3 Rank order of affinities for binding of ligands to α_1- and α_2-adrenoceptor subtypes of compounds discussed in Sect. 6. Binding affinities were estimated using CHO cells expressing each of the human adrenoceptor subtypes. ">>" indicates a difference in affinity of 10-fold, at least. ">" indicates a difference in affinity of between 3- and 10-fold. The ranks are based on information provided in full datasets in Proudman et al. (2020), Proudman et al. (2022)

Drug class	Compound	Rank	
		α1-adrenoceptors	α2-adrenoceptors
First generation	Chlorpromazine	α1A > α1D = α1B (α1A >> α1D)	α2B > α2C = α2A (α2B >> α2A)
	Haloperidol	α1A >α1B > α1D (α1A >> α1D)	α2C = α2B = α2A
Second generation	Clozapine	α1A > α1B > α1D (α1A >> α1D)	α2C > α2B = α2A (α2C >> α2A)
	Lurasidone	α1D = α1A > α1B (α1D > α1B)	α2B = α2C = α2A
	Olanzapine	α1A > α1B = α1D	α2C = α2A = α2B
	Paliperidone	α1A > α1D = α1B (α1A >> α1B)	α2C > α2B = α2A
	Quetiapine	α1A > α1B > α1D (α1A >> α1D)	α2B = α2C > α2A
	Risperidone	α1A > α1B > α1D (α1A >> α1D)	α2A > α2B = α2C
	Ziprasidone	α1A >> α1B > α1D (α1A >> α1D)	α2C = α2B = α2A
Third generation	Aripiprazole	α1A > α1B > α1D (α1A >> α1D)	α2C > α2A = α2B

for α_2-adrenoceptors (i.e., a difference in the K_D of less than 10-fold) (Richelson and Souder 2000).

The binding of antipsychotics to α_1-adrenoceptors was also measured by Proudman et al. (2020), using CHO cells transfected with the human gene for each of the subtypes. These studies confirmed the high affinity of antipsychotics for this subgroup, especially the α_{1A}-subtype. For all licensed compounds, the K_D for binding to these receptors was consistently higher than for α_{1B}-adrenoceptors, but the difference reached the criterion for selectivity (10-fold difference) only with ziprasidone and paliperidone (Table 3). Apart from lurasidone, another consistent finding was that binding to α_{1A}-adrenoceptors was higher than that to the α_{1D}-subtype, but none of the compounds showed any α_{1B}-/α_{1D}-adrenoceptor selectivity (Table 3).

Data from a later study (Proudman et al. 2022) indicate that the K_D for binding of these antipsychotics to α_{2A}-adrenoceptors was in the μM range but, apart from clozapine, none showed any selectivity for any of the α_2-adrenoceptor subtypes (Table 3). These findings broadly confirm an earlier radioligand meta-analysis in which binding affinities were compared with that of haloperidol, as a standard (Minzenberg and Yoon 2011).

As with the antidepressants, neither the first- or second-generation antipsychotics bind appreciably to either the β_1- or β_2-adrenoceptors, but the lead compound for the third generation of antipsychotics, aripiprazole, is an interesting exception (with μM affinity for both subtypes: Proudman et al. 2022); this compound shares the benefits of the atypical antipsychotics on Type 2 symptoms and cognitive impairment, but carries an appreciably lower risk of obesity and its comorbidities.

6.4 The Binding Profile of Antipsychotics and the Therapeutic Response

It is not surprising that the K_Ds for binding of antipsychotics to α_{1A}-adrenoceptors is higher than for other subtypes, as is the case for antidepressants, because they have a similar molecular heritage. However, it is striking that whereas the rank order for antipsychotics is typically $\alpha_{1A} > \alpha_{1B} > \alpha_{1D}$, that for antidepressants is usually $\alpha_{1A} > \alpha_{1D} \geq \alpha_{1B}$. Also, whereas the rank order of binding of antidepressants to α_2-subtypes is not consistent (but often $\alpha_{2C} \geq \alpha_{2A} \geq \alpha_{2B}$), this is not the case with antipsychotics, for which there is little difference in binding to these three subtypes. Whether (and, if so, how) these different rank profiles affect overall noradrenergic transmission in ways that could contribute to the different therapeutic applications of antidepressants and antipsychotics merits consideration.

Although, like the tricyclics, all antipsychotics have cardiovascular side effects, for the "atypicals," these have been eclipsed by the incidence of harmful weight gain and metabolic syndrome (see Heal et al. 2012a, b; Bernardo et al. 2021). There is some evidence that this is associated with polymorphism of α_{2A}- and β_3-adrenoceptor genes, *ADRA2A* and *ADRB3* (Sickert et al. 2009; Zhang et al. 2016, but see Tsai et al. 2004), which would be consistent with evidence that activation of α_{2A}-adrenoceptors inhibits lipolysis, glycolysis, and thermogenesis, whereas activation of β_3-adrenoceptor has the opposite effect. It is tempting to speculate that the exceptional binding of the third-generation (atypical) antipsychotic, aripiprazole, to β-adrenoceptors has some bearing on the lower incidence of weight gain and other metabolic side effects associated with this compound, compared with its predecessors.

Unfortunately, binding of antipsychotics to β_3-adrenoceptors was not included in the Proudman study (Proudman et al. 2022), but this is an obvious candidate for future research. The relative affinities of antipsychotics for α- and β-adrenoceptor subtypes could be an important factor in determining their overall effect on body weight.

7 Adrenoceptors, Neurogenesis, and Cognition in Treatment of Depression and Schizophrenia

Following reports that depression is associated with reduced hippocampal volume (Sheline et al. 1996; Bremner et al. 2000) and that prolonged, but not acute, administration of antidepressants promotes hippocampal neurogenesis in rats (Malberg et al. 2000), this response has been investigated extensively as an explanation for the therapeutic response. However, the functional consequences of an increase in neurogenesis are uncertain. The undisputed role of the hippocampus in cognition gives rise to the possibility that increased neurogenesis helps to resolve the cognitive impairment that is prominent in depression, which has been therapeutically challenging. A recent suggestion is that neurogenesis augments cognitive flexibility and that this has beneficial effects on stress resilience and mood (see Anacker and Hen 2017; Tartt et al. 2022).

However, cognitive impairment is also a prominent feature of schizophrenia. Given the similar chemical provenance of antidepressant and antipsychotic drugs, it is surprising that comparatively little research has focused on the effects of antipsychotics on neurogenesis (but see, for example; Kusumi et al. 2014; Carli et al. 2021), not least because atypical antipsychotics, unlike their predecessors, are noted for their beneficial effects on cognitive impairment in schizophrenia (but see Clissold and Crowe 2019). Isolated preclinical studies have suggested that atypical antipsychotics, like antidepressants, increase hippocampal neurogenesis (e.g., Chikama et al. 2017; Chen and Nasrallah 2019), but there has been little research of the role of adrenoceptors in this response.

Extensive evidence has accumulated to suggest that noradrenergic transmission in the brain influences long-term potentiation (LTP) (Maity et al. 2020), cognition (Perez 2021), focused attention (see Vazey et al. 2018), and neurogenesis (Kulkarni et al. 2002). There is also evidence that neurogenesis is required for expression of the effects of antidepressants in preclinical screens using rats (Santarelli et al. 2003). Furthermore, activation of α_1-adrenoceptors (particularly the α_{1A}-subtype, but not the α_{1B}-subtype (reviewed by Perez 2021) improves cognitive performance and augments LTP and neurogenesis (e.g., Doze et al. 2011). This is interesting because, as discussed above, antidepressants and antipsychotics bind to the former subtype (Tables 1 and 2), but as antagonists, which gives cause to question their contribution to the beneficial effects of antidepressants and antipsychotics on cognition.

A role for α_2-adrenoceptors in the effects of antidepressants and antipsychotics on neurogenesis is also uncertain. There are reports that the α_2-adrenoceptor antagonist, yohimbine, accelerates neurogenesis (Yanpallewar et al. 2010), but another study did not find any change in proliferation of neural precursor cells from the dentate gyrus after treatment with either yohimbine (Jhaveri et al. 2014) or a range of antidepressants (Masuda et al. 2012). Yet another study, using the more selective α_2-adrenoceptor antagonist, idazoxan, concluded that α_2-adrenoceptor activation promotes proliferation (Bortolotto et al. 2021). The explanation for these disparate findings is unknown, but it is possible that antagonism of α_2-adrenoceptors by either antidepressants or antipsychotics promotes neurogenesis.

An early finding was that norepinephrine has a direct effect on proliferation *in vitro* of neurosphere cultures derived from the hippocampus (Jhaveri et al. 2010): this response was attributed to activation of β_3-adrencoceptors on pluripotent neural precursors from the hippocampal subgranular zone and could be replicated by administration of norepinephrine reuptake inhibitors, but not serotonin or SSRIs, *in vivo.* This is an interesting finding because, as noted above, there is little, if any, detectable β_3-adrenoceptor protein in the brain (Sugama et al. 2019), but their mRNA is denser in the hippocampus than elsewhere (Summers et al. 1995). There is also conflicting evidence regarding the effects of other β-adrenoceptor subtypes on neurogenesis: whereas one study found no change (Jhaveri et al. 2010), more recent evidence suggests that activation of β_2-adrenoceptors promotes neurogenesis of adult hippocampal progenitor cells (Masuda et al. 2012; Bortolotto et al. 2019, 2021).

Clearly, more research is needed to improve our understanding of the role of different adrenoceptor subtypes in neurogenesis and whether this response is relevant to the actions of antidepressants and antipsychotics on cognition, or other aspects of depression and schizophrenia.

8 Anxiety

8.1 Norepinephrine and Anxiety

Because some of the symptoms and signs of anxiety resemble the sympathoadrenal stress response, an obvious explanation for the cause of anxiety is excessive noradrenergic transmission in the brain. Evidence apparently supporting that proposal emerged from experiments in which the locus coeruleus in non-human primates was stimulated directly. The behavioral changes that ensued were ethologically similar to those expressed when these animals experience threatening stimuli and so were interpreted as an indication that they were anxious (Redmond and Huang 1979). Despite evidence that direct stimulation of the locus coeruleus in humans caused a sensation of relaxation, not anxiety or even fear, in human subjects (Libet and Gleason 1994), the theory that excessive noradrenergic transmission in the brain causes anxiety has dominated the field ever since (e.g., Morris et al. 2020). However, a complicating factor is that anxiety comprises a family of heterogenous disorders. Although they all share features of an inappropriate stress response (sympathetic hyperarousal), the subjective symptoms and diagnostic criteria differ markedly from one to another, as do their treatment strategies.

8.2 Do Adrenoceptor Agonists and Antagonists Induce or Prevent Anxiety?

There is now a great deal of evidence that undermines the proposal that excessive noradrenergic transmission in the brain causes anxiety. One line of research has been

to study the response of humans who have been given the α_2-adrenoceptor antagonist, yohimbine, which binds with high affinity to all α_2-adrenoceptor subtypes (Proudman et al. 2022). Although this compound causes sympathetic arousal and exacerbates anxiety in patients with a pre-existing anxiety disorder, evidence that this drug induces anxiety in healthy subjects is equivocal (e.g., Charney et al. 1982, 1983). Similarly, the more selective α_2-adrenoceptor antagonist, idazoxan, which binds to all three subtypes, does not induce anxiety in healthy human subjects (Glue et al. 1991), with the possible exception of a transient increase in anxiety after a high dose of this drug (Schmidt et al. 1997). However, in both cases, it should be borne in mind that antagonism of presynaptic α_2-adrenoceptors will not only increase release of norepinephrine (and other neurotransmitters) but will also block transmission mediated by postysynaptic α_2-adrenoceptors and so the net effect of this drug on noradrenergic transmission is hard to predict.

By contrast, α_2-adrenoceptor agonists have established anxiolytic effects, but only at doses that also induce sedation. The α_2-adrenoceptor partial agonist, clonidine, is used to treat anxiety in the context of supervised alcohol and opiate withdrawal: this drug is not a viable for routine treatment of anxiety on account of its profound hypotensive and sedative effects. Both clonidine and the more selective, full agonist, dexmedetomidine are also used preoperatively because, in this context, the sedation is beneficial. A sedative dose of guanfacine has been used off-label to treat anxiety in post-operative critical care (Srour et al. 2018) and an extended-release formulation has been used to treat pediatric anxiety (Strawn et al. 2017).

There is evidence that activation of presynaptic α_2-adrenoceptors, which will blunt norepinephrine release and the firing rate of neurons in the locus coeruleus, contributes to the sedative effects of these drugs (Heal 1990). This is most likely because neurons project from this nucleus to the ventral preoptic area of the hypothalamus, which governs arousal state. However, the extent to which activation of α_2-heteroceptors, which blunt release of the many other transmitters that influence arousal, is unknown.

The proposal that excessive noradrenergic transmission is a cause of anxiety has been used as a rationale for using the β-adrenoceptor antagonists to treat anxiety in humans. It is clear that these compounds blunt the peripheral sympathoadrenal hyperarousal during a stressful experience ("situational anxiety" / "competition nerves": see Anon 1985), which could serve as an interoceptive, anxiogenic cue, but whether or not β-adrenoceptor antagonists prevent the subjective elements of anxiety is controversial and their long-term use is not recommended. For instance, recent metanalyses advise that propranolol should not be used to treat any anxiety disorder on the grounds of lack of clear efficacy (Steenen et al. 2016; Raut et al. 2022)

In summary, there is plenty of evidence that challenges the theory that excessive noradrenergic transmission, mediated by adrenoceptor activation, in the brain causes anxiety. It is also clear that blockade of any adrenoceptor subtype is not an effective strategy for treating this family of disorders. For all these reasons, evidence gathered from preclinical studies in which adrenoceptor ligands have been used to study the neurobiology of anxiety and its treatment should be interpreted with caution.

9 Attention-Deficit Hyperactivity Disorder (ADHD)

Attention-deficit hyperactivity disorder is a common developmental disorder that is characterized by its core symptoms of inattentiveness, distractibility, impulsiveness, and hyperactivity. ADHD is a heterogeneous disorder, but it is currently broadly classified as either "predominantly inattentive" subtype (low level of hyperactivity) or "combined predominantly hyperactive-impulsive" subtype. As a developmental disorder, ADHD should strictly be supported by a diagnosis in childhood (before the age of 7 years). However, because ADHD is considerably under-diagnosed, many cases go unrecognized leading to an ADHD diagnosis later in life.

It was originally believed that ADHD was exclusively a disorder of childhood and adolescence that gradually resolved as individuals reached adulthood. It is now recognized that in many instances ADHD persists in adults. Although the symptoms may reduce in adulthood and be partly mitigated by individuals developing coping strategies for the disorder, persistent ADHD symptoms have a substantial negative impact on the mental health, wellbeing, and life opportunities of adult sufferers. The clinical case for continued treatment has now been accepted, and many ADHD drugs are approved for adults in addition to children and adolescents.

Although it widely believed that an imbalance between noradrenergic and dopaminergic neurotransmission in the PFC plays an important role in the psychopathology of ADHD (Heal and Pierce 2006; Arnsten 2006; Heal et al. 2008, 2009, 2012a, b, 2022), no evidence has so far emerged from brain imaging experiments in humans to suggest that alterations in adrenoceptor density or function are responsible for this imbalance. Yet, despite a lack of any evidence that ADHD can be ascribed to an abnormality in the number or function of any adrenoceptor subtype(s), the α_{2A}-adrenoceptor has been unequivocally implicated in the therapeutic effect of ADHD drugs.

The history of pharmacotherapy in ADHD started with racemic amphetamine in the 1930s, followed by methylphenidate in the 1950s; these drugs are catecholaminergic stimulants. Their powerful effect on dopaminergic neurotransmission has led to a persistent erroneous belief that dopamine is the primary mediator of efficacy in ADHD with norepinephrine relegated to a minor supporting role (Volkow et al. 2012; del Campo et al. 2011, 2013; Aarts et al. 2015). The introduction of the selective norepinephrine reuptake inhibitor (NARI), atomoxetine, in 2002 failed to resolve the matter because it potentiates both noradrenergic and dopaminergic neurotransmission in the PFC (Bymaster et al. 2002), which is the primary site of action for ADHD drugs (Heal et al. 2008, 2009, 2012a, b, 2022; Arnsten 2009; Arnsten and Pliszka 2011; Berridge and Devilbiss 2011). The PFC has highly unusual neuroanatomy with a low density of dopamine reuptake transporter (DAT) sites (Hitri et al. 1991; Sesack et al. 1998). For this reason, a substantial proportion of released dopamine is transported into noradrenergic neurons via norepinephrine reuptake transporters (NET) (Morón et al. 2002; Stahl 2003) and, as a consequence, selective NARIs increase the synaptic concentrations of norepinephrine and dopamine (Bymaster et al. 2002; Yu et al. 2020), thereby potentiating signaling of both catecholamines.

A selective role for norepinephrine, and α_2-adrenoceptors specifically, came to light with the 1985 report by Hunt et al. (1985) of the therapeutic benefit of clonidine in treating children with ADHD. Later, the ability of α_2-adrenoceptor agonists to improve cognitive function was demonstrated in primates by Arnsten and colleagues (1988; Cai et al. 1993), which ultimately led to the conduct of several small, open-label, clinical trials that provided preliminary proof of efficacy for guanfacine in ADHD (reviewed by Arnsten et al. 2007). These initial findings for positive effects of α_{2A}-adrenoceptor agonists on cortical level cognitive function in primates have been replicated in subjects with ADHD (Schulz et al. 2013; Logemann et al. 2013; Bédard et al. 2015). Moreover, when tested in the 5-choice serial reaction-time (5-CSRT) test, the attention deficit of mice with functional ablation of neurokinin-1 receptors (NK1R), which express all core features of ADHD, is ameliorated by low (non-sedative) doses of guanfacine (Pillidge et al. 2014a).

A long-acting formulation of guanfacine (guanfacine-XR) has been shown to reduce ADHD symptoms in pivotal clinical trials in children and adolescents and adults (Biederman et al. 2008; Sallee et al. 2009; Wilens et al. 2012; Iwanami et al. 2020), and it was approved for use in this psychiatric indication in 2010. Although the potential value of clonidine as an ADHD treatment had been reported many years earlier, it was only in approximately 2005 that development of a long-acting formulation of clonidine (clonidine-XR) in ADHD was initiated. The results of these studies have not been published, but the FDA approval of clonidine-XR was supported by efficacy demonstrated in two pivotal trials, one as monotherapy and one as an adjunct to stimulant therapy.

Evidence from animal experiments (Arnsten and Leslie. 1991; Arnsten and Cai. 1993) supports the hypothesis that the α_2-adrenoceptor agonists produce their primary therapeutic effect on ADHD symptoms by activating postsynaptic α_2-adrenoceptors in the PFC. Unlike the NARIs and stimulants that increase synaptic concentrations of both dopamine and norepinephrine in the PFC, the α_2-adrenoceptor agonists actually decrease exocytotic (impulse-dependent) release of both these catecholamines (Gresch et al. 1995; Tanda et al. 1996) via their inhibitory and autoreceptor actions. Nonetheless, the α_2-agonists are unquestionably efficacious in ADHD providing clear evidence that dopamine is not a critical effector of efficacy in ADHD. This point is further illustrated by the moderate efficacy of the DAT inhibitor, bupropion, in ADHD trials (see Heal et al. 2012a, b) and discontinuation of several drug candidates that preferentially enhance dopaminergic neurotransmission (see Heal et al. 2012a, 2012b, 2022).

These findings demonstrate a role for α_2-adrenoceptors as a mediator of efficacy in ADHD, but they do not identify which subtype is responsible. Although there can be no absolute certainty on this point, it is highly likely to be the α_{2A}-subtype because almost all of the key effects of α_2-adrenoceptor agonists in the central nervous system (CNS) (e.g., monoamine turnover, locomotion, sedation, and analgesia) are abolished in animals lacking functional α_{2A}-adrenoceptors (MacMillan et al. 1998; Lähdesmäki et al. 2002, 2003). Also, the affinity of guanfacine for this (human) subtype is higher than that for α_{2B}- or α_{2C}-adrenoceptors (Audinot et al. 2002; Table 4).

Table 4 Alpha-adrenergic receptor subtype profiles of various α_2-adrenoceptor agonists

Receptor	Species	Source	*Ki* (nM)		
			Clonidine	Guanfacine	Lofexidine
α2-Adrenoceptor subtypes					
α2A-adrenoceptor	Human	Cloned	32[a]	50[a]	7.2[b]
α2A-adrenoceptor	Human	Cloned	–	–	4.9[b,c]
α2A-adrenoceptor	Rat	Salivary gland	25[a]	–	–
α2A-adrenoceptor	Mouse	Cloned	–	20[a]	–
α2B-adrenoceptor	Human	Cloned	7.2[a]	>1000[a]	88[b,c]
α2B-adrenoceptor	Human	Cloned	40[a]	–	>1000[c, d]
α2C-adrenoceptor	Human	Cloned	63[a]	>1000[a]	0.9[b,c]
α1-Adrenoceptor subtypes					
α1A-adrenoceptor	Human	Cloned	>300[a]	N.D.	287[b]
α1A-adrenoceptor	Rat	Salivary gland	100[a]	N.D.	–
α1B-adrenoceptor	Human	Cloned	>300[a]	N.D.	45[b]
α1B-adrenoceptor	Rat	Liver	>300[a]	N.D.	–
α1D-adrenoceptor	Human	Cloned	126[a]	N.D.	N.D.

N.D. not determined
Data sources:
[a] *Ki* database (The PDSP Ki Database n.d.; https://pdsp.unc.edu/databases/kidb.php)
[b] FDA Lofexidine Hydrochloride Lucemyra® FDA Multi-disciplinary Evaluation (2017) (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/209229Orig1s000MultidisciplineR.pdf)
[c] EC_{50} determined in a functional assay
[d] Raffa et al. (2019)

One key question with respect to efficacy is whether or not the α_2-adrenoceptor agonists genuinely modulate PFC function to improve cognitive control or merely dampen aberrant behavior as a result of their powerful sedative properties? Huss et al. (2019) addressed this question by stratifying patient populations from pooled trials with guanfacine-XR and showed that efficacy was significantly greater in subjects without sedative side effects than in those with them and, moreover, the drug was equally effective in treating the combined/predominantly hyperactive-impulsive and predominantly inattentive (non-hyperactive) forms of ADHD. Together, these findings clearly support the hypothesis that activation of central α_2-adrenoceptors rectifies the psychopathological symptoms of ADHD.

On the basis of what has been learned about the α_2-adrenoceptor agonists, it is safe to assume that the activation of postsynaptic α_{2A}-adrenoceptors also mediates a substantial part of ADHD effects of the NARIs, atomoxetine and viloxazine, and also the catecholaminergic stimulants, methylphenidate and the amphetamines (*d*-amphetamine, lisdexamfetamine, and enantiomer-mixed salts of amphetamine) (see Fig. 1). The involvement of other adrenergic subtypes in the actions of these indirect agonists is unclear. It has been suggested that activation of β_1- and α_1-adrenoceptors in the PFC impairs cognitive function (Arnsten and Jentsch 1997; Arnsten and Dudley 2005; Arnsten 2006), but the evidence is based on experiments in normal rats and, therefore, has debatable translational relevance to humans with ADHD.

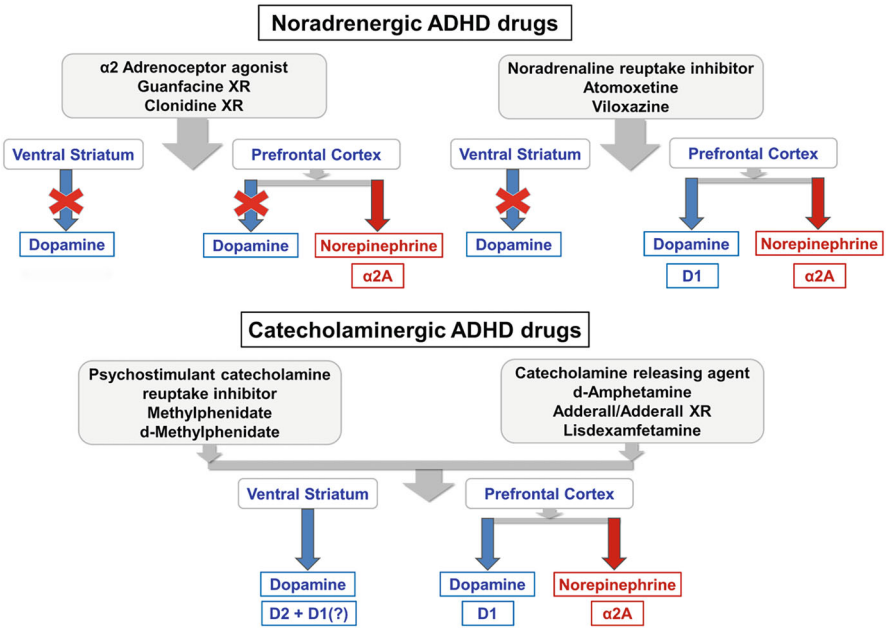

Fig. 1 ADHD drugs – proposed primary and secondary pharmacological mechanisms. In pharmacological terms, ADHD drugs are either norepinephrine-selective or catecholamine (norepinephrine + dopamine)-selective. The sedative ADHD drugs, guanfacine and clonidine, increase noradrenergic transmission via α_{2A}-adrenoceptors. These drugs decrease noradrenergic signaling via other adrenoceptor subtypes and either attenuate or are inactive on dopaminergic neurotransmission. The non-sedative, norepinephrine reuptake inhibitors, atomoxetine and viloxazine, indirectly increase noradrenergic and dopaminergic neurotransmission in the PFC via α_{2A}-adrenergic and D_1 receptors, respectively. They do not potentiate dopaminergic neurotransmission in the striatum or accumbens. The stimulant ADHD drugs, the amphetamines and methylphenidate, indirectly increase noradrenergic and dopaminergic neurotransmission in the PFC via α_{2A}-adrenergic and D_1 receptors, respectively. They also have a secondary therapeutic action to normalize deficits in reward pathways by increasing dopaminergic neurotransmission in the ventral striatum, including the nucleus accumbens, via D_2 and possibly also D_1receptors

Given that the α_2-agonists are considered to be no more effective as ADHD treatments than the NARIs, and to have weaker efficacy than the catecholaminergic stimulants (Taylor and Russo 2001; Bilder et al. 2016), the clinical evidence indicates that non-selective activation of central adrenoceptors has no deleterious outcome, and as discussed later, may contribute to the benefits of atomoxetine.

Atomoxetine, which is a selective NARI, is often considered to be less effective in ADHD than the stimulants, but this opinion is open to debate. For example, a comparison against methylphenidate revealed that although it was superior to atomoxetine in some trials (Kemner et al. 2005; Starr and Kemner 2005), it showed no advantage over atomoxetine in others (Kratochvil et al. 2002; Wang et al. 2007). The picture may also be distorted by the short duration of many ADHD trials, which favors drugs with a rapid trajectory of efficacy. A significant proportion of patients

prescribed atomoxetine have a notably gradual rate of clinical improvement (Sobanski et al. 2015) putting it at a disadvantage in such comparisons. A meta-analysis of trials ≥12-weeks in duration showed no superiority of methylphenidate over atomoxetine (Bushe et al. 2016; Elliott et al. 2020).

Atomoxetine not only differs from the stimulants by virtue of its slower onset of action, but it also maintains efficacy for much longer after discontinuation. Terminating treatment with amphetamine- or methylphenidate-based stimulants results in a rapid relapse to pre-medication status (e.g., Arnold et al. 2004; Brams et al. 2012; Matthijssen et al. 2019). A similarly rapid relapse has also been reported after guanfacine-XR discontinuation (Newcorn et al. 2016). In contrast, efficacy after discontinuing atomoxetine is maintained at high levels for many weeks or months (Michelson et al. 2004; Upadhyaya et al. 2013; Buitelaar et al. 2015; Tanaka et al. 2017). Following 6-month open-label treatment, adults randomized to placebo showed >90% maintenance of efficacy for the following 6 months (Upadhyaya et al. 2013).

NET inhibition by atomoxetine produces sustained activation of all subtypes of adrenoceptor in the brain. Although this pharmacological mechanism generally requires 2–3 months of treatment to achieve maximum efficacy, the benefit is maintained for many months after discontinuation. It raises the intriguing possibility that atomoxetine works through a neuro-adaption mechanism to produce a more permanent resetting of catecholaminergic function in the brain leading to remission in patients for substantial periods. In contrast, the efficacy produced by the stimulants or α_2-agonists is directly driven by the concentration of drug in plasma and brain: i.e., these drugs merely provide daily symptom relief that rapidly dissipates when treatment is discontinued.

Interestingly, despite all blunting reuptake of catecholamines, atomoxetine, methylphenidate, and amphetamine have strikingly different effects on the performance of neurokinin-1 receptor (NK1R) knockout mice in 5-CSRT test. Whereas atomoxetine reduced their excessive expression of premature responses (an index of motor impulsivity), but not inattention or perseveration (Pillidge et al. 2014b), both d-amphetamine and methylphenidate reduced perseveration, but did not reduce inattention or premature responses (Yan et al. 2011; Pillidge et al. 2016; reviewed by Stanford 2022). These findings support the view that direct activation of α_2-adrenoceptors accounts for the beneficial effect of guanfacine on attention but suggest that activation of different adrenoceptor subtypes is needed to effect a reduction in impulsivity and perseveration. The disparate responses to drugs with confirmed efficacy in treating ADHD further suggest that, although all these compounds increase noradrenergic transmission indirectly, they have different effects on each of the core diagnostic elements of ADHD, likely through activation of different combinations of catecholamine receptors.

Although the focus has been on cortical mechanisms, numerous studies have implicated abnormal reward processing in sub-cortical brain regions and dysregulated dopaminergic connectivity with the PFC (Teicher et al. 2000; Paloyelis et al. 2010; Costa Dias et al. 2013) in the psychopathology of ADHD. It is this secondary dopaminergic mechanism which pharmacologically differentiates the

catecholaminergic stimulants from the NARIs and α_2-adrenergic agonists (see Fig. 1).

The α_2-adrenoceptor agonists have also gained a role as adjunctive treatments in ADHD to augment the efficacy of stimulant drugs, particularly in situations when ADHD coexists with other conditions, e.g., oppositional-defiant disorder, autism, and tics. Clonidine-XR and guanfacine-XR are both approved for use in ADHD as either monotherapy or adjunctive therapy with stimulant medications (Clonidine-XR – US Product Label; Intuniv® – US Product Label). These drugs have been clinically evaluated in combination with methylphenidate- or amphetamine-based stimulants in which the combinations were shown to be significantly superior in reducing ADHD severity than treatment with stimulants alone (Wilens et al. 2012; McCracken et al. 2016).

The pharmacological mechanism responsible for the increased efficacy of the α_2-adrenergic agonists + stimulant combination has not been elucidated. Based on our knowledge of the pharmacology of these drugs, the former would be predicted to decrease the exocytotic release of catecholamines in the PFC (Gresch et al. 1995; Tanda et al. 1996; Devoto et al. 2003) and also the release of monoamines in many other regions, including dopamine in the striatum (Devoto et al. 2003; Sood et al. 2012). The result would be to increase α_2-adrenoceptor-mediated transmission in the PFC while simultaneously attenuating the effect of the stimulant on dopaminergic transmission in sub-cortical regions, such as the striatum. A supplementary therapeutic effect derived from activation of α_2-adrenoceptors in other areas modulating the function of the striato-thalamo-cortical pathway also cannot be discounted.

All of the drugs used to treat ADHD illustrated in Fig. 1 are "clean" molecules with no potential to cause side effects due to off-target interactions. Therefore, the pharmacology that delivers efficacy is the same as the one producing side effects and adverse events. From a prescribing perspective, it means that the selection of drug dose in ADHD will often be a balance between optimizing efficacy while maintaining an acceptable level of safety and tolerability.

Side effects and adverse events resulting from activation of α_{2A}-adrenoceptor and other CNS and peripheral adrenergic receptor subtypes stated in the "Warnings and Precautions" sections of the Product Labels include sedation, hypotension and bradycardia, syncope, and rebound hypertension on discontinuation (Clonidine-XR – US Product Label; Intuniv® – US Product Label), all of which are α_{2A}-adrenoceptor-mediated CNS adverse events (MacMillan et al. 1998; Lähdesmäki et al. 2002, 2003). Their impact on patients can be mitigated by staged dose titration. In addition, tolerance to the sedative and cardiovascular effects of the α_{2A}-adrenergic agonists develops within a few weeks, hence the warning about rebound hypertension.

The NARIs activate all adrenoceptor subtypes indirectly and have a spectrum of adverse events that differs from the α_{2A}-adrenergic agonists. They are non-sedative, but their use comes with the risk of hypertension and tachycardia, aggression and hostility, and mania/hypomania, and they carry a Black Box Warning for inducing suicidal ideation (Strattera® – US Product Label; Qelbree® – US Product Label). In addition, atomoxetine carries a specific warning for causing sudden death and

pre-existing structural cardiac abnormalities or other serious heart problems (Strattera® – US Product Label). With the exception of sudden death, these adverse events are consistent with the sympathomimetic effects of the NARIs.

The side-effect profiles of the stimulants reflect their sympathomimetic properties with Warnings and Precautions for hypertension, stroke, and myocardial infarction in adults, and sudden death in children and adolescents (Concerta® [methylphenidate] – US Product Label; Adderall-XR® [mixed enantiomer–mixed salts amphetamine] – US Product Label; Vyvanse® [lisdexamfetamine] – US Product Label). It is their powerful dopaminergic effects that are associated with the emergence of psychotic or manic symptoms, seizures, and the Black Box Warning for drug dependence (Concerta® [methylphenidate] – US Product Label; Adderall-XR® [mixed enantiomer-mixed salts amphetamine] – US Product Label; Vyvanse® [lisdexamfetamine] – US Product Label).

In summary, central adrenoceptors have an important role in mediating the therapeutic effects of drugs used to treat ADHD. Agonism of central α_{2A}-adrenoceptors is, of itself, sufficient to ameliorate the severity of ADHD systems not only for the α_2-adrenergic agonists but also for the NARIs and stimulants. However, caution should be exercised when prescribing these drugs because indirect or indirect activation of these receptors is also responsible for many of their CNS and cardiovascular side effects.

10 Binge-Eating Disorder

Binge-eating disorder (BED) is characterized by loss of control leading to frequent, compulsive episodes of excessive eating (binges). It is now recognized that, like ADHD, BED is an impulse-control disorder (Kessler et al. 2016; Reinblatt. 2015; Ural et al. 2017; Heal and Smith 2022; Heal and Gosden 2022). BED can be differentiated from bulimia nervosa (BN) or anorexia nervosa (AN) because individuals do not indulge in compensatory behavior such as purging, fasting, or excessive exercising. BED is the commonest eating disorder with a lifetime prevalence rate in young individuals >1% vs. 0.3% and ~1% for AN and BN, respectively (Hoek and van Hoeken 2003). Although BED is a predisposing factor for the development of obesity (Goldschmidt et al. 2011; Kessler et al. 2013; Micali et al. 2015), it is a psychiatric disorder, not a metabolic disease, and BED is unresponsive to treatment with appetite suppressants or anti-obesity drugs (Heal and Gosden 2022). The efficacy goal for drug treatment in BED is to enable the individual to regain self-control, to reduce the impulsive, compulsive, and perseverative drive to binge-eat, and to decrease the frequency and severity of binge-eating episodes.

The similarities between BED and ADHD extend to drug treatments where the only two pharmacological interventions to have demonstrated efficacy in pivotal clinical trials are lisdexamfetamine and dasotraline (Heal and Gosden 2022). Both these drugs are effective in ADHD (Heal et al. 2022). In the USA and some other countries, lisdexamfetamine has been approved to treat BED as well as ADHD. After showing efficacy in phase 3 trials in BED and ADHD, development of

dasotraline was discontinued in both indications after the Food and Drug Administration declined to approve it without additional clinical studies to support its safety for human use (Sunovion Press Release 2020).

Allowing female rats repeated, intermittent, limited access to palatable food over a period of weeks induces a binge-eating phenotype that mimics many of the core psychopathological symptoms of BED (Vickers et al. 2015, 2017; Heal et al. 2016, 2017; Heal et al. 2022). The clinically effective drugs, lisdexamfetamine and dasotraline, reduce binge-eating in these rats (Vickers et al. 2015; Heal et al. 2018, Heal and Smith 2022). In addition, lisdexamfetamine has been shown to decrease their compulsive, perseverative, and impulsive responding to the presentation of palatable foods (Heal et al. 2016, Vickers et al. 2017). In translationally valid rat models of BED, single-unit electrophysiological activity recorded in the locus coeruleus showed no differences in spontaneous or tonic activity compared with normal chow-fed controls, but significantly reduced locus coeruleus discharge rates in response to sciatic nerve stimulation (Bello et al. 2019).

In a previous study, Bello et al. (2014) observed that binge-eating rats showed greater neuronal activation in the medial PFC (mPFC) and paraventricular nucleus (PVN) in response to immobilization stress than chow-fed controls. Both studies suggest that the binge-eating phenotype is associated with dysregulation of noradrenergic neurotransmission in the CNS.

Experiments with selective antagonists revealed the reduction of binge-eating produced by lisdexamfetamine was partially reversed by prazosin (α_1-adrenoceptor antagonist) and SCH23390 (a D_1 dopamine receptor antagonist) but was unaffected by RX821002 (α_2-adrenoceptor antagonist) or raclopride (a D_2 dopamine receptor antagonist) (Vickers et al. 2015). Consistent with the non-involvement of α_2-adrenoceptors as efficacy mediators, prolonged administration of guanfacine not only failed to decrease palatable food consumption by binge-eating rats, but it significantly increased it (Bello et al. 2014). The latter effect may be explained by the observation that activation of α_2-adrenoceptors in the PVN stimulates food intake (Wellman 2000).

Ascending fibers from the locus coeruleus innervate the neocortex and thalamus, and dysregulation of the striato-thalamo-cortical pathway regulating cognitive control and reward processing is implicated in both BED and ADHD (see Heal and Smith 2022; Heal et al. 2022). We have reported that the density of D_1 dopamine receptors was substantially decreased, and μ-opioid receptors increased, in the striata of binge-eating rats (Heal et al. 2017). There were no changes in D_1 or μ-opioid receptors in the PFC, or D_1 dopamine receptors nucleus accumbens or D_2 dopamine receptors in the PFC and striatum (Heal et al. 2017). Unfortunately, we could find no published investigations on noradrenergic function or adrenoceptors in the brains of binge-eating rats.

When the totality of non-clinical evidence is considered, it points to BED being linked to a deficit in cognitive control at the PFC level resulting from reduced α_1-adrenergic and D_1 dopamine receptor-mediated neurotransmission. At the sub-cortical level, reward processing deficits due to D_1 and μ-opioid receptors are likely to be an important secondary driver of BED psychopathology.

A number of brain imaging studies have been performed in individuals with BED. Although there are subtle differences between the findings, there is a broad consensus that PFC executive function is significantly attenuated in BED and it exerts diminished control over reward processing at the striatal level which, in turn, is abnormally under-functional (Balodis et al. 2013; Stopyra et al. 2019; Fleck et al. 2019; see reviews by Steward et al. 2018; Heal and Smith 2022). Balodis et al. (2013) conducted fMRI scans on groups of subjects who were performing a monetary reward/loss task. Subject cohorts were BED/obese individuals, obese individuals without BED, and lean controls. Compared with BMI-matched controls, the BED/obese group exhibited a generalized pattern of diminished fronto-striatal processing of both rewards and losses revealing a psychopathology specific to BED that is unrelated to the metabolic condition of obesity.

It is important to emphasize that abnormal brain functioning in BED is not only not linked to obesity but is also different from the psychopathology of BN, which is another binge-related eating disorder. Stopyra et al. (2019) conducted resting-state fMRI experiments to compare functional connectivity in the default mode network (DMN), salience network (SN), and executive network (EN) in groups of subjects with BED, BN, and normal-weight controls. Compared with normal-weight controls, the eating disorder groups showed aberrant functional connectivity in the dorsal anterior cingulate cortex (dACC) within the SN, as well as in the mPFC within the DMN. Within each of these networks, the aberrant functional connectivity differed between the BED and BN groups. The BN group also exhibited stronger synchronous dACC-retrosplenial cortex activity than the BED group.

Having identified the deficits in cognitive control and reward processing in the striato-thalamo-cortical network in individuals with BED, Fleck et al. (2019) took the next logical step to investigate whether lisdexamfetamine alleviated these abnormalities. BED/obese women were treated with lisdexamfetamine for 12 weeks; the obese control group received no pharmacological intervention. fMRI scans focusing on the ventral PFC (vPFC) and striatum were taken at baseline and at the end of treatment. At baseline, the BED/obese women with moderate/severe BED symptoms showed greater activation of the vPFC and globus pallidus than the obese controls when presented with pictures of palatable food.

Lisdexamfetamine, which produced remission from BED in 87% of the subjects, significantly reduced these exaggerated responses. Treatment-associated decreases in binge-eating scores correlated with reductions in vPFC activity, while decreases in obsessive-compulsive symptoms correlated with reductions in thalamus activation. The effect sizes of lisdexamfetamine in different brain regions suggest it exerts a greater influence on cortical control than in sub-cortical regions. The findings indicate that exaggerated vPFC-sub-cortical brain response to palatable foods may be a causal factor in BED, and this abnormality is at least partially prevented by lisdexamfetamine treatment.

The non-clinical and clinical evidence consistently supports the hypothesis that BED is due to deficits of α_1-adrenergic and D_1 signaling in PFC and hypoactive dopaminergic neurotransmission in the striatum. A comprehensive re-evaluation of the results from drug trials in BED revealed the catecholamine reuptake inhibitors

and releasing agents are the only pharmacological classes with clinically proven efficacy in BED; drugs acting on other neurotransmitters were ineffective or showed equivocal efficacy (Heal and Gosden 2022).

To date, the pharmacology of efficacious drugs to treat BED and ADHD is highly specific and almost identical. Lisdexamfetamine (a norepinephrine + dopamine releasing agent) and dasotraline (norepinephrine + dopamine reuptake inhibitor) have been clinically proven to be effective treatments for BED and ADHD (see reviews by Heal and Smith 2022; Heal et al. 2022) but what about other ADHD drugs? The non-clinical evidence predicts that the α_2-adrenoceptor agonists will not be effective BED treatments, but it should be emphasized that there are no clinical data to support that prediction. NARIs would increase α_1-adrenergic and D_1 receptor-mediated signaling in the PFC but would not alleviate diminished dopamine signaling in sub-cortical regions, e.g., the striatum.

Atomoxetine was investigated in a small, double-blind, placebo-controlled trial in subjects with moderate/severe BED (McElroy et al. 2007). The results were confounded by high drop-out rates in both arms (atomoxetine = 30%; placebo = 45%) and a very high placebo response rate. With this *caveat*, the results suggested that atomoxetine reduced binge-eating frequency and severity and decreased Yale-Brown Obsessive-Compulsive scale, modified for Binge-Eating (YBOCS-BE scores). Reboxetine has been investigated in a small open trial in BED where it showed substantial efficacy (Silveira et al. 2005); however, given the high rate of placebo responding in BED trials, this result carries little weight. Solriamfetol (Sunosi®) is a weak micromolar potency dopamine and norepinephrine reuptake inhibitor (IC_{50}s: dopamine = 2.9 μM; norepinephrine = 4.4 μM) (Baladi et al. 2018) that is approved to treat excessive daytime sleepiness associated with narcolepsy or obstructive sleep apnea (Sunosi® – US Product Label). A clinical trial of solriamfetol in BED was planned (Guerdjikova et al. 2021), but at this time no results are available. The preliminary evidence suggests that NARIs might be effective treatments in BED. What also emerges is the efficacy of NARIs appears to be substantially lower than lisdexamfetamine or dasotraline, indicating that the secondary dopaminergic actions of the latter drugs are an important contributor to efficacy.

In summary, adrenoceptors have an important role in the therapeutic actions of drugs used to treat BED. Although catecholaminergic stimulants are effective in both BED and ADHD, in the former, it is the α_1-adrenoceptor rather than the α_2-adrenoceptor that is the mediator of efficacy. Increasing dopaminergic neurotransmission in the PFC is almost certainly therapeutically essential. The ability of drugs to alleviate deficits in striatal dopaminergic neurotransmission appears to be more important in BED than ADHD.

11 Opiate/Opioid Withdrawal and Detoxification

It is an established fact that tolerance rapidly develops to the pharmacological effects of opiates (naturally occurring compounds derived from opium) and opioids (synthetic opioid agonists). Attempts to maintain the pharmacological effects of μ-opioid

receptor agonists can lead to dose escalation by patients when prescribed as analgesics, and by abusers when self-administering these drugs to experience their euphoriant "highs." In both situations, the result is the establishment of opiate/opioid dependence and discontinuing μ-opioid agonists produces a withdrawal syndrome involving craving and physical signs including restlessness, aching, cramps, fever, hyperventilation, hypertension, seizures, and even hallucinations. The physical withdrawal symptoms last for 5–7 days and peak around the second or third day of abstinence. The psychological effects of withdrawal, such as anhedonia, anxiety, and drug craving, can persist for many weeks or months after quitting opiate/opioid abuse.

Experiments in animals and humans have demonstrated that many of these withdrawal symptoms are mediated by hyperactivity of sympathetic drive in the peripheral and central nervous systems (Chang and Dixon 1990; Delle et al. 1990; Milanés et al. 2001; Hoffman et al. 1998; McDonald et al. 1999). α_2-Adrenoceptors are located presynaptically on sympathetic neurons where they regulate neurotransmitter release and postsynaptically in the CNS where they play an important role in emotional and cognitive function and central regulation of blood pressure and heart rate.

Although clonidine and dexmedetomidine have been used off-label to alleviate the severity of withdrawal symptoms, or as an adjunct to opioid antagonist detoxification (Spencer and Gregory 1989; Cuthill et al. 1990; Senft 1991; Upadhyay et al. 2011; Nasr et al. 2011), lofexidine has shown greater therapeutic potential because of its reduced propensity to cause cardiovascular side effects (Kahn et al. 1997; Lin et al. 1997; Carnwath and Hardman 1998; Gerra et al. 2001). In the UK, lofexidine was approved to treat opiate/opioid withdrawal in 1992, but it is no longer marketed in this territory. In the USA, a collaborative development program between US WorldMeds and the National Institute of Drug Abuse (NIDA) led to the approval of lofexidine to mitigate opioid withdrawal symptoms and facilitate abrupt opioid discontinuation in adults (Lucemyra® – US Product Label).

This partnership investigated the efficacy and safety of lofexidine in treating opiate/opioid withdrawal in three multi-site, pivotal, clinical trials (Yu et al. 2008; Gorodetzky et al. 2017; Fishman et al. 2019). The initial trial to study the efficacy and safety of lofexidine on withdrawal symptoms in 68 heavy opiate/opioid abusers was relatively small (Yu et al. 2008). It was followed by two very large trials using the same inclusion/exclusion criteria: the second involving 264 subjects (Gorodetzky et al. 2017) and the third in 603 subjects (Fishman et al. 2019) which evaluated lofexidine at two different doses. Lofexidine met its primary efficacy endpoint in all of these trials and the results consistently demonstrated that lofexidine markedly reduced the withdrawal symptoms at their peak and accelerated their disappearance.

Opiate/opioid withdrawal is an exceptionally unpleasant physical and psychological experience and the very high discontinuation rates are testament to this fact; drop-out rates ranged between 72 and 83% in the placebo groups and 59 and 65% in the lofexidine groups (Yu et al. 2008; Gorodetzky et al. 2017; Fishman et al. 2019). When there are high drop-out rates, the intention to treat/last observation carried

forward (LOCF-ITT) analysis can be misleading and discontinuation for lack of efficacy can provide a more realistic perspective on the efficacy of the intervention. Discontinuations for lack of efficacy in the lofexidine and placebo arms were 15% and 30%, respectively (Yu et al. 2008), and 22% and 33% for the two lofexidine dose groups and 49% for placebo (Fishman et al. 2019).

Overall, lofexidine effectively reduces the severity of opiate/opioid withdrawal symptoms in a significant proportion of opiate/opioid-dependent individuals and is a useful aid to detoxification. The findings also reveal that, despite successfully undergoing detoxification, many individuals relapse to opiate/opioid abuse; this high rate of relapse highlights the negative effect of psychological dependence, which usually persists for months after the physical symptoms of opiate/opioid dependence have resolved.

Lofexidine has also been evaluated as an adjunct to naltrexone therapy to maintain abstinence in a 12-week, placebo-controlled trial in 69 detoxified previously opiate/opioid-dependent subjects (Hermes et al. 2019). Although lofexidine did not meet the co-primary efficacy endpoints of increasing the number of naltrexone treatment-compliant days, opioid craving, or days of opiate/opioid use, the subgroup of lofexidine/naltrexone subjects who completed the trial reported greater naltrexone compliance, and a lower number of positive urine tests than the placebo/naltrexone group. Drop-out rates were ~40% in both groups.

When assessing the efficacy of lofexidine as an aid to detoxification or as an adjunct to abstinence therapy, it is important to appreciate not only the grip of opiate/opioid dependence but also the challenges of other comorbid psychiatric disorders. Using the subjects from the trial by Hermes et al. (2019) as an example, at the time of entry into the trial 15% were suffering anxiety, 2% had PTSD, 20% were cannabis dependent, and 7% alcohol dependent; therefore, any positive outcome in such challenging treatment population should be regarded as a major success.

The adverse cardiovascular (increased blood pressure and heart rate) and physical effects of opiate/opioid withdrawal result from central and peripheral sympathetic hyperactivity. The attenuation of these effects by the α_2-adrenoceptor agonists is most probably mediated by activation of the α_{2A}-adrenoceptor subtype (MacMillan et al. 1998; Lähdesmäki et al. 2002, 2003). The most common adverse effects of lofexidine and clonidine reported in these trials were tiredness/fatigue, lightheadedness/dizziness, and decreased blood pressure and heart rate; these side effects are also mediated by α_{2A}-adrenoceptor agonism (MacMillan et al. 1998; Lähdesmäki et al. 2002, 2003). Therefore, the efficacy and adverse effects of the α_2-adrenoceptor agonists are predominantly mediated by the same pharmacological mechanism.

Lofexidine is a potent agonist of the α_{2A}- and α_{2C}-adrenoceptor subtypes, whereas clonidine is a moderately potent agonist at all three α_2-adrenoceptor subtypes (Table 4). Experiments in mice showed that the ability of clonidine and dexmedetomidine to attenuate naloxone-precipitated morphine withdrawal was absent in the α_{2A}-adrenoceptor knockout genotype (Ozdogan et al. 2004). Other research has revealed that α_{2C}-adrenoceptor agonism has no effect on blood pressure or heart rate (Link et al. 1995), and co-agonism of α_{2A}- and α_{2C}-subtypes produced

greater inhibition of CNS monoamine turnover than selective α_{2A}-adrenoceptor activation (Bücheler et al. 2002). Activation of α_{2B}-adrenoceptors increases blood pressure, which partly counteracts the hypotensive effect of α_{2A}-adrenoceptor agonism (Link et al. 1995). These results are, therefore, difficult to reconcile with the clinical observation that clonidine produced more cardiovascular adverse events than lofexidine. One possible explanation is potent α_{2A}/α_{2C}-adrenoceptor co-agonism by lofexidine leads to profound inhibition of peripheral and central sympathetic drive before its postsynaptic α_{2A}-adrenoceptor-mediated central hypotensive and bradycardic effects reach a problematic level.

In summary, sympathetic hyperactivity is an important driver of opiate/opioid withdrawal symptoms, which can be substantially reduced by administration of α_2-adrenoceptor agonists. In this pharmacological class, lofexidine has the best therapeutic profile. Experimental evidence indicates that its efficacy in mitigating withdrawal symptoms is probably mediated by α_{2A}/α_{2C}-adrenoceptor co-agonism, while its side effects predominantly result from α_{2A}-adrenoceptor agonism.

12 Concluding Remarks

Given that the acceptance of norepinephrine as a neurotransmitter in the CNS was still a subject of debate until the 1960s, there has been dramatic transition over the last 60 years, not only to ascribing a role for this catecholamine in the neurobiology of many psychiatric disorders, but also in the therapeutic actions of drugs used in their treatment.

Adrenoceptors are the molecular effectors of norepinephrine signaling with specific adrenoceptor subtypes in the central and peripheral nervous systems responsible for a wide spectrum of behavioral, emotional, cognitive, and physiological functions. We now know that the most, or all, drugs used in psychiatry interact with adrenoceptors to some extent. Direct or indirect activators of specific adrenoceptor subtypes have been exploited as therapeutic strategies to treat disorders such as depression, ADHD, BED, and opiate/opioid withdrawal. The interaction of drugs with various adrenoceptor subtypes is also a probable contributor to enhancing their therapeutic efficacy and mitigating side effects in other disorders, e.g., schizophrenia and anxiety. However, drug/adrenoceptor interactions are not always beneficial; they are unequivocally implicated as mediators of the cardiovascular and CNS side effects of drugs used in psychiatry and they limit the clinically tolerated doses of others, e.g., α_2-adrenoceptor agonists.

The substantial progress that has been achieved in basic and clinical research on central adrenoceptors has addressed many of these problems. However, the task is not complete, and there are still unanswered questions that need to be resolved before it will be feasible to explain how changes in the function of any adrenoceptor subtype affect mood and behavior in humans and other animals.

References

Aarts E, van Holstein M, Hoogman M et al (2015) Reward modulation of cognitive function in adult attention-deficit/hyperactivity disorder: a pilot study on the role of striatal dopamine. Behav Pharmacol 26:227–240. https://doi.org/10.1097/FBP.0000000000000116

Adderall® US Product Label (n.d.). https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=aff45863-ffe1-4d4f-8acf-c7081512a6c0

Akinaga J, García-Sáinz JA, S Pupo A (2019) Updates in the function and regulation of α_1 -adrenoceptors. Br J Pharmacol 176(14):2343-2357. doi: https://doi.org/10.1111/bph.14617

Alluri SR, Kim SW, Volkow ND, Kil K-E (2020) PET radiotracers for CNS-adrenergic receptors: developments and perspectives. Molecules 25(17):4017. https://doi.org/10.3390/molecules25174017

Anacker C, Hen R (2017) Adult hippocampal neurogenesis and cognitive flexibility – linking memory and mood. Nat Rev Neurosci 18(6):335–346. https://doi.org/10.1038/nrn.2017.45

Andrade C (2021) β-blockers and the risk of new-onset depression: meta-analysis reassures, but the Jury is still out. J Clin Psychiatry 82(3):21f14095. https://doi.org/10.4088/JCP.21f14095

Anon (1985) Beta-blockers in situational anxiety [Editorial]. Lancet 2(8448):193

Aoki C, Zemcik BA, Strader CD, Pickel VM (1989) Cytoplasmic loop of beta-adrenergic receptors: synaptic and intracellular localization and relation to catecholaminergic neurons in the nuclei of the solitary tracts. Brain Res 493(2):331–347. https://doi.org/10.1016/0006-8993(89)91168-2

Arnold LE, Lindsay RL, Conners CK et al (2004) A double-blind, placebo-controlled withdrawal trial of dexmethylphenidate hydrochloride in children with attention deficit hyperactivity disorder. J Child Adolesc Psychopharmacol 14:542–554. https://doi.org/10.1089/cap.2004.14.542

Arnsten AF (2006) Fundamentals of attention-deficit/hyperactivity disorder: circuits and pathways. J Clin Psychiatry 67(Suppl 8):7–12

Arnsten AF (2009) Toward a new understanding of attention-deficit hyperactivity disorder pathophysiology: an important role for prefrontal cortex dysfunction. CNS Drugs 23(Suppl 1):33–41. https://doi.org/10.2165/00023210-200923000-00005

Arnsten AF, Cai JX (1993) Postsynaptic alpha-2 receptor stimulation improves memory in aged monkeys: indirect effects of yohimbine versus direct effects of clonidine. Neurobiol Aging 14(6):597–603. https://doi.org/10.1016/0197-4580(93)90044-c

Arnsten AF, Dudley AG (2005) Methylphenidate improves prefrontal cortical cognitive function through alpha2 adrenoceptor and dopamine D1 receptor actions: relevance to therapeutic effects in attention deficit hyperactivity disorder. Behav Brain Funct 1(1):2. https://doi.org/10.1186/1744-9081-1-2

Arnsten AF, Jentsch JD (1997) The alpha-1 adrenergic agonist, cirazoline, impairs spatial working memory performance in aged monkeys. Pharmacol Biochem Behav 58:55–59. https://doi.org/10.1016/s0091-3057(96)00477-7

Arnsten AF, Leslie FM (1991) Behavioral and receptor binding analysis of the alpha 2-adrenergic agonist, 5-bromo-6 [2-imidazoline-2-yl amino] quinoxaline (UK-14304): evidence for cognitive enhancement at an alpha 2-adrenoceptor subtype. Neuropharmacology 30(12A):1279–1289. https://doi.org/10.1016/0028-3908(91)90024-6

Arnsten AF, Pliszka SR (2011) Catecholamine influences on prefrontal cortical function: relevance to treatment of attention deficit/hyperactivity disorder and related disorders. Pharmacol Biochem Behav 99:211–216. https://doi.org/10.1016/j.pbb.2011.01.020

Arnsten AF, Cai JX, Goldman-Rakic PS (1988) The alpha-2 adrenergic agonist guanfacine improves memory in aged monkeys without sedative or hypotensive side effects: evidence for alpha-2 receptor subtypes. J Neurosci 8:4287–4298. https://doi.org/10.1523/JNEUROSCI.08-11-04287.1988

Arnsten AF, Scahill L, Findling RL (2007) Alpha2-adrenergic receptor agonists for the treatment of attention-deficit/hyperactivity disorder: emerging concepts from new data. J Child Adolesc Psychopharmacol:393–406. https://doi.org/10.1089/cap.2006.0098

Audinot V, Fabry N, Nicolas JP, Beauverger P, Newman-Tancredi A, Millan MJ, Try A, Bornancin F, Canet E, Boutin JA (2002) Ligand modulation of [35S]GTPgammaS binding at human alpha(2A), alpha(2B) and alpha(2C) adrenoceptors. Cell Signal 14(10):829–837. https://doi.org/10.1016/s0898-6568(02)00030-x

Baladi MG, Forster MJ, Gatch MB et al (2018) Characterization of the neurochemical and behavioral effects of solriamfetol (JZP-110), a selective dopamine and norepinephrine reuptake inhibitor. J Pharmacol Exp Ther 366:367–376. https://doi.org/10.1124/jpet.118.248120

Balodis IM, Kober H, Worhunsky PD et al (2013) Monetary reward processing in obese individuals with and without binge eating disorder. Biol Psychiatry 73:877–886. https://doi.org/10.1016/j.biopsych.2013.01.014

Banerjee SP, Kung LS, Riggi SJ, Chanda SK (1977) Development of beta-adrenergic receptor subsensitivity by antidepressants. Nature 268(5619):455–456. https://doi.org/10.1038/268455a0

Bédard AC, Schulz KP, Krone B et al (2015) Neural mechanisms underlying the therapeutic actions of guanfacine treatment in youth with ADHD: a pilot fMRI study. Psychiatry Res 231(3):353–356. https://doi.org/10.1016/j.pscychresns.2015.01.012

Bello NT, Walters AL, Verpeut JL et al (2014) Dietary-induced binge eating increases prefrontal cortex neural activation to restraint stress and increases binge food consumption following chronic guanfacine. Pharmacol Biochem Behav 125:21–28. https://doi.org/10.1016/j.pbb.2014.08.003

Bello NT, Yeh CY, James MH (2019) Reduced sensory-evoked locus coeruleus-norepinephrine neural activity in female rats with a history of dietary-induced binge eating. Front Psychol 10:1966. https://doi.org/10.3389/fpsyg.2019.01966

Bennett JP Jr, Enna SJ, Bylund DB, Gillin JC, Wyatt RJ, Snyder SH (1979) Neurotransmitter receptors in frontal cortex of schizophrenics. Arch Gen Psychiatry 36(9):927–934. https://doi.org/10.1001/archpsyc.1979.01780090013001

Berridge CW, Devilbiss DM (2011) Psychostimulants as cognitive enhancers: the prefrontal cortex, catecholamines, and attention-deficit/hyperactivity disorder. Biol Psychiatry 69:e101–e111. https://doi.org/10.1016/j.biopsych.2010.06.023

Bernardo M, Rico-Villademoros F, García-Rizo C, Rojo R, Gómez-Huelgas R (2021) Real-world data on the adverse metabolic effects of second-generation antipsychotics and their potential determinants in adult patients: a systematic review of population-based studies. Adv Ther 38(5):2491–2512. https://doi.org/10.1007/s12325-021-01689-8

Biederman J, Melmed RD, Patel A et al (2008) SPD503 Study Group. A randomized, double-blind, placebo-controlled study of guanfacine extended release in children and adolescents with attention- deficit/hyperactivity disorder. Pediatrics 121(1):e73–e84. https://doi.org/10.1542/peds.2006-3695

Bilder RM, Loo SK, McGough JJ et al (2016) Cognitive effects of stimulant, guanfacine, and combined treatment in child and adolescent attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 55(8):667–673. https://doi.org/10.1016/j.jaac.2016.05.016

Bochorishvili G, Nguyen T, Coates MB, Viar KE, Stornetta RL, Guyenet PG (2014) The orexinergic neurons receive synaptic input from C1 cells in rats. J Comp Neurol 522(17):3834–3846. https://doi.org/10.1002/cne.23643

Booij L, Van der Does AJ, Riedel WJ (2003) Monoamine depletion in psychiatric and healthy populations: review. Mol Psychiatry 8(12):951–973. https://doi.org/10.1038/sj.mp.4001423

Bortolotto V, Bondi H, Cuccurazzu B, Rinaldi M, Canonico PL, Grilli M (2019) Salmeterol, a β2 adrenergic agonist, promotes adult hippocampal neurogenesis in a region-specific manner. Front Pharmacol 10:1000. https://doi.org/10.3389/fphar.2019.01000

Bortolotto V, Canonico PL, Grilli M (2021) β_2 and α_2 adrenergic receptors mediate the proneurogenic *in vitro* effects of norquetiapine. Neural Regen Res 16(10):2041–2047. Published online 2021 Feb 19. https://doi.org/10.4103/1673-5374.308097

Brams M, Weisler R, Findling RL et al (2012) Maintenance of efficacy of lisdexamfetamine dimesylate in adults with attention-deficit/hyperactivity disorder: randomized withdrawal design. J Clin Psychiatry 73:977–983. https://doi.org/10.4088/JCP.11m07430

Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL, Charney DS (2000) Hippocampal volume reduction in major depression. Am J Psychiatry 157(1):115–118. https://doi.org/10.1176/ajp.157.1.115

Brocos-Mosquera I, Gabilondo AM, Diez-Alarcia R, Muguruza C, Erdozain AM, Meana JJ, Callado LF (2021) α_{2A}- and α_{2C}-adrenoceptor expression and functionality in postmortem prefrontal cortex of schizophrenia subjects. Eur Neuropsychopharmacol 52:3–11. https://doi.org/10.1016/j.euroneuro.2021.05.012

Bücheler MM, Hadamek K, Hein L (2002) Two alpha2-adrenergic receptor subtypes, alpha2A and alpha2C, inhibit transmitter release in the brain of gene-targeted mice. Neuroscience 109(4):819–826. https://doi.org/10.1016/s0306-4522(01)00531-0

Buitelaar J, Asherson P, Soutullo C et al (2015) Differences in maintenance of response upon discontinuation across medication treatments in attention-deficit/hyperactivity disorder. Eur Neuropsychopharmacol 25:1611–1621. https://doi.org/10.1016/j.euroneuro.2015.06.003

Bushe C, Day K, Reed V et al (2016) A network meta-analysis of atomoxetine and osmotic release oral system methylphenidate in the treatment of attention-deficit/hyperactivity disorder in adult patients. J Psychopharmacol 30:444–458. https://doi.org/10.1177/0269881116636105

Bymaster FP, Katner JS, Nelson DL et al (2002) Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: a potential mechanism for efficacy in attention deficit/hyperactivity disorder. Neuropsychopharmacology 27:699–711. https://doi.org/10.1016/S0893-133X(02)00346-9

Cai JX, Ma YY, Xu L et al (1993) Reserpine impairs spatial working memory performance in monkeys: reversal by the alpha 2-adrenergic agonist clonidine. Brain Res 614(1-2):191–196. https://doi.org/10.1016/0006-8993(93)91034-p

Carli M, Aringhieri S, Kolachalam S, Longoni B, Grenno G et al (2021) Is adult hippocampal neurogenesis really relevant for the treatment of psychiatric disorders? Curr Neuropharmacol 19(10):1640–1660. https://doi.org/10.2174/1570159X18666200818194948

Carnwath T, Hardman J (1998) Randomised double-blind comparison of lofexidine and clonidine in the out-patient treatment of opiate withdrawal. Drug Alcohol Depend 50(3):251–254. https://doi.org/10.1016/s0376-8716(98)00040-4

Chang AP, Dixon WR (1990) Role of plasma catecholamines in eliciting cardiovascular changes seen during naloxone-precipitated withdrawal in conscious, unrestrained morphine-dependent rats. J Pharmacol Exp Ther 254(3):857–863

Charney DS, Heninger GR, Sternberg DE (1982) Assessment of alpha 2 adrenergic autoreceptor function in humans: effects of oral yohimbine. Life Sci 30(23):2033–2041. https://doi.org/10.1016/0024-3205(82)90444-1

Charney DS, Heninger GR, Redmond DE (1983) Yohimbine induced anxiety and increased noradrenergic function in humans: effects of diazepam and clonidine. Life Sci 33(1):19–29. https://doi.org/10.1016/0024-3205(83)90707-5

Chen AT, Nasrallah HA (2019) Neuroprotective effects of the second generation antipsychotics. Schizophr Res 208:1–7. https://doi.org/10.1016/j.schres.2019.04.009

Chikama K, Yamada H, Tsukamoto T, Kajitani K, Nakabeppu Y, Uchimura N (2017) Chronic atypical antipsychotics, but not haloperidol, increase neurogenesis in the hippocampus of adult mouse. Brain Res 1676:77–82. https://doi.org/10.1016/j.brainres.2017.09.006

Clark DA, Mancama D, Kerwin RW, Arranz MJ (2006) Expression of the alpha1A-adrenergic receptor in schizophrenia. Neurosci Lett 401(3):248–251. https://doi.org/10.1016/j.neulet.2006.03.025

Claustre Y, Leonetti M, Santucci V, Bougault I, Desvignes C, Rouquier L, Aubin N, Keane P, Busch S, Chen Y, Palejwala V, Tocci M, Yamdagni P, Didier M, Avenet P, Le Fur G, Oury-Donat F, Scatton B, Steinberg R (2008) Effects of the beta3-adrenoceptor (Adrb3) agonist SR58611A (amibegron) on serotonergic and noradrenergic transmission in the rodent: relevance

to its antidepressant/anxiolytic-like profile. Neuroscience 156(2):353–364. https://doi.org/10.1016/j.neuroscience.2008.07.011

Clissold M, Crowe SF (2019) Comparing the effect of the subcategories of atypical antipsychotic medications on cognition in schizophrenia using a meta-analytic approach. J Clin Exp Neuropsychol 41(1):26–42. https://doi.org/10.1080/13803395.2018.1488952

Clonidine-XR – US Product Label (n.d.). https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=152bf066-722d-4a26-83c4-69f77dfbd432

Cohen BM, Lipinski JF (1986) In vivo potencies of antipsychotic drugs in blocking alpha 1 noradrenergic and dopamine D2 receptors: implications for drug mechanisms of action. Life Sci 39(26):2571–2580. https://doi.org/10.1016/0024-3205(86)90111-6

Concerta® US Product Label (n.d.). https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=1a88218c-5b18-4220-8f56-526de1a276cd

Costa Dias TG, Wilson VB, Bathula DR et al (2013) Reward circuit connectivity relates to delay discounting in children with attention-deficit/hyperactivity disorder. Eur Neuropsychopharmacol 23:33–45. https://doi.org/10.1016/j.euroneuro.2012.10.015

Cuthill JD, Beroniade V, Salvatori VA et al (1990) Evaluation of clonidine suppression of opiate withdrawal reactions: a multidisciplinary approach. Can J Psychiatry 5:377–382. https://doi.org/10.1177/070674379003500502

Dahlström A, Fuxe K (1964) Localization of monoamines in the lower brain stem. Experientia 20(7):398–399. https://doi.org/10.1007/BF02147990

Davies MF, Tsui JY, Flannery JA, Li X, DeLorey TM, Hoffman BB (2003) Augmentation of the noradrenergic system in alpha-2 adrenergic receptor deficient mice: anatomical changes associated with enhanced fear memory. Brain Res 986(1–2):157–165. https://doi.org/10.1016/s0006-8993(03)03248-7

Day HE, Campeau S, Watson SJ, Akil H (1997) Distribution of α1a-, α1b- and α1d-adrenergic receptor mRNA in the rat brain and spinal cord. J Chem Neuroanatomy 13:115–139. https://doi.org/10.1016/s0891-0618(97)00042-2

Dean B (2003) [3H]RX 821002 in human dorsolateral prefrontal cortex: no changes in postmortem tissue from subjects with schizophrenia. Psychiatry Res 119(1–2):25–31. https://doi.org/10.1016/s0165-1781(03)00108-2

del Campo N, Chamberlain SR, Sahakian BJ et al (2011) The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. Biol Psychiatry 69(12):e145–e157. https://doi.org/10.1016/j.biopsych.2011.02.036

del Campo N, Fryer TD, Hong YT et al (2013) A positron emission tomography study of nigro-striatal dopaminergic mechanisms underlying attention: implications for ADHD and its treatment. Brain 136(Pt 11):3252–3270. https://doi.org/10.1093/brain/awt263

Delle M, Ricksten SE, Häggendal J et al (1990) Regional changes in sympathetic nerve activity and baroreceptor reflex function and arterial plasma levels of catecholamines, renin and vasopressin during naloxone-precipitated morphine withdrawal in rats. J Pharmacol Exp Ther 253(2):646–654

Devoto P, Flore G, Longu G et al (2003) Origin of extracellular dopamine from dopamine and noradrenaline neurons in the medial prefrontal and occipital cortex. Synapse 50(3):200–205. https://doi.org/10.1002/syn.10264

Doze VA, Papay RS, Goldenstein BL, Gupta MK et al (2011) Long-term α1A-adrenergic receptor stimulation improves synaptic plasticity, cognitive function, mood, and longevity. Mol Pharmacol 80(4):747–758. https://doi.org/10.1124/mol.111.073734

Drouin C, Darracq L, Trovero F, Blanc G, Glowinski J, Cotecchia S, Tassin J-P (2002) Alpha1b-adrenergic receptors control locomotor and rewarding effects of psychostimulants and opiates. J Neurosci 22(7):2873–2884. https://doi.org/10.1523/JNEUROSCI.22-07-02873.2002

Elliott J, Johnston A, Husereau D et al (2020) Pharmacologic treatment of attention deficit hyperactivity disorder in adults: a systematic review and network meta-analysis. PloS One 15(10):e0240584. https://doi.org/10.1371/journal.pone.0240584

Erdozain AM, Brocos-Mosquera I, Gabilondo AM, Meana JJ, Callado LF (2019) Differential α_{2A}- and α_{2C}-adrenoceptor protein expression in presynaptic and postsynaptic density fractions of postmortem human prefrontal cortex. J Psychopharmacol 33(2):244–249. https://doi.org/10.1177/0269881118798612

FDA Lofexidine FDA Multi-disciplinary Review (2017). https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/209229Orig1s000MultidisciplineR.pdf

Fishman M, Tirado C, Alam D, CLEEN-SLATE Team et al (2019) Safety and efficacy of lofexidine for medically managed opioid withdrawal: a randomized controlled clinical trial. J Addict Med 13(3):169–176. https://doi.org/10.1097/ADM.0000000000000474

Fleck DE, Eliassen JC, Guerdjikova AI et al (2019) Effect of lisdexamfetamine on emotional network brain dysfunction in binge eating disorder. Psychiatry Res Neuroimaging 286:53–59. https://doi.org/10.1016/j.pscychresns.2019.03.003

Franowicz JS, Kessler LE, Borja CM, Kobilka BK, Limbird LE, Arnsten AF (2002) Mutation of the alpha2A-adrenoceptor impairs working memory performance and annuls cognitive enhancement by guanfacine. J Neurosci 22(19):8771–8777. https://doi.org/10.1523/JNEUROSCI.22-19-08771.2002

Fuxe K, Agnati LF, Marcoli M, Borroto-Escuela DO (2015) Volume transmission in central dopamine and noradrenaline neurons and its astroglial targets. Neurochem Res 40(12):2600–2614. https://doi.org/10.1007/s11064-015-1574-5

Gereau RW, Conn PJ (1994) Presynaptic enhancement of excitatory synaptic transmission by beta-adrenergic receptor activation. J Neurophysiol 72(3):1438–1442. https://doi.org/10.1152/jn.1994.72.3.1438

Gerra G, Zaimovic A, Giusti F et al (2001) Lofexidine versus clonidine in rapid opiate detoxification. J Subst Abuse Treat 21(1):11–17. https://doi.org/10.1016/s0740-5472(01)00178-7

Gibbs AA, Bautista CE, Mowlem FD, Naudts KH, Duka T (2013) Alpha 2B adrenoceptor genotype moderates effect of reboxetine on negative emotional memory bias in healthy volunteers. J Neurosci 33(43):17023–17028. https://doi.org/10.1523/JNEUROSCI.2124-13.2013

Glue P, Wilson S, Lawson C, Campling GM, Franklin M, Cowen PJ, Nutt DJ (1991) Acute and chronic idazoxan in normal volunteers: biochemical, physiological and psychological effects. J Psychopharmacol 5(4):396–403. https://doi.org/10.1177/026988119100500434

Goldschmidt AB, Le Grange D, Powers P et al (2011) Eating disorder symptomatology in normal-weight vs. obese individuals with binge-eating disorder. Obesity 19:1515–1518. https://doi.org/10.1038/oby.2011.24

Gorodetzky CW, Walsh SL, Martin PR et al (2017) A phase III, randomized, multi-center, double blind, placebo-controlled study of safety and efficacy of lofexidine for relief of symptoms in individuals undergoing inpatient opioid withdrawal. Drug Alcohol Depend 176:79–88. https://doi.org/10.1016/j.drugalcdep.2017.02.020

Gresch PJ, Sved AF, Zigmond MJ et al (1995) Local influence of endogenous norepinephrine on extracellular dopamine in rat medial prefrontal cortex. J Neurochem 65:111–116. https://doi.org/10.1046/j.1471-4159.1995.65010111.x

Guerdjikova AI, Romo-Nava F, Blom TJ et al (2021) Study protocol and rationale for a randomized, placebo-controlled trial of solriamfetol to treat binge eating disorder. Contemp Clin Trials 110:106587. https://doi.org/10.1016/j.cct.2021.106587

Guo N-N, Li B-M (2007) Cellular and subcellular distributions of beta1- and beta2-adrenoceptors in the CA1 and CA3 regions of the rat hippocampus. Neuroscience 146(1):298–305. https://doi.org/10.1016/j.neuroscience.2007.01.013

Hagger C, Buckley P, Kenny JT, Friedman L, Ubogy D, Meltzer HY (1993) Improvement in cognitive functions and psychiatric symptoms in treatment-refractory schizophrenic patients receiving clozapine. Biol Psychiatry 34(10):702–712. https://doi.org/10.1016/0006-3223(93)90043-d

Harasawa I, Honda K, Tanoue A, Shinoura H, Ishida Y, Okamura H, Murao N, Tsujimoto G, Higa K, Kamiya HO, Takano Y (2003) Responses to noxious stimuli in mice lacking alpha(1d)-

adrenergic receptors. Neuroreport 14(14):1857–1860. https://doi.org/10.1097/00001756-200310060-00020

Heal DJ (1990) The effects of drugs on behavioural models of central noradrenergic function. In: Heal DJ, Marsden CA (eds) The pharmacology of noradrenaline in the central nervous system. Oxford University Press, Oxford, pp 266–315

Heal DJ, Gosden J (2022) What pharmacological interventions are effective in binge-eating disorder? Insights from a critical evaluation of the evidence from clinical trials. Int J Obes (Lond) 46:677–695. https://doi.org/10.1038/s41366-021-01032-9

Heal DJ, Pierce DM (2006) Methylphenidate and its isomers: their role in the treatment of attention-deficit hyperactivity disorder using a transdermal delivery system. CNS Drugs 20:713–738. https://doi.org/10.2165/00023210-200620090-00002

Heal DJ, Smith SL (2022) Prospects for new drugs to treat binge-eating disorder: insights from psychopathology and neuropharmacology. J Psychopharmacol 36:680–703. https://doi.org/10.1177/02698811211032475

Heal DJ, Smith SL, Kulkarni RS et al (2008) New perspectives from microdialysis studies in freely-moving, spontaneously hypertensive rats on the pharmacology of drugs for the treatment of ADHD. Pharmacol Biochem Behav 90:184–197. https://doi.org/10.1016/j.pbb.2008.03.016

Heal DJ, Cheetham SC, Smith SL (2009) The neuropharmacology of ADHD drugs in vivo: insights on efficacy and safety. Neuropharmacology 57:608–618. https://doi.org/10.1016/j.neuropharm.2009.08.020

Heal DJ, Gosden J, Jackson HC et al (2012a) Metabolic consequences of antipsychotic therapy: preclinical and clinical perspectives on diabetes, diabetic ketoacidosis, and obesity. In: Gross G, Geyer MA (eds) Current antipsychotics, handbook of experimental pharmacology, p 212. https://doi.org/10.1007/978-3-642-25761-2_6

Heal DJ, Smith SL, Findling RL (2012b) ADHD: current and future therapeutics. Curr Top Behav Neurosci 9:361–390. https://doi.org/10.1007/7854_2011_125

Heal DJ, Goddard S, Brammer RJ et al (2016) Lisdexamfetamine reduces the compulsive and perseverative behaviour of binge-eating rats in a novel food reward/punished responding conflict model. J Psychopharmacol 30:662–675. https://doi.org/10.1177/0269881116647506

Heal DJ, Hallam M, Prow M et al (2017) Dopamine and μ-opioid receptor dysregulation in the brains of binge-eating female rats – possible relevance in the psychopathology and treatment of binge-eating disorder. J Psychopharmacol 31:770–783. https://doi.org/10.1177/0269881117699607

Heal DJ, Vickers SP, Hopkins SC et al (2018) Investigation of the effects of dasotraline in a validated rat model of binge-eating disorder. Proceedings of the 57th annual meeting of the American College of Neuropsychopharmacology (ACNP), Dec 9–13, 2018, Hollywood, FL, USA. Poster presentation

Heal DJ, Gosden J, Smith SL (2022) New drugs to treat ADHD: opportunities and challenges in research and development. Curr Top Behav Neurosci 57:79–126. https://doi.org/10.1007/7854_2022_332

Hermes G, Hyman SM, Fogelman N et al (2019) Lofexidine in combination with oral naltrexone for opioid use disorder relapse prevention: a pilot randomized, double-blind, placebo-controlled study. Am J Addict 28(6):480–488. https://doi.org/10.1111/ajad.12942

Hertz L, Lovatt D, Goldman SA, Nedergaard M (2010) Adrenoceptors in brain: cellular gene expression and effects on astrocytic metabolism and [Ca(2+)]. Neurochem Int 57(4):411–420. https://doi.org/10.1016/j.neuint.2010.03.019

Hitri A, Venable D, Nguyen HQ et al (1991) Characteristics of [3H]GBR 12935 binding in the human and rat frontal cortex. J Neurochem 56:1663–1672. https://doi.org/10.1111/j.1471-4159.1991.tb02065.x

Hoek HW, van Hoeken D (2003) Review of the prevalence and incidence of eating disorders. Int J Eat Disord 34:383–396. https://doi.org/10.1002/eat.10222

Hoffman WE, McDonald T, Berkowitz R (1998) Simultaneous increases in respiration and sympathetic function during opiate detoxification. J Neurosurg Anesthesiol 10(4):205–210. https://doi.org/10.1097/00008506-199810000-00001

Holmberg M, Fagerholm V, Scheinin M (2003) Regional distribution of alpha(2C)-adrenoceptors in brain and spinal cord of control mice and transgenic mice overexpressing the alpha(2C)-subtype: an autoradiographic study with [(3)H]RX821002 and [(3)H]rauwolscine. Neuroscience 117(4):875–898. https://doi.org/10.1016/s0306-4522(02)00966-1

Howe PR, Costa M, Furness JB, Chalmers JP (1980) Simultaneous demonstration of phenylethanolamine N-methyltransferase immunofluorescent and catecholamine fluorescent nerve cell bodies in the rat medulla oblongata. Neuroscience 5(12):2229–2238. https://doi.org/10.1016/0306-4522(80)90139-6

Hunt RD, Minderaa RB, Cohen DJ (1985) Clonidine benefits children with attention deficit disorder and hyperactivity: report of a double-blind placebo-crossover therapeutic trial. J Am Acad Child Psychiatry 24(5):617–629. https://doi.org/10.1016/s0002-7138(09)60065-0

Huss M, McBurnett K, Cutler AJ et al (2019) Distinguishing the efficacy and sedative effects of guanfacine extended release in children and adolescents with attention-deficit/hyperactivity disorder. Eur Neuropsychopharmacol 29:432–443. https://doi.org/10.1016/j.euroneuro.2018.05.012

Intuniv® – US Product Label (n.d.). https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=1bb3fac5-11e8-4d1c-a5e4-2520abcdeb47

Iwanami A, Saito K, Fujiwara M et al (2020) Efficacy and safety of guanfacine extended-release in the treatment of attention-deficit/hyperactivity disorder in adults: results of a randomized, double-blind, placebo-controlled study. J Clin Psychiatry 81(3):19m12979. https://doi.org/10.4088/JCP.19m1297

Jensen CJ, Demol F, Bauwens R, Kooijman R, Massie A, Villers A, Ris L, Keyser D (2016) Astrocytic β2 adrenergic receptor gene deletion affects memory in aged mice. J PLoS One 11(10):e0164721. https://doi.org/10.1371/journal.pone.0164721

Jhaveri DJ, Mackay EW, Hamlin AS, Marathe SV, Nandam LS, Vaidya VA, Bartlett PF (2010) Norepinephrine directly activates adult hippocampal precursors via beta3-adrenergic receptors. J Neurosci 30(7):2795–2806. https://doi.org/10.1523/JNEUROSCI.3780-09.2010

Jhaveri CJ, Nanavaty I, Prosper BW, Marathe S, Husain BF, Kernie SG, Bartlett PF, Vaidya VA (2014) Opposing effects of α2- and β-adrenergic receptor stimulation on quiescent neural precursor cell activity and adult hippocampal neurogenesis. PloS One 9(6):e98736. https://doi.org/10.1371/journal.pone.0098736

Jones PK, Shaw B, Raj SR (2015) Orthostatic hypotension: managing a difficult problem. Expert Rev Cardiovasc Ther 13(11):1263–1276. https://doi.org/10.1586/14779072.2015.1095090

Kahn A, Mumford JP, Rogers GA et al (1997) Double-blind study of lofexidine and clonidine in the detoxification of opiate addicts in hospital. Drug Alcohol Depend 44(1):57–61. https://doi.org/10.1016/s0376-8716(96)01316-6

Kemner JE, Starr HL, Ciccone PE et al (2005) Outcomes of OROS methylphenidate compared with atomoxetine in children with ADHD: a multicenter, randomized prospective study. Adv Ther 22:498–512. https://doi.org/10.1007/BF02849870

Kessler RC, Berglund PA, Chiu WT et al (2013) The prevalence and correlates of binge eating disorder in the World Health Organization world mental health surveys. Biol Psychiatry 73:904–914. https://doi.org/10.1016/j.biopsych.2012.11.020

Kessler RM, Hutson PH, Herman BK et al (2016) The neurobiological basis of binge-eating disorder. Neurosci Biobehav Rev 63:223–238. https://doi.org/10.1016/j.neubiorev.2016.01.013

Knauber J, Müller WE (2000) Decreased exploratory activity and impaired passive avoidance behaviour in mice deficient for the alpha(1b)-adrenoceptor. Eur Neuropsychopharmacol 10(6):423–427. https://doi.org/10.1016/s0924-977x(00)00100-0

Kratochvil CJ, Heiligenstein JH, Dittmann R et al (2002) Atomoxetine and methylphenidate treatment in children with ADHD: a prospective, randomized, open-label trial. J Am Acad Child Adolesc Psychiatry 41:776–784. https://doi.org/10.1097/00004583-200207000-00008

Kulkarni VA, Jha S, Vaidya VA (2002) Depletion of norepinephrine decreases the proliferation, but does not influence the survival and differentiation, of granule cell progenitors in the adult rat hippocampus. Eur J Neurosci 16(10):2008–2012. https://doi.org/10.1046/j.1460-9568.2002.02268.x

Kusumi I, Boku S, Takahashi Y (2014) Psychopharmacology of atypical antipsychotic drugs: from the receptor binding profile to neuroprotection and neurogenesis. Psychiatry Clin Neurosci 69(5):243–258. https://doi.org/10.1111/pcn.12242

Lähdesmäki J, Sallinen J, MacDonald E et al (2002) Behavioral and neurochemical characterization of alpha(2A)-adrenergic receptor knockout mice. Neuroscience 113(2):289–299. https://doi.org/10.1016/s0306-4522(02)00185-9

Lähdesmäki J, Sallinen J, MacDonald E et al (2003) Alpha2-adrenergic drug effects on brain monoamines, locomotion, and body temperature are largely abolished in mice lacking the alpha2A-adrenoceptor subtype. Neuropharmacology 44(7):882–892. https://doi.org/10.1016/s0028-3908(03)00080-7

Levin BE, Biegon A (1984) Reserpine and the role of axonal transport in the independent regulation of pre- and postsynaptic beta-adrenoreceptors. Brain Res 311(1):39–50. https://doi.org/10.1016/0006-8993(84)91396-9

Libet B, Gleason CA (1994) The human locus coeruleus and anxiogenesis. Brain Res 634(1):178–180. https://doi.org/10.1016/0006-8993(94)90274-7

Lin SK, Strang J, Su LW et al (1997) Double-blind randomised controlled trial of lofexidine versus clonidine in the treatment of heroin withdrawal. Drug Alcohol Depend 48(2):127–133. https://doi.org/10.1016/s0376-8716(97)00116-6

Link RE, Stevens MS, Kulatunga M et al (1995) Targeted inactivation of the gene encoding the mouse alpha 2c-adrenoceptor homolog. Mol Pharmacol 48(1):48–55. Online ISSN 1521-0111

Lippiello P, Hoxha E, Cristiano C, Malvicini E, Stanley A, Russo R, Tempia F, Miniaci MC (2020) Role of β3-adrenergic receptor in the modulation of synaptic transmission and plasticity in mouse cerebellar cortex. J Neurosci Res 98(11):2263–2274. https://doi.org/10.1002/jnr.24712. Epub 2020 Aug 17

Logemann HN, Böcker KB, Deschamps PK et al (2013) The effect of noradrenergic attenuation by clonidine on inhibition in the stop signal task. Pharmacol Biochem Behav 110:104–111. https://doi.org/10.1016/j.pbb.2013.06.007

Lorton D, Davis JN (1987) The distribution of beta-1- and beta-2-adrenergic receptors of normal and reeler mouse brain: an in vitro autoradiographic study. Neuroscience 23(1):199–210. https://doi.org/10.1016/0306-4522(87)90283-1

Lucemyra® US Product Label (n.d.). https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=b748f308-ba71-4fd9-84ec-ec7e0f210885

Luhrs L, Manlapaz C, Kedzie K, Rao S, Cabrera-Ghayouri S, Donello J, Gil D (2016) Function of brain α_{2B}-adrenergic receptor characterized with subtype-selective α_{2B} antagonist and KO mice. Neuroscience 17(339):608–621. https://doi.org/10.1016/j.neuroscience.2016.10.024

Luijendijk HJ, van den Berg JF, Hofman A, Tiemeier H, Stricker BH (2011) β-blockers and the risk of incident depression in the elderly. J Clin Psychopharmacol 31(1):45–50. https://doi.org/10.1097/JCP.0b013e31820482c4

MacMillan LB, Lakhlani PP, Hein L et al (1998) In vivo mutation of the alpha 2A-adrenergic receptor by homologous recombination reveals the role of this receptor subtype in multiple physiological processes. Adv Pharmacol 42:493–496. https://doi.org/10.1016/s1054-3589(08)60796-6

Maggi A, U'Prichard DC, Enna SJ (1980) Differential effect of antidepressant treatment on brain monoamine receptors. Eur J Pharmacol 61:91–98. https://doi.org/10.1016/0014-2999(80)90152-1

Magistretti PJ, Allaman I (2018) Lactate in the brain: from metabolic end-product to signalling molecule. Nat Rev Neurosci 19(4):235–249. https://doi.org/10.1038/nrn.2018.19

Maity S, Chandanathil M, Millis RM, Connor SA (2020) Norepinephrine stabilizes translation-dependent, homosynaptic long-term potentiation through mechanisms requiring the cAMP

sensor Epac, mTOR and MAPK. Eur J Neurosci 52(7):3679–3688. https://doi.org/10.1111/ejn.14735

Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 20(24):9104–9110. https://doi.org/10.1523/JNEUROSCI.20-24-09104.2000

Masuda T, Nakagawa S, Boku S, Nishikawa H, Takamura N, Kato A, Inoue T, Koyama T (2012) Noradrenaline increases neural precursor cells derived from adult rat dentate gyrus through β2 receptor. Prog Neuropsychopharmacol Biol Psychiatry 36(1):44–51. https://doi.org/10.1016/j.pnpbp.2011.08.019

Matthijssen AM, Dietrich A, Bierens M et al (2019) Continued benefits of methylphenidate in ADHD after 2 years in clinical practice: a randomized placebo-controlled discontinuation study. Am J Psychiatry 176:754–762. https://doi.org/10.1176/appi.ajp.2019.18111296

McCracken JT, McGough JJ, Loo SK et al (2016) Combined stimulant and guanfacine administration in attention-deficit/hyperactivity disorder: a controlled, comparative study. J Am Acad Child Adolesc Psychiatry 55:657–666.e1. https://doi.org/10.1016/j.jaac.2016.05.015

McDonald T, Hoffman WE, Berkowitz R et al (1999) Heart rate variability and plasma catecholamines in patients during opioid detoxification. J Neurosurg Anesthesiol 11(3):195–199. https://doi.org/10.1097/00008506-199907000-00007

McElroy SL, Guerdjikova A, Kotwal R et al (2007) Atomoxetine in the treatment of binge-eating disorder: a randomized placebo-controlled trial. J Clin Psychiatry 68:390–398. https://doi.org/10.4088/jcp.v68n0306

McTavish SF, Mannie ZN, Harmer CJ, Cowen PJ (2005) Lack of effect of tyrosine depletion on mood in recovered depressed women. Neuropsychopharmacology 30(4):786–791. https://doi.org/10.1038/sj.npp.130066

Mefford IN (1988) Epinephrine in mammalian brain. Prog Neuropsychopharmacol Biol Psychiatry 12(4):365–388. https://doi.org/10.1016/0278-5846(88)90099-1

Micali N, Solmi F, Horton NJ et al (2015) Adolescent eating disorders predict psychiatric, high-risk behaviors and weight outcomes in young adulthood. J Am Acad Child Adolesc Psychiatry 54:652–659. https://doi.org/10.1016/j.jaac.2015.05.009

Michelson D, Buitelaar JK, Danckaerts M et al (2004) Relapse prevention in pediatric patients with ADHD treated with atomoxetine: a randomized, double-blind, placebo-controlled study. J Am Acad Child Adolesc Psychiatry 43:896–904. https://doi.org/10.1097/01.chi.0000125089.35109.81

Milanés MV, Martinez MD, González-Cuello A et al (2001) Evidence for a peripheral mechanism in cardiac opioid withdrawal. Naunyn Schmiedebergs Arch Pharmacol 3:193–198. https://doi.org/10.1007/s002100100451

Milner TA, Shah P, Pierce JP (2000) Beta-adrenergic receptors primarily are located on the dendrites of granule cells and interneurons but also are found on astrocytes and a few presynaptic profiles in the rat dentate gyrus. Synapse 36(3):178–193. https://doi.org/10.1002/(SICI)1098-2396(20000601)36:3<178::AID

Minzenberg MJ, Yoon JH (2011) An index of relative central α-adrenergic receptor antagonism by antipsychotic medications. Exp Clin Psychopharmacol 19(1):31–39. https://doi.org/10.1037/a0022258

Mirbolooki MR, Schade KN, Constantinescu CC, Pan M-L, Mukherjee J (2015) Enhancement of 18F-fluorodeoxyglucose metabolism in rat brain frontal cortex using a β3 adrenoceptor agonist. Synapse 69(2):96–98. https://doi.org/10.1002/syn.21789

Mishima K, Tanoue A, Tsuda M, Hasebe N, Fukue Y et al (2004) Characteristics of behavioral abnormalities in alpha1d-adrenoceptors deficient mice. Behav Brain Res 152(2):365–373. https://doi.org/10.1016/j.bbr.2003.10.038

Mishra R, Gillespie DD, Youdim MB, Sulser F (1983) Effect of selective monoamine oxidase inhibition by clorgyline and deprenyl on the norepinephrine receptor-coupled adenylate cyclase system in rat cortex. Psychopharmacology (Berl) 81(3):220–223. https://doi.org/10.1007/BF0042726

Mobley PL, Sulser F (1981) Down-regulation of the central noradrenergic receptor system by antidepressant therapies. In: Enna SJ et al (eds) Antidepressants: neurochemical, behavioral and clinical perspectives. Raven Press, New York, pp 31–51

Morón JA, Brockington A, Wise RA et al (2002) Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: evidence from knock-out mouse lines. J Neurosci 22:389–395. https://doi.org/10.1523/JNEUROSCI.22-02-00389.2002

Morris LS, McCall JG, Charney DS, Murrough JW (2020) The role of the locus coeruleus in the generation of pathological anxiety. Brain Neurosci Adv 4:2398212820930321. https://doi.org/10.1177/2398212820930321

Murchison CF, Schutsky K, Jin S-H, Thomas SA (2011) Norepinephrine and ß$_1$-adrenergic signaling facilitate activation of hippocampal CA1 pyramidal neurons during contextual memory retrieval. Neuroscience 181:109–116. https://doi.org/10.1016/j.neuroscience.2011.02.049

Nasr DA, Omran HA, Hakim SM et al (2011) Ultra-rapid opiate detoxification using dexmedetomidine under general anesthesia. J Opioid Manag 7(5):337–344

Newcorn JH, Harpin V, Huss M et al (2016) Extended-release guanfacine hydrochloride in 6-17-year-olds with ADHD: a randomised-withdrawal maintenance of efficacy study. J Child Psychol Psychiatry 57:717–728. https://doi.org/10.1111/jcpp.12492

Nicholas AP, Pieribone V, Hökfelt TJ (1993) Distributions of mRNAs for alpha-2 adrenergic receptor subtypes in rat brain: an in situ hybridization study. Comp Neurol 328(4):575–594. https://doi.org/10.1002/cne.903280409

O'Donnell J, Zeppenfeld D, McConnell E, Pena S, Nedergaard M (2012) Norepinephrine: a neuromodulator that boosts the function of multiple cell types to optimize CNS performance. Neurochem Res 37(11):2496–2512. https://doi.org/10.1007/s11064-012-0818-x

Ozdogan UK, Lähdesmäki J, Hakala K et al (2004) The involvement of alpha 2A-adrenoceptors in morphine analgesia, tolerance and withdrawal in mice. Eur J Pharmacol 497(2):161–171. https://doi.org/10.1016/j.ejphar.2004.06.051

Paloyelis Y, Asherson P, Mehta MA et al (2010) DAT1 and COMT effects on delay discounting and trait impulsivity in male adolescents with attention deficit/hyperactivity disorder and healthy controls. Neuropsychopharmacology 35:2414–2426. https://doi.org/10.1038/npp.2010.124

Papay R, Gaivin R, McCune DF, Rorabaugh BR, Macklin WB, McGrath JC, Perez DM (2004) Mouse alpha1B-adrenergic receptor is expressed in neurons and NG2 oligodendrocytes. J Comp Neurol 478(1):1–10. https://doi.org/10.1002/cne.20215

Papay R, Gaivin R, Jha A, McCune DF, McGrath JC, Rodrigo MC, Simpson PC, Doze VA, Perez DM (2006) Localization of the mouse alpha1A-adrenergic receptor (AR) in the brain: alpha1AAR is expressed in neurons, GABAergic interneurons, and NG2 oligodendrocyte progenitors. J Comp Neurol 497(2):209–222. https://doi.org/10.1002/cne.20992

Perez DM (2021) Current developments on the role of α_1-adrenergic receptors in cognition, cardioprotection, and metabolism. Front Cell Dev Biol 9:652152. https://doi.org/10.3389/fcell.2021.652152

Pillidge K, Porter AJ, Dudley JA, Tsai YC, Heal DJ, Stanford SC (2014a) The behavioural response of mice lacking NK_1 receptors to guanfacine resembles its clinical profile in treatment of ADHD. Br J Pharmacol 171(20):4785–4796. https://doi.org/10.1111/bph.12860

Pillidge K, Porter AJ, Vasili T, Heal DJ, Stanford SC (2014b) Atomoxetine reduces hyperactive/impulsive behaviours in neurokinin-1 receptor 'knockout' mice. Pharmacol Biochem Behav 127:56–61. https://doi.org/10.1016/j.pbb.2014.10.008

Pillidge K, Porter AJ, Young JW, Stanford SC (2016) Perseveration by NK1R-/- ('knockout') mice is blunted by doses of methylphenidate that affect neither other aspects of their cognitive performance nor the behaviour of wild-type mice in the 5-choice continuous performance test. J Psychopharmacol 30(9):837–847. https://doi.org/10.1177/0269881116642541. Epub 2016 Apr 19

Pratt JA, Robinson ESJ, Fernandes C, Heal D, Stanford SC (2022) BAP editorial: improving the validity and translation of preclinical research. J Psychopharmacol 36(7):779–780. https://doi.org/10.1177/02698811221104064

Proudman RGW, Pupo AS, Baker JG (2020) The affinity and selectivity of α-adrenoceptor antagonists, antidepressants, and antipsychotics for the human α1A, α1B, and α1D-adrenoceptors. Pharmacol Res Perspect 8(4):e00602. https://doi.org/10.1002/prp2.602

Proudman RGW, Baker JG (2021) The selectivity of α-adrenoceptor agonists for the human α1A, α1B, and α1D-adrenoceptors. Pharmacol Res Perspect 9(4):e00799. https://doi.org/10.1002/prp2.799

Proudman RGW, Akinaga J, Baker JG (2022) The affinity and selectivity of α-adrenoceptor antagonists, antidepressants and antipsychotics for the human α2A, α2B, and α2C-adrenoceptors and comparison with human α1 and β-adrenoceptors. Pharmacol Res Perspect 10(2):e00936. https://doi.org/10.1002/prp2.936

Qelbree ® US Product Label (n.d.). https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=aedf408d-0f84-418d-9416-7c39ddb0d29a

Raffa R, Pergolizzi J, Taylor R et al (2019) Differences in the receptor binding profile of lofexidine compared to clonidine. Pharmacol Pharmacy 10:1–10. https://doi.org/10.4236/pp.2019.101001

Raut SB, Canales JJ, Ravindran M, Eri R, Benedek DM, Ursano RJ, Johnson LR (2022) Effects of propranolol on the modification of trauma memory reconsolidation in PTSD patients: a systematic review and meta-analysis. J Psychiatr Res 150:246–256. https://doi.org/10.1016/j.jpsychires.2022.03.045

Redmond DE Jr, Huang YH (1979) Current concepts. II. New evidence for a locus coeruleus-norepinephrine connection with anxiety. Life Sci 25(26):2149–2162. https://doi.org/10.1016/0024-3205(79)90087-0

Reinblatt SP (2015) Are eating disorders related to attention deficit/hyperactivity disorder? Curr Treat Options Psychiatry 2:402–412. https://doi.org/10.1007/s40501-015-0060-7

Richelson E, Nelson A (1984) Antagonism by antidepressants of neurotransmitter receptors of normal human brain in vitro. Pharmacol Exp Ther 230(1):94–102. Online ISSN 1521-0103

Richelson E, Souder T (2000) Binding of antipsychotic drugs to human brain receptors focus on newer generation compounds. Life Sci 68(1):29–39. https://doi.org/10.1016/s0024-3205(00)00911-5

Sallee FR, Lyne A, Wigal T et al (2009) Long-term safety and efficacy of guanfacine extended release in children and adolescents with attention-deficit/hyperactivity disorder. J Child Adolesc Psychopharmacol 19:215–226. https://doi.org/10.1089/cap.2008.0080

Sánchez-Soto M, Casadó-Anguera V, Yano H, Bender BJ, Cai NS, Moreno E, Canela EI, Cortés A, Meiler J, Casadó V, Ferré S (2018) α_{2A}- and α_{2C}-adrenoceptors as potential targets for dopamine and dopamine receptor ligands. Mol Neurobiol 55(11):8438–8454. https://doi.org/10.1007/s12035-018-1004-1

Santana N, Mengod G, Artigas F (2013) Expression of α(1)-adrenergic receptors in rat prefrontal cortex: cellular co-localization with 5-HT(2A) receptors. Int J Neuropsychopharmacol 16(5):1139–1151. https://doi.org/10.1017/S1461145712001083

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301(5634):805–809. https://doi.org/10.1126/science.1083328

Scheibner J, Trendelenburg AU, Hein L, Starke K (2001) Alpha2-adrenoceptors modulating neuronal serotonin release: a study in alpha2-adrenoceptor subtype-deficient mice. Br J Pharmacol 32(4):925–933. https://doi.org/10.1038/sj.bjp.0703882

Schildkraut JJ (1965) The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am J Psychiatry 122(5):509–522. https://doi.org/10.1176/ajp.122.5.509

Schmidt ME, Risinger RC, Hauger RL, Schouten JL, Henry M, Potter WZ (1997) Responses to alpha 2-adrenoceptor blockade by idazoxan in healthy male and female volunteers. Psychoneuroendocrinology 22(3):177–188. https://doi.org/10.1016/s0306-4530(96)00045-5

Schramm NL, McDonald MP, Limbird LE (2001) The alpha(2a)-adrenergic receptor plays a protective role in mouse behavioral models of depression and anxiety. J Neurosci 21(13): 4875–4882. https://doi.org/10.1523/JNEUROSCI.21-13-04875.2001

Schulz KP, Clerkin SM, Fan J et al (2013) Guanfacine modulates the influence of emotional cues on prefrontal cortex activation for cognitive control. Psychopharmacology (Berl) 226(2):261–271. https://doi.org/10.1007/s00213-012-2893-8

Segura V, Flacco N, Oliver E, Barettino D, D'Ocon P, Ivorra MD (2010) Alpha1-adrenoceptors in the rat cerebral cortex: new insights into the characterization of alpha1L- and alpha1D-adrenoceptors. Eur J Pharmacol 641(1):41–48. https://doi.org/10.1016/j.ejphar.2010.05.016

Senft RA (1991) Experience with clonidine-naltrexone for rapid opiate detoxification. J Subst Abuse Treat 8(4):257–259. https://doi.org/10.1016/0740-5472(91)90048-f

Sesack SR, Hawrylak VA, Matus C et al (1998) Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. J Neurosci 18:2697–2708. https://doi.org/10.1523/JNEUROSCI.18-07-02697

Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW (1996) Hippocampal atrophy in recurrent major depression. Proc Natl Acad Sci U S A 93(9):3908–3913. https://doi.org/10.1073/pnas.93.9.3908

Shibao C, Okamoto LE, Gamboa A, Yu C, Diedrich A, Raj SR, Robertson D, Biaggioni I (2010) Comparative efficacy of yohimbine against pyridostigmine for the treatment of orthostatic hypotension in autonomic failure. Hypertension 56(5):847–851. https://doi.org/10.1161/HYPERTENSIONAHA.110.154898

Shen H, Peri KG, Deng XF, Chemtob S, Varma DR (2000) Distribution of alpha1-adrenoceptor subtype proteins in different tissues of neonatal and adult rats. Can J Physiol Pharmacol 78(3): 237–243

Sickert L, Müller DJ, Tiwari AK, Shaikh S, Zai C, De Souza R, De Luca V, Meltzer HY, Lieberman JA, Kennedy JL (2009) Association of the alpha 2A adrenergic receptor -1291C/G polymorphism and antipsychotic-induced weight gain in European-Americans. Pharmacogenomics 10(7):1169–1176. https://doi.org/10.2217/pgs.09.43

Silveira RO, Zanatto V, Appolinário JC et al (2005) An open trial of reboxetine in obese patients with binge eating disorder. Eat Weight Disord 10:e93–e96. https://doi.org/10.1007/BF03327498

Sobanski E, Leppämäki S, Bushe C et al (2015) Patterns of long-term and short-term responses in adult patients with attention-deficit/hyperactivity disorder in a completer cohort of 12 weeks or more with atomoxetine. Eur Psychiatry 30:1011–1020. https://doi.org/10.1016/j.eurpsy.2015.09.005

Sood P, Prow M, Rowley H et al (2012) Profound suppression of noradrenaline, dopamine and 5-ht turnover in various regions of rat brain evoked by the α2-adrenoceptor agonist, clonidine Poster TE06. Br Assoc Psychopharmacol. 22–25 July, Harrogate, UK. https://bap.org.uk/pdfs/BAP2012_abstractbook.pdf

Souza-Braga P, Lorena F, Nascimento BBP, Marcelino CP et al (2018) Adrenergic receptor β3 is involved in the memory consolidation process in mice. Braz J Med Biol Res 51(10):e7564. https://doi.org/10.1590/1414-431X20187564

Spencer L, Gregory M (1989) Clonidine transdermal patches for use in outpatient opiate withdrawal. J Subst Abuse Treat 6(2):113–117. https://doi.org/10.1016/0740-5472(89)90038-x

Spreng M, Cotecchia S, Schenk F (2001) A behavioral study of alpha-1b adrenergic receptor knockout mice: increased reaction to novelty and selectively reduced learning capacities. Neurobiol Learn Mem 75(2):214–229. https://doi.org/10.1006/nlme.2000.3965

Srour H, Pandya K, Flannery A, Hatton K (2018) Enteral guanfacine to treat severe anxiety and agitation complicating critical care after cardiac surgery. Semin Cardiothorac Vasc Anesth 22(4):403–406. https://doi.org/10.1177/1089253218768537

Stahl SM (2003) Neurotransmission of cognition, part 1 dopamine is a hitchhiker in frontal cortex: norepinephrine transporters regulate dopamine. J Clin Psychiatry 64(1):4–5. https://doi.org/10.4088/jcp.v64n0101

Stanford SC (1995) Central noradrenergic neurones and stress. Pharmacol Ther 68(2):297–242. https://doi.org/10.1016/0163-7258(95)02010-1

Stanford SC (2017) Confusing preclinical (predictive) drug screens with animal 'models' of psychiatric disorders, or 'disorder-like' behaviour, is undermining confidence in behavioural neuroscience. J Psychopharmacol 31(6):641–643. https://doi.org/10.1177/0269881116689260

Stanford SC (2020) Some reasons why preclinical studies of psychiatric disorders fail to translate: what can be rescued from the misunderstanding and misuse of animal 'Models'? Altern Lab Anim 48(3):106–115. https://doi.org/10.1177/0261192920939876

Stanford SC (2022) Animal models of ADHD? Curr Top Behav Neurosci 57:363–393. https://doi.org/10.1007/7854_2022_342

Starke K (1977) Regulation of noradrenaline release by presynaptic receptor systems. Rev Physiol Biochem Pharmacol 77:1–124. https://doi.org/10.1007/BFb0050157

Starr HL, Kemner J (2005) Multicenter, randomized, open-label study of OROS methylphenidate versus atomoxetine: treatment outcomes in African-American children with ADHD. J Natl Med Assoc 97(10 Suppl):11S–16S

Steenen SA, van Wijk AJ, van der Heijden GJMG, van Westrhenen R, de Lange J, de Jongh A (2016) Propranolol for the treatment of anxiety disorders: Systematic review and meta-analysis. J Psychopharmacol 30(2):128–139. https://doi.org/10.1177/0269881115612236

Steward T, Menchon JM, Jiménez-Murcia S et al (2018) Neural network alterations across eating disorders: a narrative review of fMRI studies. Curr Neuropharmacol 16:1150–1163. https://doi.org/10.2174/1570159X15666171017111532

Stopyra MA, Simon JJ, Skunde M et al (2019) Altered functional connectivity in binge eating disorder and bulimia nervosa: a resting-state fMRI study. Brain Behav 9:e01207. https://doi.org/10.1002/brb3.1207

Strattera® – US Product Label (n.d.). https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=96c69fdd-4a8d-4a8d-bcef-c8d2201a7980

Strawbridge R, Javed RR, Cave J, Jauhar S, Young AH (2022) The effects of reserpine on depression: a systematic review. J Psychopharmacol 24:2698811221115762. https://doi.org/10.1177/02698811221115762. Online ahead of print

Strawn JR, Compton SN, Robertson B, Albano AM, Hamdani M, Rynn MA (2017) Extended release guanfacine in pediatric anxiety disorders: a pilot, randomized, placebo-controlled trial. J Child Adolesc Psychopharmacol 27(1):29–37. https://doi.org/10.1089/cap.2016.0132

Sugama S, Takenouchi T, Hashimoto M, Ohata H, Takenaka Y, Kakinuma YJ (2019) Stress-induced microglial activation occurs through β-adrenergic receptor: noradrenaline as a key neurotransmitter in microglial activation. Neuroinflammation 16(1):266. https://doi.org/10.1186/s12974-019-1632-z

Summers RJ, Papaioannou M, Harris S, Evans B (1995) Expression of beta 3-adrenoceptor mRNA in rat brain. Br J Pharmacol 16(6):2547–2548. https://doi.org/10.1111/j.1476-5381.1995.tb17205.x

SUNOSI® (solriamfetol) – US Product Label (n.d.). https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=362f206b-73e7-4009-8c34-5a9df55679ab

Sunovion Press Release (2020). https://news.sunovion.com/press-releases/press-releases-details/2020/Sunovion-Discontinues-Dasotraline-Program/default.aspx#:~:text=(Sunovion)%20today%20announced%20that%20it,deficit%20hyperactivity%20disorder%20(ADHD)

Svensson TH (2003) Alpha-adrenoceptor modulation hypothesis of antipsychotic atypicality. Prog Neuropsychopharmacol Biol Psychiatry 27(7):1145–1158. https://doi.org/10.1016/j.pnpbp.2003.09.0

Szabadi E (2013) Functional neuroanatomy of the central noradrenergic system. J Psychopharmacol 27(8):659–693. https://doi.org/10.1177/0269881113490326

Tanaka Y, Escobar R, Upadhyaya HP (2017) Assessment of effects of atomoxetine in adult patients with ADHD: consistency among three geographic regions in a response maintenance study. Atten Defic Hyperact Disord 9:113–120. https://doi.org/10.1007/s12402-016-0212-7

Tanda G, Bassareo V, Di Chiara G (1996) Mianserin markedly and selectively increases extracellular dopamine in the prefrontal cortex as compared to the nucleus accumbens of the rat. Psychopharmacology (Berl) 123:127–130. https://doi.org/10.1007/BF02246169

Tanila H, Mustonen K, Sallinen J, Scheinin M, Riekkinen P (1999) Role of alpha2C-adrenoceptor subtype in spatial working memory as revealed by mice with targeted disruption of the alpha2C-adrenoceptor gene. Eur J Neurosci:599–603. https://doi.org/10.1046/j.1460-9568.1999.00464.x

Tartt AN, Mariani MB, Hen R, Mann JJ, Boldrini M (2022) Dysregulation of adult hippocampal neuroplasticity in major depression: pathogenesis and therapeutic implications. Mol Psychiatry 27(6):2689–2699. https://doi.org/10.1038/s41380-022-01520-y

Taylor FB, Russo J (2001) Comparing guanfacine and dextroamphetamine for the treatment of adult attention-deficit/hyperactivity disorder. J Clin Psychopharmacol 21(2):223–228. https://doi.org/10.1097/00004714-200104000-00015

Teicher MH, Anderson CM, Polcari A et al (2000) Functional deficits in basal ganglia of children with attention-deficit/hyperactivity disorder shown with functional magnetic resonance imaging relaxometry. Nat Med 6:470–473. https://doi.org/10.1038/74737

The PDSP Ki Database (n.d.) Accessed Nov 2022. https://pdsp.unc.edu/databases/kidb.php

Todorović M, Micov A, Nastić K, Tomić M, Pecikoza U, Vuković M, Stepanović-Petrović R (2022) Vortioxetine as an analgesic in preclinical inflammatory pain models: mechanism of action. Fundam Clin Pharmacol 36(2):237–249. https://doi.org/10.1111/fcp.12737

Tsai S-J, Yu YW-Y, Lin C-H, Wang Y-C, Chen J-Y, Hong C-J (2004) Association study of adrenergic beta3 receptor (Trp64Arg) and G-protein beta3 subunit gene (C825T) polymorphisms and weight change during clozapine treatment. Neuropsychobiology 50(1):37–40. https://doi.org/10.1159/000077939

Ungerstedt U (1971) Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol Scand Suppl 367:1–48. https://doi.org/10.1111/j.1365-201x.1971.tb10998.x

Upadhyay SP, Mallick PN, Elmatite WM et al (2011) Dexmedetomidine infusion to facilitate opioid detoxification and withdrawal in a patient with chronic opioid abuse. Indian J Palliat Care 17(3):251–254. https://doi.org/10.4103/0973-1075.92353

Upadhyaya H, Ramos-Quiroga JA, Adler LA et al (2013) Maintenance of response after open-label treatment with atomoxetine hydrochloride in international European and non-European adult outpatients with attention-deficit/hyperactivity disorder: a placebo-controlled, randomised withdrawal study. Eur J Psychiat 27:185–205

U'Prichard DC, Snyder SH (1977) [3H]epinephrine and [3H]norepinephrine binding to alpha-noradrenergic. Life Sci 20(3):527–533. https://doi.org/10.1016/0024-3205(77)90397-6

Ural C, Belli H, Akbudak M et al (2017) Relation of binge eating disorder with impulsiveness in obese individuals. World J Psychiatry 7:114–120. https://doi.org/10.5498/wjp.v7.i2.114

Vazey EM, Moorman DE, Aston-Jones G (2018) Phasic locus coeruleus activity regulates cortical encoding of salience information. Proc Natl Acad Sci U S A 115(40):E9439–E9448. https://doi.org/10.1073/pnas.1803716115

Vetulani J, Sulser F (1975) Action of various antidepressant treatments reduces reactivity of noradrenergic cyclic AMP-generating system in limbic forebrain. Nature 257(5526):495–496. https://doi.org/10.1038/257495a0

Vickers SP, Hackett D, Murray F et al (2015) Effects of lisdexamfetamine in a rat model of binge-eating. J Psychopharmacol 29:1290–1307

Vickers SP, Goddard S, Brammer RJ et al (2017) Investigation of impulsivity in binge-eating rats in a delay-discounting task and its prevention by the d-amphetamine prodrug, lisdexamfetamine. J Psychopharmacol 31:784–797

Vogt M (1954) Norepinephrine and epinephrine in the central nervous system. Pharmacol Rev 6(1):31–32. Online ISSN 1521-0081

Volkow ND, Wang GJ, Tomasi D et al (2012) Methylphenidate-elicited dopamine increases in ventral striatum are associated with long-term symptom improvement in adults with attention deficit hyperactivity disorder. J Neurosci 32:841–849. https://doi.org/10.1523/JNEUROSCI.4461-11.2012

Vyvanse® US Product Label (n.d.). https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=704e4378-ca83-445c-8b45-3cfa51c1ecad

Wahis J, Holt MG (2021) Astrocytes, noradrenaline, α1-adrenoreceptors, and neuromodulation: evidence and unanswered questions. Front Cell Neurosci 15:645691. https://doi.org/10.3389/fncel.2021.645691

Wang Y, Zheng Y, Du Y et al (2007) Atomoxetine versus methylphenidate in paediatric outpatients with attention deficit hyperactivity disorder: a randomized, double-blind comparison trial. Aust N Z J Psychiatry 41:222–230. https://doi.org/10.1080/00048670601057767

Webster JF, Lecca S, Wozny C (2021) Inhibition within the lateral habenula-implications for affective disorders. Front Behav Neurosci 15:786011. https://doi.org/10.3389/fnbeh.2021.786011

Wellman PJ (2000) Norepinephrine and the control of food intake. Nutrition 16:837–842. https://doi.org/10.1016/s0899-9007(00)00415-9

Wilens TE, Bukstein O, Brams M et al (2012) A controlled trial of extended-release guanfacine and psychostimulants for attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 51:74–85.e2. https://doi.org/10.1016/j.jaac.2011.10.012

Yan TC, Dudley JA, Weir RK, Grabowska EM, Peña-Oliver Y, Ripley TL, Hunt SP, Stephens DN, Stanford SC (2011) Performance deficits of NK1 receptor knockout mice in the 5-choice serial reaction-time task: effects of d-amphetamine, stress and time of day. PloS One 6(3):e17586. https://doi.org/10.1371/journal.pone.0017586

Yang M, Verfürth F, Büscher R, Michel MC (1997) Is alpha1D-adrenoceptor protein detectable in rat tissues? Naunyn Schmiedebergs Arch Pharmacol 355(4):438–446. https://doi.org/10.1007/pl00004966

Yang M, Reese J, Cotecchia S, Michel MC (1998) Murine alpha1-adrenoceptor subtypes. I. Radioligand binding studies. J Pharmacol Exp Ther 286(2):841–847

Yanpallewar SU, Fernandes K, Marathe SV, Vadodaria KC, Jhaveri D, Rommelfanger K, Ladiwala U, Jha S, Muthig V, Hein L, Bartlett P, Weinshenker D, Vaidya VA (2010) Alpha2-adrenoceptor blockade accelerates the neurogenic, neurotrophic, and behavioral effects of chronic antidepressant treatment. J Neurosci 30(3):1096–1109. https://doi.org/10.1523/JNEUROSCI.2309-09.2010

Yoshioka Y, Negoro R, Kadoi H, Motegi T, Shibagaki F, Yamamuro A, Ishimaru Y, Maeda S (2021) Noradrenaline protects neurons against H_2O_2-induced death by increasing the supply of glutathione from astrocytes via β_3-adrenoceptor stimulation. J Neurosci Res 99(2):621–637. https://doi.org/10.1002/jnr.24733. Epub 2020 Sep 20

Yu E, Miotto K, Akerele E, Montgomery A et al (2008) A Phase 3 placebo-controlled, double-blind, multi-site trial of the alpha-2-adrenergic agonist, lofexidine, for opioid withdrawal. Drug Alcohol Depend 97(1-2):158–168. https://doi.org/10.1016/j.drugalcdep.2008.04.002

Yu C, Garcia-Olivares J, Candler S et al (2020) New insights into the mechanism of action of viloxazine: serotonin and norepinephrine modulating properties. J Exp Pharmacol 12:285–300. https://doi.org/10.2147/JEP.S256586

Zhang J-P, Lencz T, Zhang RX, Nitta M, Maayan L et al (2016) Pharmacogenetic associations of antipsychotic drug-related weight gain: a systematic review and meta-analysis. Schizophr Bull 42(6):1418–1437. https://doi.org/10.1093/schbul/sbw058

Locus Coeruleus and Noradrenergic Pharmacology in Neurodegenerative Disease

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Abstract

Adrenoceptors (ARs) throughout the brain are stimulated by noradrenaline originating mostly from neurons of the locus coeruleus, a brainstem nucleus that is ostensibly the earliest to show detectable pathology in neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. The α_1-AR, α_2-AR, and β-AR subtypes expressed in target brain regions and on a range of cell populations define the physiological responses to noradrenaline, which includes activation of cognitive function in addition to modulation of neurometabolism, cerebral blood flow, and neuroinflammation. As these heterocellular functions are critical for maintaining brain homeostasis and neuronal health, combating the loss of noradrenergic tone from locus coeruleus degeneration may therefore be an effective treatment for both cognitive symptoms and disease modification in neurodegenerative indications. Two pharmacologic approaches are receiving attention in recent clinical studies: preserving noradrenaline levels (e.g., via reuptake inhibition) and direct activation of target adrenoceptors. Here, we review the expression and role of adrenoceptors in the brain, the preclinical studies which demonstrate that adrenergic stimulation can support cognitive function and cerebral health by reversing the effects of noradrenaline depletion, and the human data provided by pharmacoepidemiologic analyses and clinical trials which together identify adrenoceptors as promising targets for the treatment of neurodegenerative disease.

Keywords

Adrenergic reuptake inhibitors · Aerobic glycolysis · Alpha adrenergic receptor · Alpha-synucleinopathies · Alzheimer's disease · Beta adrenergic receptor · Cerebral blood flow · Dementia · G protein-coupled receptors · Locus coeruleus · Neurodegenerative disorders · Neuroinflammation · Neurovascular coupling · Noradrenaline · Norepinephrine · Parkinson's disease · Tauopathy

Abbreviations

2-DG	2-deoxy-D-glucose, a glucose analog which competitively inhibits glycolysis at the hexokinase enzyme
AD	Alzheimer's disease
ADAS-Cog	Alzheimer's disease assessment scale–cognitive subscale, a clinical rating scale of cognitive symptoms related to dementia, commonly used to measure drug efficacy in AD clinical trials
ADRA1A	mRNA for α1-adrenoceptor
APP	Amyloid-beta precursor protein, a neuronal protein from which Aβ is derived, mutations in which are present in humans with familial AD and recapitulated in transgenic mouse models
AR	Adrenoceptor
ASL-MRI	Arterial spin labeling MRI, a noninvasive imaging technique which uses MRI to magnetically label blood water protons for measurements of cerebral perfusion
ATP	Adenosine-5′-triphosphate
Aβ	Amyloid beta, APP-derived peptides which comprise the extracellular amyloid plaques found in AD
cAMP	3′,5′-Cyclic adenosine monophosphate
CBF	Cerebral blood flow
CMR	Cerebral metabolic rate
CNS	Central nervous system
CSF	Cerebrospinal fluid
DBH	Dopamine β-hydroxylase, an enzyme which converts dopamine to NA
DLB	Dementia with Lewy bodies
DSP-4	N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride, a neurotoxin which produces a long-lasting suppression of NA release and uptake
FDG	Fluorodeoxyglucose, a glucose analog and GLUT substrate which is transported into the cell and phosphorylated, but not hydrolyzed
FDG-PET	[^{18}F]-fluorodeoxyglucose positron emission tomography, an imaging method for measuring a glucose analog uptake into tissues, used as a proxy for glucose metabolism in determining the cerebral metabolic rate of glucose (CMRglc)
GFAP	Glial fibrillary acidic protein, an immunohistochemical marker of glia, particularly astrocytes
GLUT	Glucose transporter
LC	Locus coeruleus, a collection of brainstem cells that are the primary source of NA to the brain
L-DOPS	L-dihydroxyphenylserine, a CNS-penetrant dopamine-β-hydroxylase substrate
LPS	Lipopolysaccharide

MAO	Monoamine oxidase, an enzyme catalyzing the degradation of monoamines including NA
MCI	Mild cognitive impairment
MMSE	Mini-mental state examination, a brief clinical questionnaire commonly used in screening for and tracking longitudinal changes in cognitive impairment
MRI	Magnetic resonance imaging
NA	Noradrenaline
NET	Noradrenaline (norepinephrine) transporter
NM-MRI	Neuromelanin contrast MRI, a noninvasive technique for imaging neuromelanin pigment found in locus coeruleus and substantia nigra neurons
NRI	Noradrenaline reuptake inhibitors
NVU	Neurovascular or neurogliovascular units, a functional collection of cell types including neurons, astrocytes, vascular endothelial cells, and contractile cells, which together couple neural activity with CBF
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PD-MCI	Parkinson's disease with mild cognitive impairment
PET	Positron emission tomography
PFC	Prefrontal cortex
PS1	Presenilin 1, a protein regulator of APP processing, mutations in which are present in humans with familial AD and recapitulated in transgenic mouse models
rCBF	Regional cerebral blood flow
REM	Rapid eye movement, referring to a stage of sleep characterized by dreaming, hypertonicity of skeletal muscle, and increased visceral motor activities such as respiration and blood pressure
SMC	Smooth muscle cells
SNCA	The mRNA for α-synuclein
Tau	Tubulin-associated unit, one of six protein isoforms primarily present in the axons of neurons where they play a role in the spatial organization of microtubules
TH	Tyrosine hydroxylase, the rate-limiting enzyme for synthesis of NA
α-syn	α-Synuclein, a neuronal protein which forms pathogenic aggregates and fibrils in PD and DLB

1 Adrenoceptor Expression and Function in the Healthy Brain

Adrenoceptors (ARs) are the direct and indirect targets of a host of selective and nonselective drugs, including approved ligands (agonists and antagonists) acting directly on AR subtypes as therapies for the treatment of urological indications (terazosin, tamsulosin: α_1-AR antagonist), neurogenic orthostatic hypotension (midodrine: α_1-AR agonist), sedation, agitation, hypertension (clonidine, dexmedetomidine: α_2-AR agonists), congestive heart failure, angina, arrhythmias, and hypertension (bisoprolol, metoprolol, atenolol: β_1-AR antagonists), reactive airways disease (salbutamol, salmeterol, formoterol: β_2-AR agonists), and overactive bladder (mirabegron, vibegron: β_3-AR agonists). In addition, ARs indirectly subserve components of the therapeutic effectiveness of selective and nonselective reuptake inhibitors (atomoxetine, reboxetine, desipramine, duloxetine), central nervous system (CNS)-penetrant dopamine-β-hydroxylase substrates (L-dihydroxyphenylserine [L-DOPS] or droxidopa), noradrenaline (NA) releasers (amphetamine, pseudoephedrine), selective and nonselective monoamine oxidase-A (MAO-A) inhibitors (tranylcypromine, moclobemide), and catechol-O-methyltransferase (COMT) inhibitors (entacapone, tolcapone) that all act to prolong the effects of the endogenous catecholamine agonist by increasing its release into or inhibiting clearance from the synaptic cleft, the neurovascular space, and other receptor interfaces (Katzung 2017).

In the brain, ARs receive major inputs from neuronal projections of the locus coeruleus (LC), Latin for "blue place." The LC is the nucleus in the brainstem where most of the NA in the brain is synthesized for axonal transport throughout the mammalian CNS, including spinal cord, brainstem, thalamic nuclei, amygdala, hippocampus, and throughout the cortical regions of the cerebrum and cerebellum (reviewed in Schwarz and Luo (2015)). The copious storage of NA in the LC and the catecholamine manufacture and turnover contained therein give rise to high concentrations of neuromelanin, the dark blue metabolite of dopamine and NA biosynthesis for which the region is named (Keren et al. 2015). In the brain, NA is predominantly released by projections from the LC (the A6 nucleus), with minor contributions from smaller pontine and medullary nuclei (A1-4 nuclei) (Breton-Provencher et al. 2021). Its metabolite adrenaline is predominantly released from the adrenal glands, with little brain impact. Although some medullary neural tracts (rostroventrolateral [C1] and dorsomedial [C3] medulla) use this transmitter, they target very restricted sites (Cunningham et al. 1990; Puskás et al. 2010), mostly locally modulating sympathetic discharge, as well as hypothalamic areas regulating pituitary endocrine signal release; these latter adrenaline paths will not be part of our focus in this review.

The LC was recognized as having a role in learning and memory over 50 years ago based on the effects of targeted electrical stimulation (Crow 1973). Subsequent studies refined the understanding of the LC as playing a role in wakefulness, arousal, and particularly in salience: the emotional arousal that potentiates perception and memory of important information while inhibiting the processing of non-salient

inputs (reviewed in Harley 1987; Berridge and Waterhouse 2003; Mather et al. 2016). Salience is achieved through noradrenergic facilitation of affectively important events via both excitatory and inhibitory inputs from the LC to cortical and limbic structures that can lead to long-lasting increases or potentiation in evoked cell firing in the hippocampus (Harley 1987). In one study, phasic and tonic increases in LC activity were observed in primates during a complex reward-motivated task, coinciding with altered levels of vigilance during the task (Aston-Jones et al. 1994).

The LC sends highly arborized noradrenergic projections throughout the brain, with NA released from multitudes of varicosities that adorn the ascending axonal processes, regulating various functions including arousal, attention, mood, and memory (Samuels and Szabadi 2008; Sara 2009). Many varicosities give rise to large quanta of NA in a diffusely distributed release, distinct from that typical for classical synaptic "wiring transmission." As such, the noradrenergic innervation behaves more as "volume transmission": release of NA interacting with multiple cell types (neural, vascular, and glial cell collections described as neurovascular or neurogliovascular units, NVU) (Fuxe et al. 2015; Toyoda et al. 2022). Two noradrenergic sources innervate the brain vascular tree. Large arteries are innervated by sympathetic nerves originating outside the brain in the superior cervical and stellate ganglia. These arteries include the carotid and vertebral arteries, middle cerebral artery, and pial arteries down through the first order penetrating arterioles. As the vascular bed enters the brain and becomes more branched into intraparenchymal arterioles, which have a single smooth muscle cell (SMC) layer, noradrenergic nerves are supplied to the vascular bed from the LC (Giorgi et al. 2020). LC innervation continues along intraparenchymal capillaries that have contractile pericytes rather than smooth muscle cells to modulate perfusion under influence from noradrenergic input and local mediators of neural or astrocytic origin.

These diverse cellular functions across the brain are mediated through the family of ARs. The hippocampus, for example, is a region of the brain critically important for the acquisition and consolidation of memory and, as detailed below, receives dense noradrenergic innervation from the LC, liberating abundant NA to act on multiple ARs, expressed by many types of cells. The AR family consists of nine distinct receptors, all of which are expressed widely in mammalian peripheral tissues and organs as well as in the CNS (Bylund et al. 1994; Hieble et al. 1995; Alexander et al. 2019). α_1 subtypes (α_{1A}-AR, α_{1B}-AR, α_{1D}-AR), α_2 subtypes (α_{2A}-AR, α_{2B}-AR, α_{2C}-AR), and β subtypes (β_1-AR, β_2-AR, β_3-AR) are G protein-coupled receptors that mediate responses to the endogenous agonists, adrenaline and noradrenaline, via coupling to G-proteins and arrestins. All AR subtypes are expressed in the mammalian brain, although some have suggested a negligible presence of β_3-AR (Altosaar et al. 2021). While no evidence of β_3-AR binding is reported in the brain, pro-cognitive effects of β_3-AR agonists in chick and mouse models of cognition coupled with known species differences in β_3-AR physiology and pharmacology suggest that this story may not be fully told (Gibbs et al. 2009a, 2010; Tournissac et al. 2021). In humans, ARs are expressed on neurons, vascular cells, pericytes, and glial cells: astrocytes, oligodendrocytes, and microglia (Giorgi et al. 2020; Szabadi 2013; Morin et al. 1996) (Table 1). Each AR's relative sensitivity to the two

Table 1 Localization of adrenoceptors in the brains of humans with no neurologic disease (normal) and patients with psychiatric or neurodegenerative disease

Receptor	Localization and observation	Reference
α_1-AR	*Tissue*: Human brain: normal and Alzheimer's disease (AD) • Highest α_1-AR binding density in human hippocampus, frontal cortex, nucleus basalis of Meynert, thalamus, temporal cortex; lower expression in caudate nucleus, putamen, cerebellar hemisphere • Lower binding in corresponding AD brain regions *Method*: [^{3}H]-prazosin (nonselective) radioligand binding	Shimohama et al. (1986)
α_1-AR	*Tissue*: Human hypothalamus, LC and frontal cortex: normal and schizophrenia • α_1-AR binding in hypothalamus (high-density in paraventricular nucleus and supraoptic nucleus), LC, and frontal cortex *Method*: [^{3}H]-prazosin (nonselective) radioligand binding	Ko et al. (1989)
α_1-AR	*Tissue*: Bovine cerebral microvessels and pericyte cultures • No binding detected in cerebral microvessels or pericytes *Method*: [^{3}H]-prazosin (nonselective) radioligand binding	Elfont et al. (1989)
α_{1A}-AR, α_{1B}-AR, α_{1D}-AR	*Tissue*: Normal brain: human, rat, rabbit • ADRA1A mRNA predominates in many human tissues (heart, liver, cerebellum, and cerebral cortex), in contrast to its restricted distribution in both rats and rabbits • ADRA1B mRNA is present in highest concentrations in human spleen, kidney, and fetal brain • ADRA1D mRNA is present in highest concentrations in human aorta and cerebral cortex *Method*: mRNA extracted from central and peripheral human tissues and analyzed using RNase protection method	Price et al. (1994)
α_{1A}-AR, α_{1D}-AR, NET	*Tissue*: Human hippocampus and LC: normal, AD and dementia with Lewy bodies (DLB) • α_1-AR (nonselective binding) and noradrenaline transporter (NET) in LC • α_1-AR (nonselective binding) and ADRA1D mRNA in dorsal hippocampus, including pyramidal cell layer, dentate gyrus, and granule cell layer • Profound neuronal loss in the LC in AD and DLB vs normal based on count of tyrosine hydroxylase (TH) positive cells • Presumed compensatory increase in TH staining in hippocampus • α_1-AR binding sites elevated in hippocampus only in the molecular layer of dentate gyrus of AD and DLB. ADRA1A mRNA observed only in the granule cell layer of the dentate gyrus at similar expression levels in normal, AD and DLB • ADRA1D mRNA significantly reduced in patients vs normal in hippocampus pyramidal layer *Method*: • mRNA in situ hybridization for TH, ADRA1A,	Szot et al. (2006)

(continued)

Table 1 (continued)

Receptor	Localization and observation	Reference
	ADRA1D, ADRA2A, ADRA2B, ADRA2C • [^{3}H]-prazosin and (±)-β-([^{125}I]-iodo-4-hydroxyphenyl)-ethyl-aminomethyl-tetralone ([^{125}I]-HEAT) for nonselective α_1-AR radioligand binding • [^{3}H]-nisoxetine radioligand binding for NET	
α_{1A}-AR, α_{1D}-AR, α_{2A}-AR	*Tissue*: Human prefrontal cortex: normal, AD and DLB • α_1-AR binding through all layers of prefrontal cortex (PFC) is likely postsynaptic as similar localization for mRNA expression of ADRA1A and ADRA1D • Normal-to-elevated α_1-AR binding sites in PFC of AD and DLB compared with normal, with concomitant loss of ADRA1A, ADRA1D, and ADRA2C mRNA expression in the PFC possibly attributed to neuronal loss in dementia *Method*: • mRNA in situ hybridization for ADRA1A, ADRA1D and ADRA2C • [^{3}H]-prazosin and [^{125}I]-HEAT for α_1-AR nonselective radioligand binding	Szot et al. (2007)
α_{1A}-AR	*Tissue*: Human brain: N = 5 each of young, old, and cerebral amyloid angiopathy. Cultured human cerebrovascular smooth muscle cells • α_{1A}-AR expressed on the wall of cerebrovascular smooth muscle cells, colocalized with endothelial and smooth muscle markers in capillaries, arteries, and veins • Approximately equal expression in young, old and cerebral amyloid angiopathy patients *Method*: Immunohistochemistry and confocal immunofluorescence	Frost et al. (2020)
α_2-AR	*Tissue*: Human brain: normal and AD • Highest α_2-AR binding density in hippocampus, frontal cortex, and nucleus basalis of Meynert; lower in hippocampus, thalamus, temporal cortex, putamen, cerebellar hemisphere, and caudate nucleus • Lower and more variable binding levels in all regions in AD compared with normal *Method*: [^{3}H]-yohimbine (non α_2-AR-selective) radioligand binding	Shimohama et al. (1986)
α_2-AR	*Tissue*: Human brain: normal and schizophrenia • α_2-AR binding in the frontal cortex *Method*: [^{3}H]-p-aminoclonidine (nonselective) radioligand binding	Ko et al. (1989)
α_2-AR	*Tissue*: Bovine cerebral microvessels and their pericyte cultures • Approximately twice as many α_2-AR binding sites in cerebral microvessels compared with pericytes *Method*: [^{3}H]-rauwolscine (nonselective) radioligand binding	Elfont et al. (1989)

(continued)

Table 1 (continued)

Receptor	Localization and observation	Reference
α_2-AR	*Tissue*: Normal brain: human, rat • High α_2-AR binding in neocortex layers I and III, visual cortex, hippocampus (CA1, dentate gyrus), hypothalamus, LC, cerebellar cortex, and lower binding in the remaining layers of neocortex, amygdala, thalamus, paraventricular and ventromedial hypothalamic nuclei, substantia innominata, superior colliculus and lateral periaqueductal area at the midbrain, nucleus tractus solitarii and dorsal horn of the spinal cord • α_2-AR localization in human and rat brain is similar, however expression levels differ between species • α_2-AR expression generally consistent with predominant presynaptic localization *Method*: Quantitative autoradiography with [^{3}H]-UK-14304: • with BRL-44408 to define α_{2A}-AR • with ARC-239 to define α_{2B}-AR and α_{2C}-AR	Pascual et al. (1992)
α_2-AR	*Tissue*: Human frontal cortex, hypothalamus, cerebellum: normal and AD • No α_2-AR binding detected in white matter • Similar α_2-AR receptor density in normal vs AD in hypothalamus and orbitofrontal cortex • ~70% increase in α_2-AR receptor density in cerebellar cortex in subgroup with aggressive AD vs nonaggressive AD with similar cognitive deficit *Method*: [^{3}H]-UK-14304 (nonselective) radioligand binding	Russo-Neustadt and Cotman (1997)
α_{2C}-AR	*Tissue*: Normal human striatum, rat • α_2-AR binding in human striatum is predominantly α_{2C}-AR • α_2-AR binding in human cortex and cerebellum is non-α_{2C}-AR • Similar localization of α_{2C}-AR in rat and human *Method*: Nonselective ethyl-[^{3}H]-RS79948 autoradiography with selective α_{2C}-AR antagonist, JP-1302	Fagerholm et al. (2008)
α_{2A}-AR, α_{2B}-AR, α_{2C}-AR	*Tissue*: Normal human brain • α_{2A}-AR is predominant α-AR in the different layers of the frontal cortex, cerebellum, and hippocampal formation • α_{2B}-AR/α_{2C}-AR binding predominantly in neostriatum, less in frontal cortex • α_{2A}-AR, α_{2B}-AR/α_{2C}-AR in hippocampus (dentate gyrus and CA1) *Method*: Quantitative autoradiography and membrane binding with [^{3}H]-RX-821002: • with BRL-44408 for α_{2A}-AR • with ARC-239 for α_{2B}-AR and α_{2C}-AR	Grijalba et al. (1996)
α_{2A}-AR, α_{2B}-AR, α_{2C}-AR, NET	*Tissue*: Human hippocampus and LC: normal, AD and DLB • α_2-AR binding and mRNA for ADRA2A in LC and dorsal hippocampus including pyramidal cell layer, dentate gyrus,	Szot et al. (2006)

(continued)

Table 1 (continued)

Receptor	Localization and observation	Reference
	granule cell layer of normal human tissues. mRNA for ADRA2C was detected in the hippocampus but not the LC • Decrease in number of ADRA2A-positive cells in LC based on mRNA, but no change in number of mRNA-positive grains per cell • Increase in α_2-AR binding in hippocampus with no change in ADRA2A mRNA and decrease in ADRA2C mRNA in hippocampus granule cell layer in AD and DLB vs normal • Sprouting of peri-LC dendrites as quantified by α_2-AR and NET radioligand binding in AD and DLB vs normal • Sprouting of axonal projections to hippocampus inferred from increased α_2-AR binding in AD and DLB vs normal *Method*: • mRNA in situ hybridization for ADRA1A, ADRA1D, ADRA2A, ADRA2B, and ADRA2C • [^{3}H]-nisoxetine binding for NET • [^{3}H]-RX821002 binding for α_2-AR (nonselective)	
α_{1A}-AR, α_{1D}-AR, α_{2C}-AR	*Tissue*: Human prefrontal cortex: normal, AD and DLB • Reduction in α_2-AR binding in PFC in AD and DLB vs normal is less marked (18%) compared with LC (50–80%), and presumed presynaptic as no ADRA2A mRNA expression in PFC • ADRA2C mRNA expression in PFC, mostly in layer II. Significant decrease in mRNA expression in layer II in AD and DLB *Method*: • mRNA in situ hybridization for TH, ADRA1A, ADRA1D, and ADRA2C • [^{3}H]-RX821002 binding for α_2-AR	Szot et al. (2007)
α_1-AR, α_2-AR	*Tissue*: Human optic nerve tissue excised due to severe endophthalmitis or choroidal melanoma • No α_2-AR binding • Low-to-moderate α_1-AR binding density that collocates with GFAP surrounding optic nerve axons observed in normal and pathological tissue, consistent with localization to astrocytes *Method*: Immunohistochemistry for GFAP and nonselective radioligand binding: • [^{125}I]-HEAT for α_1-AR • 2-[(2,6-dichloro-4-[^{125}I]-iodophenyl)imino]imidazolidine autoradiography ([^{125}I]-PIC) for α_2-AR	Mantyh et al. (1995)
α_1-AR, α_2-AR	*Tissue*: PFC cerebral microvessels in human brain: normal and AD • Compared to the cerebral cortex, α_1-AR binding in cerebral microvessels was low in normal and AD • Binding to α_2-AR receptors in cerebral microvessels was ~50% of that in the cortex, and these receptors increased by ~60% in cerebral microvessels of AD subjects • Interpreted as AR 'upregulation' in response to noradrenergic deafferentation in AD	Kalaria and Harik (1989)

(continued)

Table 1 (continued)

Receptor	Localization and observation	Reference
	Method: Nonselective radioligand binding: • [^{125}I]-HEAT for α_1-AR • [^{3}H]-p-aminoclonidine for α_2-AR	
β_1-AR, β_2-AR	*Tissue*: Human brain: normal • Hippocampus, midbrain and brainstem, basal ganglia, cortex, thalamus, cerebellum, amygdala *Method*: [^{125}I]-iodopindolol (nonselective) autoradiography	Reznikoff et al. (1986)
β_1-AR, β_2-AR	*Tissue*: Human brain: normal and AD • Binding in hippocampus, cortex (multiple layers), putamen, cerebellum • Binding in hippocampus and cortex (layers I, II, III, IV, V, VI) and overall white matter, but not putamen or cerebellum, was higher in AD compared with healthy • No correlation between B_{max} of β_2-AR and aging; weak correlation between B_{max} of β_1-AR and aging *Method*: [^{125}I]-iodopindolol (nonselective) radioligand binding	Kalaria et al. (1989b)
β_1-AR, β_2-AR	*Tissue*: PFC cerebral microvessels in human brain: normal and AD • β_2-AR is the most highly expressed AR in microvessels • Total β-AR expression in cerebral microvessels, significantly *increased* in AD. Interpreted as AR 'upregulation' in response to noradrenergic deafferentation in AD *Method*: [^{125}I]-iodopindolol binding: • β_1-AR evaluated in the presence of 60 nM ICI-118,551, a selective β_2-AR antagonist • β_2-AR evaluated in the presence of 80 nM ICI-89,406, a selective β_1-AR antagonist	Kalaria and Harik (1989)
β_1-AR, β_2-AR	*Tissue*: Human frontal cortex, hypothalamus, cerebellum: normal and AD • β_1-AR and β_2-AR labeling in orbitofrontal cortex (where β_1-AR is expressed at higher density than β_2-AR), PFC (β_1-AR at higher density than β_2-AR), and hypothalamus (β_2-AR at higher density than β_1-AR), but not in subcortical white matter • Although binding density was generally similar between normal and AD, possible differences were noted between aggressive and non-aggressive AD with similar cognitive deficit: – ~25% increase in β_1-AR in cerebellar cortex in aggressive AD vs nonaggressive AD – ~20% increase in β_2-AR on white matter vs normal; lower increase vs nonaggressive AD *Method*: [^{125}I]-iodocyanopindolol autoradiography • with ICI 89,406 for selective radioligand displacement at β_1-AR • with ICI 118,551 for selective for radioligand displacement β_2-AR	Russo-Neustadt and Cotman (1997)

(continued)

Table 1 (continued)

Receptor	Localization and observation	Reference
β_1-AR, β_2-AR	*Tissue*: Human brain: normal, schizophrenic, suicide schizophrenic brain. Rat brain • Approximately 3-fold higher [^{125}I]-pindolol binding density in rat brain (55.6 fmol/mg protein) compared with human (18.7 fmol/mg protein), with different patterns of regional distribution in rat vs human. E.g., binding density in the hippocampus is β_2-AR > β_1-AR in human and β_1-AR > β2-AR in rat, whereas binding density in cortex is predominantly β_1-AR in human and rat • All regions of human brain express β_1-AR and β_2-AR with relative ratios of each receptor ranging from 70:30 to 10:90. Binding density for β_1 highest in basal ganglia (caudate nucleus, putamen, nucleus accumbens), and outer layers of the frontal cortex and occipital cortex; β_2-AR highest in hippocampus in human • β_1-AR upregulated in human striatum (nucleus accumbens and ventral putamen) of schizophrenic patients compared with normal • β_2-AR binding density in regions of the right hippocampus is higher than in the left hippocampus in normal brains. This difference is less marked in left/right hippocampal regions of patients with schizophrenia • No effect of age on β_2-AR binding density *Method*: [^{125}I]-iodocyanopindolol autoradiography • with ICI 89,406 for selective radioligand displacement at β_1-AR • with ICI 118,551 for selective for radioligand displacement β_2-AR	Joyce et al. (1992)
β_1-AR, β_2-AR	*Tissue*: Normal human brain • High β-AR binding densities in caudate, putamen, distinct cortical areas and layers, and hippocampus • Low β-AR binding densities in thalamus, hypothalamus, midbrain, and cerebellar cortex • Binding in putamen is predominantly β_1-AR in rat and human • Binding in cerebellum is predominantly β_2-AR *Method*: [^{125}I]-iodocyanopindolol autoradiography • with ICI 89,406 for selective radioligand displacement at β_1-AR • with ICI 118,551 for selective for radioligand displacement β_2-AR	Pazos et al. (1985)
β_1-AR, β_2-AR	*Tissue*: Human brain: normal and AD • β_1-AR and β_2-AR binding density observed in frontal cortex, temporal cortex, hippocampus, thalamus, putamen, caudate, nucleus basalis of Meynert, cerebellar hemisphere • Except for thalamus where receptor binding was reduced, total β-AR binding was similar in all regions evaluated from AD brains compared with normal *Method*: [^{3}H]-dihydroalprenolol binding, with metoprolol for selective binding to β_1-AR	Shimohama et al. (1987)

(continued)

Table 1 (continued)

Receptor	Localization and observation	Reference
β_1-AR, β_2-AR	*Tissue*: Human cerebral arteries: normal and after subarachnoid hemorrhage • β_1-AR and β_2-AR binding observed in human cerebral arteries with an estimated relative abundance of 60:40, respectively • β_1-AR (but not β_2-AR) binding is elevated after subarachnoid hemorrhage *Method*: [^{3}H]-dihydroalprenolol binding in homogenized cerebral arteries (mainly basilar, circle of Willis and middle cerebral arteries). β_1-AR and β_2-AR quantified using metoprolol and butoxamine, respectively	Tsukahara et al (1986)
β_1-AR, β_2-AR	*Tissue*: Human optic nerve tissue excised due to severe endophthalmitis or choroidal melanoma • No β_1-AR binding detected in human optic nerve • In normal human optic nerve, high β_2-AR binding density that collocates with GFAP surrounding optic nerve axons observed in normal and pathological tissue. Consistent with localization to astrocytes *Method*: [^{125}I]-iodocyanopindolol autoradiography with betaxolol (to compete for β_1-AR binding) or ICI 118,551 (to compete for β_2-AR binding). Immunohistochemistry for GFAP staining	Mantyh et al. (1995)
β_1-AR, β_2-AR	*Tissue*: Bovine cerebral microvessels and their pericyte cultures • More abundant β-AR binding sites in microvasculature compared with pericytes, consistent with endothelial localization *Method:* [^{125}I]-iodocyanopindolol (nonselective) radioligand binding	Elfont et al. (1989)

Abbreviations: *AD* Alzheimer's disease, *AR* adrenoceptor, *DLB* dementia with Lewy bodies, *LC* locus coeruleus, *NET* noradrenaline transporter, *PFC* prefrontal cortex, *TH* tyrosine hydroxylase

endogenous agonists and expression level across cell types provides a means of controlling the conditions under which the receptor is activated. In addition, agonist-mediated receptor desensitization provides further use-dependent control of receptor activation. Receptor desensitization at the molecular level was identified initially for β-ARs, but as reviewed elsewhere (Collins et al. 1990) is generally observed across the AR family.

The α_1-AR subtypes couple predominantly to $G_{q/11}$, resulting in activation of phospholipase C, increases in diacyl glycerol, activation of protein kinase C, and increases in inositol phosphates and intracellular calcium. However, alternate coupling of α_1-ARs to $G_{i/o}$, G_s, and $G_{\alpha 12/13}$ is also reported (Alexander et al. 2019). The α_2-ARs couple predominantly to $G_{i/o}$, which leads to adenylyl cyclase inhibition, altered potassium channel conductance, altered calcium channel conductance, or phospholipase A_2 stimulation, thereby decreasing the synthesis of 3′,5′-cyclic adenosine monophosphate (cAMP) and preventing activation of protein kinase

A. Finally, all β-AR subtypes predominantly couple to G_s and activate adenylyl cyclase to increase cAMP, activate protein kinase A, open L-type calcium channels (Alexander et al. 2019), and additionally stimulate kinase-mediated receptor phosphorylation leading to β_1-AR and β_2-AR receptor desensitization (Collins et al. 1990).

Based on radioligand binding, α_1-ARs and α_2-ARs are expressed in the hippocampus, frontal cortex, basal forebrain, thalamus, temporal cortex, caudate, putamen, and cerebellum in humans (Shimohama et al. 1986) where they mediate both stimulation of postsynaptic neurons (primarily α_1- and β-ARs) and presynaptic inhibition of NA release (via α_2-ARs; Samuels and Szabadi (2008)). Additional localization based on mRNA expression shows ADRA1A (gene name for α_{1A}-AR) expression in the LC, dorsal hippocampus including the pyramidal cell layer, dentate gyrus, and granule cell layer, and throughout the prefrontal cortex (Shimohama et al. 1986; Szot et al. 2006, 2007). While these studies reflect the location of cells that express adrenergic receptor mRNA, they do not necessarily reveal receptor protein expression or subcellular localization. The expression of mRNA for ADRA1A, ADRA1D, and ADRA2C (genes for α_{1A}-AR, α_{1D}-AR, and α_{2A}-AR, respectively) in the hippocampus and prefrontal cortex suggests that these receptors are localized postsynaptic to the ascending LC neurons. Conversely, the absence of ADRA2A mRNA in the hippocampus and prefrontal cortex is consistent with a presynaptic localization of this receptor subtype.

Similarly, widespread expression of β-ARs is observed by radioligand binding with [^{125}I]-iodocyanopindolol or [^{125}I]-iodopindolol in the human brain, including the hippocampus, midbrain and brainstem, basal ganglia, cortex, thalamus, cerebellum, amygdala, hypothalamus, and isolated cerebral blood vessels (Bacic et al. 1992; Kalaria et al. 1989a; Shimohama et al. 1987; Tsukahara et al. 1986; Kalaria and Harik 1989; Reznikoff et al. 1986; Pazos et al. 1985). Under this methodology, binding to β_1-AR and β_2-AR is detected in all regions of the human brain with relative ratios of each receptor ranging from 70:30 to 10:90, demonstrating broad regional diversity in the distribution of these two β-ARs. Binding density for β_1-AR is reported as highest in basal ganglia (caudate nucleus, putamen, nucleus accumbens) and outer layers of the frontal cortex and occipital cortex, while the binding density of β_2-AR is reported to be highest in the hippocampus in human (Joyce et al. 1992).

In the studies described above, radioligand binding sites included gray and white matter, strengthening evidence that the receptor is expressed on both neuronal and glial cells. Indeed, staining with glial fibrillary acidic protein (GFAP) and OX-42 antibody staining to identify C3b complement receptor were both found to co-localize with β_2-AR immunoreactivity in human and rat optic nerves, consistent with an expression on astrocytes and microglia (Mantyh et al. 1995). Furthermore, in cell fractions derived from the human prefrontal cortex, β_1-AR binding sites were observed predominantly in synaptosomal fractions, whereas β_2-AR binding sites were predominantly observed in glial cells (Cash et al. 1986). Several human glial cell types express β_2-AR in culture (Matt et al. 2023).

Finally, AR expression is also present in the brain vasculature. The cerebral arteries express postsynaptic α_1-AR (Frost et al. 2020; Brassard et al. 2017) and α_2-AR (Brassard et al. 2017; Wirth 2018), mediating vasoconstriction of the vascular SMCs. The expression of α_1- and α_2-ARs in primary cell cultures of endothelial cells is also inferred from observed agonist and antagonist potencies (Bacic et al. 1992). Nerves innervating cerebral vasculature express presynaptic α_2-ARs that inhibit NA release in a local negative feed-back loop (Brassard et al. 2017). The β_1-AR and β_2-AR are likewise expressed on human cerebral arteries, including basilar, middle cerebral, and circle of Willis arteries (Tsukahara et al. 1986). In addition, β-AR expression was observed using [^{125}I]-cyanopindolol binding in endothelial cells, capillary pericytes, and bovine-derived cerebral microvessels (Elfont et al. 1989). In rats, the LC neurons innervate capillaries (Cohen et al. 1997), which also show evidence for α_1-AR expression (Frost et al. 2020), as well as β-AR receptor expression on the astrocytic processes that surround them (Mantyh et al. 1995).

The observed relative expression of ARs in the mammalian brain is known to differ between rodents and humans and across brain regions (Joyce et al. 1992; Pascual et al. 1992; Price et al. 1994). For example, in one study where widespread β-AR expression was observed throughout rat and human brains, binding density in the hippocampus was β_2-AR > β_1-AR in humans but β_1-AR > β_2-AR in rats, and thus, translation of pharmacology from rodent studies needs great care. Conversely, in the same study, β_1-AR binding density in layers I/II of several cortical regions was ≥5-fold higher than β_2-AR in both human and rat (Joyce et al. 1992). Similarly, many adrenoceptor drugs are known to vary in receptor subtype selectivity between species, demanding that interpretations based on "selective" ligands require caution. Given these species differences, data for receptor localization summarized here and in Table 1 focus primarily on the human brain.

2 Decline of the Locus Coeruleus in Neurodegenerative Diseases

2.1 LC Degeneration in Mild Cognitive Impairment and Alzheimer's Disease

Loss of LC neurons in neurodegenerative disease is a common pathological feature, begins at early disease stages, and is progressive (Brunnström et al. 2011; Beardmore et al. 2021; Gannon et al. 2015; German et al. 1992). There are several suggested reasons why the LC is unusually vulnerable to damage in neurodegenerative diseases. Its proximity to the vascularized floor of the fourth ventricle and dense contact with capillary circulation, with each LC neuron innervating an estimated 20 m of capillaries, is hypothesized to increase the risk of LC exposure to circulating reactive substances, pathogens, and toxins (Pamphlett 2014). It has also been shown that chronic wakefulness and inflammation, factors normally under adrenergic control, can lead to oxidative damage and neurodegeneration of the LC (Song et al. 2019; Wang et al. 2020; Zhang et al. 2014; Zhu et al. 2016, 2018). Proteomic

evidence for mitochondrial stress is seen in the LC of the aged mouse (Evans et al. 2021). Furthermore, LC neurons project significant distances and their axons are thin due to limited myelination, driving higher metabolic demand and allowing greater potential exposure to oxidative factors, which may contribute to the LC vulnerability (reviewed in Matchett et al. (2021)).

Tau (tubulin associated unit) proteins are microtubule-associated intrinsically disordered proteins present in neurons. Upon hyperphosphorylation, tau proteins aggregate to form intracellular cytotoxic neurofibrillary tangles. The LC is reportedly the first brain region to develop tau pathology in neurodegenerative disease (Kelly et al. 2017; Braak et al. 2011) with this site being the potential nidus for seeding damage to distal brain sites (discussed in Jacobs et al. 2021). In Alzheimer's disease (AD), LC degeneration may weaken the blood-brain barrier, allowing peripheral proteins to gain greater access to the brain to seed the canonical amyloid-β (Aβ) plaques and neurofibrillary tangles (reviewed in Giorgi et al. (2020)). Years before neuronal death and atrophy, LC neurons contain hyperphosphorylated tau, the earliest detectable pathology of AD (Chalermpalanupap et al. 2017; Weinshenker 2018). The LC is also among the first areas in the brain to show early tau-positive pre-tangle lesions, present in AD patients without evident neurofibrillary tangles or signs of cognitive decline (Braak et al. 2011). While the tangle pathology is typically present in the prodromal stage, sometimes decades before cognitive symptoms, a decline in neuronal number begins later in AD progression, where it has been suggested the LC volume decreases by ~8% with each Braak stage (Braak et al. 2011; Jacobs et al. 2021; Theofilas et al. 2017). Compared with cognitively normal people, those with mild cognitive impairment (MCI) retain 30% fewer LC neurons, and an additional 25% of LC neurons are lost in patients progressing to AD (Kelly et al. 2017). It is postulated that LC axons projecting to cortical and limbic areas are regressing prior to loss of their neuronal soma (Matchett et al. 2021). Therefore, degeneration of the LC and the extended LC network may precipitate a more widespread neuronal decline in AD patients (Fig. 1) (Matchett et al. 2021; Weinshenker 2018; Jacobs et al. 2019; Mather and Harley 2016; Ross et al. 2015).

The death of LC neurons, reflected by loss of neuromelanin contrast in magnetic resonance imaging (NM-MRI; Galgani et al. (2021)), correlates with cognitive decline and memory impairment (Kelly et al. 2017; Jacobs et al. 2021; Bolton and Tam 2021; Wilson et al. 2013). This correlation holds in both late-onset (sporadic) and early-onset (familial) forms of AD, where significant LC degradation is associated with cognitive deficits, including impaired attentional function (Bolton and Tam 2021) and mood (Zweig et al. 1989). AD patients show reduced scores on the mini-mental state exam (MMSE) in correlation with the fraction of LC neurons displaying abnormal tau pathology (Grudzien et al. 2007). Even among cognitively healthy older subjects, reduced LC integrity correlates with deficits in emotional memory, a key noradrenergic function (Hämmerer et al. 2018). In summary, pathologic changes in the LC, loss of noradrenergic neurons, and increases in numbers of plaques and tangles correlate with the severity and duration of dementia in patients

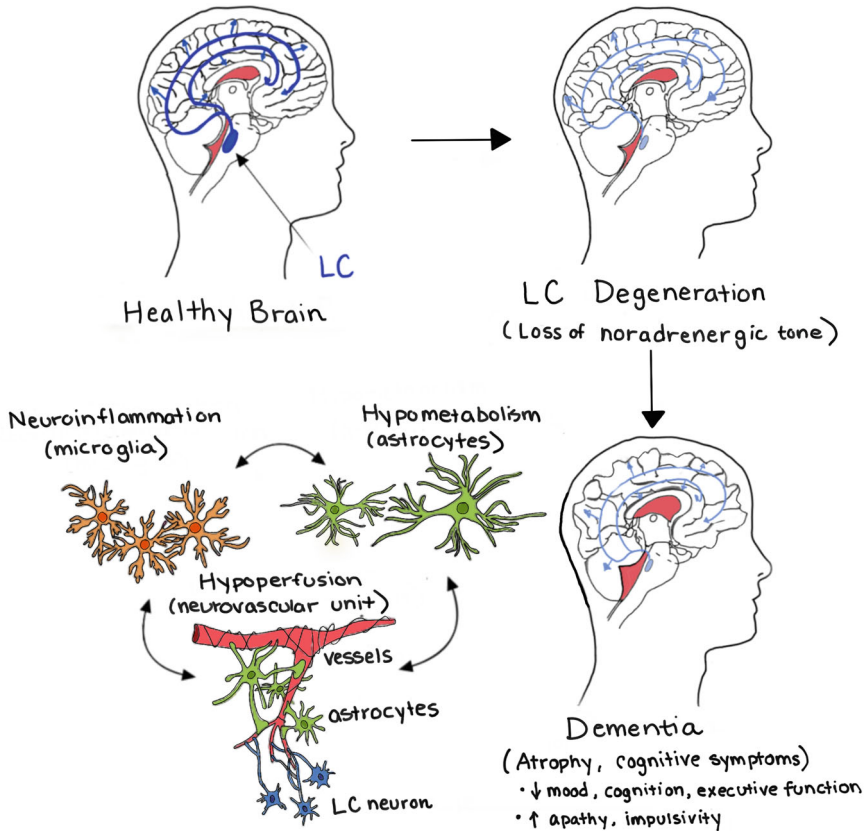

Fig. 1 The pontine locus coeruleus (LC) noradrenergic neurons are among the first to decline in neurodegenerative disease. As the LC sends axonal projections throughout the brain, this results in broad loss of NA influence on multiple cell types: neurons, astrocytes, microglia, vascular endothelial and contractile cells. Many neurodegenerative diseases display disruption of brain metabolic, immune, and vascular systems, each of which has the potential for modulation by adrenergic receptors. Restoring this lost noradrenergic tone to support heterocellular brain functions may prevent both neuronal atrophy and cognitive symptoms of neurodegenerative disease

with MCI or AD (Kelly et al. 2017; Bondareff et al. 1987a) and support a role of the noradrenergic system in disease pathology.

2.2 LC Degeneration in Parkinson's Disease

As in AD, patients with Parkinson's disease (PD) display major reductions in noradrenergic neurons, measured in living patients by NM-MRI or positron emission tomography (PET) using [^{11}C]-MeNER, a ligand for the noradrenaline transporter (NET), and in postmortem brain tissues by tyrosine hydroxylase (TH, the rate-

limiting enzyme for synthesis of NA) immunohistochemistry (German et al. 1992; García-Lorenzo et al. 2013; Sommerauer et al. 2017; Chan-Palay and Asan 1989; Sulzer et al. 2018). PD patients also display noradrenergic system dysfunction (Rommelfanger and Weinshenker 2007), α-synuclein (α-syn) inclusions (the precursor to Lewy bodies and the pathologic hallmark of PD formed by intracellular aggregates of α-syn, which, like tau, is a disordered protein) (Weinshenker 2018), and degeneration of the LC neurons (McMillan et al. 2011). Neuropathological α-syn-immunopositive Lewy neurites and Lewy bodies are observed in the LC, the first supramedullary brain area to witness α-syn pathology, from stages 2 through 6 of the six histologic disease stages of PD where, like cognitive impairment, they generally increase with disease severity (Braak et al. 2003, 2005). Some studies report a greater degree of LC atrophy in those PD patients with more non-motor symptoms, such as depression and reduced vigilance (Wang et al. 2018; Solopchuk et al. 2018); this PD population often has a common prodromal history of REM-sleep behavior disorder, a parasomnia in which sufferers act out their dreams due to loss of descending REM-sleep-associated atonia (Sommerauer et al. 2017). In a neuroblastoma cell line, stimulation of β_2-AR apparently downregulates the expression of α-syn, the protein found in pathologic Lewy bodies in PD, dementia with Lewy bodies (DLB), and other parkinsonian diseases (Mittal et al. 2017), although a recent publication has raised questions about the magnitude and durability of these findings (Patterson et al. 2022). Disease pathology, be it due to misfolded α-syn, toxins, or energy demand, appears to be rescued by exogenous AR agonists, as several pharmacoepidemiologic associational studies show that the risk of developing PD may be reduced by β_2-AR agonists and increased by β-AR antagonists (Mittal et al. 2017; Gronich et al. 2018; Nielsen et al. 2018), as discussed later.

2.3 Noradrenaline Levels in Neurodegenerative Disease

Consistent with deterioration of LC noradrenergic neurons, there are reduced NA levels in the brain and cerebrospinal fluid (CSF) of AD patients. In a comparison of tissues from demented and age-matched controls, NA concentrations were lower in ten different brain regions, with reductions of 50% or more in the putamen and cortex gyrus frontalis (Adolfsson et al. 1979). Another study compared 19 AD brains with those from 21 nondemented and eight multi-infarct dementia subjects. The number of LC neurons was similar in the non-AD brains overall, but there was a reduction of 54.8% of LC neurons in the AD brains with concomitant reduction in NA concentrations ranging from 15.5% in temporal cortex and 29.5% in hippocampus to 39.7% in frontal cortex and 50.9% in hypothalamus (Mann et al. 1982). Further evidence from a larger study of 46 AD subjects and 34 healthy subjects concluded that NA and serotonin levels were reduced by about a third in AD frontal cortex and about half in temporal cortex while dopamine levels were unchanged (Palmer et al. 1987). A postmortem analysis of ventricular CSF from 15 AD patients and 15 nondemented subjects showed a significant reduction in NA concentration (0.76 versus 2.0 ng/mL, respectively) (Kaddurah-Daouk et al. 2011). Decline in NA

progresses with disease; of the neurotransmitters and metabolites observed to be significantly different in AD patients, the one most strongly associated with disease stage was NA (Kaddurah-Daouk et al. 2011). Similarly, reduced NA levels in mid-temporal and orbital-frontal cortices correlated with cognitive impairment among AD patients as measured by the MMSE score (Matthews et al. 2002).

PD is well-known as a disease of dopamine deficit, but NA is also depleted in the brains of PD patients. In a small comparison of nine control and seven parkinsonian brains, levels of dopamine and NA were significantly lower in the substantia nigra and ventral tegmental area of diseased brains (Taquet et al. 1982). Another report found NA to be reduced by about 50% in five regions (caudate, putamen, substantia nigra, nucleus accumbens, and hypothalamus) of parkinsonian brains compared to controls; in the hypothalamus, the NA reduction was >80% (Jenner et al. 1983). A separate study confirmed these findings by immunostaining for TH and dopamine β-hydroxylase (DBH) in postmortem brains of control and PD subjects, finding significant diffuse depletion of noradrenergic innervation in all cortical laminae (Gaspar et al. 1991).

2.4 Adrenoceptor Expression Changes in Neurodegenerative Disease

Adrenoceptor expression levels vary with disease pathology, likely due to the reductions in noradrenergic neurons bearing ARs and the loss of stimulation of target cells arising from depleted NA release (Szot et al. 2006). Interpretation of the effect of disease pathology on adrenoceptor expression is challenging as the reported observations are sometimes conflicting and attribution of the observed effects to pathophysiological changes is confounded by the typically small numbers of subjects in a study, postmortem delays in sample handling, or differences in the precision and sensitivity of the methods, e.g., due to radioligand specificity or the energy of radioisotope decay. These observations are summarized in Table 1 and described here.

Localization of TH and NET reflects noradrenaline synthesis and transport, providing insight into the presence of LC projections. Compared with controls of similar ages, AD or DLB patients have significant decreases in the number of LC cells expressing TH mRNA detected by in situ hybridization, with significant increases in the amount of TH expression per cell (Szot et al. 2006). This reflects a disease-related decrease in the number of LC projections, with a possible compensatory increase in neurotransmitter synthesis in the remaining cells. In keeping with this, significant decreases in [^{3}H]-nisoxetine autoradiography (to quantify NET binding sites) were observed in the LC cell bodies of these patients, correlating with the number of TH positive cells (Szot et al. 2006). In the dentate gyrus of AD and DLB patients, increases in TH mRNA with concomitant increases in NET and α_1-AR binding sites were observed compared with age-matched controls (Szot et al. 2006). Similarly, normal-to-elevated α_1-AR binding sites were observed in specific layers of the prefrontal cortex of these patients (Szot et al. 2007). These findings are

consistent with preservation of postsynaptic α_1-AR receptors in the context of LC neuronal loss. This also supports the model that lost NA would decrease the potential for agonist-mediated desensitization and thereby increase receptor proteins at the target cell surface, a phenomenon known as denervation supersensitivity.

Analogous observations with α_2-AR support this interpretation. Specifically, in the above AD and PD patient populations, the number of cells that stained positive for ADRA2A mRNA were decreased *in the LC* compared to control subjects without change in the number of immuno-positive grains per cell (Szot et al. 2006), likely reflecting the lower number of LC soma. At axon terminals, α_2-AR binding sites were unchanged in the prefrontal cortex (Szot et al. 2007) and increased in the dentate gyrus (Szot et al. 2006). The investigators suggest that the latter data point to axonal sprouting, presumably to compensate for the lost incoming LC projections.

Conversely, in separate studies, α_1-AR and α_2-AR binding densities were generally lower in patients with AD compared with controls in hippocampus, frontal cortex, nucleus basalis, thalamus, temporal cortex, caudate nucleus, putamen, and cerebellar hemisphere (Shimohama et al. 1986). Observed α_2-AR binding sites were increased in the microvessels from prefrontal cortex of patients with AD compared with age-matched controls (Kalaria and Harik 1989), and α_1-AR immunoreactivity was unchanged in the cerebral vasculature of the occipital lobe of patients with cerebral amyloid angiopathy compared with controls (Frost et al. 2020).

Notwithstanding the above confounds, it seems possible that there are genuine changes in AR expression at different stages of disease progression or in different brain regions. In contrast to the observations described earlier in this section, reductions in α_1- and α_2-AR binding sites were observed in postmortem brains from patients with AD or schizophrenia compared with controls (Shimohama et al. 1986; Ko et al. 1989) based on radioligand binding with [^{3}H]-yohimbine, [^{3}H]-prazosin or [^{3}H]p-aminoclonidine, while in a separate study, increased α_2-AR expression was observed in the hippocampus and amygdala at the early stages of AD that diminished as neurofibrillary tangle pathology in the LC increased (Andrés-Benito et al. 2017).

The reported changes in brain β-ARs also varied across different studies and may be influenced differentially by subregion of interest, labeling method and disease progression status. In one study, a decrease in the number of β-AR binding sites labeled nonselectively with [^{3}H]-dihydroalprenolol was observed in the thalamus of patients with AD compared with age-matched controls, but other all other brain regions examined showed preserved total β-AR binding in patients with AD, with a shift from β_1-AR (control subjects) to β_2-AR (AD patients) predominating in the hippocampus (Shimohama et al. 1987). Other studies reported increased total β-AR expression by [^{125}I]-iodopindolol binding in multiple layers of the hippocampus, in cerebral microvessels in the prefrontal cortex, and in white matter of the AD brain (Russo-Neustadt and Cotman 1997; Kalaria et al. 1989b). In the latter study, AD patients showed similar frontal cortex β-AR expression to control subjects, despite patients having decreased cell number in the LC and decreased brain NA levels (Kalaria et al. 1989b). Taken together, these studies provide evidence that AD patients have preserved postsynaptic AR binding sites in the context of LC

degeneration and that receptors undergo changes in expression influenced by disease stage and brain region. A summary of comparisons of AR binding densities or mRNA expression in patients with neurodegenerative or neuropsychiatric diseases and control subjects with no neurological disease is presented in Table 1.

2.5 Locus Coeruleus and Noradrenergic Dysfunction as a Therapeutic Target in Neurodegenerative Disease

The consistent observation of LC decline in neurodegenerative disease suggests that targeted enhancement of signaling from the noradrenergic system may be a viable therapeutic approach, with the potential to be disease modifying while also improving poorly addressed symptoms of depression, loss of executive function, and cognitive decline (Kelly et al. 2017; Vermeiren and Deyn 2017; Leanza et al. 2018; Tredici and Braak 2013). The utility of this approach may extend beyond AD and PD, with potential benefit in amyotrophic lateral sclerosis (Bartus et al. 2016) and progressive supranuclear palsy (Kaalund et al. 2020), as well as acute ischemic conditions of the brain, such as traumatic brain injury (Jenkins et al. 2016) and stroke (Sternberg and Schaller 2020).

Therapeutic strategies to compensate for the loss of noradrenergic neurons and endogenous agonist in neurodegeneration follow two major themes: globally increasing NA levels (O'Callaghan et al. 2021; Chalermpalanupap et al. 2013), or specifically targeting distinct ARs on neurons and glia that would normally receive adequate noradrenergic stimulus from the LC (Zorec et al. 2018). The former can be achieved by use of various existing CNS adrenergic drugs (noradrenaline reuptake inhibitors [NRIs], monoamine oxidase inhibitors [MAOIs], NA pro-drugs such as L-DOPS) but likely will become less effective as endogenous NA concentrations decline and axonal projections from the LC recede. Importantly, restoration of adrenergic function with exogenous agonists should, by contrast, remain effective regardless of the state of LC projections, as long as the target cell populations and their AR expression remains intact. The best-studied AR targets in the brain include α_1-ARs (Perez 2020), α_2-ARs (Samuels and Szabadi 2008), β_1-AR (Coutellier et al. 2014), and β_2-AR (Abdelmotilib and West 2017; Peterson et al. 2014). As these receptors are expressed on a variety of cell types, especially glia, such a therapeutic strategy may ultimately confer a heterocellular impact across neuronal, glial, and vascular cells hosting the receptor populations and can be achieved without modifying NA release from LC neurons (Fig. 1). In this manner, it is possible to direct signals to receptor populations mediating specific excitatory processes and avoid agonist action for receptors (such as auto-inhibitory α_2-AR subtypes) that generally confer negative impact on adrenergic neurotransmission.

In the following sections, we review the experimental methodology to study LC deficit and reversal that defined the role of NA and ARs in regulating critical homeostatic brain functions: perfusion, metabolism, and inflammation. We then discuss epidemiological and clinical data exploring the strategies to restore noradrenergic function in neurodegenerative disease.

3 Experimental Modulation of LC Function

Some of the earliest work to examine LC function utilized unilateral or bilateral electrolytic lesions of the LC in rats to demonstrate impaired learning and memory after LC degeneration (Anlezark et al. 1973); subsequent studies confirmed these general findings (Compton et al. 1995). However, electrolytic lesions destroy non-adrenergic neuronal populations, so specific neurotoxins capable of selectively reducing noradrenergic signaling emanating from the LC were developed. A single dose of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4) produces a long-lasting suppression of NA release and uptake throughout the brain while having little or no effect on dopaminergic or serotonergic signaling (Ross and Renyi 1976; Ross et al. 1973) as long as uptake into non-noradrenergic terminals is blocked (Ross and Stenfors 2015). DSP-4 appears to cyclize to a brain-penetrant aziridinium derivative that enters noradrenergic nerve terminals through NET and results in a rapid decline of central NA concentrations, loss of terminal axons, retrograde degeneration, and reduction of cell bodies in the LC (Ross and Stenfors 2015). The degeneration of signaling from the LC is long-lasting but not permanent; 50 mg/kg DSP-4 produced an 80% reduction of NA in rat cerebral cortex 7 days after treatment but at 90, 240, and 300 days following treatment the reduction was 50, 41, and 25%, respectively (Wolfman et al. 1994).

Another method to selectively reduce noradrenergic signaling is by the stereotactic injection of non-brain-penetrant neurotoxins directly into the LC. 6-Hydroxydopamine depletes both dopaminergic and noradrenergic nerve terminals and cell bodies as early as 1 day after treatment (Ungerstedt 1968; Kostrzewa and Jacobowitz 1974), possibly via uptake by dopamine and NA transporters and the generation of toxic reactive oxygen species (Varešlija et al. 2020). Selectivity for degeneration of dopaminergic or noradrenergic nerves has been accomplished through the coadministration of 6-hydroxydopamine with selective reuptake inhibitors, such as desipramine or GBR 12909 for noradrenergic or dopaminergic uptake inhibition, respectively (Luthman et al. 1989).

A technique to more selectively degenerate noradrenergic nerves employs a monoclonal antibody targeting DBH attached to a cytotoxic payload, the ribosome-inactivating protein saporin. Upon release of NA, the membrane-bound form of DBH is exposed to the extracellular space containing anti-DBH-saporin, leading to endocytosis and retrograde axonal transport of the toxin (Wrenn et al. 1996). Anti-DBH-saporin is not CNS-penetrant, and thus depletion of noradrenergic nerves in the brain with this toxin is accomplished through intraventricular injection (Wrenn et al. 1996).

Recent advances in optogenetic (Carter et al. 2010; Takeuchi et al. 2016) and chemogenetic (Borodovitsyna et al. 2020; Cope et al. 2019; Rorabaugh et al. 2017) targeting of genetically defined subpopulations of cells in the brain have led to a greater understanding of the function of the noradrenergic system in learning and memory, sleep and wakefulness, sensory salience, and the identification of functionally distinct subpopulations of LC noradrenergic neurons. Using these innovative

new techniques, LC noradrenergic neurons can be selectively activated or silenced in behaving animals without permanent lesion or gene deletion.

3.1 Effects of LC in Neurobehavior of Healthy and Neurodegeneration Model Animals

To explore the role of the LC in reference and working memory, anti-DBH-saporin was bilaterally administered into the LC of healthy young rats subsequently assessed in the Morris water maze task 4 weeks later. Reference memory was unimpaired, but there were substantial deficits in working memory in lesioned animals compared to vehicle-treated controls, accompanied by a marked decrease in the proliferation of neural progenitor cells in the dentate gyrus of the hippocampus (Coradazzi et al. 2016). Healthy adult rats also show both impaired short- and long-term memory in the novel object recognition test after LC lesion with 6-hydroxydopamine; this impairment was almost completely reversed by infusion of NA into the prefrontal cortex through surgically installed cannulae (Sampaio et al. 2020).

The role of the LC in neurodegeneration has been studied by producing lesions in transgenic animals expressing mutated forms of proteins known to be linked to early-onset (familial) forms of these dementias, such as amyloid precursor protein (APP) or tau (for AD) or α-syn (for PD (Song et al. 2020)). For example, APP23 mice expressing the Swedish double mutation, K670N-M671L, reflect human neuropathology, as they have significant depositions of Aβ plaques throughout the brain, increases in hyperphosphorylated tau, and activation of microglia and astrocytes compared to control animals (Sturchler-Pierrat et al. 1997). APP23 mice have cognitive deficits (Morris water maze, passive avoidance), particularly in aged mice, and LC degeneration further exacerbates cognitive deficits in reference and working memory (radial arm maze, social partner recognition test, and novel object recognition) in the APP23 model (Heneka et al. 2006). DSP-4 treatment of APP23 mice produced a 50–60% loss of LC neurons and substantially increased signs of microglial and astrocytic activation and amyloid plaque load and increased neuronal loss in the frontal cortex and hippocampus (Heneka et al. 2006). Micro-PET imaging of DSP-4 treated APP23 mice also demonstrated marked reductions in cerebral glucose metabolism ([^{18}F]-fluorodeoxyglucose), neuronal integrity ([^{11}C]-flumazenil), and cholinergic function ([^{11}C]-methylpiperidin-4-yl acetate) relative to transgenic controls, suggesting that LC deterioration worsened many of the hallmark signs linked to those known to occur in human dementias.

A double transgenic model with APP and presenilin 1 overexpression (APP/PS1 mouse) shows synergistic increases in amyloid burden, neuroinflammation, and memory deficits compared to the single transgenic animals (Borchelt et al. 1997; Holcomb et al. 1998; Matsuoka et al. 2001). APP/PS1 mice treated with DSP-4 have higher levels of Aβ, increased expression of the inflammatory genes MIP-1α and MIP-1β, and cognitive deficits in spatial learning and memory (Morris water maze) compared to non-lesioned APP/PS1 mice (Jardanhazi-Kurutz et al. 2010). The relevance of the noradrenergic system in spatial and working memory is supported

by a pharmacological study where APP/PS1 mice were treated with daily intraperitoneal injections of clenbuterol, a β_2-AR agonist, for 2 months, which reversed the memory deficits measured in the Morris water maze (Chai et al. 2016). Furthermore, LC ablation with anti-DBH-saporin reduced neurogenesis in the dentate gyrus of the hippocampus, whereas chronic clenbuterol treatment promoted neurogenesis in the dentate gyrus of APP/PS1 mice (Chai et al. 2016). Because clenbuterol was administered systemically, it is unknown whether any of these effects on cognition and neurogenesis may have been mediated in part through peripheral effects, such as cardiovascular modulation by β-ARs in the heart and blood vessels.

Because LC neurons release other neuromodulators besides NA, a subsequent study crossbred APPswe/PS1ΔE9 mice with DBH knockout mice to produce animals with AD-like phenotypes and a more targeted depletion of noradrenergic signaling (Hammerschmidt et al. 2013). Both APP/PS1 and DBH KO mice showed significant impairment of spatial memory in the Morris water maze and the combination of the two deficits in DBH (−/−)APP/PS1 mice produced further impairment of spatial memory that was partially reversed by treatment with the NA pro-drug, L-DOPS (Hammerschmidt et al. 2013), a DBH substrate that is converted to NA. Similarly, increasing adrenergic tone with chemogenetic activation of LC neurons in an APP/PS1 rat model (TgF344-AD) rescued reversal learning deficits in Morris water maze (Rorabaugh et al. 2017).

A commonly used model for tauopathies is the transgenic mouse expressing a P301S mutation of MAPT, producing a phenotype characterized by hippocampal atrophy, inflammatory activation of astrocytes and microglia, cognitive impairment, and shortened life spans (Yoshiyama et al. 2007). When the LC of P301S mice were lesioned with DSP-4, there were further increases in neuroinflammation (Iba1 and GFAP immunoreactivity for microglial and astrocytic inflammatory activation, respectively), hippocampal atrophy, tau hyperphosphorylation and paired helical tau pathology, and impairments in spatial learning and memory (Chalermpalanupap et al. 2018). In fear conditioning experiments, LC-lesioned P301S mice showed impairments in contextual memory (thought to be largely hippocampal-dependent) but not in cued memory (thought to be largely amygdala-dependent). Importantly, the role of the LC in maintaining overall brain health can be inferred from the significant reduction of the already short life span of P301S mice (Chalermpalanupap et al. 2018) produced by LC lesioning.

4 Cerebral Blood Flow Regulation

4.1 Impaired Brain Perfusion in Neurodegenerative Disease

Maintaining cerebral blood flow (CBF) and regional cerebral perfusion critically supports brain health, and reversing limited perfusion may benefit patients with neurodegenerative disease (Iadecola 2017). The link between CBF and cognitive function has been demonstrated in both healthy aging subjects and those with cognitive decline. Among healthy older individuals participating in a longitudinal

arterial spin labeling MRI (ASL-MRI; Haller et al. (2016)) study, those with higher regional perfusion at baseline had better executive function, and those with declining whole-brain perfusion over 2–3 years performed worse in cognitive tests (Staffaroni et al. 2019). Decreased CBF is seen in subjects experiencing early cognitive decline (van der Thiel et al. 2019) and rodent models recapitulate the link between aging, CBF, and brain function (Lourenço et al. 2018). Reduced CBF is evident in early and late-stage PD patients, where it is correlated with disease severity (Lin et al. 2017). Compared to healthy subjects, PD patients have decreased CBF across widespread regions, including regions involved in supporting adrenergic autonomic function (Lin et al. 2017; Borghammer et al. 2010; Heron et al. 2014; Kamagata et al. 2011). Impaired CBF is a common finding in frontal temporal dementia, DLB, and amyotrophic lateral sclerosis (reviewed in Iadecola (2017); Haller et al. (2016)).

Vascular deficits of aging also manifest clearly in AD, including breakdown of the blood-brain barrier (Sweeney et al. 2018), vascular hypertrophy and remodeling (Kelly et al. 2019), and reduced CBF (Giorgi et al. 2020; Iadecola 2017; Haller et al. 2016; Girouard and Iadecola 2006; Verclytte et al. 2016). A cohort from the Alzheimer's Disease Neuroimaging Initiative (Mueller et al. 2005), a multi-site partnership formed to track AD biomarkers, shows that vascular dysregulation is a very early occurrence in AD progression (Iturria-Medina et al. 2016). Longitudinal ASL-MRI studies in subjects with stable cognition show decreased CBF in the hippocampus with age; patients who convert to MCI or AD have additional CBF reduction in cerebellum and prefrontal cortex, respectively, combined with further hippocampal CBF reduction (Camargo et al. 2021). AD is in turn intimately coupled to cardiovascular disease, with intertwined risk factors (Iadecola 2017; Stampfer 2006; Arvanitakis et al. 2016; Malek-Ahmadi et al. 2021). The term "cardiogenic dementia" was coined in 1977 to describe cognitive decline associated with acute or chronic cardiac trauma (Lancet 1977; Ovsenik et al. 2021). Cardiovascular disease is now recognized as a major risk factor for developing AD (Stampfer 2006; Cortes-Canteli and Iadecola 2020). This is likely one of the many contributing factors to neurodegenerative disease progression (Iturria-Medina et al. 2016) but highlights the importance of adrenergic regulation of function in both the brain and cardiovascular system. As vascular dysfunction and LC pathology (Grudzien et al. 2007; Iturria-Medina et al. 2016; Robinson et al. 2020; Malek-Ahmadi et al. 2020) both occur at similar prodromal stages (Braak and Braak 1991) of AD, the noradrenergic component of CBF maintenance is likely a key driver of the cerebrovascular deficits of neurodegenerative disease and thus a potentially important therapeutic intervention point.

4.2 LC and Adrenoceptor Function in Regulation of Cerebral Blood Flow

Adrenergic drugs broadly affect cerebrovascular regulation in humans. Recent conference abstracts report that the selective β_2-AR agonist clenbuterol selectively increases regional cerebral blood flow (rCBF) in several limbic regions, including

hippocampus, thalamus, and amygdala, both in healthy adults and in patients with MCI or PD (Lodeweyckx et al. 2021, 2023; Bishop et al. 2022b; Vargas et al. 2023). In contrast, dexmedetomidine, a centrally active α_2-AR agonist, decreases CBF in healthy human subjects (Zornow et al. 1992). Other studies confirm the dexmedetomidine-induced decrease in CBF with a concomitant decrease in the cerebral metabolic rate, an outcome that may serve to preserve brain oxygenation (Drummond et al. 2008; Farag et al. 2017). These drugs may be acting by binding receptors on multiple cell types of the NVU (neurons, astrocytes, vascular endothelial cells, contractile cells) to regulate CBF.

Redistribution of rCBF to activated regions requires that the brain coordinates both "supply" and "demand" regulation, also known as "feed-forward" vs "feedback" regulation, respectively (Iadecola 2017). On the supply side, neuronal activity directly signals to the vasculature: one mechanism for noradrenergic regulation of CBF. There is debate as to the degree of noradrenergic CBF regulation by *peripheral* sympathetic nerves that originate outside the brain and innervate the larger brain arteries (Purves 2018; Ter Laan et al. 2013), because experimental observations may be confounded by reflex changes in blood pressure and arterial blood gases (Brassard et al. 2017; Purkayastha and Raven 2011). However, there is a clear role for CBF supply regulation through *intrinsic* noradrenergic innervation from LC neurons that project to the smaller intraparenchymal brain vessels: arterioles, metarterioles, and capillaries (Giorgi et al. 2020; Peppiatt et al. 2006). Blood vessels within the brain (versus external or on the brain surface) account for 40% of the brain's total vascular resistance and therefore have a significant potential to modulate blood flow (Iadecola 2017). These vessels also provide a mechanism for regional specificity of blood delivery, allowing the neurovascular coupling between localized brain activity and CBF, also known as functional hyperemia (Iadecola 2017).

LC neurons project directly onto capillaries (Cohen et al. 1997; Paspalas and Papadopoulos 1996), which have a critical role in the functional hyperemia response (Nippert et al. 2018; Hall et al. 2014). NA application causes pericytes, the contractile cell type surrounding capillaries, to constrict, affecting 50% of capillaries in cerebellar slices (Peppiatt et al. 2006; Oishi et al. 2007) possibly through α_1-AR involvement. This is consistent with multiple observations that LC transmission can produce global decreases CBF in the brain (reviewed in Giorgi et al. 2020). However, opposite effects where LC stimulation increased CBF have also been reported (Toussay et al. 2013). The mixed findings for LC stimulation and lesion on CBF may be explained by contextual differences in experimental methodology, the selective involvement of distinct AR subtypes, and regional differences in CBF regulation mechanisms.

ARs are expressed on brain vessels (Table 1) and alter their constrictive properties. In one study, phentolamine and propranolol phenocopied the LC lesion-induced loss of functional hyperemia, pointing to a role for both α-ARs and β-ARs in the central NA-evoked CBF increase (Toussay et al. 2013). Most evidence of AR function on brain vessels mirrors their roles in the periphery with α_1-ARs mediating smooth muscle contraction and β-ARs causing vasodilation, but the resulting changes in CBF may be dependent on brain region, local environment,

model species, and anesthesia conditions (Brassard et al. 2017; Purkayastha and Raven 2011; Bekar et al. 2012; Froese et al. 2020). In line with this, early animal studies demonstrated regionally increased CBF (particularly in the caudate nucleus) in response to NA (MacKenzie et al. 1976) or isoprenaline (Edvinsson et al. 1979; Seylaz et al. 1975; Aubineau et al. 1973) indicating β-AR-regulation, presumably dilatory. There is evidence for β_1-AR-mediated dilation of small cerebral arteries and arterioles where the receptor participates in a supramolecular complex with voltage-gated potassium channels on SMCs, activation of which causes K+ efflux and SMC relaxation (Rhee and Rusch 2018). Metoprolol, a β-AR antagonist, inhibits vasodilation in rat cerebral arteries at therapeutic doses (Moore et al. 2021). However, in other studies, β-AR-mediated vascular responses have been small or insignificant suggesting that regulation of cerebral vessels by the adrenergic system may be dependent on regional or physiologic conditions (Dahlgren et al. 1981; Asano et al. 2020). There also is functional evidence for α_2-AR regulation of blood flow; receptor antagonism with atipamezole increased carotid artery blood flow in anesthetized pigs (Wirth 2018).

In multiple studies, the role of α_1-AR activation has phenocopied the effects of NA or LC stimulation in causing a global CBF decrease consistent with the well-understood peripheral function of α_1-AR agonism mediating vasoconstriction. Phentolamine, a nonselective α-AR antagonist, almost completely inhibited the decreases in CBF mediated by NA or adrenaline infusion in many regions of the rat brain (Edvinsson et al. 1979). In a multiphoton imaging study, prazosin (α_1-AR-selective antagonist) reversed the constriction of pial and penetrating arterioles by NA (Bekar et al. 2012). The α_1-ARs may also be involved in a neuroprotective process coupling oxygen saturation to CBF in subcortical brain regions shown by treatment of sheep with urapidil, an α_1-AR antagonist (Schiffner et al. 2018). It has been suggested that these vasoactive mechanisms, particularly for α_1-ARs, are helping to satisfy higher activity-driven demand by redistribution of blood from regions of low demand for which some vessels must relax and others constrict. This is supported by data showing an α_1-AR dependent vasoconstriction throughout broad regions of the mouse brain, coincident with an increased functional hyperemia in the hind limb area of the motor cortex, a region receiving sensory stimulation (Bekar et al. 2012). Examining the temporal dynamics of regional hemoglobin redistribution, the authors attributed the initial CBF decrease upon sensory stimulation to the effects of global NA and surmised that the subsequent CBF increase in stimulated brain regions was due to local mechanisms overriding the global blood flow decrease, a form of noradrenergic fine-tuning of the CBF response (Bekar et al. 2012).

In addition to direct vasoactive "*supply-side*" regulatory mechanisms controlling regional perfusion, actions at the many cell types of the NVU driving metabolic *demand* are likely equally important components of CBF regulation (Iadecola 2017). LC neuronal axons primarily release NA from varicosities versus at axon terminals, a phenomenon known as volume transmission (Fuxe et al. 2015; O'Donnell et al. 2012). Immunogold labeling of rat noradrenergic fibers in the visual cortex demonstrated that these noradrenergic boutons contact oligodendrocytes, astrocytic end feet, and the basal lamina directly (Paspalas and Papadopoulos 1996) allowing

NA delivery to the other cell types of the NVU (Giorgi et al. 2020; Cohen et al. 1997). As discussed in detail below, ARs greatly influence cellular metabolism, particularly for astrocytes. Therefore, increased metabolic needs to support the cellular functions stimulated by NA at the NVU may be driving a responsive CBF increase in active brain regions. Indeed, a combined imaging and microelectrode study in cats demonstrated high correlation between glucose utilization and neural activity with lagging increases in CBF (Freeman and Li 2016). The importance of the noradrenergic system in this form of neurovascular coupling deserves further study.

5 Neurometabolism

5.1 Hypometabolism in Neurodegenerative Disease

The NVU coordinates energy supply to brain regions under increased demand and is therefore tightly coupled to neurometabolism, the utilization of those energy substrates (Gordon et al. 2007, 2008; Buxton and Frank 1996). The brain's primary fuel is glucose supplied directly to neurons and glia via glucose transporters (GLUT) or liberated by breakdown of astrocyte-stored glycogen (Camandola and Mattson 2017). The optimal utilization of glucose is critical for neuronal health and is required for cell survival and maintenance of prolonged synaptic functions such as protein turnover and glutamate clearance (Camandola and Mattson 2017; Vlassenko et al. 2010).

In the diseased brain, deficits of glucose uptake and utilization occur. Measurement of glucose uptake using [^{18}F]-fluorodeoxyglucose positron emission tomography (FDG-PET) reveals that in both familial (Verclytte et al. 2016; Gordon et al. 2018) and sporadic (Iturria-Medina et al. 2016) AD, hypometabolism precedes clinical symptoms and brain atrophy by many years and can be a predictive biomarker of disease diagnosis and monitoring of progression (Drzezga et al. 2005). Reduced FDG-PET signal is matched by reduced regional perfusion as measured by ASL-MRI (Musiek et al. 2012; Dolui et al. 2020; Tosun et al. 2016). Impaired FDG uptake correlates with lower cognitive scores on the MMSE (Mishina et al. 2007), and hypometabolism progresses in parallel with cognitive decline in AD (Mosconi et al. 2008). Extensive cortical hypometabolism is also seen in PD (Borghammer et al. 2010; Anandhan et al. 2017). Metabolic changes, both central and peripheral, are also implicated in ALS (Velebit et al. 2020; Floare and Allen 2020). Taken together, observations of dysfunctional metabolism in multiple neurodegenerative diseases have led to an "energy rescue" hypothesis (Cunnane et al. 2020), positing that therapeutics which improve energy delivery and utilization could be disease-modifying by slowing or reversing the progressive disruption in optimal glucose metabolism. The ARs are potential therapeutic targets for this strategy, as NA is a key regulator of glucose supply and processing in the brain (Fig. 2).

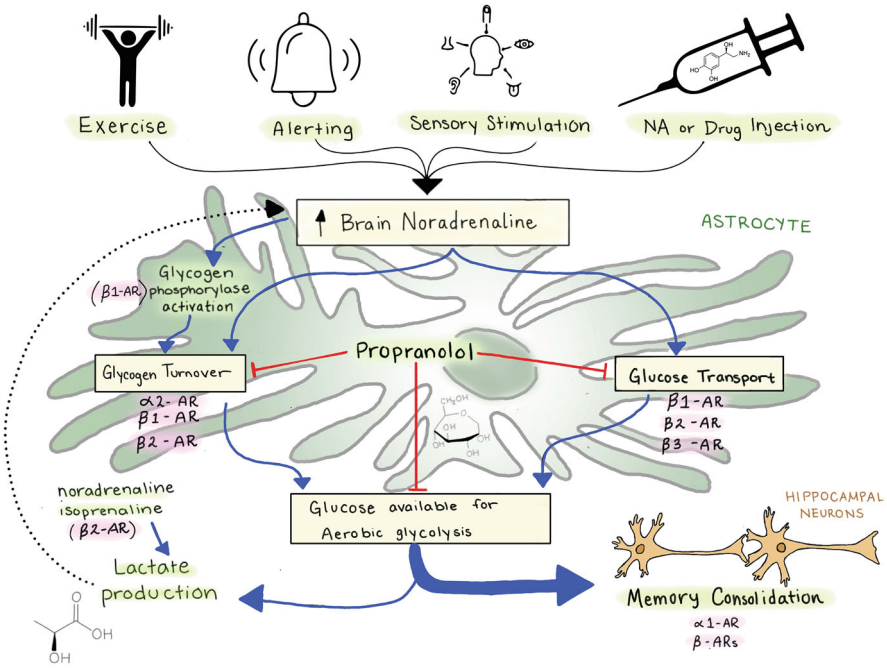

Fig. 2 Cellular and animal models have demonstrated a role for ARs in regulation of brain metabolism. In particular, β-AR activation facilitates glycogenolysis and glucose transport processes critical for memory consolidation. AR agonists stimulate aerobic glycolysis in rodents and humans, producing lactate and generating ATP in order to meet brain energy demands. The translation of β-AR brain metabolism regulation (particularly for β_3-AR) from model organisms to humans requires further study to understand relevant species-specific differences

5.2 Adrenoceptor Regulation of Aerobic Glycolysis

Aerobic glycolysis is defined as the use of glucose through pathways other than oxidative phosphorylation, so the term encompasses glycolysis via the tricarboxylic acid cycle, the pentose phosphate pathway, and glycogen turnover. In cancerous tissues, aerobic glycolysis is known as the Warburg effect: upregulation of cytoplasmic glycolysis despite sufficient oxygen to meet energy needs by mitochondrial oxidative phosphorylation (Leanza et al. 2018). In the brain, aerobic glycolysis is a normal physiologic response to increased neuronal demand, allowing the brain to quickly couple synaptic activity with increased glucose metabolism (Pellerin and Magistretti 1994). In the brains of living subjects, aerobic glycolysis can be quantified with PET-based methods measuring CBF and glucose utilization (CMRglc) relative to oxygen consumption ($CMRO_2$), which have revealed that aerobic glycolysis in both whole brain and specific sub-regions is associated with memory formation and plasticity (Vlassenko et al. 2010; Shannon et al. 2016; Madsen et al. 1994; Vaishnavi et al. 2010).

At the cellular and tissue level, aerobic glycolysis is often quantified via lactate release. After neuronal firing, astrocytes respond to increased glutamate in the synaptic cleft by increasing their glucose uptake, which is followed by glycolysis and lactate release (Pellerin and Magistretti 1994). Astrocyte-derived lactate can be taken up into neurons through monocarboxylate transporters where it likely serves as a fuel to support persistent neural activity, a phenomenon known as the astrocyte-neuron lactate shuttle (Pellerin and Magistretti 1994; Suzuki et al. 2011). Lactate is produced in the brain under conditions of increasing alertness and sensory stimulation, linking aerobic glycolysis to these known noradrenergic system functions. However, the relevance of lactate as an oxidative fuel during aerobic glycolysis conditions (when, by definition, oxygen consumption is limited) remains a matter of some debate (Dienel 2017).

Adrenoceptors regulate aerobic glycolysis on many levels (for extensive review, see Dienel and Cruz (2016)). In humans, the β blocker, propranolol, blunts aerobic glycolysis (as measured by a decreased oxygen-carbohydrate index) during exercise, a physiologic state in which central NA is elevated (reviewed in Dienel and Cruz (2016)). In rat cultured astrocytes, both isoprenaline and NA stimulate lactate production that correlates with the production of cAMP, also pointing to the β-ARs as regulators of glycolysis (Vardjan et al. 2018). In brainstem slices from adult or juvenile rats, the reciprocal effect was also observed: lactate application stimulated NA release from nearby LC neurons (Tang et al. 2014). The effect of lactate was dependent on L-lactate, the endogenous enantiomer, and on the presence of adenylyl cyclase. These data further support β-AR regulation and suggest a possible feed-forward mechanism coupling LC NA release and astrocytic glycolysis.

There have been several papers in recent years reporting the effects of α_1-AR antagonists historically used to treat cardiovascular disorders (the quinazolines terazosin, prazosin, and doxazosin) in comparison with newer α_{1A}-AR selective antagonists such as the non-quinazoline antagonist tamsulosin. In preclinical models, the α_1-AR antagonist terazosin raises brain ATP and slows neuron loss, apparently via enhancing PGK-1 activity that upregulates glycolysis through an AR-independent mechanism (Cai et al. 2019). These authors also found an epidemiological association with terazosin therapy and reduced PD diagnosis (Cai et al. 2019; Simmering et al. 2021) pointing to potential therapeutic application of this mechanism that is just beginning to be explored (Schultz et al. 2021). In counterpoint, other retrospective findings, which have also been disputed (Andrade 2018), suggest that the more selective α_{1A}-AR blocker tamsulosin is associated with increased dementia or PD risk (Duan et al. 2018; Sasane et al. 2021).

5.3 Adrenoceptor Regulation of Glucose Uptake

The β-ARs mediate glucose uptake. In CHO cells expressing heterologous β_2-AR and GLUT-4, treatment with isoprenaline and zinterol (β_2-AR-selective agonist) stimulated glucose uptake and GLUT-4 translocation (Dehvari et al. 2012). Both β-AR agonists also showed a dose-dependent increase in glucose uptake in mouse

astrocytes (Catus et al. 2011). At the same time, β_3-AR knockout astrocytes showed reduced glucose uptake in response to agonist treatment compared to astrocytes from a background strain, suggesting that β_3-AR, though expressed at low levels, also contributes to glucose uptake in mouse astrocytes (Catus et al. 2011). In chick cultured astrocytes, isoprenaline caused β_2-AR-mediated glucose uptake via G_s, β_3-AR-mediated glucose uptake via G_i, but no effect through β_1-AR due to lack of expression (Hutchinson et al. 2007). Further studies are needed to characterize how expression, signaling, and metabolic function are conserved between humans and model organisms, particularly for β_3-AR, which has known species-specific differences.

Glucose uptake can be translated from in vitro models to measurements in living animals and human subjects with the use of FDG-PET imaging. In a mouse model of ascending neurodegeneration, FDG-PET signal is reduced in several cortical regions 10 months after LC lesioning with DSP-4 neurotoxin that results in reduction in brain NA (Song et al. 2019). This dampened glucose uptake may reflect hypometabolism due to both noradrenergic dysfunction and decreased energy demand from neurons lost in response to DSP-4 treatment (Song et al. 2019). The link between noradrenergic function and FDG-PET has also been shown in patients whose essential tremor responded to β-AR blockers; responders showed lower baseline FDG-PET signal in the left basal ganglia, compared with non-responders (Song et al. 2015).

However, findings from the use of the FDG-PET technique face challenges in interpreting results for adrenergic drug treatment. Firstly, the technique measures just the first step of glucose metabolism: uptake and phosphorylation to glucose-6-phosphate. The radiotracer accumulates in cells and thus does not track or identify the fate of glucose. Without complementary studies (such as those that measure cerebral metabolic rate of oxygen consumption to track oxidative phosphorylation), information about detailed mechanism is unavailable, such as contributions to glucose metabolism from aerobic glycolysis or glycogen synthesis (Vlassenko et al. 2010).

Secondly, brain FDG-PET results are difficult to interpret when drugs alter peripheral glucose uptake or metabolism. This is the likely explanation for an observed decrease in whole-brain FDG-PET signal upon treatment with CL-316,243, a β_3-AR agonist: drug-stimulated uptake of FDG itself in peripheral brown adipose tissue would decrease the availability of PET tracer to the brain (Mirbolooki et al. 2015). In the same study, rat whole-brain uptake was decreased relative to control conditions after stimulating with atomoxetine (a noradrenaline reuptake inhibitor) or mirabegron (β_3-AR agonist with mixed pharmacology). However, the frontal cortex showed a region-specific increase in FDG-PET signal when rats were treated with these drugs; mirabegron stimulated frontal cortex glucose uptake in a dose-dependent manner (Mirbolooki et al. 2015). This suggests that noradrenergic regulation of glucose uptake can be region-specific in the brain. Since β_2-AR stimulation also alters peripheral glucose handling (Boyda et al. 2013), these effects could similarly confound interpretation of central FDG-PET studies, as exemplified in recent conference reports where clenbuterol treatment of human

subjects increased CBF without significant effects on FDG-PET signal (Lodeweyckx et al. 2023; Bishop et al. 2022a).

5.4 Adrenoceptor Regulation of Astrocytic Metabolism

Noradrenaline stimulates astrocytic glycogenolysis via stimulation of α_1-AR and β-AR subtypes (Dong et al. 2012; Gibbs 2016; Hertz et al. 2013; Sorg and Magistretti 1991). Both α_1-AR and β-AR on rat astrocytes are activated by NA, but with different time constants and periodicity displayed by their second messengers (Horvat et al. 2016). cAMP is a key second messenger in regulating the astrocyte functions of energy supply, trophic support, gliosis, and immune activation (Zhou et al. 2019), and there is an important role for intracellular calcium in activating glycogen phosphorylase, thus enhancing glycogenolysis (Hertz et al. 2014). Different receptor subtypes are responsible for effects on glycogenolysis depending on the species; in mice, β_1-AR regulates brain glycogenolysis, whereas chick astrocytes utilize β_2-AR (reviewed in Hertz et al. (2010)). In cultured rat astrocytes expressing a cytosolic glucose biosensor, treatment with adrenaline or NA increases intracellular glucose and is sensitive to an inhibitor of glycogenolysis, suggesting at least part of the response is due to glucose liberation from cellular glycogen stores (Prebil et al. 2011). In rat brain slices, optogenetic activation of a transgenic β_2-AR protein in astrocytes elevates cAMP and caused a release of NA from nearby LC neurons (Tang et al. 2014). The NA release is again sensitive to adenylyl cyclase and glycogenolysis inhibitors, consistent with β_2-AR-mediated astrocytic glycogenolysis supporting neuronal function. In LC-lesioned rats, both cAMP production and glycogen breakdown are impaired under conditions where there is a demand on glycogen stores (Harik et al. 1982). In this study, there was no metabolic impairment in the resting state suggesting that noradrenergic glycogenolysis may be utilized only in stressed conditions such as cerebral ischemia or seizure (Harik et al. 1982).

Other central functions of astrocytic metabolism are regulated by α-ARs. Clonidine and dexmedetomidine, both α-AR agonists, increase glutamine uptake and metabolism in astrocytes, an effect which could be neuroprotective, as glutamine is energetically costly for neurons to deal with (Huang et al. 2000). Clonidine and dexmedetomidine also stimulate glycogen formation and pyruvate dehydrogenase (Hertz et al. 2010). Glycogen turnover is enhanced by NA and α_2-AR agonists (G_i-mediated), as well as β_3-AR agonists, in chick astrocytes (Hutchinson et al. 2011). The α_2-ARs play a role in maintaining glycogen synthesis (reviewed in Gibbs 2016). α_{2A}-ARs may also play a role in non-metabolic brain function; dexmedetomidine has reported neuroprotective effects in ischemia-reperfusion models, possibly via an anti-inflammatory mechanism (Cheng et al. 2018) or through regulation of cerebral blood flow, as mentioned above (Zornow et al. 1992; Drummond et al. 2008; Farag et al. 2017).

5.5 An Adrenergic Link Between Cognition and Neurometabolism

Several compelling studies have been performed in animal models demonstrating the role of astrocytic β-ARs in learning and memory. One proposed mechanism linking astrocyte metabolism to memory formation is the stimulation of the astrocyte-neuron lactate shuttle by astrocytic glycogenolysis, thus fueling neurons with astrocyte-derived lactate (Suzuki et al. 2011; Zhou et al. 2019; Coggan et al. 2018). This hypothesis is supported by work in chicks and rats demonstrating a role of astrocytic β_2-AR in promoting glycogenolysis (Gibbs et al. 2007) and lactate release (Gao et al. 2016), both of which contribute to memory consolidation and long-term potentiation of synaptic strength in hippocampal neurons. In a rat inhibitory avoidance task, lactate rescued propranolol-induced deficits in long-term memory, and conversely, the β_2-AR antagonist ICI-118,551 inhibited lactate release during learning (Gao et al. 2016). In a chick memory reinforcement model, day-old chicks discriminate against colored beads coated with an unpleasant tasting substance (for reviews, see Gibbs 2016; Hertz et al. 2013). Repeated exposure to these beads consolidates the initial memory formation, which would otherwise be forgotten after about 30 min. Treating chicks with central NA improves the memory consolidation (Gibbs et al. 2007). In the chick, both β_2-AR and β_3-AR have a demonstrated role from pharmacological studies employing selective agonists (zinterol and CL-316,243, respectively). Both drugs cause a dose-dependent improvement in bead discrimination which is sensitive to iodoacetate, a glycolysis inhibitor, showing that the learning is dependent on glycolytic activity. β_3-AR acts by regulating glucose supply via uptake through the GLUT transporters, as bead discrimination is potentiated by higher plasma glucose levels and is sensitive to 2-deoxyglucose (2-DG) or treatment with cytochalasin B, a pan-GLUT inhibitor. In contrast, β_2-AR appears to act by supplying glucose from glycogenolysis, as its pharmacologic effects are insensitive to glucose and 2-DG treatment, but the zinterol dose-response is right-shifted by 1,4-dideoxy-1,4-imino-D-arabinitol (DAB), a glycogenolysis inhibitor (Gibbs et al. 2007). The same chick memory model was combined with an acute treatment of Aβ(1–42), where Aβ inhibits memory consolidation, apparently by interfering with glycogen synthesis (Gibbs 2015). The Aβ-induced memory impairment was rescued by treatment with the β_3-AR agonist CL-316,243 (Gibbs 2015; Gibbs et al. 2009b).

The same β_3-AR agonist (CL-316,243, 1 mg/kg) improved novel object recognition and increased the hippocampal Aβ42/Aβ40 ratio in the 3xTg mouse model of AD (a model that shows Aβ pathology) after month-long treatment. Agonist treatment also lowered peripheral glucose and insulin (Tournissac et al. 2021). These correlations support a picture of improved peripheral metabolism and cognitive function in a mouse model of AD but did not evaluate whether the cognitive effect was secondary to the known peripheral effects of β_3-AR agonism or was a result of any direct central effects of the agonist.

Taken together, NA stimulates both energy conserving (glycogen storage, glutamate, potassium uptake) and energy generating processes (glycolysis, tricarboxylic

acid cycle regulation), via distinct AR subtypes to couple energy production and utilization in the brain (Hertz et al. 2010). The impact of drugs on the regulation of aerobic glycolysis holds therapeutic potential, given the hypometabolism seen in multiple neurodegenerative diseases (Cunnane et al. 2020) and the regional correlation between aerobic glycolysis in healthy subjects and Aβ deposition in AD patients (Vlassenko et al. 2010).

6 Neuroinflammation

6.1 Adrenoceptors and Neuroinflammation in the Context of Neurodegenerative Disease

In neurodegenerative disorders such as AD and PD and in ischemic stroke, the loss of neurons and synapses is associated with neuroinflammation. Neuroinflammation can accelerate neuronal and synaptic loss but is also an essential player in the repair and recovery process (Heneka et al. 2010a; Wyss-Coray and Rogers 2012). Resident microglia and astrocytes are critical modulators of the inflammatory response in the brain. Additionally, peripheral monocytes are recruited to the brain and, alongside microglia, play a dynamic role in the modulation of the neuroimmune response, neuronal and synaptic loss, clearance of cellular debris, protein aggregates, and repair processes (Lampron et al. 2013; Schwartz and Shechter 2010). Noradrenaline and CNS ARs have been shown to play a crucial role in the modulation of the neuroimmune responses in both experimental models and clinical disease (Fig. 3) (Heneka et al. 2010a; Feinstein et al. 2002, 2016; Weinshenker 2008). The role of the noradrenergic system in modulating neuroinflammation in neurodegenerative disorders and stroke is still not entirely understood and is under intense investigation at the clinical and preclinical levels. In this section, we review the contribution of NA and its receptors in the modulation of neuroinflammation in the context of neurodegenerative disorders and stroke, with a focus on preclinical studies.

6.2 β-Adrenoceptors Regulate Microglial Phagocytosis

Phagocytosis is an important function of macrophages such as microglia for clearance of both misfolded proteins and cellular debris as well as maintenance of healthy functional synaptic networks through synaptic pruning. NA stimulates phagocytosis of Aβ in mouse microglia cultures (Heneka et al. 2010b; Kalinin et al. 2007) and a partial agonist at the β_1-AR, xamoterol, reduces plaque loads in 5XFAD mouse model of AD, a transgenic model known for early and aggressive plaque presentation (Ardestani et al. 2017). Furthermore, lesion of LC noradrenergic neurons impairs microglial phagocytosis of Aβ in vivo, and this impairment is reversed with NA pro-drug supplementation (Heneka et al. 2010b), suggesting that NA released from the LC may play a role in facilitating phagocytosis of Aβ.

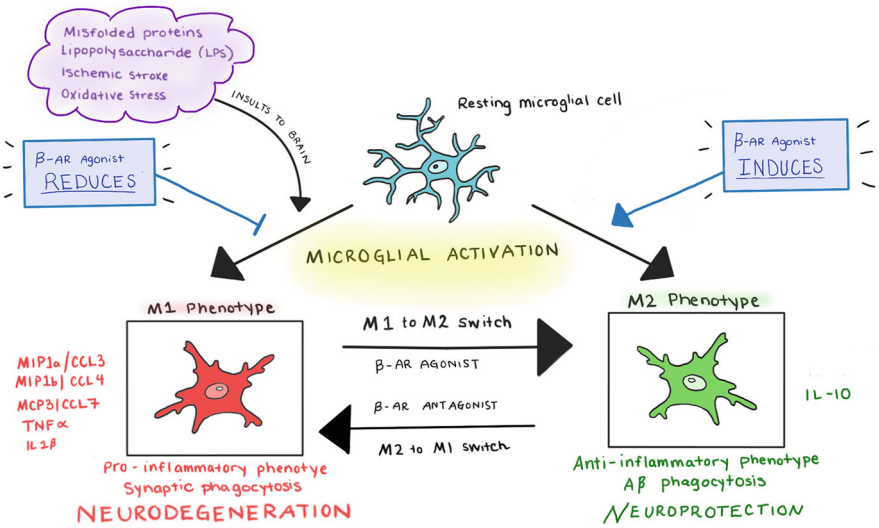

Fig. 3 AR drugs can modulate induction of M1-type versus M2-type microglial phenotypes in response to brain insults, such as misfolded proteins, bacterial infection, oxidative stress, and damage following ischemic stroke. While the M1 vs. M2 designation is likely an oversimplification, in general, resting microglia assume an M1-type activation state in response to multiple insults and this is associated with release of pro-inflammatory cytokines, synaptic phagocytosis, and neurodegeneration. β-AR agonists can attenuate M1-type activation and drive transition to an M2-type activation state associated with reduction in pro-inflammatory cytokines, IL-10 release, phagocytosis of misfolded proteins and an anti-inflammatory neuroprotective phenotype. Reciprocally, antagonism of β-ARs can bias microglia toward an M1-type pro-inflammatory activation state

On the other hand, the β-AR agonists xamoterol and isoprenaline suppress phagocytosis of synaptosomes or beads in immortalized and primary microglial cell cultures (Evans et al. 2020; Steininger et al. 2011), which may be relevant for overactive synaptic pruning and synaptic degeneration associated with neurodegenerative disorders. Effects of xamoterol on suppression of phagocytosis were reversed with a highly selective β_1-AR antagonist, CGP-20712A. Conversely, the β-AR blocker metoprolol potentiates phagocytosis of synaptosomes in microglia cell culture (Evans et al. 2020). In addition, propranolol increases the phagocytotic activity of spinal cord microglia in an experimental autoimmune encephalomyelitis model of multiple sclerosis in rats (Pilipović et al. 2022).

The differential regulation of Aβ versus synaptosome phagocytosis suggests that β-AR signaling could regulate microglial activity in a contextual fashion depending on environmental stimuli and pathological stressors. It is also possible that these functional differences are mediated by two independent populations of cells. In summary, in the context of neurodegenerative diseases, β-AR agonism of microglia may enhance the clearance of Aβ aggregates, while at the same time, preventing excessive synaptic degeneration and pruning. Further experimental evidence will be

needed to fully validate this hypothesis of bidirectional control and modulation of phagocytic activity of microglia in neurodegeneration.

6.3 Adrenoceptor Drugs in Rodent Models of Neuroinflammation in AD and PD

The LC is the primary source of forebrain NA and plays a critical role in regulating cognition, arousal, and neuroinflammation (Sara 2009; Matchett et al. 2021; Feinstein et al. 2016). Under experimental conditions, the loss of noradrenergic tone resulting from LC degeneration or experimental lesion exacerbates the behavioral deficits, neuroinflammation, and pathology observed in animal models of AD (Heneka et al. 2006, 2010b; Kalinin et al. 2007). Pharmacological lesion of noradrenergic neurons in the LC in transgenic mice overexpressing human APP results in elevated Aβ plaque load, increased neuronal loss, elevated markers of inflammation, impaired migration of microglia to plaque sites, impaired microglial phagocytosis of Aβ, and deficits in social and spatial memory tasks (Heneka et al. 2006, 2010b; Kalinin et al. 2007). Many of these deficits are reversed by replacing NA (or its influence on ARs). For example, the NA pro-drug L-DOPS decreases plaque load in transgenic mice overexpressing human APP (Heneka et al. 2010b; Kalinin et al. 2012), and β-AR agonists improve cognition and attenuate Aβ load, neuroinflammation, and tau pathology in mouse models of AD (Coutellier et al. 2014; Heneka et al. 2010b; Ardestani et al. 2017). Specifically, in the 5XFAD transgenic mouse model of AD, chronic administration of xamoterol, a partial agonist at the β_1-AR, attenuates neuroinflammation across both early and late stages of disease pathology and reduces both Aβ and tau pathology (Ardestani et al. 2017). Soluble Aβ oligomers have been shown to impair hippocampal long-term potentiation and this impairment is rescued by isoprenaline or selective agonists activating either β_1-AR or β_2-AR (Jin et al. 2022). Like the APP models, in a mouse model of Down's syndrome (Ts65Dn with overexpression of mouse APP gene), in which degeneration of LC is also observed, acute treatment with L-DOPS or xamoterol reverses cognitive behavioral deficits (Salehi et al. 2009; Faizi et al. 2011). Treatment of APP/PS1 mice with a selective β_2-AR agonist, clenbuterol, also enhances hippocampal neurogenesis, attenuates behavioral deficits, and increases dendritic branching and spine density (Chai et al. 2016). β_2-AR activation with clenbuterol also decreases amyloid plaques (Chai et al. 2017). Conversely, β-AR blockers such as propranolol or metoprolol impair cognition in rodent models of learning and memory and potentiate neuroinflammation both in mouse APP models of AD and in models of neuroinflammation induced by systemic inflammation with lipopolysaccharide (LPS) (Evans et al. 2020; Roozendaal et al. 2008). Similarly, pharmacological blockade of β-ARs with a highly selective β_2-AR antagonist (ICI-118,551) in a mouse model of AD exacerbates cognitive deficits and neuroinflammation and increases Aβ and plaque load (Branca et al. 2014). The effectiveness of adrenergic drugs in AD models and the impairment induced by β-AR antagonists highlights the significant role LC noradrenergic neurons and

noradrenergic tone may have on AD progression. Genetic evidence also points to a role for the β_1-AR as polymorphisms in the human ADRB1 gene which encodes β_1-AR contribute to a genetic risk factor for the development of AD (Bullido et al. 2004). However, whether these purported polymorphic forms of the β_1-AR are associated with either function gain or loss, or altered cell receptor expression, is not yet clear.

Effects of AR modulation can also be seen in in vitro models of CNS inflammation. Noradrenaline directly suppresses cytokine and chemokine responses to Aβ in microglia cultures in vitro, including suppression of major histocompatibility complex class II (MHCII), tumor necrosis factor-alpha (TNF-α), interleukin 1-beta (IL-1β), and inducible nitric oxide synthase (iNOS) signaling (Heneka et al. 2010b). Xamoterol, a partial agonist at β_1-AR, and other β-AR agonists have anti-inflammatory effects on LPS-induced TNF-α production in rodent primary microglia culture (Ardestani et al. 2017; Yi et al. 2017). Conversely, β-AR blockers have been shown to potentiate LPS-induced inflammation in microglial cultures (Ardestani et al. 2017; Evans et al. 2020).

Some of the CNS effects of adrenergic drugs may result from an interaction with a systemic effect on inflammation as adrenergic systems broadly regulate both systemic and CNS inflammation. One of the most robust anti-inflammatory effects of β-AR agonism in the periphery is upregulation of the anti-inflammatory factor, interleukin-10. This has previously been demonstrated with the β_2-AR agonist clenbuterol in the CNS (McNamee et al. 2010; Ryan et al. 2016). In an LPS model in which peripheral immune activation leads to neuroinflammation, mabuterol, a selective β_2-AR agonist, potentiates interleukin-10 and attenuates MIP-1α in the periphery. Conversely, a nonselective β-AR antagonist, propranolol, downregulates interleukin-10 and potentiates MIP-1α in the periphery, showing bidirectional modulation of these cytokines in the periphery with β-AR agonism versus antagonism (Evans et al. 2020). Mabuterol also attenuates systemic protein levels of the pro-inflammatory mediators, monocyte chemoattractant protein-1 (MCP-1), TNF-α, interleukin-27, macrophage colony-stimulating factor (MCSF), and interferon-alpha (IFN-α) in the LPS model and attenuates interferon gamma-induced protein 10 (IP-10), macrophage inflammatory protein-1beta (MIP-1β), and interleukin-27 in brain homogenate in the LPS model.

Overall, activation of ARs, mainly β_2-AR receptors, leads to both central and peripheral reduction in pro-inflammatory markers, with implications for recruitment of peripheral immune cells to the brain following acute brain injury or in chronic neurodegenerative disorders. This dynamic, multifaceted modulation of the immune response by the adrenergic system may be leveraged as a neuroprotective strategy in neurodegenerative disorders.

6.4 β-AR Agonists and Antagonists in Stroke

Ischemic stroke is one of the leading causes of death worldwide and is characterized by cerebral infarction, neuronal degeneration, neuroinflammation, and the

development of sensorimotor and cognitive impairments (Kochanek et al. 2017). As with AD, the degree to which neuroinflammation, as a consequent or contributory factor, modulates ischemic brain injury remains under investigation. It has been suggested that chronic neuroinflammation contributes to secondary neuronal injury in the ischemic penumbra and that microglia, the primary immune cells of the CNS, play a key role in processes leading to cellular and synaptic loss (Marien et al. 2004; Trapp et al. 2007). Conversely, the recruitment of peripheral immune cells to the brain, specifically monocyte-derived macrophages, may aid in reparative processes following brain injury (Wattananit et al. 2016). As discussed above, degeneration of the LC that occurs with normal aging may reduce noradrenergic tone in the aging brain and increase the risk of ischemic stroke, autonomic dysfunction, and neurological impairments (Matchett et al. 2021; Jacobs et al. 2021; Mather and Harley 2016). β-AR antagonists are associated with reduced risk for early death in ischemic stroke patients, although the timing of the drug treatment in relation to the onset of ischemic stroke can influence whether the neuroinflammatory response in the brain is neuroprotective versus contributing to secondary neuronal injury (Dziedzic et al. 2007).

β-AR antagonists augment the neuroimmune response in terms of cytokine expression post-stroke yet are overall considered as neuroprotective (Lechtenberg et al. 2019), supporting the idea that there may be a window of time post-stroke where immune response plays a neuroprotective role. The α_1/β-AR antagonist, carvedilol, also promotes neuroprotection (Savitz et al. 2000), which could lead to a reduction in inflammation secondary to the neuroprotection. Pretreatment with the β-AR blocker, propranolol, reduces infarct and inflammation post-stroke in rat models of ischemia (Lin et al. 2020). Furthermore, blocking ARs broadly with a combination of propranolol, prazosin, and atipamezole are also neuroprotective as they normalize abnormal neural activity (cortical spreading depolarizations) and extracellular K+ concentrations associated with neuronal damage after photothrombotic stroke (Monai et al. 2019, 2021). These neuroprotective effects in rats are observed both with pretreatment and when antagonists were administered up to but not beyond 3 h post-stroke (Monai et al. 2019).

The effects of β-AR agonists in stroke models are somewhat mixed: while overall showing anti-inflammatory effects, some studies report neuroprotection (Junker et al. 2002; Semkova et al. 1996) and others report increased damage. For example, clenbuterol is neuroprotective and also reduces inflammation in kainic acid-induced excitotoxicity and neuronal injury models (Gleeson et al. 2010). However, when administered post-stroke, clenbuterol increases infarct size while again reducing the neuroimmune response based on reduced microglial cell counts and suppressed cytokine expression (Lechtenberg et al. 2019). Further detailed exploration of the therapeutic windows and temporal profiles for β-AR agonism and antagonism are warranted to clearly map and understand the role of NA and neuroinflammation in the pathophysiology of ischemic stroke, and especially any potential benefit of AR-targeted therapeutics.

7 Clinical Strategies to Rescue Loss of Noradrenergic Impact Following LC Decline

The noradrenergic system, and β-ARs in particular, are promising therapeutic targets for neurodegenerative diseases such as AD and PD. Through its binding to both α- and β-ARs, NA plays a key role in a variety of essential CNS functions such as learning and memory, mood, arousal, attention, and cognition (Samuels and Szabadi 2008; Sara 2009). In neurodegenerative disorders, degradation of the LC occurs early in the disease process and leads to a reduction in the integrity of this important brainstem nucleus. This reduced LC integrity leads to a decrease in NA and the resultant clinically significant impairments in mood and cognition, which are hallmarks of these disorders.

7.1 Noradrenaline Levels and Positive Mood

One clinical approach to rescue the adrenergic deficit caused by LC degeneration is through indirect modulation of NA levels, rather than directly targeting the ARs. In the case of depression, several agents acting as indirect agonists of adrenergic neurotransmission have been developed over the last 70 years (David et al. 2022). Of these, the most selective noradrenergic agents are the NRIs including reboxetine (approved for treatment of depression) and atomoxetine (approved for treatment of attention deficit/hyperactivity disorder).

The clinical effects of NRIs arise from their inhibition of NA reuptake via NETs which leads to increased concentration of this neurotransmitter in the synaptic cleft and thus facilitates increased noradrenergic neurotransmission (Bunney and Davis 1965). Additionally, due to crosstalk between noradrenergic and serotonergic and dopaminergic neurotransmitter systems, there may be a secondary effect on serotonin or dopamine neuronal transmission through increased NA release (Blier 2001; Castelli et al. 2016; Williams et al. 2014; Baraban and Aghajanian 1980). Adrenergic receptors expressed on dopaminergic terminals in the prefrontal cortex regulate dopamine release (Castelli et al. 2016), and noradrenergic input from the LC facilitates tonic firing rates of stress-responsive dopaminergic and serotonergic neurons of the raphe nucleus projecting to forebrain target sites (Williams et al. 2014; Baraban and Aghajanian 1980). Increased levels of NA at the synapse have a resultant positive effect on mood (Delgado and Moreno 2000). Positive mood effects may be due to these agents having a strong effect on social cognitive processes. Depressed individuals commonly have a negative emotional bias, which means that they misinterpret social cognitive signals that are neutral as being negative (Surguladze et al. 2004). This can be reliably demonstrated in the facial expression recognition task (FERT; Harmer et al. (2009)) in which subjects are shown faces with a range of expressions ranging from neutral to highly emotive. It is a consistent finding that when subjects with depression are shown images of faces with a neutral expression, they categorize them as having a negative expression.

Negative emotional bias can be reduced by treatment with a variety of NRI antidepressants even after only a single dose. Reboxetine, for example, has been demonstrated to increase the recognition of happy faces in both healthy volunteers and subjects with depression (Harmer et al. 2003, 2009). This effect on increased recognition of happy faces might be particularly specific to noradrenergic modulation and might be relevant for subjects with anhedonia. Anhedonia, a loss in the ability to feel pleasure, is a core feature of depression and a common symptom for depressed AD and PD patients. The NRI-mediated reduction of negative emotional bias produces a change in the perspective of how these patients view the world, improving their mood over time. This combined pharmacological/neuropsychological hypothesis serves as an explanation for the disconnect seen in the treatment of depression, where the pharmacological effect on the reuptake transporters occurs in a matter of hours, but the antidepressant effects take weeks (Godlewska and Harmer 2021).

Since the deficits seen in AD and PD have a strong adrenergic component, several medications that target this system have been used to treat depression in these patient populations. Depression is common in PD, impairs quality of life, and has been estimated to occur in 40–50% of patients (Reijnders et al. 2008). However, to date no NRIs have convincingly shown a strong effect on depressive symptoms for AD and PD patients. One study tested the NRI, atomoxetine, in 55 PD patients in an 8-week, randomized, double-blind, placebo-controlled study (Weintraub et al. 2010). Subjects were randomized to atomoxetine 40 mg/day or placebo for 2 weeks at which point the dosage of atomoxetine or placebo was increased to 80 mg/day for the duration of the study. Interestingly, while the effects on the primary endpoint of depression (Inventory for Depressive Symptomatology–Clinician Rated) were not statistically significant, subjects on atomoxetine experienced an improvement in global cognition as measured by the MMSE relative to patients on placebo (Weintraub et al. 2010).

7.2 Noradrenaline and Cognition

Noradrenaline is essential for arousal and attention, which are the fundamental substrates of cognition (Berridge and Waterhouse 2003). The role of the noradrenergic system in arousal is being applied in clinical studies of aggression and agitation in neurodegenerative disease, where α-AR antagonists (NCT03710642; Wang et al. (2009)) and β-AR blockers (Peskind et al. 2005; Herrmann et al. 2004; Yu et al. 2011) show promise in treating disruptive behavior. However, the use of adrenoceptor antagonists may have unintended negative consequences on aspects of cognition, including attention. Attention is affected in a variety of neurodegenerative disorders, including in both PD and AD, and can be defined as: “a selection mechanism that allows for the preferential processing of task-relevant information over irrelevant (distracting) information, i.e., it is a filter mechanism” (Thiele and Bellgrove 2018).

While PD is commonly viewed as a motor disorder, many patients also experience cognitive deficits (Santangelo et al. 2015). At the time of PD diagnosis approximately 20–33% of patients experience PD-mild cognitive impairment (PD-MCI). Eventually up to 60–80% develop Parkinson's disease dementia (PDD) within 12 years of disease duration (Hely et al. 2008). Currently, the only drug approved to treat the cognitive deficits associated with PD is the cholinesterase inhibitor rivastigmine, which is approved for PDD but not PD-MCI.

Several studies have examined the use of NRIs for the treatment of cognitive deficits in PD, mostly using the NRI, atomoxetine. The largest of these studies with a cognition-based primary endpoint was a 12-week, single-site, double-blind, placebo-controlled, parallel-group design with 30 patients receiving 80 mg atomoxetine or placebo (Hinson et al. 2017). There was no statistically significant difference between treatment groups in the primary outcome measures of cognition. However, there were significant improvements on subjective measures of attention and impulsivity using the Conners Adult Attention Deficit Hyperactivity Disorder Rating Scale. These results on this rating scale were similar to a previous study that examined cognition and impulsivity (Marsh et al. 2009). In this open-label study, 12 patients were administered flexible doses of atomoxetine over 8 weeks starting at 25 mg/day on week one and reaching 100 mg at week six. Clinically significant improvements in executive function in addition to the improvement in impulsivity and attention were noted (Marsh et al. 2009).

In AD there have been several attempts to treat the underlying NA deficit with NRIs. A study conducted by Lilly used doses of 25–80 mg/day of atomoxetine in 92 patients with mild to moderate AD. There was no benefit in the primary endpoint, defined as a change from baseline in the Alzheimer's Disease Assessment Scale–Cognitive Subscale (ADAS-Cog) (Mohs et al. 2009). However, a recently published study of MCI patients with probable AD found biomarker changes following treatment with atomoxetine that are consistent with disease modification (Levey et al. 2021). This was a single-center, phase 2 randomized, double-blind, placebo-controlled, crossover trial. A total of 39 subjects with MCI were randomized to atomoxetine or placebo for 6 months, and then crossed over to receive the alternative intervention for 6 months. Among the biomarkers were plasma and CSF NA levels, which increased, consistent with target engagement and the mechanism of action of the drug. There was also a significant reduction in CSF tau and tau phosphorylated at threonine 181 compared to placebo. FDG-PET and functional MRI imaging detected changes consistent with increased metabolism and network connectivity, respectively, following treatment with atomoxetine (Levey et al. 2021). The latter observation is similar to findings with atomoxetine intervention in a PD cohort (Borchert et al. 2016).

7.3 Direct Receptor Targeting to Rescue Cognitive and Depressive Symptoms

The observed effects of NRIs on cognitive and depression scales in clinical studies are clearly somewhat mixed in outcomes. One likely reason is that these drugs act as indirect agonists (David et al. 2022), and their efficacy depends on the underlying levels of NA still available despite ongoing neurodegeneration and noradrenergic neuronal loss. This phenomenon has been observed with cholinesterase inhibitors (Sabbagh and Cummings 2011). As neurodegenerative disease progresses and LC declines, it inevitably means that the endogenous levels of NA are decreasing and therefore the efficacy of NRIs wanes as a function of disease progression.

Direct agonism of ARs to replace lost noradrenergic tone may be a more promising approach. For instance, in a small placebo-controlled crossover study of nine PD patients there was an improvement in some attentional tasks after treatment with naphtoxazine (SDZ NVI-085), an α_1-AR agonist (Bédard et al. 1998). Besipirdine, a molecule with multiple pharmacologic properties including metabolism to an α_1-AR agonist/α_2-AR antagonist, trended toward maintaining ADAS-Cog scores in a larger 12-week, double-blind, parallel-group, placebo-controlled AD study of 275 patients, but did not attain statistical significance (Huff et al. 1996).

As has been shown for several neurotransmitters, NA stimulation follows a "Yerkes-Dodson" (or inverted U-shaped) dose-response (David et al. 2022; Baldi and Bucherelli 2005). Excessive amounts of endogenous NA coupled with exogenous noradrenergic stimulation may lead to a state of too much arousal. Direct imaging of the LC may aid identification of patients with diminished LC integrity who may be most likely to respond to direct noradrenergic therapeutic approaches. Elegant work using NM-MRI to quantify LC integrity suggests that in patients with PD, response to atomoxetine is dependent on LC integrity (O'Callaghan et al. 2021). Those patients with greater LC integrity do not respond as well to atomoxetine as those with more neurodegeneration at this nucleus. AD patients also display heterogeneity in the degree of LC degeneration (Bondareff et al. 1987b). Therefore, for optimal treatment, it may be necessary to identify patients who have low LC integrity (Betts et al. 2019) to select those patients who are most likely to respond to intervention with a direct agonist.

7.4 Pharmacoepidemiological Studies of β-Adrenoceptor Ligands in Parkinson's Disease

Pharmacoepidemiological studies exploit prescription database information from known drugs to furnish additional evidence for roles of drug target mechanisms in the management of many pathological conditions and symptoms, as well as in potential adverse drug reactions. Study popularity has flourished due to the broad availability of well-curated databases housing prescription and diagnostic histories across large cohorts of patients through health care provider databases and country-wide public authority registries.

Within the AR family, one receptor has received notable attention in neurological pharmacoepidemiology over the last 5 years, triggered by findings related to β_2-AR agonists in Parkinson's and other degenerative neurological conditions. Scherzer and coworkers (Mittal et al. 2017) made an intriguing discovery when screening a library of over 1,100 approved and former drugs, health supplements, vitamins, and related substances in a human neuroblastoma cell line SK-N-MC that expresses α-syn mRNA (SNCA) and protein. The team sought to identify from a large range of known pharmacological mechanisms ones that may have activity at reducing expression of SNCA or its protein (α-syn) production. The β_2-AR agonist metaproterenol was found to produce a $> 35\%$ reduction in SNCA, and the potent and selective β_2-AR agonists, clenbuterol and salbutamol, were then also found to be active, suppressing the production of α-syn protein. The activity of these agonists was investigated in a range of tissue and animal-based models of dopaminergic neuronal function and PD, yielding evidence that this mechanism may offer neuroprotective properties and influence the pathological hallmarks of PD. A collaboration with the Norwegian health care authority's prescription database (NorPD) allowed interrogation of the relationship between PD diagnosis and the use of the β_2-AR agonist salbutamol (commonly used in respiratory disorders), and the nonselective β-AR blocker, propranolol (commonly used for treatment of migraine or anxiety), over the years 2005–2014. The key finding was that chronic use of salbutamol (albuterol) over several years was associated with a significantly reduced incidence of PD diagnosis. Consistent with this, the use of the highly CNS-penetrant nonselective β-AR blocker propranolol was associated with an increased incidence of PD, seemingly extending the cell based and preclinical findings all the way through to impact on human Parkinson's pathology. The intimation was that a β_2-AR mediated transcriptional regulation suppressed SNCA and thereby α-syn production, thereby attenuating disease pathology.

Such studies in broad databases documenting the use of pharmacological agents can be instructive, just as can be achieved with genome-wide association studies, but it is always clearly cautioned that these observations cannot provide proof of causality: they are simply associational studies, and the occurrence of such associations could be driven by a variety of confounding factors. A flurry of similar analyses across a range of cohorts and geographical populations, including those in Israel, UK, Denmark, and several US database populations, subsequently examined the impact of β_2-AR agonists and β-AR blockers, and in some cases additional non-adrenergic treatments for respiratory, cardiovascular, and neurologic disorders (Gronich et al. 2018; Nielsen et al. 2018; Koren et al. 2019; Hopfner et al. 2019; Cepeda et al. 2019). All of these studies set out to identify and control for confounds or biases that may otherwise skew associations in a non-causal manner.

In essence, the sequence of studies (Table 2) have all demonstrated findings that are, on the surface, qualitatively consistent with and supportive of those described in the original paper, but that in some studies, associations could have resulted to an extent from inadequate correction for the use of β_2-AR agonists (including salbutamol) in patients who were smokers, reflective of a well-described (but poorly explained) protective association of tobacco smoking with PD diagnosis (Hopfner

Table 2 Pharmacoepidemiology studies investigating association between β_2-AR and risk of Parkinson's disease

Reference	Study
Mittal et al. (2017)	*Method*: Longitudinal Analysis (2005–2014) of 4.6M Norway population (NorPD), 10K PD cases: salbutamol & propranolol use. *Findings*: Of 620K salbutamol users, PD diagnosis rate ratio (RR) 0.65 (95% CI 0.57 to 0.74), dose/use dependent; inhaled corticosteroid (ICS) users had no risk reduction. Smoking status seemingly not a clear confounder. Of 63K propranolol users, PD diagnosis rate ratio 2.16 (1.59 to 2.94), non-neurological cases (essential tremor excluded). *Interpretation*: Subjects taking β_2-AR agonist bronchodilators, but not other asthma/COPD products have reduced PD risk. Drugs blocking brain β-ARs may increase risk, correcting for use in tremor.
Gronich et al. (2018)	*Method*: Nested case-control study (2004–2017) in 1.8M Israel Clalit HS population; 11.3K PD pts with 113K nested controls: Multiple β_2-AR agonists & blockers *Findings*: β_2-AR agonists associated with lower PD diagnosis. RR and 95% CI: • RR 0.89 [0.82–0.96] for short-acting β_2-AR, • RR 0.84 [0.76–0.93] for long-acting β_2-AR, • RR 0.49 [0.25–0.92] for ultra-long-acting β_2-AR. Corrections for smoking and COPD did not change outcome. Non-selective β-AR blockers again associated with increased risk: RR 2.04 [1.90–2.20], but not use of selective β1-preferring antagonists: RR 1.00 [0.95–1.05]. Exclusion of migraine & tremor use of β-AR blockers was *without* impact on findings. *Interpretation*: β_2-AR agonist bronchodilators associated with lower PD incidence, especially those with higher lipophilicity and receptor residence time. Increase RR seen with β-AR blockers, unless showing weaker affinity at β_2-ARs.
Searles Nielsen et al. (2018)	*Method*: Case control study in US Medicare population. 48K PD cases, 52K controls: Salbutamol compared with ICS use; propranolol, carvedilol & metoprolol compared with primidone. *Findings*: Salbutamol use inversely associated *modestly* with PD diagnosis: RR 0.89 (0.86–0.92; similar data for ICS, & both associations lost following exclusion of smokers. However, metered-dose (commonly used) salbutamol use yielded RR 0.81 (0.77–0.84) that was not influenced by smoker exclusion. Of the antagonists, propranolol alone was associated with increased PD diagnosis: RR (3.31–3.96); association lost when excluding those with essential and other forms of tremor. *Interpretation*: Smoking is well-known to inversely associate with PD risk & may be a confounder in this study for protective potential of β_2-AR agonists or ICS. The finding with metered-dose salbutamol is an interesting anomaly. Use of β-AR blockers in tremor accounts for increased associational effect of propranolol.
Koren et al. (2019)	*Method*: Case control study of 2M population Maccabi HS EMRs in Israel; 1998–2016, 145K on β-AR blockers, plus paired control subjects. *Findings*: β-AR blocker use associated with a 1.55 (1.28–1.77) Cox

(continued)

Table 2 (continued)

Reference	Study
	proportional hazard ratio for PD diagnosis. Patients were excluded from analysis if benign tremor was reported in history or if β_2-AR agonist use for asthma. Suggestion of a threshold impact of β-AR blocker based on cumulative dose over time. *Interpretation*: β-AR blocker use association with PD diagnosis not lost by exclusion of subjects with tremor.
Hopfner et al. (2019)	*Method*: Case control study 2000–2012—Danish Rx Registries (5.6M population) with 2.8K PD & 11.2K controls. Examined short & long acting β_2-AR agonists and several β-AR blockers. *Findings*: Found significant inverse association with β_2-AR agonist use & PD diagnosis RR 0.66 (0.52–0.85), with individual agonists consistent, and long-acting β_2-AR agonists possibly more prominent than short-acting β_2-AR agonists. Similar associations seen with ICS and long-acting muscarinic antagonists; correction for COPD diagnosis & related factors eliminated finding for ICS but not completely for β_2-agonists. Antagonist use again associated with increased risk of PD diagnosis, especially for propranolol RR 2.26 (1.48–3.46); finding that shorter term propranolol use was as effective as longer-term use raised suspicion that shorter term use for essential tremor may have resulted in biased association with early PD patients. *Interpretation*: Findings generally consistent with other reports, though formal correction for smoking status (for agonists), tremor as prodromal (antagonists), not undertaken.
Cepeda et al. (2019)	*Method*: Self-controlled cohort study examining >2K medicines (incl. salbutamol & β-blockers) & PD incidence, 4 Db & 117M subjects, 430K PD cases profiled. Design superior for control of bias, improved accuracy, vast cohort population. *Findings*: Five drugs associated with >30% PD diagnosis risk reduction, in ≥2 of 4 Db: modafinil, armodafinil, methylphenidate, isradipine, diphenhydramine; β_2-AR agonist salbutamol associated with lower PD diagnosis in all 4 Db, ranging 11–31%, combine RR 0.69 (0.5–0.96). Of 3 β-AR blockers, propranolol use alone was associated with increased PD diagnosis; metoprolol (β_1-AR-preferring) had no association, and carvedilol (α_1- & β-AR antagonist) show weakly reduced incidence association. These drugs vary in degree of BBB penetration. *Interpretation*: Confirmation of the directional findings of salbutamol and propranolol seen in previous association studies, and with approach less prone to influence of confounders. Finding that modafinil (and its 'eutomer'), & methylphenidate, both with adrenergic facilitating activity, show similar association with lower incidence of PD Rx provides further spotlight on the LC & adrenergic pathway.
Hopfner et al. (2020)	*Method*: Literature review *Findings*: Examination of the six studies above provided appraisal of the risk or odds ratios for use of β_2-AR agonists or antagonists, the impact of suspected confounds (esp. smoking and tremor, respectively), and provided context for other known or suspected risk factors for PD diagnosis.

(continued)

Table 2 (continued)

Reference	Study
	Interpretation: Concluded that evidence is varied for the associations β_2-AR ligand use and PD, with ostensible confounds attenuating and sometimes nullifying odd ratios. Level of risk does not clearly justify changing β_2-AR ligand drug use in highly prevalent diseases where benefit is well established.

Abbreviations: *COPD* congestive obstructive pulmonary disease, *Db* database(s), *ICS* inhaled corticosteroid, *RR* rate ratio, *Rx* prescription

et al. 2020). Likewise, association of increased PD diagnosis with propranolol and other β-AR antagonists could have been confounded by the use of propranolol in treatment of essential tremor, which can itself be prodromal for PD. Examination of the many papers in totality highlights that these studies fail to fully or consistently remove associational evidence by correcting for smoking or tremor. It leaves one suspecting that the real potential for benefit is not adequately robust or conclusive to justify modifying current medical use of these drugs. What is reasonable, however, is that these associational studies do raise the level of mechanistic evidence in support of β_2-AR agonist therapeutic potential that deserves additional, prospective clinical examination. This latter point is stated most especially in the context not so much of regulation of SNCA transcription, per se, but upon more broad heterocellular evidence, described elsewhere in this review, implicating loss of LC neurons and noradrenergic function in the early decline in many degenerative conditions, the evidence for neuroprotective influence of β-AR activation on neuronal, metabolic, and inflammatory cell populations in the brain, as well as evidence from pharmacological studies using such agonists in transgenic models of neurodegenerative disease.

A further twist in this puzzle comes from a recent paper challenging the SNCA regulating effects of β_2-AR activation (Patterson et al. 2022). These investigators examined the original Scherzer team findings on regulation of SNCA transcription, however, unlike the original paper, Patterson and colleagues found that clenbuterol produced only *marginal* and *transient* suppression of SNCA transcription in rodents, and no changes in α-syn protein in various key brain areas, raising questions of the strength of the initial findings. Interestingly, in both papers, clenbuterol was used at supratherapeutic exposures, with findings of least activity in some instances at the highest doses and longest durations of treatment. Given the sub-nanomolar agonist potency of clenbuterol at β_2-ARs (its clinical dose for oral use in man indicates 20–80 μg once daily), use of concentrations 5–20 μM in vitro and doses of 10–40 mg/kg in rodents are not selective for β_2-AR. Such doses make interpretation difficult due to potential off-target (nonselective doses) and on-target (homologous desensitization at β_2-AR) confounds. Further studies will hopefully shed light on this, though perhaps not limited solely to regulation of α-syn biology given the established breadth of β_2-AR function in the brain.

A final question on the salbutamol PD association topic arises from an assertion in the initial paper that salbutamol is brain-penetrant. Although salbutamol is available as an oral agent, its dominant usage is by inhalation locally into the airways, where it is distributed and eliminated rather quickly. There is evidence that the compound can gain access to the brain, though only sparingly so, with estimates for CNS penetration in rats of ~5% versus plasma, and even less than that versus pineal or pituitary gland levels (these areas reside outside the blood-brain barrier) (Caccia and Fong 1984). These data are consistent with predictions based on salbutamol physicochemical properties (Seelig et al. 1994). It seems *unlikely* therefore that systemic exposures of salbutamol, even when administered at high or frequent doses, would afford permeation of pharmacologically significant concentrations into the CNS to generate much receptor activation. An alternative explanation could be nasal entry of salbutamol to the brain; it is well-known that the inhalation route can be highly inefficient in regular use and that a large proportion of inhaled product can become trapped in upper airways tissues. If this occurred readily, then ostensibly this could deliver salbutamol to brain tissues via specialized mucosal to trigeminal or upper nasal/olfactory neural permeation processes that have been hypothesized as being a feasible route for CNS access of drug substances. Indeed, one report suggests that good CNS regional access to salbutamol was achieved after nasal administration in rats (Zhang et al. 2020). Accordingly, as low levels of CNS β_2-AR activation seen in patients using bronchodilators can furnish consistent evidence for even a modest level of neuroprotection (versus PD), then what if one could fashion the ideal β_2-AR agonist to introduce strong activation of brain receptors expressed on a range of cell types, while protecting from significant adverse effects in the periphery? We look forward to seeing that approach find its place in clinical exploration.

8 Conclusion

In summary, there is clear evidence for noradrenergic deficit associated with LC loss occurring in AD, PD, and other neurodegenerative disorders. The pathology of LC loss is consistent with the known biology of brain noradrenergic transmission in supporting arousal, cognition, and mood. Noradrenergic mechanisms mediating this functional decline may include modulation of neurometabolism, neuroinflammation, and regional cerebral blood flow. Attempts to restore the lost adrenergic input with existing reuptake inhibitor drugs show some benefit for attention and cognition, and some hints of potential impact on disease pathology. However, these effects are diminished in patients with more progressed neurodegeneration in whom the endogenous levels of NA are likely to be reduced dramatically due to LC degradation. In these situations, an alternative approach using direct agonists to activate ARs in target cells has the promise to restore the lost noradrenergic input. This approach also offers the ability to fine-tune activity at select AR subtypes, for instance by employing partial agonism or polypharmacology. Further study of expression levels and function for each AR, in both neuronal and glial cells will aid in selecting drugs with optimal pharmacology. For the direct agonism approach to succeed clinically,

brain penetration must be achieved and the peripheral effects of AR agonists must be acceptably controlled. Fortunately, the deep history of AR study provides an excellent foundation for understanding drug effects in humans, providing a bright outlook for the translation of noradrenergic pharmacology into effective therapeutics for neurodegenerative disease.

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References

Abdelmotilib H, West AB (2017) Breathing new life into an old target: pulmonary disease drugs for Parkinson's disease therapy. Genome Med 9:88

Adolfsson R, Gottfries CG, Roos BE, Winblad B (1979) Changes in the brain catecholamines in patients with dementia of Alzheimer type. Brit J Psychiat 135:216–223

Alexander SPH et al (2019) THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: G protein-coupled receptors. Brit J Pharmacol 176:S21–S141

Altosaar K et al (2021) Adrenoceptors (version 2019.3) in the IUPHAR/BPS guide to pharmacology database. https://doi.org/10.2218/gtopdb/F4/2021.3

Anandhan A et al (2017) Metabolic dysfunction in Parkinson's disease: bioenergetics, redox homeostasis and central carbon metabolism. Brain Res Bull 133:12–30

Andrade C (2018) How to read a research paper: an exercise in critical thinking in the context of an epidemiologic study on tamsulosin and the risk of dementia. J Clin Psychiatry 79

Andrés-Benito P et al (2017) Locus coeruleus at asymptomatic early and middle Braak stages of neurofibrillary tangle pathology. Neuropath Appl Neuro 43:373–392

Anlezark GM, Crow TJ, Greenway AP (1973) Impaired learning and decreased cortical norepinephrine after bilateral locus coeruleus lesions. Science 181:682–684

Ardestani PM et al (2017) Modulation of neuroinflammation and pathology in the 5XFAD mouse model of Alzheimer's disease using a biased and selective beta-1 adrenergic receptor partial agonist. Neuropharmacology 116:371–386

Arvanitakis Z, Capuano AW, Leurgans SE, Bennett DA, Schneider JA (2016) Relation of cerebral vessel disease to Alzheimer's disease dementia and cognitive function in elderly people: a cross-sectional study. Lancet Neurol 15:934–943

Asano N, Hishiyama S, Ishiyama T, Kotoda M, Matsukawa T (2020) Effects of β1-adrenergic receptor blockade on the cerebral microcirculation in the normal state and during global brain ischemia/reperfusion injury in rabbits. BMC Pharmacol Toxicol 21:13

Aston-Jones G, Rajkowski J, Kubiak P, Alexinsky T (1994) Locus coeruleus neurons in monkey are selectively activated by attended cues in a vigilance task. J Neurosci 14:4467–4480

Aubineau P-F, Seylaz J, Sercombe R, Mamo H (1973) Evidence for regional differences in the effect of beta-adrenergic stimulation on cerebral blood flow. Brain Res 61:153–161

Bacic F, McCarron RM, Uematsu S, Spatz M (1992) Adrenergic receptors coupled to adenylate cyclase in human cerebromicrovascular endothelium. Metab Brain Dis 7:125–137

Baldi E, Bucherelli C (2005) The inverted "U-shaped" dose-effect relationships in learning and memory: modulation of arousal and consolidation. Dose-Response 3:nonlin.003.01.002

Baraban JM, Aghajanian GK (1980) Suppression of firing activity of 5-HT neurons in the dorsal raphe by alpha-adrenoceptor antagonists. Neuropharmacology 19:355–363

Bartus RT et al (2016) β2-adrenoceptor agonists as novel, safe and potentially effective therapies for amyotrophic lateral sclerosis (ALS). Neurobiol Dis 85:11–24

Beardmore R, Hou R, Darekar A, Holmes C, Boche D (2021) The locus coeruleus in aging and Alzheimer's disease: a postmortem and brain imaging review. J Alzheimers Dis 83:5–22

Bédard MA et al (1998) Attentional deficits in Parkinson's disease: partial reversibility with naphtoxazine (SDZ NVI-085), a selective noradrenergic alpha 1 agonist. Clin Neuropharmacol 21:108–117

Bekar LK, Wei HS, Nedergaard M (2012) The locus coeruleus-norepinephrine network optimizes coupling of cerebral blood volume with oxygen demand. J Cereb Blood Flow Metabolism 32: 2135–2145

Berridge CW, Waterhouse BD (2003) The locus coeruleus–noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. Brain Res Rev 42:33–84

Betts MJ et al (2019) Locus coeruleus imaging as a biomarker for noradrenergic dysfunction in neurodegenerative diseases. Brain 142:2558–2571

Bishop C et al (2022a) Disentangling apparent discordance between ASL-MRI and [18F]-FDG PET following a single dose of the β2-agonist clenbuterol. In: Conference reports: international society for magnetic imaging in medicine

Bishop C et al (2022b) Dose-dependent response of cerebral blood flow in healthy volunteers following administration of β2-adrenergic receptor agonist clenbuterol. In: Conference reports: international society for magnetic imaging in medicine

Blier P (2001) Crosstalk between the norepinephrine and serotonin systems and its role in the antidepressant response. J Psychiatry Neurosci Jpn 26(Suppl):S3–S10

Bolton CJ, Tam JW (2021) Differential involvement of the locus coeruleus in early- and late-onset Alzheimer's disease: a potential mechanism of clinical differences? J Geriatr Psych Neur:089198872110447. https://doi.org/10.1177/08919887211044755

Bondareff W et al (1987a) Neuronal degeneration in locus ceruleus and cortical correlates of Alzheimer disease. Alzheimer Dis Assoc Disord 1:256–262

Bondareff W et al (1987b) Age and histopathologic heterogeneity in Alzheimer's disease: evidence for subtypes. Arch Gen Psychiat 44:412–417

Borchelt DR et al (1997) Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. Neuron 19:939–945

Borchert RJ et al (2016) Atomoxetine enhances connectivity of prefrontal networks in Parkinson's disease. Neuropsychopharmacology 41:2171–2177

Borghammer P et al (2010) Cortical hypometabolism and hypoperfusion in Parkinson's disease is extensive: probably even at early disease stages. Brain Struct Funct 214:303–317

Borodovitsyna O, Duffy BC, Pickering AE, Chandler DJ (2020) Anatomically and functionally distinct locus coeruleus efferents mediate opposing effects on anxiety-like behavior. Neurobiology Stress 13:100284

Boyda HN, Procyshyn RM, Pang CCY, Barr AM (2013) Peripheral adrenoceptors: the impetus behind glucose dysregulation and insulin resistance. J Neuroendocrinol 25:217–228

Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82:239–259

Braak H et al (2003) Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 24:197–211

Braak H, Rüb U, Steur ENHJ, Tredici KD, de Vos RAI (2005) Cognitive status correlates with neuropathologic stage in Parkinson disease. Neurology 64:1404–1410

Braak H, Thal DR, Ghebremedhin E, Tredici KD (2011) Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. J Neuropathol Exp Neurol 70:960–969

Branca C, Wisely EV, Hartman LK, Caccamo A, Oddo S (2014) Administration of a selective β2 adrenergic receptor antagonist exacerbates neuropathology and cognitive deficits in a mouse model of Alzheimer's disease. Neurobiol Aging 35:2726–2735

Brassard P, Tymko MM, Ainslie PN (2017) Sympathetic control of the brain circulation: appreciating the complexities to better understand the controversy. Autonomic Neurosci 207: 37–47

Breton-Provencher V, Drummond GT, Sur M (2021) Locus coeruleus norepinephrine in learned behavior: anatomical modularity and spatiotemporal integration in targets. Front Neural Circuit 15:638007
Brunnström H, Friberg N, Lindberg E, Englund E (2011) Differential degeneration of the locus coeruleus in dementia subtypes. Clin Neuropathol 30:104–110
Bullido MJ et al (2004) Polymorphism in genes involved in adrenergic signaling associated with Alzheimer's. Neurobiol Aging 25:853–859
Bunney WE, Davis JM (1965) Norepinephrine in depressive reactions: a review. Arch Gen Psychiat 13:483–494
Buxton RB, Frank LR (1996) A model for the coupling between cerebral blood flow and oxygen metabolism during neural stimulation. J Cereb Blood Flow Metabolism 17:64–72
Bylund DB et al (1994) International Union of pharmacology nomenclature of adrenoceptors. Pharmacology Reviews 46:121–136
Caccia S, Fong MH (1984) Kinetics and distribution of the β-adrenergic agonist salbutamol in rat brain. J Pharm Pharmacol 36:200–202
Cai R et al (2019) Enhancing glycolysis attenuates Parkinson's disease progression in models and clinical databases. J Clin Invest 129:4539–4549
Camandola S, Mattson MP (2017) Brain metabolism in health, aging, and neurodegeneration. EMBO J 36:1474–1492
Camargo A, Wang Z, Initiative ADN (2021) Longitudinal cerebral blood flow changes in normal aging and the Alzheimer's disease continuum identified by arterial spin labeling MRI. J Alzheimers Dis 81:1727–1735
Carter ME et al (2010) Tuning arousal with optogenetic modulation of locus coeruleus neurons. Nat Neurosci 13:1526–1533
Cash R, Raisman R, Lanfumey L, Ploska A, Agid Y (1986) Cellular localization of adrenergic receptors in rat and human brain. Brain Res 370:127–135
Castelli MP et al (2016) α2A adrenergic receptors highly expressed in mesoprefrontal dopamine neurons. Neuroscience 332:130–139
Catus SL, Gibbs ME, Sato M, Summers RJ, Hutchinson DS (2011) Role of β-adrenoceptors in glucose uptake in astrocytes using β-adrenoceptor knockout mice. Brit J Pharmacol 162:1700–1715
Cepeda MS, Kern DM, Seabrook GR, Lovestone S (2019) Comprehensive real-world assessment of marketed medications to guide Parkinson's drug discovery. Clin Drug Invest 39:1067–1075
Chai G, Wang Y, Yasheng A, Zhao P (2016) Beta 2-adrenergic receptor activation enhances neurogenesis in Alzheimer's disease mice. Neural Regen Res 11:1617–1624
Chai G, Wang Y, Zhu D, Yasheng A, Zhao P (2017) Activation of β2-adrenergic receptor promotes dendrite ramification and spine generation in APP/PS1 mice. Neurosci Lett 636:158–164
Chalermpalanupap T et al (2013) Targeting norepinephrine in mild cognitive impairment and Alzheimer's disease. Alzheimers Res Ther 5:21
Chalermpalanupap T, Weinshenker D, Rorabaugh JM (2017) Down but not out: the consequences of pretangle tau in the locus coeruleus. Neural Plast 2017:1–9
Chalermpalanupap T et al (2018) Locus coeruleus ablation exacerbates cognitive deficits, neuropathology, and lethality in P301S tau transgenic mice. J Neurosci:1483–1417. https://doi.org/10.1523/jneurosci.1483-17.2017
Chan-Palay V, Asan E (1989) Alterations in catecholamine neurons of the locus coeruleus in senile dementia of the Alzheimer type and in Parkinson's disease with and without dementia and depression. J Comp Neurol 287:373–392
Cheng J et al (2018) Dexmedetomidine attenuates cerebral ischemia/reperfusion injury in neonatal rats by inhibiting TLR4 signaling. J Int Medical Res 46:2925–2932
Coggan JS et al (2018) Norepinephrine stimulates glycogenolysis in astrocytes to fuel neurons with lactate. PLoS Comput Biol 14:e1006392
Cohen Z, Molinatti G, Hamel E (1997) Astroglial and vascular interactions of noradrenaline terminals in the rat cerebral cortex. J Cereb Blood Flow Metabolism 17:894–904

Collins S et al (1990) Mechanisms involved in adrenergic receptor desensitization. Biochem Soc T 18:541–544
Compton DM, Dietrich KL, Smith JS, Davis BK (1995) Spatial and non-spatial learning in the rat following lesions to the nucleus locus coeruleus. Neuroreport 7:177–182
Cope ZA, Vazey EM, Floresco SB, Jones GSA (2019) DREADD-mediated modulation of locus coeruleus inputs to mPFC improves strategy set-shifting. Neurobiol Learn Mem 161:1–11
Coradazzi M et al (2016) Selective noradrenaline depletion impairs working memory and hippocampal neurogenesis. Neurobiol Aging 48:93–102
Cortes-Canteli M, Iadecola C (2020) Alzheimer's disease and vascular aging. J Am Coll Cardiol 75: 942–951
Coutellier L, Ardestani PM, Shamloo M (2014) β1-adrenergic receptor activation enhances memory in Alzheimer's disease model. Ann Clin Transl Neur 1:348–360
Crow T (1973) The coeruleo-cortical norepinephrine system and learning. In: Proceedings of the third international catecholamine symposium vol. frontiers in catecholamine research, pp 723–726
Cunnane SC et al (2020) Brain energy rescue: an emerging therapeutic concept for neurodegenerative disorders of ageing. Nat Rev Drug Discov:1–25. https://doi.org/10.1038/s41573-020-0072-x
Cunningham ET, Bohn MC, Sawchenko PE (1990) Organization of adrenergic inputs to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. J Comp Neurol 292: 651–667
Dahlgren N, Ingvar M, Siesjö BK (1981) Effect of propranolol on local cerebral blood flow under normocapnic and hypercapnic conditions. J Cereb Blood Flow Metabolism 1:429–436
David MCB et al (2022) Cognitive and neuropsychiatric effects of noradrenergic treatment in Alzheimer's disease: systematic review and meta-analysis. J Neurol Neurosurg Psychiatry:jnnp-2022-329136. https://doi.org/10.1136/jnnp-2022-329136
Dehvari N et al (2012) β2-adrenoceptors increase translocation of GLUT4 via GPCR kinase sites in the receptor C-terminal tail. Brit J Pharmacol 165:1442–1456
Delgado PL, Moreno FA (2000) Role of norepinephrine in depression. J Clin Psychiatry 61 Suppl 1: 5–12
Dienel GA (2017) Noradrenergic signaling and astroglia:145–166. https://doi.org/10.1016/b978-0-12-805088-0.00007-4
Dienel GA, Cruz NF (2016) Aerobic glycolysis during brain activation: adrenergic regulation and influence of norepinephrine on astrocytic metabolism. J Neurochem 138:14–52
Dolui S, Li Z, Nasrallah IM, Detre JA, Wolk DA (2020) Arterial spin labeling versus 18F-FDG-PET to identify mild cognitive impairment. Neuroimage Clin 25:102146
Dong J et al (2012) Beta2-adrenergic receptor and astrocyte glucose metabolism. J Mol Neurosci 48:456–463
Drummond JC et al (2008) Effect of dexmedetomidine on cerebral blood flow velocity, cerebral metabolic rate, and carbon dioxide response in normal humans. Anesthesiology 108:225–232
Drzezga A et al (2005) Prediction of individual clinical outcome in MCI by means of genetic assessment and (18)F-FDG PET. J Nucl Medicine Official Publ Soc Nucl Medicine 46:1625–1632
Duan Y, Grady JJ, Albertsen PC, Wu ZH (2018) Tamsulosin and the risk of dementia in older men with benign prostatic hyperplasia. Pharmacoepidem Dr S 27:340–348
Dziedzic T, Slowik A, Pera J, Szczudlik A (2007) Beta-blockers reduce the risk of early death in ischemic stroke. J Neurol Sci 252:53–56
Edvinsson L, Lacombe P, Owman CH, Reynier-Rebuffel AM, Seylaz J (1979) Quantitative changes in regional cerebral blood flow of rats induced by alpha-and beta-adrenergic stimulants. Acta Physiol Scand 107:289–296
Elfont RM, Sundaresan PR, Sladek CD (1989) Adrenergic receptors on cerebral microvessels: pericyte contribution. Am J Physiology-regulatory Integr Comp Physiology 256:R224–R230

Evans AK et al (2020) Beta-adrenergic receptor antagonism is proinflammatory and exacerbates neuroinflammation in a mouse model of Alzheimer's disease. Neurobiol Dis 146:105089
Evans AK et al (2021) Age-related neuroinflammation and pathology in the locus coeruleus and hippocampus: beta-adrenergic antagonists exacerbate impairment of learning and memory in aged mice. Neurobiol Aging 106:241–256
Fagerholm V et al (2008) Autoradiographic characterization of α2C-adrenoceptors in the human striatum. Synapse 62:508–515
Faizi M et al (2011) Comprehensive behavioral phenotyping of Ts65Dn mouse model of Down syndrome: activation of β1-adrenergic receptor by xamoterol as a potential cognitive enhancer. Neurobiol Dis 43:397–413
Farag E et al (2017) The relative effects of dexmedetomidine and propofol on cerebral blood flow velocity and regional brain oxygenation. Eur J Anaesthesiol 34:732–739
Feinstein DL et al (2002) Noradrenergic regulation of inflammatory gene expression in brain. Neurochem Int 41:357–365
Feinstein DL, Kalinin S, Braun D (2016) Causes, consequences, and cures for neuroinflammation mediated via the locus coeruleus: noradrenergic signaling system. J Neurochem 139:154–178
Floare M-L, Allen SP (2020) Why TDP-43? Why not? Mechanisms of metabolic dysfunction in amyotrophic lateral sclerosis. Neurosci Insights 15:2633105520957302
Freeman RD, Li B (2016) Neural–metabolic coupling in the central visual pathway. Philos Trans R Soc B Biological Sci 371:20150357
Froese L, Dian J, Gomez A, Unger B, Zeiler FA (2020) The cerebrovascular response to norepinephrine: a scoping systematic review of the animal and human literature. Pharmacol Res Perspectives 8:e00655
Frost M et al (2020) Vascular α1A adrenergic receptors as a potential therapeutic target for IPAD in Alzheimer's disease. Pharm 13:261
Fuxe K, Agnati LF, Marcoli M, Borroto-Escuela DO (2015) Volume transmission in central dopamine and noradrenaline neurons and its astroglial targets. Neurochem Res 40:2600–2614
Galgani A et al (2021) Locus coeruleus magnetic resonance imaging in neurological diseases. Curr Neurol Neurosci 21:2
Gannon M et al (2015) Noradrenergic dysfunction in Alzheimer's disease. Front Neurosci 9:220
Gao V et al (2016) Astrocytic β2-adrenergic receptors mediate hippocampal long-term memory consolidation. Proc Natl Acad Sci 113:8526–8531
García-Lorenzo D et al (2013) The coeruleus/subcoeruleus complex in rapid eye movement sleep behaviour disorders in Parkinson's disease. Brain 136:2120–2129
Gaspar P, Duyckaerts C, Alvarez C, Javoy-Agid F, Berger B (1991) Alterations of dopaminergic and noradrenergic innervations in motor cortex in Parkinson's disease. Ann Neurol 30:365–374
German DC et al (1992) Disease-specific patterns of locus coeruleus cell loss. Ann Neurol 32:667–676
Gibbs M (2015) Reflections on glycogen and β-amyloid: why does glycogenolytic β2-adrenoceptor stimulation not rescue memory after β-amyloid? Metab Brain Dis 30:345–352
Gibbs ME (2016) Role of glycogenolysis in memory and learning: regulation by noradrenaline, serotonin and ATP. Frontiers Integr Neurosci 9:70
Gibbs ME, Hutchinson DS, Summers RJ (2007) Role of β-adrenoceptors in memory consolidation: β3-adrenoceptors act on glucose uptake and β2-adrenoceptors on glycogenolysis. Neuropsychopharmacology 33:1301629
Gibbs ME, Rodricks CL, Hutchinson DS, Summers RJ, Miller SL (2009a) Importance of adrenergic receptors in prenatally induced cognitive impairment in the domestic chick. Int J Dev Neurosci 27:27–35
Gibbs ME, Gibbs Z, Hertz L (2009b) Rescue of Aβ1–42-induced memory impairment in day-old chick by facilitation of astrocytic oxidative metabolism: implications for Alzheimer's disease. J Neurochem 109:230–236
Gibbs ME et al (2010) Memory loss caused by β-amyloid protein is rescued by a β3-adrenoceptor agonist. Neurobiol Aging 31:614–624

Giorgi FS et al (2020) Locus coeruleus and neurovascular unit: from its role in physiology to its potential role in Alzheimer's disease pathogenesis. J Neurosci Res. https://doi.org/10.1002/jnr.24718
Girouard H, Iadecola C (2006) Neurovascular coupling in the normal brain and in hypertension, stroke, and Alzheimer disease. J Appl Physiol 100:328–335
Gleeson LC, Ryan KJ, Griffin ÉW, Connor TJ, Harkin A (2010) The β2-adrenoceptor agonist clenbuterol elicits neuroprotective, anti-inflammatory and neurotrophic actions in the kainic acid model of excitotoxicity. Brain Behav Immun 24:1354–1361
Godlewska BR, Harmer CJ (2021) Cognitive neuropsychological theory of antidepressant action: a modern-day approach to depression and its treatment. Psychopharmacology 238:1265–1278
Gordon GRJ, Mulligan SJ, MacVicar BA (2007) Astrocyte control of the cerebrovasculature. Glia 55:1214–1221
Gordon GRJ, Choi HB, Rungta RL, Ellis-Davies GCR, MacVicar BA (2008) Brain metabolism dictates the polarity of astrocyte control over arterioles. Nature 456:745–749
Gordon BA et al (2018) Spatial patterns of neuroimaging biomarker change in individuals from families with autosomal dominant Alzheimer's disease: a longitudinal study. Lancet Neurol 17:241–250
Grijalba B, Callado LF, Meana JJ, García-Sevilla JA, Pazos A (1996) α2-adrenoceptor subtypes in the human brain: a pharmacological delineation of [3H]RX-821002 binding to membranes and tissue sections. Eur J Pharmacol 310:83–93
Gronich N et al (2018) β2-adrenoceptor agonists and antagonists and risk of Parkinson's disease. Movement Disord 33:1465–1471
Grudzien A et al (2007) Locus coeruleus neurofibrillary degeneration in aging, mild cognitive impairment and early Alzheimer's disease. Neurobiol Aging 28:327–335
Hall CN et al (2014) Capillary pericytes regulate cerebral blood flow in health and disease. Nature 508:55–60
Haller S et al (2016) Arterial spin labeling perfusion of the brain: emerging clinical applications. Radiology 281:337–356
Hämmerer D et al (2018) Locus coeruleus integrity in old age is selectively related to memories linked with salient negative events. Proc Natl Acad Sci 115:201712268
Hammerschmidt T et al (2013) Selective loss of noradrenaline exacerbates early cognitive dysfunction and synaptic deficits in APP/PS1 mice. Biol Psychiatry 73:454–463
Harik S, Busto R, Martinez E (1982) Norepinephrine regulation of cerebral glycogen utilization during seizures and ischemia. J Neurosci 2:409–414
Harley CW (1987) A role for norepinephrine in arousal, emotion and learning?: limbic modulation by norepinephrine and the Kety hypothesis. Prog Neuro-psychopharmacol Biol Psychiatry 11:419–458
Harmer CJ et al (2003) Acute SSRI administration affects the processing of social cues in healthy volunteers. Neuropsychopharmacology 28:148–152
Harmer CJ et al (2009) Effect of acute antidepressant administration on negative affective bias in depressed patients. Am J Psychiat 166:1178–1184
Hely MA, Reid WGJ, Adena MA, Halliday GM, Morris JGL (2008) The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years. Movement Disord 23:837–844
Heneka MT et al (2006) Locus ceruleus degeneration promotes Alzheimer pathogenesis in amyloid precursor protein 23 transgenic mice. J Neurosci 26:1343–1354
Heneka MT, O'Banion MK, Terwel D, Kummer MP (2010a) Neuroinflammatory processes in Alzheimer's disease. J Neural Transm 117:919–947
Heneka MT et al (2010b) Locus ceruleus controls Alzheimer's disease pathology by modulating microglial functions through norepinephrine. Proc Natl Acad Sci 107:6058–6063
Heron CJL et al (2014) Comparing cerebral perfusion in Alzheimer's disease and Parkinson's disease dementia: an ASL-MRI study. J Cereb Blood Flow Metabolism 34:964–970

Herrmann N, Lanctôt KL, Eryavec G, Khan LR (2004) Noradrenergic activity is associated with response to pindolol in aggressive Alzheimer's disease patients. J Psychopharmacol 18:215–220

Hertz L, Lovatt D, Goldman SA, Nedergaard M (2010) Adrenoceptors in brain: cellular gene expression and effects on astrocytic metabolism and [Ca2+]i. Neurochem Int 57:411–420

Hertz L et al (2013) Brain glycogenolysis, adrenoceptors, pyruvate carboxylase, Na+, K+-ATPase and Marie E. Gibbs' pioneering learning studies. Frontiers Integr Neurosci 7:20

Hertz L et al (2014) Astrocytic glycogenolysis: mechanisms and functions. Metab Brain Dis 30: 317–333

Hieble JP et al (1995) International Union of pharmacology for nomenclature of adrenoceptors: consensus update. Pharmacol Rev 47:267–270

Hinson VK, Delambo A, Elm J, Turner T (2017) A randomized clinical trial of atomoxetine for mild cognitive impairment in Parkinson's disease. Mov Disord Clin Pract 4:416–423

Holcomb L et al (1998) Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. Nat Med 4:97–100

Hopfner F et al (2019) Use of β2-adrenoreceptor agonist and antagonist drugs and risk of Parkinson disease. Neurology 93:e135–e142

Hopfner F et al (2020) β-adrenoreceptors and the risk of Parkinson's disease. Lancet Neurol 19: 247–254

Horvat A, Zorec R, Vardjan N (2016) Adrenergic stimulation of single rat astrocytes results in distinct temporal changes in intracellular Ca2+ and cAMP-dependent PKA responses. Cell Calcium 59:156–163

Huang R, Chen Y, Yu ACH, Hertz L (2000) Dexmedetomidine-induced stimulation of glutamine oxidation in astrocytes: a possible mechanism for its neuroprotective activity. J Cereb Blood Flow Metabolism 20:895–898

Huff F et al (1996) A treatment and withdrawal trial of besipirdine in Alzheimer disease. Alz Dis Assoc Dis 10:93–102

Hutchinson DS, Summers RJ, Gibbs ME (2007) β2- and β3-adrenoceptors activate glucose uptake in chick astrocytes by distinct mechanisms: a mechanism for memory enhancement? J Neurochem 103:997–1008

Hutchinson DS, Catus SL, Merlin J, Summers RJ, Gibbs ME (2011) α2-adrenoceptors activate noradrenaline-mediated glycogen turnover in chick astrocytes. J Neurochem 117:915–926

Iadecola C (2017) The neurovascular unit coming of age: a journey through neurovascular coupling in health and disease. Neuron 96:17–42

Iturria-Medina Y et al (2016) Early role of vascular dysregulation on late-onset Alzheimer's disease based on multifactorial data-driven analysis. Nat Commun 7:11934

Jacobs HIL, Riphagen JM, Ramakers IHGB, Verhey FRJ (2019) Alzheimer's disease pathology: pathways between central norepinephrine activity, memory, and neuropsychiatric symptoms. Mol Psychiatry:1–10. https://doi.org/10.1038/s41380-019-0437-x

Jacobs HIL et al (2021) In vivo and neuropathology data support locus coeruleus integrity as indicator of Alzheimer's disease pathology and cognitive decline. Sci Transl Med 13:eabj2511

Jardanhazi-Kurutz D et al (2010) Induced LC degeneration in APP/PS1 transgenic mice accelerates early cerebral amyloidosis and cognitive deficits. Neurochem Int 57:375–382

Jenkins PO, Mehta MA, Sharp DJ (2016) Catecholamines and cognition after traumatic brain injury. Brain 139:2345–2371

Jenner P, Sheehy M, Marsden C (1983) Noradrenaline and 5-hydroxytryptamine modulation of brain dopamine function: implications for the treatment of Parkinson's disease. Brit J Clin Pharmaco 15:277S–289S

Jin S-X, Liu L, Li S, Meunier AL, Selkoe DJ (2022) Aβ oligomers from human brain impair mossy fiber LTP in CA3 of hippocampus, but activating cAMP-PKA and cGMP-PKG prevents this. Neurobiol Dis 172:105816

Joyce JN et al (1992) Distribution of beta-adrenergic receptor subtypes in human post-mortem brain: alterations in limbic regions of schizophrenics. Synapse 10:228–246

Junker V et al (2002) Stimulation of β-adrenoceptors activates astrocytes and provides neuroprotection. Eur J Pharmacol 446:25–36
Kaalund SS et al (2020) Locus coeruleus pathology in progressive supranuclear palsy, and its relation to disease severity. Acta Neuropathologica Commun 8:11
Kaddurah-Daouk R et al (2011) Metabolomic changes in autopsy-confirmed Alzheimer's disease. Alzheimers Dement 7:309–317
Kalaria RN, Harik SI (1989) Increased α2- and β2-adrenergic receptors in cerebral microvessels in Alzheimer disease. Neurosci Lett 106:233–238
Kalaria RN, Andorn AC, Harik SI (1989a) Alterations in adrenergic receptors of frontal cortex and cerebral microvessels in Alzheimer's disease and aging. Prog Clin Biol Res 317:367–374
Kalaria RN et al (1989b) Adrenergic receptors in aging and Alzheimer's disease: increased β2-receptors in prefrontal cortex and hippocampus. J Neurochem 53:1772–1781
Kalinin S et al (2007) Noradrenaline deficiency in brain increases β-amyloid plaque burden in an animal model of Alzheimer's disease. Neurobiol Aging 28:1206–1214
Kalinin S et al (2012) The noradrenaline precursor L-DOPS reduces pathology in a mouse model of Alzheimer's disease. Neurobiol Aging 33:1651–1663
Kamagata K et al (2011) Posterior hypoperfusion in Parkinson's disease with and without dementia measured with arterial spin labeling MRI. J Magn Reson Imaging 33:803–807
Katzung BG (2017) Chapter 6: Introduction to autonomic pharmacology cholinergic transmission adrenergic transmission cotransmitters in cholinergic & adrenergic nerves central integration. In: Katzung B (ed) Basic & clinical pharmacology
Kelly SC et al (2017) Locus coeruleus cellular and molecular pathology during the progression of Alzheimer's disease. Acta Neuropathologica Commun 5:8
Kelly SC et al (2019) Locus coeruleus degeneration induces forebrain vascular pathology in a transgenic rat model of Alzheimer's disease. J Alzheimers Dis 70:371–388
Keren NI et al (2015) Histologic validation of locus coeruleus MRI contrast in post-mortem tissue. NeuroImage 113:235–245
Ko GN et al (1989) Localization and measurement of neurotransmitter receptors in rat and human brain by quantitative autoradiography. Comput Med Imag Grap 13:37–45
Kochanek KD, Murphy S, Xu J, Arias E (2017) Mortality in the United States, 2016. NCHS Data Brief:1–8
Koren G, Norton G, Radinsky K, Shalev V (2019) Chronic use of β-blockers and the risk of Parkinson's disease. Clin Drug Invest 39:463–468
Kostrzewa RM, Jacobowitz DM (1974) Pharmacological actions of 6-hydroxydopamine. Pharmacol Rev 26:199–288
Lancet (1977) Cardiogenic dementia. Lancet 309:27–28
Lampron A, Pimentel-Coelho PM, Rivest S (2013) Migration of bone marrow-derived cells into the central nervous system in models of neurodegeneration. J Comp Neurol 521:3863–3876
Leanza G, Gulino R, Zorec R (2018) Noradrenergic hypothesis linking neurodegeneration-based cognitive decline and astroglia. Front Mol Neurosci 11:254
Lechtenberg KJ, Meyer ST, Doyle JB, Peterson TC, Buckwalter MS (2019) Augmented β2-adrenergic signaling dampens the neuroinflammatory response following ischemic stroke and increases stroke size. J Neuroinflamm 16:112
Levey AI et al (2021) A phase II study repurposing atomoxetine for neuroprotection in mild cognitive impairment. Brain. https://doi.org/10.1093/brain/awab452
Lin W-C et al (2017) Autonomic function impairment and brain perfusion deficit in Parkinson's disease. Front Neurol 8:246
Lin S-Y et al (2020) Effects of β-adrenergic blockade on metabolic and inflammatory responses in a rat model of ischemic stroke. Cell 9:1373
Lodeweyckx T et al (2021) Safety, tolerability and cerebral blood flow after single doses of the β2-AR agonist, clenbuterol, in patients with mild cognitive impairment or Parkinson's disease. In: Conference reports: clinical trials in Alzheimer's disease

Lodeweyckx T et al (2023) A phase 1 safety, pharmacokinetics and pharmacodynamics study of CST-2032: a novel, selective, CNS-penetrating beta-2 adrenoceptor agonist for treatment of cognitive impairment. In: Conference report #338: AD/PD Alzheimer's and Parkinson's diseases conference

Lourenço CF, Ledo A, Caetano M, Barbosa RM, Laranjinha J (2018) Age-dependent impairment of neurovascular and neurometabolic coupling in the hippocampus. Front Physiol 9:913

Luthman J, Fredriksson A, Sundström E, Jonsson G, Archer T (1989) Selective lesion of central dopamine or noradrenaline neuron systems in the neonatal rat: motor behavior and monoamine alterations at adult stage. Behav Brain Res 33:267–277

MacKenzie E, McCulloch J, Harper A (1976) Influence of endogenous norepinephrine on cerebral blood flow and metabolism. Am J Physiology-legacy Content 231:489–494

Madsen PL et al (1994) Persistent resetting of the cerebral oxygen/glucose uptake ratio by brain activation: evidence obtained with the Kety – Schmidt technique. J Cereb Blood Flow Metabolism 15:485–491

Malek-Ahmadi M, Perez SE, Chen K, Mufson EJ (2020) Braak stage, cerebral amyloid angiopathy, and cognitive decline in early Alzheimer's disease. J Alzheimers Dis 74:189–197

Malek-Ahmadi M, Su Y, Jansen WJ (2021) Editorial: vascular factors and vascular lesions in pre-clinical Alzheimer's disease. Front Neurol 12:738465

Mann DM, Yates PO, Hawkes J (1982) The noradrenergic system in Alzheimer and multi-infarct dementias. J Neurol Neurosurg Psychiatry 45:113

Mantyh P et al (1995) Beta 2-adrenergic receptors are expressed by glia in vivo in the normal and injured central nervous system in the rat, rabbit, and human. J Neurosci 15:152–164

Marien MR, Colpaert FC, Rosenquist AC (2004) Noradrenergic mechanisms in neurodegenerative diseases: a theory. Brain Res Rev 45:38–78

Marsh L, Biglan K, Gerstenhaber M, Williams JR (2009) Atomoxetine for the treatment of executive dysfunction in Parkinson's disease: a pilot open-label study. Movement Disord 24: 277–282

Matchett BJ, Grinberg LT, Theofilas P, Murray ME (2021) The mechanistic link between selective vulnerability of the locus coeruleus and neurodegeneration in Alzheimer's disease. Acta Neuropathol 141:631–650

Mather M, Harley CW (2016) The locus coeruleus: essential for maintaining cognitive function and the aging brain. Trends Cogn Sci 20:214–226

Mather M, Clewett D, Sakaki M, Harley CW (2016) Norepinephrine ignites local hotspots of neuronal excitation: how arousal amplifies selectivity in perception and memory. Behav Brain Sci 39:e200

Matsuoka Y et al (2001) Inflammatory responses to amyloidosis in a transgenic mouse model of Alzheimer's disease. Am J Pathol 158:1345–1354

Matt RA, Westhorpe FG, Romuar RF, Rana P, Gever JR, Ford AP (2023) Fingerprinting heterocellular β-adrenoceptor functional expression in the brain using agonist activity profiles. Front Mol Biosci 10:1214102. https://doi.org/10.3389/fmolb.2023.1214102

Matthews KL et al (2002) Noradrenergic changes, aggressive behavior, and cognition in patients with dementia. Biol Psychiatry 51:407–416

McMillan PJ et al (2011) Differential response of the central noradrenergic nervous system to the loss of locus coeruleus neurons in Parkinson's disease and Alzheimer's disease. Brain Res 1373: 240–252

McNamee EN et al (2010) Noradrenaline acting at β-adrenoceptors induces expression of IL-1β and its negative regulators IL-1ra and IL-1RII, and drives an overall anti-inflammatory phenotype in rat cortex. Neuropharmacology 59:37–48

Mirbolooki MR, Schade KN, Constantinescu CC, Pan M, Mukherjee J (2015) Enhancement of 18F-fluorodeoxyglucose metabolism in rat brain frontal cortex using a β3 adrenoceptor agonist. Synapse 69:96–98

Mishina M et al (2007) Correlation between each task of the mini-mental state examination and regional glucose hypometabolism in at-rest Alzheimer's disease patients. Geriatr Gerontol Int 7: 124–130
Mittal S et al (2017) β2-adrenoreceptor is a regulator of the α-synuclein gene driving risk of Parkinson's disease. Science 357:891–898
Mohs RC et al (2009) Atomoxetine augmentation of cholinesterase inhibitor therapy in patients with Alzheimer disease: 6-month, randomized, double-blind, placebo-controlled, parallel-trial study. Am J Geriatric Psychiatry 17:752–759
Monai H et al (2019) Adrenergic receptor antagonism induces neuroprotection and facilitates recovery from acute ischemic stroke. Proc Natl Acad Sci 116:11010–11019
Monai H et al (2021) Adrenergic inhibition facilitates normalization of extracellular potassium after cortical spreading depolarization. Sci Rep-UK 11:8150
Moore CL et al (2021) Metoprolol impairs beta1 adrenergic receptor-mediated vasodilation in rat cerebral arteries: implications for beta-blocker therapy. J Pharmacol Exp Ther 376:JPET-AR-2020-000176
Morin D, Sapena R, Zini R, Onteniente B, Tillement J-P (1996) Characterization of β-adrenergic receptors of freshly isolated astrocytes and neurons from rat brain. Life Sci 60:315–324
Mosconi L, Pupi A, Leon MJD (2008) Brain glucose hypometabolism and oxidative stress in preclinical Alzheimer's disease. Ann N Y Acad Sci 1147:180–195
Mueller SG et al (2005) The Alzheimer's disease neuroimaging initiative. Neuroimaging Clin N Am 15:869–877
Musiek ES et al (2012) Direct comparison of fluorodeoxyglucose positron emission tomography and arterial spin labeling magnetic resonance imaging in Alzheimer's disease. Alzheimers Dement 8:51–59
Nielsen SS, Gross A, Camacho-Soto A, Willis AW, Racette BA (2018) β2-adrenoreceptor medications and risk of Parkinson disease. Ann Neurol 84:683–693
Nippert AR, Biesecker KR, Newman EA (2018) Mechanisms mediating functional hyperemia in the brain. Neurosci 24:73–83
O'Callaghan C et al (2021) Locus coeruleus integrity and the effect of atomoxetine on response inhibition in Parkinson's disease. Brain:awab142. https://doi.org/10.1093/brain/awab142
O'Donnell J, Zeppenfeld D, McConnell E, Pena S, Nedergaard M (2012) Norepinephrine: a neuromodulator that boosts the function of multiple cell types to optimize CNS performance. Neurochem Res 37:2496–2512
Oishi K, Kamiyashiki T, Ito Y (2007) Isometric contraction of microvascular pericytes from mouse brain parenchyma. Microvasc Res 73:20–28
Ovsenik A, Podbregar M, Fabjan A (2021) Cerebral blood flow impairment and cognitive decline in heart failure. Brain Behav 11:e02176
Palmer AM, Wilcock GK, Esiri MM, Francis PT, Bowen DM (1987) Monoaminergic innervation of the frontal and temporal lobes in Alzheimer's disease. Brain Res 401:231–238
Pamphlett R (2014) Uptake of environmental toxicants by the locus ceruleus: a potential trigger for neurodegenerative, demyelinating and psychiatric disorders. Med Hypotheses 82:97–104
Pascual J, del Arco C, González AM, Pazos A (1992) Quantitative light microscopic autoradiographic localization of α2-adrenoceptors in the human brain. Brain Res 585:116–127
Paspalas CD, Papadopoulos GC (1996) Ultrastructural relationships between noradrenergic nerve fibers and non-neuronal elements in the rat cerebral cortex. Glia 17:133–146
Patterson JR et al (2022) Beta2-adrenoreceptor agonist clenbuterol produces transient decreases in alpha-synuclein mRNA but no long-term reduction in protein. Npj Park Dis 8:61
Pazos A, Probst A, Palacios JM (1985) β-Adrenoceptor subtypes in the human brain: autoradiographic localization. Brain Res 358:324–328
Pellerin L, Magistretti PJ (1994) Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc Natl Acad Sci 91:10625–10629

Peppiatt CM, Howarth C, Mobbs P, Attwell D (2006) Bidirectional control of CNS capillary diameter by pericytes. Nature 443:700–704
Perez DM (2020) α1-adrenergic receptors in neurotransmission, synaptic plasticity, and cognition. Front Pharmacol 11:581098
Peskind ER et al (2005) Propranolol for disruptive behaviors in nursing home residents with probable or possible Alzheimer disease. Alz Dis Assoc Dis 19:23–28
Peterson L, Ismond KP, Chapman E, Flood P (2014) Potential benefits of therapeutic use of β2-adrenergic receptor agonists in neuroprotection and Parkinsonus disease. J Immunol Res 2014: 103780
Pilipović I et al (2022) β-adrenoceptor blockade moderates neuroinflammation in male and female EAE rats and abrogates sexual dimorphisms in the major neuroinflammatory pathways by being more efficient in males. Cell Mol Neurobiol:1–29. https://doi.org/10.1007/s10571-022-01246-z
Prebil M, Vardjan N, Jensen J, Zorec R, Kreft M (2011) Dynamic monitoring of cytosolic glucose in single astrocytes. Glia 59:903–913
Price DT, Lefkowitz RJ, Caron MG, Berkowitz D, Schwinn DA (1994) Localization of mRNA for three distinct alpha 1-adrenergic receptor subtypes in human tissues: implications for human alpha-adrenergic physiology. Mol Pharmacol 2:171–175
Purkayastha S, Raven PB (2011) The functional role of the alpha-1 adrenergic receptors in cerebral blood flow regulation. Indian J Pharmacol 43:502–506
Purves MJ (2018) Do vasomotor nerves significantly regulate cerebral blood flow? Circ Res 43: 485–493
Puskás N, Papp RS, Gallatz K, Palkovits M (2010) Interactions between orexin–immunoreactive fibers and adrenaline or noradrenaline-expressing neurons of the lower brainstem in rats and mice. Peptides 31:1589–1597
Reijnders JSAM, Ehrt U, Weber WEJ, Aarsland D, Leentjens AFG (2008) A systematic review of prevalence studies of depression in Parkinson's disease. Movement Disord 23:183–189
Reznikoff GA, Manaker S, Rhodes CH, Winokur A, Rainbow TC (1986) Localization and quantification of beta-adrenergic receptors in human brain. Neurology 36:1067–1067
Rhee SW, Rusch NJ (2018) Molecular determinants of beta-adrenergic signaling to voltage-gated K + channels in the cerebral circulation. Microcirculation 25:e12425
Robinson AC et al (2020) The contribution of vascular pathology toward cognitive impairment in older individuals with intermediate Braak stage tau pathology. J Alzheimers Dis 77:1005–1015
Rommelfanger KS, Weinshenker D (2007) Norepinephrine: the redheaded stepchild of Parkinson's disease. Biochem Pharmacol 74:177–190
Roozendaal B, Castello NA, Vedana G, Barsegyan A, McGaugh JL (2008) Noradrenergic activation of the basolateral amygdala modulates consolidation of object recognition memory. Neurobiol Learn Mem 90:576–579
Rorabaugh JM et al (2017) Chemogenetic locus coeruleus activation restores reversal learning in a rat model of Alzheimer's disease. Brain J Neurology 140:3023–3038
Ross SB, Renyi AL (1976) On the long-lasting inhibitory effect of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP 4) on the active uptake of noradrenaline. J Pharm Pharmacol 28:458–459
Ross SB, Stenfors C (2015) DSP4, a selective neurotoxin for the locus coeruleus noradrenergic system. A review of its mode of action. Neurotox Res 27:15–30
Ross SB, Johansson JG, Lindborg B, Dahlbom R (1973) Cyclizing compounds. I. Tertiary N-(2-bromobenzyl)-N-haloalkylamine with adrenergic blocking action. Acta Pharm Suec 10: 29–42
Ross JA, McGonigle P, Bockstaele EJV (2015) Locus coeruleus, norepinephrine and Aβ peptides in Alzheimer's disease. Neurobiology Stress 2:73–84
Russo-Neustadt A, Cotman CW (1997) Adrenergic receptors in Alzheimer's disease brain: selective increases in the cerebella of aggressive patients. J Neurosci 17:5573–5580
Ryan KM et al (2016) Clenbuterol activates the central IL-1 system via the β2-adrenoceptor without provoking inflammatory response related behaviours in rats. Brain Behav Immun 56:114–129

Sabbagh M, Cummings J (2011) Progressive cholinergic decline in Alzheimer's disease: consideration for treatment with donepezil 23 mg in patients with moderate to severe symptomatology. BMC Neurol 11:21–21

Salehi A et al (2009) Restoration of norepinephrine-modulated contextual memory in a mouse model of Down syndrome. Sci Transl Med 1:7ra17

Sampaio TB, Marques NF, Binder LB, Tasca CI, Prediger RD (2020) Role of prefrontal cortex on recognition memory deficits in rats following 6-OHDA-induced locus coeruleus lesion. Oxidative Med Cell Longev 2020:1–10

Samuels ER, Szabadi E (2008) Functional neuroanatomy of the noradrenergic locus coeruleus: its roles in the regulation of arousal and autonomic function part ii: physiological and pharmacological manipulations and pathological alterations of locus coeruleus activity in humans. Curr Neuropharmacol 6:254–285

Santangelo G et al (2015) Mild cognitive impairment in newly diagnosed Parkinson's disease: a longitudinal prospective study. Parkinsonism Relat D 21:1219–1226

Sara SJ (2009) The locus coeruleus and noradrenergic modulation of cognition. Nat Rev Neurosci 10:nrn2573

Sasane R et al (2021) Parkinson disease among patients treated for benign prostatic hyperplasia with α1 adrenergic receptor antagonists. J Clin Invest 131

Savitz SI et al (2000) The novel β-blocker, carvedilol, provides neuroprotection in transient focal stroke. J Cereb Blood Flow Metabolism 20:1197–1204

Schiffner R et al (2018) Underlying mechanism of subcortical brain protection during hypoxia and reoxygenation in a sheep model – influence of α1-adrenergic signalling. PLoS One 13:e0196363

Schultz JL et al (2021) A pilot to assess target engagement of terazosin in Parkinson's disease. Parkinsonism Relat D 94:79–83

Schwartz M, Shechter R (2010) Systemic inflammatory cells fight off neurodegenerative disease. Nat Rev Neurol 6:405–410

Schwarz LA, Luo L (2015) Organization of the locus coeruleus-norepinephrine system. Curr Biol 25:R1051–R1056

Seelig A, Gottschlich R, Devant RM (1994) A method to determine the ability of drugs to diffuse through the blood-brain barrier. Proc Natl Acad Sci 91:68–72

Semkova I, Schilling M, Henrich-Noack P, Rami A, Krieglstein J (1996) Clenbuterol protects mouse cerebral cortex and rat hippocampus from ischemic damage and attenuates glutamate neurotoxicity in cultured hippocampal neurons by induction of NGF. Brain Res 717:44–54

Seylaz J et al (1975) Cerebral circulation and metabolism:454–458. https://doi.org/10.1007/978-3-642-65814-3_116

Shannon BJ et al (2016) Brain aerobic glycolysis and motor adaptation learning. Proc Natl Acad Sci 113:E3782–E3791

Shimohama S, Taniguchi T, Fujiwara M, Kameyama M (1986) Biochemical characterization of α-adrenergic receptors in human brain and changes in Alzheimer-type dementia. J Neurochem 47:1294–1301

Shimohama S, Taniguchi T, Fujiwara M, Kameyama M (1987) Changes in β-adrenergic receptor subtypes in Alzheimer-type dementia. J Neurochem 48:1215–1221

Simmering JE, Welsh MJ, Liu L, Narayanan NS, Pottegård A (2021) Association of glycolysis-enhancing α-1 blockers with risk of developing Parkinson disease. JAMA Neurol 78:1–7

Solopchuk O et al (2018) Locus coeruleus atrophy doesn't relate to fatigue in Parkinson's disease. Sci Rep-UK 8:12381

Sommerauer M et al (2017) Evaluation of the noradrenergic system in Parkinson's disease: an 11C-MeNER PET and neuromelanin MRI study. Brain 141:496–504

Song I-U, Ha S-W, Yang Y-S, Chung Y-A (2015) Differences in regional glucose metabolism of the brain measured with F-18-FDG-PET in patients with essential tremor according to their response to beta-blockers. Korean J Radiol 16:967–972

Song S et al (2019) Loss of brain norepinephrine elicits neuroinflammation-mediated oxidative injury and selective caudo-rostral neurodegeneration. Mol Neurobiol 56:2653–2669

Song S, Liu J, Zhang F, Hong J-S (2020) Norepinephrine depleting toxin DSP-4 and LPS alter gut microbiota and induce neurotoxicity in α-synuclein mutant mice. Sci Rep-UK 10:15054
Sorg O, Magistretti PJ (1991) Characterization of the glycogenolysis elicited by vasoactive intestinal peptide, noradrenaline and adenosine in primary cultures of mouse cerebral cortical astrocytes. Brain Res 563:227–233
Staffaroni AM et al (2019) A longitudinal characterization of perfusion in the aging brain and associations with cognition and neural structure. Hum Brain Mapp 40:3522–3533
Stampfer MJ (2006) Cardiovascular disease and Alzheimer's disease: common links. J Intern Med 260:211–223
Steininger TS, Stutz H, Kerschbaum HH (2011) Beta-adrenergic stimulation suppresses phagocytosis via Epac activation in murine microglial cells. Brain Res 1407:1–12
Sternberg Z, Schaller B (2020) Central noradrenergic agonists in the treatment of ischemic stroke – an overview. Transl Stroke Res 11:165–184
Sturchler-Pierrat C et al (1997) Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. Proc Natl Acad Sci 94:13287–13292
Sulzer D et al (2018) Neuromelanin detection by magnetic resonance imaging (MRI) and its promise as a biomarker for Parkinson's disease. Npj Park Dis 4:11
Surguladze SA et al (2004) Recognition accuracy and response bias to happy and sad facial expressions in patients with major depression. Neuropsychology 18:212–218
Suzuki A et al (2011) Astrocyte-neuron lactate transport is required for long-term memory formation. Cell 144:810–823
Sweeney MD, Sagare AP, Zlokovic BV (2018) Blood–brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol 14:133
Szabadi E (2013) Functional neuroanatomy of the central noradrenergic system. J Psychopharmacol 27:659–693
Szot P et al (2006) Compensatory changes in the noradrenergic nervous system in the locus ceruleus and hippocampus of postmortem subjects with Alzheimer's disease and dementia with Lewy bodies. J Neurosci 26:467–478
Szot P et al (2007) Changes in adrenoreceptors in the prefrontal cortex of subjects with dementia: evidence of compensatory changes. Neuroscience 146:471–480
Takeuchi T et al (2016) Locus coeruleus and dopaminergic consolidation of everyday memory. Nature 537:357–362
Tang F et al (2014) Lactate-mediated glia-neuronal signalling in the mammalian brain. Nat Commun 5:3284
Taquet H et al (1982) Microtopography of methionine-enkephalin, dopamine and noradrenaline in the ventral mesencephalon of human control and Parkinsonian brains. Brain Res 235:303–314
Ter Laan M, van Dijk JMC, Elting JWJ, Staal MJ, Absalom AR (2013) Sympathetic regulation of cerebral blood flow in humans: a review. Brit J Anaesth 111:361–367
Theofilas P et al (2017) Locus coeruleus volume and cell population changes during Alzheimer's disease progression: a stereological study in human postmortem brains with potential implication for early-stage biomarker discovery. Alzheimers Dement 13:236–246
Thiele A, Bellgrove MA (2018) Neuromodulation of attention. Neuron 97:769–785
Tosun D et al (2016) Diagnostic utility of ASL-MRI and FDG-PET in the behavioral variant of FTD and AD. Ann Clin Transl Neur 3:740–751
Tournissac M et al (2021) Repurposing beta-3 adrenergic receptor agonists for Alzheimer's disease: beneficial effects in a mouse model. Alzheimers Res Ther 13:103
Toussay X, Basu K, Lacoste B, Hamel E (2013) Locus coeruleus stimulation recruits a broad cortical neuronal network and increases cortical perfusion. J Neurosci 33:3390–3401
Toyoda H et al (2022) The nature of noradrenergic volume transmission from locus coeruleus to brainstem mesencephalic trigeminal sensory neurons. Front Cell Neurosci 16:841239
Trapp BD et al (2007) Evidence for synaptic stripping by cortical microglia. Glia 55:360–368
Tredici KD, Braak H (2013) Dysfunction of the locus coeruleus–norepinephrine system and related circuitry in Parkinson's disease-related dementia. J Neurol Neurosurg Psychiatry 84:774

Tsukahara T, Taniguchi T, Shimohama S, Fujiwara M, Handa H (1986) Characterization of beta adrenergic receptors in human cerebral arteries and alteration of the receptors after subarachnoid hemorrhage. Stroke 17:202–207
Ungerstedt U (1968) 6-hydroxy-dopamine induced degeneration of central monoamine neurons. Eur J Pharmacol 5:107–110
Vaishnavi SN et al (2010) Regional aerobic glycolysis in the human brain. Proc Natl Acad Sci 107: 17757–17762
van der Thiel M, Rodriguez C, Ville DVD, Giannakopoulos P, Haller S (2019) Regional cerebral perfusion and cerebrovascular reactivity in elderly controls with subtle cognitive deficits. Front Aging Neurosci 11:19
Vardjan N et al (2018) Enhancement of astroglial aerobic glycolysis by extracellular lactate-mediated increase in cAMP. Front Mol Neurosci 11:148
Varešlija D, Tipton KF, Davey GP, McDonald AG (2020) 6-hydroxydopamine: a far from simple neurotoxin. J Neural Transm 127:213–230
Vargas G et al (2023) Effects of a beta-2 adrenoceptor agonist on cognition in Parkinson's disease patients with REM sleep behavior disorder. In: Conference report #368: AD/PD Alzheimer's and Parkinson's diseases conference
Velebit J et al (2020) Astrocytes with TDP-43 inclusions exhibit reduced noradrenergic cAMP and Ca2+ signaling and dysregulated cell metabolism. Sci Rep-UK 10:6003
Verclytte S et al (2016) Cerebral hypoperfusion and hypometabolism detected by arterial spin labeling MRI and FDG-PET in early-onset Alzheimer's disease. J Neuroimaging 26:207–212
Vermeiren Y, Deyn PPD (2017) Targeting the norepinephrinergic system in Parkinson's disease and related disorders: the locus coeruleus story. Neurochem Int 102:22–32
Vlassenko AG et al (2010) Spatial correlation between brain aerobic glycolysis and amyloid-β (Aβ) deposition. Proc Natl Acad Sci 107:17763–17767
Wang LY et al (2009) Prazosin for the treatment of behavioral symptoms in patients with Alzheimer disease with agitation and aggression. Am J Geriatric Psychiatry 17:744–751
Wang J et al (2018) Neuromelanin-sensitive magnetic resonance imaging features of the substantia nigra and locus coeruleus in de novo Parkinson's disease and its phenotypes. Eur J Neurol 25: 949–e73
Wang Q et al (2020) Locus coeruleus neurons are most sensitive to chronic neuroinflammation-induced neurodegeneration. Brain Behav Immun 87:359–368
Wattananit S et al (2016) Monocyte-derived macrophages contribute to spontaneous long-term functional recovery after stroke in mice. J Neurosci 36:4182–4195
Weinshenker D (2008) Functional consequences of locus coeruleus degeneration in Alzheimer's disease. Curr Alzheimer Res 5:342–345
Weinshenker D (2018) Long road to ruin: noradrenergic dysfunction in neurodegenerative disease. Trends Neurosci. https://doi.org/10.1016/j.tins.2018.01.010
Weintraub D et al (2010) Atomoxetine for depression and other neuropsychiatric symptoms in Parkinson disease (LOE classification). Neurology 75:448–455
Williams MA, Li C, Kash TL, Matthews RT, Winder DG (2014) Excitatory drive onto dopaminergic neurons in the rostral linear nucleus is enhanced by norepinephrine in an α1 adrenergic receptor-dependent manner. Neuropharmacology 86:116–124
Wilson RS et al (2013) Neural reserve, neuronal density in the locus ceruleus, and cognitive decline. Neurology 80:1202–1208
Wirth KJ (2018) Role of noradrenergic brain nuclei in the regulation of carotid artery blood flow: pharmacological evidence from anesthetized pigs with alpha-2 adrenergic receptor modulator drugs. J Alzheimers Dis:1–13
Wolfman C et al (1994) Recovery of central noradrenergic neurons one year after the administration of the neurotoxin DSP4. Neurochem Int 25:395–400
Wrenn CC, Picklo MJ, Lappi DA, Robertson D, Wiley RG (1996) Central noradrenergic lesioning using anti-DBH-saporin: anatomical findings. Brain Res 740:175–184

Wyss-Coray T, Rogers J (2012) Inflammation in Alzheimer disease – a brief review of the basic science and clinical literature. Csh Perspect Med 2:a006346

Yi B et al (2017) Discovery of novel brain permeable and G protein-biased beta-1 adrenergic receptor partial agonists for the treatment of neurocognitive disorders. PLoS One 12:e0180319

Yoshiyama Y et al (2007) Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. Neuron 53:337–351

Yu J-T et al (2011) Roles of β-adrenergic receptors in Alzheimer's disease: implications for novel therapeutics. Brain Res Bull 84:111–117

Zhang J et al (2014) Extended wakefulness: compromised metabolics in and degeneration of locus ceruleus neurons. J Neurosci 34:4418–4431

Zhang R et al (2020) Spatial distribution of (R)-salbutamol in rat brain following nasal and intravenous administration using DESI-MS. Pharm 12:35

Zhou Z, Ikegaya Y, Koyama R (2019) The astrocytic cAMP pathway in health and disease. Int J Mol Sci 20:779

Zhu Y et al (2016) Intermittent short sleep results in lasting sleep wake disturbances and degeneration of locus coeruleus and orexinergic neurons. Sleep 39:1601–1611

Zhu Y et al (2018) Chronic sleep disruption advances the temporal progression of tauopathy in P301S mutant mice. J Neurosci 38:10255–10270

Zorec R, Parpura V, Verkhratsky A (2018) Preventing neurodegeneration by adrenergic astroglial excitation. FEBS J 285:3645–3656

Zornow MH, Maze M, Dyck JB, Shafer SL (1992) Dexmedetomidine decreases cerebral blood flow velocity in humans. J Cereb Blood Flow Metabolism 13:350–353

Zweig RM et al (1989) Neuropathology of aminergic nuclei in Alzheimer's disease. Prog Clin Biol Res 317:353–365

Clinical Use of Adrenergic Receptor Ligands in Acute Care Settings

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Contents

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Abstract

In this chapter, we review how ligands, both agonists and antagonists, for the major classes of adrenoreceptors, are utilized in acute care clinical settings. Adrenergic ligands exert their effects by interacting with the three major classes of adrenoceptors. Adrenoceptor agonists and antagonists have important applications, ranging from treatment of hypotension to asthma, and have proven to be extremely useful in a variety of clinical settings of acute care from the operating room to the critical care environment. Continued research interpreting the mechanisms of adrenoreceptors may help the discovery of new drugs with more desirable clinical profiles.

Keywords

Adrenergic receptor · Agonists · Antagonists · Dexmedetomidine

1 Introduction

Adrenergic agonists and antagonists exert their effects by interacting with three major classes of adrenoreceptors (ARs), namely alpha$_1$ (α_1), α_2, and beta (β); these classes are further classified by pharmacological and molecular biological techniques into subgroups that are comprehensively addressed in other chapters (cf **X**). The acute care use and systemic effects of ligands acting at these sites are the subject of this chapter (Table 1). The ligands are referred to as selective when they bind to a single AR subgroup or non-selective when multiple AR subtypes are involved.

Table 1 Activity at adrenoceptors and systemic effects

	Receptor activity	Action expected	Clinical application	Example medication
α_1	Agonism Antagonism	Vasoconstriction Smooth muscle relaxation	Treatment of hypotension Treatment of hypertension	Phenylephrine Prazosin
α_2	Agonism	Sedation	Sedation, analgesia, and anxiolysis	Dexmedetomidine
β_1	Agonism Antagonism	Increased heart rate Decreased heart rate	Treatment of hypotension and bradycardia Treatment of hypertension	Dobutamine Metoprolol
β_2	Agonism	Bronchodilation	Treatment of bronchospasm	Albuterol

2 Non-selective Adrenoceptor Ligands

Non-selective adrenergic drugs bind to more than one adrenoceptor (AR) and as a result have a multitude of actions; the non-selective nature may be quite beneficial depending on the clinical application. Adrenaline is an example of an extremely useful non-selective adrenoceptor agonist that activates each of nine AR subtypes although not with equal affinity (Simons and Simons 2010). Several acute care conditions including cardiac arrest, anaphylaxis, hypotension, and shock can have deleterious outcomes, which can be mitigated by the non-selective actions of adrenaline (Soar 2020). Adrenaline is considered the gold standard in treating anaphylaxis (Shaker et al. 2020); the clinical benefits are largely due to the activation of α_1, β_1, and β_2 ARs as a full agonist. As a result of these properties, adrenaline improves hypotension via vasoconstriction due to activation of α_1-AR, increases heart rate, contractility, and cardiac output via β_1 and β_2 ARs, and relieves bronchospasm via β_2-AR (Simons and Simons 2010). Adrenaline is most frequently administered intravenously, and the dose can be titrated to a clinical endpoint depending on which of the adrenoceptor subtype properties needs to be emphasized based upon its disparate subtype binding affinities (Sacha et al. 2019). The route of administration may also favor one property over another; for example, adrenaline can be administered by inhalation to combat bronchospasm and reduce other systemic effects although more selective β_2-AR agonists are preferable by this route for this indication. Noradrenaline is another commonly administered non-selective adrenoceptor ligand in acute care settings and activates each of α_1, α_2, and β_1 ARs (Practice Parameters 1999); noradrenaline is useful in many of the acute care settings for which adrenaline is used apart from anaphylaxis because of the relative lack of action of noradrenaline at β_2-ARs and hence little effect on bronchospasm. Noradrenaline is particularly effective at mitigating hypotension in the setting of septic shock (Avni et al. 2015).

3 Selective Ligands of α_1 Adrenoceptors

α_1-ARs are located postsynaptically on a variety of tissue types including smooth muscle cells located on blood vessels, lungs, uterus, eyes, and the male genitourinary system.

3.1 α_1-AR Agonists

The activation of α_1-ARs results in smooth muscle contraction. Phenylephrine is a relatively selective α_1-AR agonist that can be administered intravenously, orally, nasally, or in ophthalmic solutions. Common adverse effects include bradycardia, which is vagally mediated by the baroreceptor reflex (Richards et al. 2022).

3.2 α_1-AR Agonists Clinical Uses

Phenylephrine, while α_1-AR selective, is non-selective for the different α_1-AR subtypes and may be used in the treatment of hypotension when given intravenously through its vasoconstrictive action on smooth muscle of the blood vessels. Phenylephrine has a rapid onset of action (~1 to 2 min) and is therefore particularly useful to combat a sudden precipitous decline in blood pressure as can occur from vasodilation following induction of general anesthesia (Lonjaret et al. 2014) or from the sympatholysis of neuraxial anesthesia (Kinsella et al. 2018). While these anesthesia-related hypotensive events are usually transient, the accompanying hypoperfusion of critical organs, such as the brain, heart, kidneys, and spinal cord, can result in multiple organ dysfunction even when transient (Lonjaret et al. 2014). In addition, other qualities such as the short duration of action of phenylephrine and its dose-dependent effect make this drug ideal for careful titration when there are dynamic changes in blood pressure (Lonjaret et al. 2014). Other unique clinical scenarios include hypotensive patients with aortic stenosis, as the baroreflex-induced bradycardia is a favorable property (Samarendra and Mangione 2015; Goertz et al. 1993). Spinal cord injury patients may also uniquely benefit as they require strict blood pressure control to optimize spinal cord perfusion (Consortium for Spinal Cord Medicine 2008). Additional clinical uses include intranasal administration for nasal decongestion, and in ophthalmic formulations to induce mydriasis (Stavert et al. 2015).

3.3 α_1-AR Antagonists

Antagonists of α_1-ARs inhibit smooth muscle contraction and can be administered either intravenously or orally. Common adverse effects are reflex tachycardia and orthostatic hypotension through an extension of the pharmacological action (Docherty 2019).

3.4 α_1-AR Antagonists: Clinical Uses

α_1-AR antagonists are most commonly used for the treatment of hypertension and to facilitate urine flow in patients with benign prostatic hyperplasia (BPH). Relaxation of the vascular smooth muscle produces vasodilation and thereby lowers blood pressure although the frequent occurrence of side effects precludes the use of non-subtype selective drugs such as prazosin and tamsulosin, as first-line drugs for essential hypertension (Chapman et al. 2010). The hypertension accompanying pheochromocytoma represents a unique clinical challenge that requires α_1-AR blockade for blood pressure management (Ahmed 2007; Naranjo et al. 2017) as these rare tumors secrete catecholamines, such as noradrenaline. The definitive treatment for these tumors is surgical resection, but to avoid blood pressure lability during surgery prehabilitation is required for several weeks with an α_1-AR

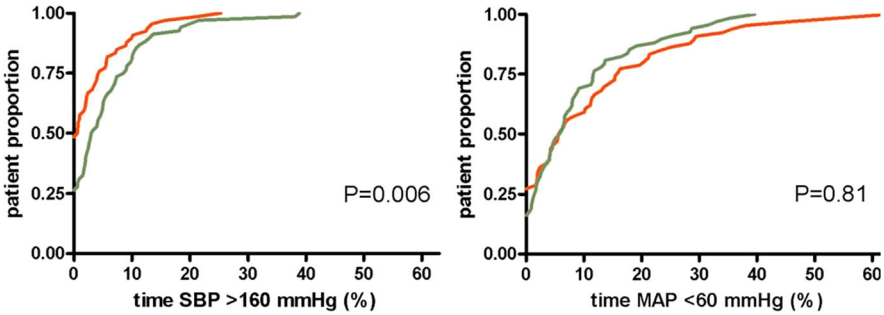

Fig. 1 Cumulative distribution of the percentage of total intraoperative time with blood pressure outside the target values (i.e., systolic blood pressure [SBP] >160 mmHg and mean arterial pressure [MAP] <60 mmHg) during resection of phaeochromocytoma in patients receiving perioperative α_1-adrenergic receptor blockade with phenoxybenzamine vs doxazosin. Patients for surgical resection of phaeochromocytoma were randomized to receive perioperative α_1-AR blockade with either phenoxybenzamine or doxazosin beginning 3 weeks prior to surgery at a dose titrated to control blood pressure close to 130/80. Patients randomized to phenoxybenzamine spent significantly ($p = 0.006$) less time with SBP greater than 160 mmHg with no greater likelihood of a MAP <60 mmHg when compared to patients receiving doxazosin. Reproduced with permission (Buitenwerf et al. 2020)

antagonist, not only to negate the vasoconstrictive effects of the secreted catecholamines but also to uncover and reverse the underlying secondary hypovolemia, which can contribute to hemodynamic instability (Ramakrishna 2015). Phenoxybenzamine, administered orally, irreversibly binds to α_1-ARs and is used, together with fluid resuscitation, to optimize blood pressure preoperatively. The tachycardia associated with excessive catecholamines can be managed with a β_1-AR antagonist (see below), but only after complete α_1-AR blockade has been achieved as an excessive increase in blood pressure may occur through relative blockade of β_2-AR-mediated vasodilation. After resection of the tumor, the sudden withdrawal of the source of catecholamines may precipitate acute hypotension that can be reversed with volume resuscitation and vasopressin; the latter non-adrenoceptor ligand is used because α_1-AR agonists, such as phenylephrine (see above), would be ineffective in the presence of irreversible binding of phenoxybenzamine to α_1-AR. Because of the high costs (~$442) of daily phenoxybenzamine (Zhu et al. 2022), investigators have compared the comparative efficacy of hemodynamic control with other α_1-AR antagonists such as doxazosin (Buitenwerf et al. 2020); in such studies, phenoxybenzamine is superior when the duration of intraoperative hemodynamic instability is compared (Fig. 1).

Through its relaxant effects on the smooth muscle of the genitourinary tract, α_1-AR antagonists such as tamsulosin and silodosin are used to combat obstruction to urine flow from BPH ("Alpha 1 Adrenergic, 2018"; Lee and Sharifi 2017) and also after lithotripsy of ureteral calculi (Oestreich et al. 2020). The use of these α_1-AR antagonists may be complicated by orthostatic hypotension that may be particularly troublesome in elderly patients who suffer from BPH or calculi because of the

relative hypovolemia (Semplicini et al. 1981); in this setting, the α_{1a} AR selectivity of silodosin may confer a putative clinical advantage over tamsulosin that non-selectivity blocks all α_1-AR subtypes although this has yet to be rigorously confirmed (Dell'Atti 2015).

As stated above, non-selective adrenergic agonists are frequently used by continuous infusion for the management of shock and sepsis. Inadvertent extravasation of these agonists from a peripheral intravenous cannula can result in tissue injury related to the vasoconstrictive properties of these AR agonists. In these circumstances, the direct infiltration of the α_1-AR antagonist, phentolamine, in a saline solution diluted to 1 mg/ml, is the antidote of choice to limit and even reverse tissue injury (Ong and Van Gerpen 2020).

4 Selective Ligands of α_2-AR

α_2-Adrenoceptor agonists (α_2-AR agonists) were initially developed for nasal decongestion; however, when the first-in-man studies were performed with clonidine, other important hemodynamic and sedative properties were noted. These findings led to the market authorization of clonidine by the FDA as an anti-hypertensive in 1974 (Muir et al. 1969). Dexmedetomidine, which has eight times greater α_{2-} to α_1-AR selectivity ratio than clonidine (Virtanen et al. 1988), was subsequently developed for its sedative properties for use in acute care settings and received market authorization in the USA in 1999. Because the focus of the chapter is the utility of adrenergic ligands in acute care settings, we will deal exclusively with recent findings involving dexmedetomidine in our discussion of α_2-AR agonists.

4.1 Dexmedetomidine and Sedation

As the use of dexmedetomidine in the intensive care setting required careful dose titration to minimize adverse hemodynamic consequences, a parenteral route was chosen, and the original studies were performed with an intravenous formulation (Bloor et al. 1992; Belleville et al. 1992). While parenteral administration remains the predominant route, newer studies, especially in pediatric populations, have resorted to other routes that will now be considered.

Intranasal: The use of nasal drops and nasal atomization of dexmedetomidine have been studied to obviate the use of intravenous cannulation in children. A pharmacokinetic/pharmacodynamic (PK/PD) study in adults demonstrated that bioavailability of nasal administration of 84 μg of dexmedetomidine was 65% of the same parenteral dose with peak levels achieved in 38 min (Iirola et al. 2011). In another PK/PD study, the bioavailability was noted to be 40% of the same parenteral dose (1 $\mu g.kg^{-1}$); the onset of sedation was 3–4 times slower than for intravenous (i.v.) dexmedetomidine (i.v. = 15 min vs nasal = 48–60 min (Li et al. 2018). In a pediatric population, the onset of sedation was achieved within 20 min following atomized delivery of 1 $\mu g.kg^{-1}$ with peak concentration at 47 min and 84%

bioavailability (Miller et al. 2018); the ED95 of intranasal dexmedetomidine was 2.64 $\mu g.kg^{-1}$(Li et al. 2020a). Peak plasma concentration corresponded to the peak sedative effect (Uusalo et al. 2020). Procedures for which intranasal dexmedetomidine has been reported to be safe and effective run a wide gamut from sophisticated imaging studies requiring no movement (Yu et al. 2017: Gu et al. 2020; Sulton et al. 2020; Miller et al. 2016a, b) to dental procedures (Rehman et al. 2021; Qiu and Luo 2019; Wang et al. 2020; Patel et al. 2018). A systematic review and meta-analysis of appropriate studies revealed that intranasal administration of dexmedetomidine was safe and provided effective sedation for procedures in children (Tervonen et al. 2020; Jun et al. 2017) and is superior to chloral hydrate (Li et al. 2020). The only putative safety concern is bradycardia that occurred in 2.3% of children sedated with intranasal dexmedetomidine (Lei et al. 2020). Intranasal application of dexmedetomidine has also been investigated in adult endoscopic nasal surgical patients in which the drug was impregnated into the nasal packing postoperatively; a dose of 2 $\mu g.kg^{-1}$ proved effective as an analgesic and sleep promoter (Wang et al. 2021).

4.2 Dexmedetomidine as a Sleep Aid

Dexmedetomidine has come to the fore in this setting both because it does not depress ventilatory efforts and because the dexmedetomidine-sedated patient can be easily aroused and responds to commands. The foundational basis for these unusual features is that the mechanism of action for sedation by dexmedetomidine involves the same endogenous pathways that produce non-REM (nREM) sleep (Nelson et al. 2003; Lu et al. 2008). Investigators have taken advantage of this feature by exploring the utility of oral dexmedetomidine to induce nREM sleep (Chamadia et al. 2020a) and to explore the neurophysiological basis for awake/sleep state transitions (Song et al. 2017; Purdon et al. 2015; Akeju et al. 2014) by comparing the electroencephalographic signatures (Purdon et al. 2015; Akeju et al. 2014; Scheinin et al. 2018). Beyond semantic constraints, understanding the biological basis for unconsciousness continues to be challenging and drugs like dexmedetomidine are becoming more useful as pharmacological tools to address this problem (Scheinin et al. 2021). Because of its similarity to natural sleep, that produced by dexmedetomidine lacks the post-hypnotic psychomotor retardation that accompanies commonly prescribed hypnotics, such as zolpidem (Akeju et al. 2018). To further explore the utility of dexmedetomidine to facilitate sleep, studies have been conducted with an oral formulation; initial pharmacokinetic studies indicated that a formulation containing 700 μg of dexmedetomidine produced sedative levels (Chamadia et al. 2020b). In a pharmacodynamic study, it was noted that REM sleep was reduced with a corresponding increase in nREM stage 2 sleep (Chamadia et al. 2020a); the increase in N2 sleep has also been noted in non-intubated ICU patients (Romagnoli et al. 2020). Although REM sleep is reduced, a volunteer study involving i.v. dexmedetomidine indicated that dreaming was reported upon recovery of responsiveness (Radek et al. 2018). The benefits of intraoperative administration

of dexmedetomidine on the quality of postoperative sleep have also been noted (Wu et al. 2022).

4.3 Dexmedetomidine and Mechanical Ventilation

In most cases, mechanical ventilation is delivered via a "breathing" or endotracheal tube that is inserted while the patient is in a deeply sedated or anesthetized state. The continuing presence of the endotracheal tube in the postoperative or intensive care setting causes discomfort that can be manifested by "bucking" or "fighting the ventilator" that interferes with gas exchange because of the ventilation asynchrony that it creates. Therefore, sedation is usually provided for the patient to tolerate mechanical ventilation, but it can be complicated by delayed weaning because the patient may be too sedated to sustain voluntary respiratory efforts.

Compared with other sedatives for mechanical ventilation, dexmedetomidine has a better profile than either midazolam or propofol as it avoids the adverse effects of delirium and propofol infusion syndrome, respectively (Jakob et al. 2012); however, mortality was not different (Kawazoe et al. 2017). Early sedation with dexmedetomidine reduced 90-day mortality in critically ill older patients but had an opposite effect in younger patients (Shehabi et al. 2021); the worse outcome in the younger patients was not due to greater vasopressor requirement nor difficulty maintaining hemodynamic control (Cioccari et al. 2020). Sedation of mechanically ventilated COVID-19 patients with dexmedetomidine was associated with a high incidence (~30%) of bradycardia (<45 bpm), but oxygenation was markedly improved (Uusalo et al. 2021).

4.4 Dexmedetomidine and Perioperative Neurocognitive Disorders

A feared complication for older surgical patients is the onset of a perioperative neurocognitive disorder (PND) (Matthey et al. 2001) that includes conditions from delirium to dementia, which affect ~7 million patients annually in the USA (Silva et al. 2021), and that threatens both functional independence and life of the surgical patient. Within the first postoperative month, the most prevalent PND is postoperative delirium (POD), an acute confusional state characterized by inattention, diminished level of consciousness, thought disorganization, and a fluctuating course (Marcantonio 2012) that is established by routine clinical testing. Whether and how dexmedetomidine reduces PNDs has been the subject of several clinical trials that have yielded meta-analyses. We have tabulated the results of the meta-analyses providing relevant details regarding the number of trials and patients, the effect that dexmedetomidine had on the selected cognitive outcome, type of surgery (cardiac or non-cardiac), and comments on the strengths/weaknesses of the meta-analysis (Table 2). Of the fifteen meta-analyses considered, only one arrived at equipoise that perioperative dexmedetomidine does not reduce postoperative cognitive impairment

Table 2 Meta-analysis of dexmedetomidine and cognitive outcomes in surgical populations

Study name	Trials	Patients	Surgery type	Control	OR or RR	Significance	Comments
Govêia et al. (2021)	15	2,183	Non-cardiac	Placebo	0.36	0.001	
Yang et al. (2019)	26	2,018	Non-cardiac	Placebo	0.49	0.001	
Yu et al. (2022)	14	1,626	All types	Placebo	0.47	0.001	
Li et al. (2021a)	21	2,902	All types	Placebo	0.36–0.45	0.00001	Multiple days
Zhou et al. (2016)	13	1,347	All types	Placebo	0.59	0.0001	
Lin et al. (2021)	21	6,328	All types	Anesthetics	0.55	0.001	
Singh et al. (2022)	9	945	Cardiac	No mention	0.39	0.0001	
Bi et al. (2021)	16	4,376	Non-cardiac	Placebo	0.53	0.0001	Elderly population
Duan et al. (2018)	18	3,309	All types	No mention	0.35	0.00001	
Ming et al. (2020)	10	2,286	Non-cardiac	Placebo	0.53	0.02	
Qin et al. (2021)	13	4,015	Non-cardiac	No mention	0.60	0.0001	
Liu et al. (2017)	8	969	Cardiac	Propofol	0.40	0.0002	
Shen et al. (2020)	16	4,534	Non-cardiac	No mention	0.51	0.01	
Li et al. (2021b)	15	2,813	Cardiac	Mixed	0.56	0.0004	
Patel et al. (2022)	30	4,090	Cardiac	No mention	0.62	0.005	

and that was after sub-analysis rejected several trials (Patel et al. 2022); each of the other meta-analyses concluded that dexmedetomidine conferred benefit. However, it should be noted that two recent trials have disputed the conclusions from the meta-analyses (Deiner et al. 2017; Turan et al. 2020); it is notable that each of those trials was curtailed for futility. In preclinical studies, a possible mechanism for the efficacy of dexmedetomidine in preventing PNDs relates to its vagomimetic action in promoting the resolution of the inflammatory response to injury (Hu et al. 2018).

4.5 Xylazine and Substance Abuse Disorders

Xylazine is a selective α_2-AR agonist that is widely used in veterinary practice to provide sedation and analgesia for diagnostic and invasive procedures (Valverde and Skelding 2019). While not approved for human use, xylazine is frequently used as an adulterant ("tranq") together with drugs of abuse including opiate narcotics (especially fentanyl and heroin), cocaine ("speedball"), and methamphetamine, each of which it can synergize with resulting in acute overdose (Ruiz-Colón et al. 2014). Xylazine toxicity in humans is characterized by drowsiness, bradycardia, hypotension/hypertension, and slurred speech (Pergolizzi et al. 2023) and is present in more than 25% of overdose deaths in major US cities (Friedman et al. 2022). Reversal of the opioid component of the illicit drug combination with naloxone can be effective in treating overdoses containing xylazine. When used parenterally, xylazine can result in skin ulceration because of its vasoconstrictor properties resulting in infection, necrosis, and ultimately amputation (Pergolizzi et al. 2023). Xylazine when used alone can be addictive and its withdrawal symptoms can be treated with parenteral dexmedetomidine (Ehrman-Dupre et al. 2022).

4.6 α_2-AR Agonists for Analgesia

Postoperative analgesia: Dexmedetomidine does not have the analgesic efficacy of opiate narcotics (Angst et al. 2004) but has proven to be quite effective as an adjuvant especially when combined with opioids (Maze and Angst 2004). A meta-analysis of nine studies involving 907 patients revealed that, compared with sufentanil alone, the addition of dexmedetomidine reduced postoperative pain intensity on days one and two, and limited the amount of sufentanil required without adverse events (Feng et al. 2019).

Neuraxial/nerve blocks: Clonidine had been shown to enhance the action potential-reducing effects of local anesthetics without having an effect on its own (Butterworth and Strichartz 1993). These findings have provoked a series of studies in which the addition of dexmedetomidine as an adjuvant has been found to prolong nerve and neuraxial block provided by local anesthetics in settings from intravenous regional (Karam et al. 2022), perineural (Ouchi and Sugiyama 2016; Andersen et al. 2019; Hussain et al. 2021), spinal (Azemati et al. 2022; Liu et al. 2019; Breebaart et al. 2021; Fares et al. 2020; Li et al. 2020b), and epidural (Liu et al. 2020) anesthesia/analgesia. A meta-analysis involving 32 trials and 2007 patients concluded that dexmedetomidine significantly prolonged sensory (57%) and motor (58%) blockade and increased duration of analgesia. The mechanism for prolongation of the duration, and the quality, of the block by dexmedetomidine does not exclusively involve delayed clearance of the local anesthetic by vasoconstriction because dexmedetomidine enhances hyperpolarization of local anesthetics in isolated nerve preparations (Butterworth and Strichartz 1993).

4.7 α_2-AR Agonists for Neuroprotection and Organ Protection

Building on the efficacy of dexmedetomidine for PNDs, preclinical investigations have explored its utility in other CNS disorders as well as in conditions in which unresolved inflammation features in the pathogenesis. To date, there have been reports that dexmedetomidine mitigates (i) anesthesia-induced developmental neurotoxicity (Andropoulos 2023), (ii) lung injury in a cecal ligation/perforation model of sepsis in mice (Zhang et al. 2023; Li et al. 2021c), and (iii) lipopolysaccharide-induced brain injury (Wu et al. 2020), liver injury (Tong et al. 2021), and kidney injury (Kiyonaga et al. 2020). Off-label, dexmedetomidine has been shown to prevent colistin-induced acute kidney injury (Kucuk et al. 2023) and toxicity from a variety of toxins (Baumgartner et al. 2022).

5 α_2-AR Antagonists

While there are no labeled pharmacons in this category as yet for human use, highly selective α_2-AR antagonists such as atipamezole have been successfully used in veterinary anesthesia for rapid re-emergence following dexmedetomidine-induced sedation (Pertovaara et al. 2005). In PK/PD studies in human volunteers, atipamezole increased blood pressure and alertness but was associated with tremor, shivering, increase in salivation, and sweating of hands and feet (Karhuvaara et al. 1990). Despite these side effects, there are studies planned to compare the effects of atipamezole and caffeine for arousal after dexmedetomidine sedation (Fox and Xie 2022). Vatinoxan (L650,066; MK-467) is another antagonist that is currently being developed for clinical use and, unlike atipamezole, is peripherally selective (Honkavaara et al. 2020; Clineschmidt et al. 1988). Because the immediate cardiovascular effects of dexmedetomidine (hypertension and bradycardia) preclude its utility in non-acute care settings, combination with vatinoxan may overcome this limitation as the adverse cardiovascular properties are attenuated, while the sedative effects are maintained (Honkavaara et al. 2017).

6 Selective β_1-AR Ligands

β_1-AR agonists and antagonists exert their effect by binding to the cognate receptor in tissues innervated by the sympathetic nervous system including the heart, kidneys, and fat cells. β_1-AR agonism is clinically used to increase heart contractility and heart rate, while antagonism is most frequently used to decrease heart rate and lower blood pressure.

6.1 β_1-AR Agonists: Clinical Uses

Activation of both β_1 and β_2-ARs in the heart increases sinoatrial and atrioventricular nodal firing, which results in an increased heart rate and contractility and an increase in cardiac output. Dobutamine is a β_1 AR agonist that can be used in acute care settings of refractory cardiogenic heart failure to assist in treating low cardiac output (Tariq and Aronow 2015) and also may be utilized in septic shock as a second- or third-line pressor (Hollenberg 2011; Rhodes et al. 2017; Al-Hesayen et al. 2002). However, for palliative treatment of chronic heart failure dobutamine is not as effective as the phosphodiesterase inhibitor milrinone (Eaton et al. 2022). Isoproterenol, a non-selective β-AR agonist, is used in clinical scenarios for its chronotropic effects. Clinical settings that utilize isoproterenol include heart block, which may require pharmacological pacing while awaiting pacemaker insertion, and improving heart rate in patients who have undergone a heart transplant (Kaplan 2008; Field et al. 2010). In the kidney, increased β_1-AR activation results in an increase in renin release (Kopp et al. 1980). Renin impacts blood pressure by modulating blood volume, sodium retention, and water absorption via the renin-angiotensin-aldosterone system.

6.2 β_1-AR Antagonists: Clinical Uses

Selectivity of antagonists for β_1-ARs is a goal to avoid blockade of β_2-ARs that attenuates bronchodilation and can provoke asthmatic attacks in vulnerable subjects (Huang et al. 2021). This is especially desirable for those with chronic obstructive pulmonary disease for as many as 40% of these patients also have concomitant heart disease for whom a β-AR antagonist may be a treatment option (Feary et al. 2010). While β_1-AR antagonists are frequently referred to as "cardioselective" this is a misnomer for two reasons; firstly, there are functional β_2-ARs in the heart and, secondly, even bisoprolol, considered to be the most selective drug available for β_1-ARs, also blocks β_2-ARs (Baker 2005). Of the relatively selective β_1-AR antagonists, intravenously administered esmolol has shown particular utility in acute care settings for the treatment of tachyarrhythmias (both sinus and supraventricular) because it has a fast onset and is short acting, both of which facilitate titratability (Sung et al. 1986; Benfield and Sorkin 1987). In addition, these qualities make it a safe choice in patients in whom reducing the aortic wall stress is critical, such as in the setting of acute aortic dissection (Krenz et al. 2021).

Examples of non-selective β-blockers include propranolol and timolol. Common clinical uses of β-blockers include treatment of hypertension, arrhythmias, coronary artery disease, and heart failure and for post-myocardial infarction care (López-Sendón et al. 2004). Other uses of β-blockers include treatment of essential tremor and treatment of anxiety disorders that are associated with increased sympathetic nervous system activity. Because of the multitude of conditions for which β-blockers are used, many surgical patients are chronically treated so that treatment with these drugs should not be suspended perioperatively because of the benefits that this class

of drug provides to surgical patients (Mangano et al. 1996; Wallace et al. 2010). Whether perioperative initiation of β-blockade confers a morbidity or mortality benefit for those undergoing non-cardiac surgery is not a settled issue (Blessberger et al. 2019). Surgical patients treated with timolol eye drops for open angle glaucoma may have systemic β-blockade which may only be recognized perioperatively (Samuels and Maze 1980). Common adverse effects of β-blockers include bradycardia and low blood pressure.

7 β_2-AR Agonists

β_2-AR agonists exert their effect by binding to their cognate receptors in the smooth muscle of the airway and uterus, the heart, brain, liver, skeletal muscle, and adipose tissues.

7.1 β_2-AR Agonists: Clinical Uses

β_2-AR agonists are used clinically for the treatment of reactive airway disease, such as asthma or chronic obstructive bronchopulmonary disease (Barisione et al. 2010). These drugs are clinically categorized as short-acting or long-acting β-agonists. Short-acting medications, such as inhaled or nebulized albuterol, are used to rapidly treat acute reactive airway disease (Barisione et al. 2010). Albuterol has a rapid onset of minutes, which is useful for acute bronchospasm that can occur after airway instrumentation or aspiration following induction of anesthesia. For management of subacute reactive airway disease as occurs in critical care settings, long-acting β_2-AR agonists are used, such as salmeterol as their therapeutic effects last 12 h. Another clinical property of β_2-AR agonists is uterine relaxation or tocolysis and is indicated for inhibition of preterm labor. For decades, ritodrine has been administered for myometrial relaxation to inhibit premature contractions. However, studies have failed to prove that ritodrine has a clinically significant benefit for prolongation of pregnancy beyond 48 h (Ledger 1992), and its use is now controversial.

8 Conclusion

In this chapter, we reviewed the major classes of adrenoreceptors and how ligands acting at these receptors are utilized in acute care clinical settings (Table 2). In summary, adrenoceptor agonists and antagonists have important clinical applications, ranging from treatment of hypotension to asthma, and have proven useful in a variety of clinical settings. Continued research interpreting the mechanisms whereby adrenoreceptors produce their actions may help to discover new drugs with desirable clinical effects.

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References

Ahmed A (2007) Perioperative management of pheochromocytoma: anaesthetic implications. J Pak Med Assoc 57(3):140–146

Akeju O, Pavone KJ, Westover MB, Vazquez R, Prerau MJ, Harrell PG, Purdon PL (2014) A comparison of propofol- and dexmedetomidine-induced electroencephalogram dynamics using spectral and coherence analysis. Anesthesiology 121:978–989

Akeju O, Hobbs LE, Gao L, Burns SM, Pavone KJ, Plummer GS, Brown EN (2018) Dexmedetomidine promotes biomimetic non-rapid eye movement stage 3 sleep in humans: a pilot study. Clin Neurophysiol 129:69–78

Al-Hesayen A, Azevedo ER, Newton GE, Parker JD (2002) The effects of dobutamine on cardiac sympathetic activity in patients with congestive heart failure. J Am Coll Cardiol 39:1269–1274

Andersen JH, Jaeger P, Grevstad U, Estrup S, Geisler A, Vilhelmsen F, Mathiesen O (2019) Systemic dexmedetomidine is not as efficient as perineural dexmedetomidine in prolonging an ulnar nerve block. Reg Anesth Pain Med 44:333–340

Andropoulos DB (2023) Neuroprotective strategies in anesthesia-induced neurotoxicity. Best Pract Res Clin Anaesthesiol 37:52–62

Angst MS, Ramaswamy B, Davies MF, Maze M (2004) Comparative analgesic and mental effects of increasing plasma concentrations of dexmedetomidine and alfentanil in humans. Anesthesiology 101:744–752

Avni T, Lador A, Lev S, Leibovici L, Paul M, Grossman A (2015) Vasopressors for the treatment of septic shock: systematic review and meta-analysis. PloS One 10(8):e0129305

Azemati S, Zarghami A, Jouybar R, Naderi-Boldaji V (2022) Analgesic characteristics of bupivacaine alone and in combination with dexmedetomidine or meperidine in spinal anesthesia during cesarean section: a double-blind randomized clinical trial study. Pain Res Manag 2022: 5111214

Baker JG (2005) The selectivity of beta-adrenoceptor antagonists at the human beta1, beta2 and beta3 adrenoceptors. Br J Pharmacol 144:317–322

Barisione G, Baroffio M, Crimi E, Brusasco V (2010) Beta-adrenergic agonists. Pharmaceuticals (Basel) 3:1016–1044

Baumgartner K, Doering And M, Mullins ME (2022) Toxicology Investigators Consortium. Dexmedetomidine in the treatment of toxicologic conditions: a systematic review and review of the toxicology investigators consortium database. Clin Toxicol 60:1356–1375

Belleville JP, Ward DS, Bloor BC, Maze M (1992) Effects of intravenous dexmedetomidine in humans. I. Sedation, ventilation, and metabolic rate. Anesthesiology 77:1125–1133

Benfield P, Sorkin EM (1987) Esmolol. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. Drugs 33:392–412

Bi X, Wei J, Zhang X (2021) Effects of dexmedetomidine on neurocognitive disturbance after elective non-cardiac surgery in senile patients: a systematic review and meta-analysis. J Int Med Res 49:3000605211014294

Blessberger H, Lewis SR, Pritchard MW, Fawcett LJ, Domanovits H, Schlager O, Wildner B, Kammler J, Steinwender C (2019) Perioperative beta-blockers for preventing surgery-related mortality and morbidity in adults undergoing non-cardiac surgery. Cochrane Database Syst Rev 26:CD013438

Bloor BC, Ward DS, Belleville JP, Maze M (1992) Effects of intravenous dexmedetomidine in humans. II. Hemodynamic changes. Anesthesiology 77:1134–1142

Breebaart MB, Saerens L, Branders J, Casaer S, Sermeus L, Van Houwe P (2021) Spinal or intravenous dexmedetomidine for spinal anesthesia with chloroprocaine in ambulatory knee arthroscopies: a double-blind randomized trial. Local Reg Anesth 14:153–160

Buitenwerf E, Osinga TE, Timmers HJLM, Lenders JWM, Feelders RA, Eekhoff EMW, Haak HR, Corssmit EPM, Bisschop PHLT, Valk GD, Veldman RG, Dullaart RPF, Links TP, Voogd MF, Wietasch GJKG, Kerstens MN (2020) Efficacy of α-blockers on hemodynamic control during pheochromocytoma resection: a randomized controlled trial. J Clin Endocrinol Metab 105: 2381–2391

Butterworth JF 5th, Strichartz GR (1993) The alpha 2-adrenergic agonists clonidine and guanfacine produce tonic and phasic block of conduction in rat sciatic nerve fibers. Anesth Analg 76:295–301

Chamadia S, Hobbs L, Marota S, Ibala R, Hahm E, Gitlin J, Akeju O (2020a) Oral dexmedetomidine promotes non-rapid eye movement stage 2 sleep in humans. Anesthesiology 133:1234–1243

Chamadia S, Pedemonte JC, Hobbs LE, Deng H, Nguyen S, Cortinez LI, Akeju O (2020b) A pharmacokinetic and pharmacodynamic study of oral dexmedetomidine. Anesthesiology 133: 1223–1233

Chapman N, Chen CY, Fujita T, Hobbs FD, Kim SJ, Staessen JA, Tanomsup S, Wang JG, Williams B (2010) Time to re-appraise the role of alpha-1 adrenoceptor antagonists in the management of hypertension? J Hypertens 28:1796–1803

Cioccari L, Luethi N, Bailey M, Shehabi Y, Howe B, Messmer AS, ANZICS Clinical Trials Group and the SPICE III Investigators (2020) The effect of dexmedetomidine on vasopressor requirements in patients with septic shock: a subgroup analysis of the Sedation Practice in Intensive Care Evaluation [SPICE III] Trial. Crit Care 24:441

Clineschmidt BV, Pettibone DJ, Lotti VJ, Hucker HB, Sweeney BM, Reiss DR, Lis EV, Huff JR, Vacca J (1988) A peripherally acting alpha-2 adrenoceptor antagonist: L-659,066. J Pharmacol Exp Ther 245:32–40

Consortium for Spinal Cord Medicine (2008) Early acute management in adults with spinal cord injury: a clinical practice guideline for health-care professionals. J Spinal Cord Med 31:403–479

Deiner S, Luo X, Lin H-M, Sessler DI, Saager L, Sieber FE, Rock P (2017) Intraoperative infusion of dexmedetomidine for prevention of postoperative delirium and cognitive dysfunction in elderly patients undergoing major elective noncardiac surgery: a randomized clinical trial. JAMA Surg 152:e171505

Dell'Atti L (2015) Silodosin versus tamsulosin as medical expulsive therapy for distal ureteral stones: a prospective randomized study. Urologia 82:54–57

Docherty JR (2019) The pharmacology of α1-adrenoceptor subtypes. Eur J Pharmacol 855:305–320

Duan X, Coburn M, Rossaint R, Sanders RD, Waesberghe JV, Kowark A (2018) Efficacy of perioperative dexmedetomidine on postoperative delirium: systematic review and meta-analysis with trial sequential analysis of randomised controlled trials. Br J Anaesth 121:384–397

Eaton RE, Kissling KT, Haas GJ, McLaughlin EM, Pickworth KK (2022) Rehospitalization of patients with advanced heart failure receiving continuous, palliative dobutamine or milrinone. Am J Cardiol 184:80–89

Ehrman-Dupre R, Kaigh C, Salzman M, Haroz R, Peterson LK, Schmidt R (2022) Management of xylazine withdrawal in a hospitalized patient: a case report. J Addict Med 16:595–598

Fares KM, Mohamed SA-B, Abd El-Rahman AM, AbdeLemam RM, Osman AMM (2020) Analgesic effect of intrathecal fentanyl vs dexmedetomidine as adjuvants to bupivacaine following abdominal surgery for cancer in children, a randomized trial. Pain Med 21:2634–2641

Feary JR, Rodrigues LC, Smith CJ, Hubbard RB, Gibson JE (2010) Prevalence of major comorbidities in subjects with COPD and incidence of myocardial infarction and stroke: a comprehensive analysis using data from primary care. Thorax 65:956–962

Feng M, Chen X, Liu T, Zhang C, Wan L, Yao W (2019) Dexmedetomidine and sufentanil combination versus sufentanil alone for postoperative intravenous patient-controlled analgesia: a systematic review and meta-analysis of randomized controlled trials. BMC Anesthesiol 19:81

Field JM, Hazinski MF, Sayre MR, Chameides L, Schexnayder SM, Hemphill R, Vanden Hoek TL (2010) Part 1: executive summary: American heart association guidelines for cardiopulmonary resuscitation and emergency cardiovascular care. Circulation 122:S640–S656

Fox A, Xie J (2022) Turning dexmedetomidine into a powerful anesthetic that can be rapidly and completely reversed. Identifier: NCT04942340

Friedman J, Montero F, Bourgois P, Wahbi R, Dye D, Goodman-Meza D, Shover C (2022) Xylazine spreads across the US: a growing component of the increasingly synthetic and polysubstance overdose crisis. Drug Alcohol Depend 233:109380

Goertz AW, Lindner KH, Seefelder C, Schirmer U, Beyer M, Georgieff M (1993) Effect of phenylephrine bolus administration on global left ventricular function in patients with coronary artery disease and patients with valvular aortic stenosis. Anesthesiology 78:834–841

Govêia CS, de Miranda DB, Oliveira LV d B, Praxedes FB, Moreira LG, Guimarães GMN (2021) Dexmedetomidine reduces postoperative cognitive and behavioral dysfunction in adults submitted to general anesthesia for non-cardiac surgery: meta-analysis of randomized clinical trials. Braz J Anesthesiol 71:413–420

Gu H, Song Y, Bai J (2020) ED50 of intranasal dexmedetomidine sedation for transthoracic echocardiography in children with or without a history of cardiac surgery for cyanotic congenital heart disease. Biomed Res Int 2020:1349432

Hollenberg SM (2011) Vasoactive drugs in circulatory shock. Am J Respir Crit Care Med 183:847–855

Honkavaara J, Pypendop B, Ilkiw J (2017) The impact of MK-467 on sedation, heart rate and arterial blood pressure after intramuscular coadministration with dexmedetomidine in conscious cats. Vet Anaesth Analg 44:811–822

Honkavaara JM, Raekallio MR, Syrja PM, Pypendop BH, Knych HK, Kallio-Kujala IJ, Vainio OM (2020) Concentrations of medetomidine enantiomers and vatinoxan, an α_2-adrenoceptor antagonist, in plasma and central nervous tissue after intravenous coadministration in dogs. Vet Anaesth Analg 47:47–52

Hu J, Vacas S, Feng X, Lutrin D, Uchida Y, Lai IK, Maze M (2018) Dexmedetomidine prevents cognitive decline by enhancing resolution of high mobility group box 1 protein-induced inflammation through a vagomimetic action in mice. Anesthesiology 128:921–931

Huang KY, Tseng PT, Wu YC, Tu YK, Stubbs B, Su KP, Matsuoka YJ, Hsu CW, Lin CH, Chen YW, Lin PY (2021) Do beta-adrenergic blocking agents increase asthma exacerbation? A network meta-analysis of randomized controlled trials. Sci Rep 11:452

Hussain N, Brummett CM, Brull R, Alghothani Y, Moran K, Sawyer T, Abdallah FW (2021) Efficacy of perineural versus intravenous dexmedetomidine as a peripheral nerve block adjunct: a systematic review. Reg Anesth Pain Med 46:704–712

Iirola T, Vilo S, Manner T, Aantaa R, Lahtinen M, Scheinin M, Olkkola KT (2011) Bioavailability of dexmedetomidine after intranasal administration. Eur J Clin Pharmacol 67:825–831

Jakob SM, Ruokonen E, Grounds RM, Sarapohja T, Garratt C, Pocock SJ (2012) Dexmedetomidine for long-term sedation investigators. Dexmedetomidine vs midazolam or propofol for sedation during prolonged mechanical ventilation: two randomized controlled trials. JAMA 307:1151–1160

Jun JH, Kim KN, Kim JY, Song SM (2017) The effects of intranasal dexmedetomidine premedication in children: a systematic review and meta-analysis. Can J Anaesth 64:947–961

Kaplan JA (2008) Essentials of cardiac anesthesia. Saunders/Elsevier

Karam C, Al Assadi S, Kanazi G, Zeeni C (2022) A sequential allocation study to determine the ED50 of dexmedetomidine as an adjuvant to lidocaine intravenous regional anesthesia. BMC Anesthesiol 22:165

Karhuvaara S, Kallio A, Scheinin M, Anttila M, Salonen JS, Scheinin H (1990) Pharmacological effects and pharmacokinetics of atipamezole, a novel alpha 2-adrenoceptor antagonist—a

randomized, double-blind cross-over study in healthy male volunteers. Br J Clin Pharmacol 30: 97–106
Kawazoe Y, Miyamoto K, Morimoto T, Yamamoto T, Fuke A, Hashimoto A, Dexmedetomidine for Sepsis in Intensive Care Unit Randomized Evaluation (DESIRE) Trial Investigators (2017) Effect of dexmedetomidine on mortality and ventilator-free days in patients requiring mechanical ventilation with sepsis: a randomized clinical trial. JAMA 317:1321–1328
Kinsella SM, Carvalho B, Dyer RA, Fernando R, McDonnell N, Mercier FJ, Consensus Statement Collaborators (2018) International consensus statement on the management of hypotension with vasopressors during caesarean section under spinal anaesthesia. Anaesthesia 73:71–92
Kiyonaga N, Moriyama T, Kanmura Y (2020) Effects of dexmedetomidine on lipopolysaccharide-induced acute kidney injury in rats and mitochondrial function in cell culture. Biomed Pharmacother 125:109912
Kopp U, Aurell M, Nilsson IM, Ablad B (1980) The role of beta-1-adrenoceptors in the renin release response to graded renal sympathetic nerve stimulation. Pflugers Arch 387:107–113
Krenz JR, O'Brien ME, Lee J, Hayes BD (2021) Evaluation of esmolol for heart rate control in patients with acute aortic dissection. Am J Emerg Med 44:312–314
Kucuk M, Heybeli C, Ozturk MC, Ergun B, Yakar MN, Gokmen AN, Comert B, Ergan B (2023) Dexmedetomidine may reduce the risk of acute kidney injury development in critically ill patients during colistin therapy. J Infect Chemother 29:673–677
Ledger WJ (1992) Treatment of preterm labor with the beta-adrenergic agonist ritodrine. N Engl J Med 327:1758–1759
Lee M, Sharifi R (2017) Benign prostatic hyperplasia. In: DiPiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey L (eds) Pharmacotherapy: a pathophysiologic approach, vol 10e. McGraw Hill
Lei H, Chao L, Miao T, Ya Jun L, Shen Ling L, Yan Ying P, Xin T (2020) Incidence and risk factors of bradycardia in pediatric patients undergoing intranasal dexmedetomidine sedation. Acta Anaesthesiol Scand 64:464–471
Li A, Yuen VM, Goulay-Dufaÿ S, Sheng Y, Standing JF, Kwok PCL, Irwin MG (2018) Pharmacokinetic and pharmacodynamic study of intranasal and intravenous dexmedetomidine. Br J Anaesth 120(5):960–968
Li L, Zhou J, Yu D, Hao X, Xie Y, Zhu T (2020) Intranasal dexmedetomidine versus oral chloral hydrate for diagnostic procedures sedation in infants and toddlers: a systematic review and meta-analysis. Medicine 99:e19001
Li S, Liu H, Zhang J, Liu Y, Yu Q, Sun M, Tu S (2020a) The 95% effective dose of intranasal dexmedetomidine sedation for pulmonary function testing in children aged 1–3 years: a biased coin design up-and-down sequential method. J Clin Anesth 63:109746
Li X, Li Y, Lv X et al (2020b) The efficacy and safety of intrathecal dexmedetomidine for parturients undergoing cesarean section: a double-blind randomized controlled trial. BMC Anesthesiol 20:190
Li J, Yin Q, Xun X, He J, Yu D, Wang Z, Rong J (2021a) The effect of intraoperative dexmedetomidine on cognitive dysfunction after surgery: an updated meta-analysis. J Cardiothorac Surg 16:351
Li P, Li L-X, Zhao Z-Z, Xie J, Zhu C-L, Deng X-M, Wang J-F (2021b) Dexmedetomidine reduces the incidence of postoperative delirium after cardiac surgery: a meta-analysis of randomized controlled trials. BMC Anesthesiol 21(1):153. https://doi.org/10.1186/s12871-021-01370-1
Li ZB, Li GC, Qin J (2021c) Dexmedetomidine attenuates lung injury in toxic shock rats by inhibiting inflammation and autophagy. Arch Med Res 52:277–283
Lin C, Tu H, Jie Z, Zhou X, Li C (2021) Effect of dexmedetomidine on delirium in elderly surgical patients: a meta-analysis of randomized controlled trials. Ann Pharmacother 55:624–636
Liu X, Xie G, Zhang K, Song S, Song F, Jin Y, Fang X (2017) Dexmedetomidine vs propofol sedation reduces delirium in patients after cardiac surgery: a meta-analysis with trial sequential analysis of randomized controlled trials. J Crit Care 38:190–196

Liu L, Qian J, Shen B, Xiao F, Shen H (2019) Intrathecal dexmedetomidine can decrease the 95% effective dose of bupivacaine in spinal anesthesia for cesarean section: a prospective, double-blinded, randomized study. Medicine 98:e14666

Liu L, Drzymalski D, Xu W, Zhang W, Wang L, Xiao F (2020) Dose dependent reduction in median effective concentration (EC50) of ropivacaine with adjuvant dexmedetomidine in labor epidural analgesia: an up-down sequential allocation study. J Clin Anesth 68:110115

Lonjaret L, Lairez O, Minville V, Geeraerts T (2014) Optimal perioperative management of arterial blood pressure. Integr Blood Press Control 7:49–59

López-Sendón J, Swedberg K, McMurray J, Tamargo J, Maggioni AP, Dargie H, Tendera M, Waagstein F, Kjekshus J, Lechat P, Torp-Pedersen C, Task ForceOn Beta-Blockers of the European Society of Cardiology (2004) Expert consensus document on beta-adrenergic receptor blockers. Eur Heart J 25(1341–1):362

Lu J, Nelson LE, Franks N, Maze M, Chamberlin NL, Saper CB (2008) Role of endogenous sleep-wake and analgesic systems in anesthesia. J Comp Neurol 508:648–662

Mangano DT, Layug EL, Wallace A, Tateo I (1996) Effect of atenolol on mortality and cardiovascular morbidity after noncardiac surgery. Multicenter Study of Perioperative Ischemia Research Group. N Engl J Med 335:1713–1720

Marcantonio ER (2012) Postoperative delirium: a 76-year-old woman with delirium following surgery. JAMA 308:73–81

Matthey P, Finucane BT, Finegan BA (2001) The attitude of the general public towards preoperative assessment and risks associated with general anesthesia. Can J Anaesth 48:333–339

Maze M, Angst MS (2004) Review of dexmedetomidine and opioid interactions: defining the role of dexmedetomidine for intensive care unit sedation. Anesthesiology 101:1059–1061

Miller JW, Divanovic AA, Hossain MM, Mahmoud MA, Loepke AW (2016a) Dosing and efficacy of intranasal dexmedetomidine sedation for pediatric transthoracic echocardiography: a retrospective study. Can J Anaesth 63:834–841

Miller J, Xue B, Hossain M, Zhang M-Z, Loepke A, Kurth D (2016b) Comparison of dexmedetomidine and chloral hydrate sedation for transthoracic echocardiography in infants and toddlers: a randomized clinical trial. Paediatr Anaesth 26:266–272

Miller JW, Balyan R, Dong M, Mahmoud M, Lam JE, Pratap JN, Loepke AW (2018) Does intranasal dexmedetomidine provide adequate plasma concentrations for sedation in children: a pharmacokinetic study. Br J Anaesth 120:1056–1065

Ming S, Zhang X, Gong Z, Xie Y, Xie Y (2020) Perioperative dexmedetomidine and postoperative delirium in non-cardiac surgery: a meta-analysis. Ann Palliat Med 9:264–271

Muir AL, Burton JL, Lawrie DM (1969) Circulatory effects at rest and exercise of clonidine, an imidazoline derivative with hypotensive properties. Lancet 2(7613):181–184

Naranjo J, Dodd S, Martin YN (2017) Perioperative management of pheochromocytoma. J Cardiothorac Vasc Anesth 31(1427–1):439

Nelson LE, Lu J, Guo T, Saper CB, Franks NP, Maze M (2003) The alpha2-adrenoceptor agonist dexmedetomidine converges on an endogenous sleep-promoting pathway to exert its sedative effects. Anesthesiology 98:428–436

Oestreich MC, Vernooij RW, Sathianathen NJ, Hwang EC, Kuntz GM, Koziarz A, Scales CD, Dahm P (2020) Alpha-blockers after shock wave lithotripsy for renal or ureteral stones in adults. Cochrane Database Syst Rev 11:CD013393

Ong J, Van Gerpen R (2020) Recommendations for management of noncytotoxic vesicant extravasations. J Infus Nurs 43:319–343

Ouchi K, Sugiyama K (2016) Dexmedetomidine dose dependently enhances the local anesthetic action of lidocaine in inferior alveolar nerve block: a randomized double-blind study. Reg Anesth Pain Med 41:348–355

Patel V, Singh N, Saksena AK, Singh S, Sonkar SK, Jolly SM (2018) A comparative assessment of intranasal and oral dexmedetomidine for procedural sedation in pediatric dental patients. J Indian Soc Pedod Prev Dent 36:370–375

Patel M, Onwochei DN, Desai N (2022) Influence of perioperative dexmedetomidine on the incidence of postoperative delirium in adult patients undergoing cardiac surgery. Br J Anaesth 129:67–83

Pergolizzi J Jr, LeQuang JAK, Magnusson P, Miller TL, Breve F, Varrassi G (2023) The new stealth drug on the street: a narrative review of xylazine as a street drug. Cureus 15:e40983

Pertovaara A, Haapalinna A, Sirviö J, Virtanen R (2005) Pharmacological properties, central nervous system effects, and potential therapeutic applications of atipamezole, a selective alpha2-adrenoceptor antagonist. CNS Drug Rev 11:273–288

Practice Parameters for Hemodynamic Support of Sepsis in Adult Patients in Sepsis (1999) Task force of the American College of Critical Care Medicine, Society of Critical Care Medicine. Crit Care Med 27:639–660

Purdon PL, Sampson A, Pavone KJ, Brown EN (2015) Clinical electroencephalography for anesthesiologists: Part I: background and basic signatures. Anesthesiology 123:937–960

Qin C, Jiang Y, Lin C, Li A, Liu J (2021) Perioperative dexmedetomidine administration to prevent delirium in adults after non-cardiac surgery: a systematic review and meta-analysis. J Clin Anesth 73:110308

Qiu J, Luo Z (2019) The comparison of dexmedetomidine and ketamine for pediatric dental surgery: a meta-analysis of randomized controlled studies. Medicine 98:e15068

Radek L, Kallionpää RE, Karvonen M, Scheinin A, Maksimow A, Långsjö J, Valli K (2018) Dreaming and awareness during dexmedetomidine- and propofol-induced unresponsiveness. Br J Anaesth 121:260–269

Ramakrishna H (2015) Pheochromocytoma resection: current concepts in anesthetic management. J Anaesthesiol Clin Pharmacol 31:317–323

Rehman F, Goyal A, Gauba K, Jain K, Kapur A (2021) Safety and efficacy of IV dexmedetomidine as an adjunct to propofol to sedate anxious and uncooperative pediatric dental patients: a randomized controlled trial. J Clin Pediatr Dent 45:428–432

Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Dellinger RP (2017) Surviving sepsis campaign: international guidelines for management of sepsis and Septic Shock: 2016. Intensive Care Med 43:304–377

Richards E, Lopez MJ, Maani CV (2022) Phenylephrine. StatPearls

Romagnoli S, Villa G, Fontanarosa L, Tofani L, Pinelli F, De Gaudio AR, Ricci Z (2020) Sleep duration and architecture in non-intubated intensive care unit patients: an observational study. Sleep Med 70:79–87

Ruiz-Colón K, Chavez-Arias C, Díaz-Alcalá JE, Martínez MA (2014) Xylazine intoxication in humans and its importance as an emerging adulterant in abused drugs: a comprehensive review of the literature. Forensic Sci Int 240:1–8

Sacha GL, Bauer SR, Lat I (2019) Vasoactive agent use in septic shock: beyond first-line recommendations. Pharmacotherapy 39:369–381

Samarendra P, Mangione MP (2015) Aortic stenosis and perioperative risk with noncardiac surgery. J Am Coll Cardiol 65:295–302

Samuels SI, Maze M (1980) Beta-receptor blockade following the use of eye drops. Anesthesiology 52:369–370

Scheinin A, Kallionpää RE, Li D, Kallioinen M, Kaisti K, Långsjö J, Scheinin H (2018) Differentiating drug-related and state-related effects of dexmedetomidine and propofol on the electroencephalogram. Anesthesiology 129:22–36

Scheinin A, Kantonen O, Alkire M, Långsjö J, Kallionpää RE, Kaisti K, Scheinin H (2021) Foundations of human consciousness: imaging the twilight zone. J Neurosci 41:1769–1778

Semplicini A, Pessina AC, Palatini P, Hlede M, Palù CD (1981) Orthostatic hypotension after the first administration of prazosin in hypertensive patients: role of the plasma volume. Clin Exp Pharmacol Physiol 8:1–10

Shaker MS, Wallace DV, Golden DBK, Oppenheimer J, Bernstein JA, Campbell RL, Wang J (2020) Anaphylaxis-a 2020 practice parameter update, systematic review, and grading of

recommendations, assessment, development and evaluation (GRADE) analysis. J Allergy Clin Immunol 145:1082–1123
Shehabi Y, Serpa Neto A, Howe BD, Bellomo R, Arabi YM, Bailey M, SPICE III Study Investigators (2021) Early sedation with dexmedetomidine in ventilated critically ill patients and heterogeneity of treatment effect in the SPICE III randomised controlled trial. Intensive Care Med 47:455–466
Shen Q-H, Li H-F, Zhou X-Y, Yuan X-Z (2020) Dexmedetomidine in the prevention of postoperative delirium in elderly patients following non-cardiac surgery: a systematic review and meta-analysis. Clin Exp Pharmacol Physiol 47:1333–1341
Silva AR, Regueira P, Albuquerque E, Baldeiras I, Cardoso AL, Santana I, Cerejeira J (2021) Estimates of geriatric delirium frequency in noncardiac surgeries and its evaluation across the years: a systematic review and meta-analysis. J Am Med Dir Assoc 22:613–620
Simons KJ, Simons FER (2010) Epinephrine and its use in anaphylaxis: current issues. Curr Opin Allergy Clin Immunol 10:354–361
Singh A, Brenna CTA, Broad J, Kaustov L, Choi S (2022) The effects of dexmedetomidine on perioperative neurocognitive outcomes after cardiac surgery: a systematic review and meta-analysis of randomized controlled trials. Ann Surg 275:864–871
Soar J (2020) Epinephrine for cardiac arrest: knowns, unknowns and controversies. Curr Opin Crit Care 26:590–595
Song AH, Kucyi A, Napadow V, Brown EN, Loggia ML, Akeju O (2017) Pharmacological modulation of noradrenergic arousal circuitry disrupts functional connectivity of the locus ceruleus in humans. J Neurosci 37:6938–6945
Stavert B, McGuinness MB, Harper CA, Guymer RH, Finger RP (2015) Cardiovascular adverse effects of phenylephrine eyedrops: a systematic review and meta-analysis. JAMA Ophthalmol 133:647–652
Sulton C, Kamat P, Mallory M, Reynolds J (2020) The use of intranasal dexmedetomidine and midazolam for sedated magnetic resonance imaging in children: a report from the Pediatric Sedation Research Consortium. Pediatr Emerg Care 36:138–142
Sung RJ, Blanski L, Kirshenbaum J, MacCosbe P, Turlapaty P, Laddu AR (1986) Clinical experience with esmolol, a short-acting beta-adrenergic blocker in cardiac arrhythmias and myocardial ischemia. J Clin Pharmacol 26:A15–A26
Tariq S, Aronow WS (2015) Use of inotropic agents in treatment of systolic heart failure. Int J Mol Sci 16:29060–29068
Tervonen M, Pokka T, Kallio M, Peltoniemi O (2020) Systematic review and meta-analysis found that intranasal dexmedetomidine was a safe and effective sedative drug during paediatric procedural sedation. Acta Paediatr 109:2008–2016
Tong F, Shen W, Song P, Song J, Hu Y, Liu F, Meng Z, Liu J (2021) Dexmedetomidine attenuates lipopolysaccharide-induced acute liver injury in rats by inhibiting caveolin-1 downstream signaling pathway. Biosci Rep 41:BSR20204279
Turan A, Duncan A, Leung S, Karimi N, Fang J, Mao G, DECADE Study Group (2020) Dexmedetomidine for reduction of atrial fibrillation and delirium after cardiac surgery (DECADE): a randomised placebo-controlled trial. Lancet 396:177–185
Uusalo P, Guillaume S, Siren S, Manner T, Vilo S, Scheinin M, Saari TI (2020) Pharmacokinetics and sedative effects of intranasal dexmedetomidine in ambulatory pediatric patients. Anesth Analg 130(4):949–957. https://doi.org/10.1213/ANE.0000000000004264
Uusalo P, Valtonen M, Järvisalo MJ (2021) Hemodynamic and respiratory effects of dexmedetomidine sedation in critically ill Covid-19 patients: a retrospective cohort study. Acta Anaesthesiol Scand 65:1447–1456
Valverde A, Skelding AM (2019) Alternatives to opioid analgesia in small animal anesthesia: alpha-2 agonists. Vet Clin North Am Small Anim Pract 49:1013–1027
Virtanen R, Savola JM, Saano V, Nyman L (1988) Characterization of the selectivity, specificity and potency of medetomidine as an alpha 2-adrenoceptor agonist. Eur J Pharmacol 150:9–14

Wallace AW, Au S, Cason BA (2010) Association of the pattern of use of perioperative β-blockade and postoperative mortality. Anesthesiology 113:794–805

Wang L, Huang L, Zhang T, Peng W (2020) Comparison of intranasal dexmedetomidine and oral midazolam for premedication in pediatric dental patients under general anesthesia: a randomised clinical trial. Biomed Res Int 2020:5142913

Wang Y, Shuai Y, Qiu F, He J, Zhuang S (2021) Dexmedetomidine-soaked nasal packing can reduce pain and improve sleep quality after nasal endoscopic surgery: a double-blind, randomized, controlled clinical trial. Sleep Breath 25:2045–2052

Wu Z, Xue H, Zhang Y, Zhao P (2020) Dexmedetomidine alleviates neurobehavioral impairments and myelination deficits following lipopolysaccharide exposure in early postnatal rats. Life Sci 263:118556

Wu Y, Miao Y, Chen X, Wan X (2022) A randomized placebo-controlled double-blind study of dexmedetomidine on postoperative sleep quality in patients with endoscopic sinus surgery. BMC Anesthesiol 22:172

Yang W, Kong L-S, Zhu X-X, Wang R-X, Liu Y, Chen L-R (2019) Effect of dexmedetomidine on postoperative cognitive dysfunction and inflammation in patients after general anaesthesia: a PRISMA-compliant systematic review and meta-analysis. Medicine 98:e15383

Yu Q, Liu Y, Sun M, Zhang J, Zhao Y, Liu F, Tu S (2017) Median effective dose of intranasal dexmedetomidine sedation for transthoracic echocardiography in pediatric patients with noncyanotic congenital heart disease: an up-and-down sequential allocation trial. Paediatr Anaesth 27:1108–1114

Yu H, Kang H, Fan J, Cao G, Liu B (2022) Influence of dexmedetomidine on postoperative cognitive dysfunction in the elderly: a meta-analysis of randomized controlled trials. Brain Behav 12:e2665

Zhang Q, Huang Y, Gong C, Tang Y, Xiong J, Wang D, Liu X (2023) Dexmedetomidine attenuates inflammation and organ injury partially by upregulating Nur77 in sepsis. Immun Inflamm Dis 11:e883

Zhou C, Zhu Y, Liu Z, Ruan L (2016) Effect of dexmedetomidine on postoperative cognitive dysfunction in elderly patients after general anaesthesia: a meta-analysis. J Int Med Res 44: 1182–1190

Zhu CY, Hong JC, Kamdar NV, Hu MY, Tseng CH, Lee JS, Kuo EJ, Yu R, Isorena J, Yeh MW, Livhits MJ (2022) Comparison of preoperative alpha-blockade for resection of paraganglioma and pheochromocytoma. Endocr Pract 28:889–896

Adrenoceptor Expression and Function in the Endocrine Pancreas

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Contents

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Abstract

The sympathetic nervous system plays an important role in the regulation of endocrine pancreatic function, most importantly insulin release. Among the nine adrenoceptor (AR) subtypes, the α_{2A}-AR appears to be the subtype most abundantly expressed in the human pancreas. While α_2- and β-AR have opposing effects, the net response to sympathetic stimulation is inhibition of insulin secretion mediated by α_2-AR located in the plasma membrane of pancreatic β cells. This inhibition may be present physiologically as evidenced by increased insulin secretion in healthy and diabetic humans and animals in response to α_2-AR antagonists, a finding that was confirmed in all studies. Based on such data and on an association of an α_{2A}-AR polymorphism, that increases receptor expression levels, with an elevated risk for diabetes, increased α_{2A}-AR signaling in the pancreatic β cells has been proposed as a risk factor for the development of type 2 diabetes. Thus, the α_{2A}-AR was proposed as a drug target for the treatment of some forms of type 2 diabetes. Drug research and development programs leveraging this mechanism have reached the clinical stage, but none have resulted in an approved medicine due to a limited efficacy. While β-AR agonists can increase circulating insulin levels in vivo, it remains controversial whether this includes a direct effect on β cells or occurs secondary to general metabolic effects. Therefore, the regulation of endocrine pancreatic function is physiologically interesting but may be of limited therapeutic relevance.

Keywords

Diabetes · Glucagon release · Insulin · Pancreas · α_2-Adrenoceptor · β-Adrenoceptor

Abbreviations

AR	Adrenoceptor
GPCR	G protein-coupled receptor
T1DM	Type 1 diabetes
T2DM	Type 2 diabetes

1 Introduction

The sympathetic nervous system plays a major role in the regulation of glucose and energy homeostasis. This mainly involves effects on adipose tissue, liver, skeletal muscle, and the pancreas. In this regard, modulation of adipose tissue is involved in energy storage and thermogenesis and at least partly serves long-term homeostasis. In contrast, effects on liver (glycogen storage and glycogenolysis) and pancreas (regulation of insulin and glucagon release) are more oriented towards acute glucose

homeostasis. The sympathetic regulation of adipocyte function has been reviewed elsewhere (Chan 1993; Dwaib and Michel 2023; Fagerholm et al. 2011; Ruffolo et al. 1991). Briefly, α-adrenoceptors (AR), mostly of the α_{2A} subtype inhibit lipolysis whereas β-AR stimulate it. In this regard, the effect of α-AR is only partly a direct effect and occurs largely indirectly by inhibiting insulin release from the pancreas (see below) and modulating the release of multiple hormones by acting on hypothalamic neurons. The role of β-adrenoceptors differs between white and brown adipose tissue and between species; while effects in rodents are largely carried by β_3-AR, those in humans are not. Moreover, adult humans (in contrast to rodents) have little brown adipose tissue.

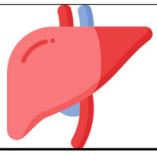
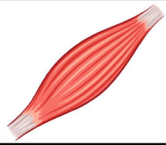
The pancreas is the second largest accessory gland in the digestive system (Yuan et al. 2021). It is situated in the retroperitoneal upper part of the abdominal cavity between the duodenum (right side) and the spleen (left side) (Mahadevan 2019; Talathi et al. 2023). It has a distinct lobular morphology (Bockman 1993) with two major cell types. This peculiar anatomy of the pancreas makes the dual exocrine and endocrine functions possible, that use acinar cells and the islets of Langerhans, respectively (Pandol 2011; Yuan et al. 2021). The various functions are also reflected by this distinct morphology, as the acinar cells are responsible for exocrine excretion of the pancreatic digestive enzymes and bicarbonate to the duodenum, while the islets of Langerhans are mainly responsible for blood glucose regulation through glucagon (from the α cells) and insulin (from the β cells) (Cade and Hanison 2017; Lammert et al. 2014; Pandol 2011; Yuan et al. 2021). The pancreas is innervated by both arms of the autonomic nervous system. As only limited evidence for a role of the sympathetic system in the regulation of exocrine pancreatic function has been provided (Barlow et al. 1974; Nagain et al. 1995), this chapter will focus on the sympathetic regulation of the endocrine pancreas.

Reports on the regulation of insulin release by AR in humans date back more than 50 years (Porte 1967) and previous reviews in this area are explicitly acknowledged (Chan 1993; Fagerholm et al. 2011; Ruffolo et al. 1991). An overview of the role of the three AR subfamilies of α_1-, α_2-, and β-AR is provided in Fig. 1. The role of other G protein-coupled receptors (GPCR) on the pancreas has recently been reviewed (Varney and Benovic 2024). In this chapter, we will first discuss the expression of AR subtypes in the pancreas and their signal transduction mechanisms. We will then discuss the role of α_2- and β-AR in the regulation of insulin and glucagon release.

2 Adrenoceptor Expression Data

2.1 α_1-Adrenoceptor Expression

AR expression in the pancreas has been studied in multiple species at the mRNA and protein level. In rat pancreas, mRNA for α_{1A}-, α_{1B}-, and α_{1D}-AR was detected as 244, 0.5, and 0.4 molecules/ng total RNA, respectively; while the α_{1A}-AR had the highest relative expression, it was the fifth lowest among 19 tissues being tested (Scofield et al. 1995). A semi-quantitative study in a panel of 13 human tissues did

Tissue / AR Subfamily				
AR-α_1		Enhancement of lipolysis (main effect)	Gluconeogenesis (indirect effect) Glycogenesis	
AR-α_2	Inhibition of insulin secretion (main effect)	Inhibition of lipolysis		
AR-β	Enhancement of insulin secretion	Enhancement of lipolysis (main effect)	Gluconeogenesis (indirect effect) Glycogenesis	Release of gluconeogenic precursors
Unclear ?	Enhancement of glucagon secretion			

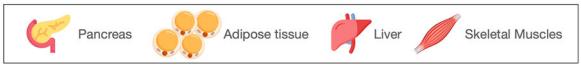


Fig. 1 A schematic overview of the involvement of AR subfamilies in the regulation of glucose homeostasis

not detect α_{1A}-AR mRNA in the pancreas and only weak signals for α_{1B}- and α_{1D}-AR (Price et al. 1994). Arguably, the methodologically strongest study explored the overall human transcriptome in a panel of more than 30 tissues and also failed to detect α_{1A}-AR mRNA and only weak signals for the other two subtypes. A limitation of this study is that samples from only two people were assessed (Uhlen et al. 2015) (Fig. 2). Thus, α_1-AR subtypes appear to have limited expression in the pancreas and importantly, although the α_{1A}-AR appears somewhat expressed in rats it is largely undetectable in humans.

2.2 α_2-Adrenoceptor Expression

The α_{2A}-AR is one of the most abundantly expressed receptors in mouse pancreas within a panel of 373 non-odorant GPCR (Regard et al. 2007). Another interesting study explored α_2-AR subtype mRNA expression in rat pancreas (Chan et al. 1997).

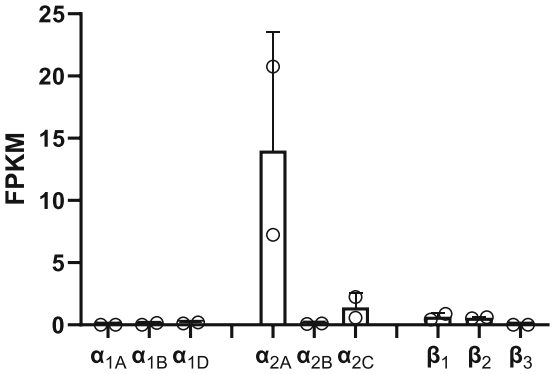


Fig. 2 Quantification of adrenoceptor subtype mRNA expression in whole human pancreas. All data are shown as fragments per kilobase of transcript length per million mapped reads (F, a transcript abundance unit and represent samples from two patients). Generated based on data in (Uhlen et al. 2015)

While mRNA coding for all three α_2-AR subtypes was found in total pancreas and in α cells purified by fluorescence-assisted cell sorting, only α_{2A}- and α_{2C}-AR mRNA was detected in β cells. Early studies in human pancreas provided a semi-quantitative assessment of α_2-AR mRNA expression. One report found all three subtypes in the pancreas tail, whereas α_{2A}-AR mRNA was not detected in the pancreas head (Eason and Liggett 1993). Another study examining total pancreas reported α_{2A}-AR mRNA expressed moderately, α_{2C}-AR less, and α_{2B}-AR only barely (Berkowitz et al. 1994). All three α_2-AR subtypes in the human pancreas were detected using in situ hybridization, although expression levels were lower in the islets than in the acinar cells of the exocrine pancreas (Lacey et al. 1996). The full transcriptomic study found α_{2A}-AR to be predominant with α_{2C}-AR less but still greater than any other AR subtypes (Uhlen et al. 2015) (Fig. 1). Of note, the expression of α_{2A}-AR appears increased in individuals with certain polymorphisms of the gene and is associated with impairment of glucose-stimulated insulin secretion that can be corrected with an α_2-AR antagonist (Varney and Benovic 2024).

mRNA expression of α_{2A}-AR (Hamamdzic et al. 1995) and other α_2-AR subtypes (Lacey et al. 1996) was reported in HIT-T15 cells, a cell line derived from Syrian hamster β cells. Within the same study, α_{2A}-AR protein was also detected based on radioligand binding in HIT-T15 and in RIN-5AH cells (derived from rat insulinoma).

Presence of α_2-AR protein in rat isolated pancreatic islets was demonstrated with saturation radioligand binding using [^{3}H]rauwolscine (Urano et al. 2004). Autoradiographic studies using [ethyl-^{3}H]RS-79948-197 detected α_2-AR protein in mouse pancreatic islets. Binding was blocked in wild-type mice by phentolamine and was not detected in α_2-AR knock-out mice (Fagerholm et al. 2004). Similarly, α_2-AR binding was detected in rat RINm5F insulinoma cells using [^{3}H]RX 821002 (Chan et al. 1994). [^{3}H]RX 821002 binding sites were also found in hamster pancreatic islets, where they exhibited a greater abundance in 14–15 week-old as compared to 6–7-week-old animals (Lacombe et al. 1993).

2.3 β-Adrenoceptor Expression

The mRNA expression of β-AR subtypes in the pancreas has been explored in mice, rats, and humans. β_2-AR were detected in mouse pancreas at the mRNA and protein level (Santulli et al. 2012). However, expression was greater in female than in male mice (Ceasrine et al. 2018). mRNA expression of β_3-AR was detected in lean control rats and downregulated in Zucker diabetic fatty rats. The insulin secretion inhibitor diazoxide partly restored reduced β_3-AR expression in diabetic but did not change that in control rats (Alemzadeh and Tushaus 2004). A similar picture was observed in adipose tissue within that study. In contrast, uncoupling protein 2 and insulin mRNA expression were greater in diabetic than in control rats with diazoxide mitigating the increase for uncoupling protein 2 but increasing it for insulin. Early semi-quantitative studies detected β_2- and β_3-AR mRNA in human pancreas (Thomas and Liggett 1993). Later studies reported only low expression of β_2-AR mRNA in the human pancreas. In contrast to the findings on α_2-AR within the same study, limited β_2-AR mRNA expression was mainly found in the islets (Lacey et al. 1996). Another study also detected β_3-AR mRNA in human pancreas and in rat insulinoma cells RIN 1040-38 (Perfetti et al. 2001). Applying a validated antibody, that study also detected β_3-AR protein in the islets of Langerhans. The study exploring expression across about 30 tissues reported low expression of β_1- and β_2-AR mRNA, whereas that of β_3-AR was undetected (Uhlen et al. 2015). Thus, the expression data on β-AR subtypes, particularly in species other than humans are limited and not fully conclusive.

A study in isolated rat pancreatic islets using [^{3}H]CGP 12177 as the radioligand detected a similar density of β-AR as of α_2-AR labeled by [^{3}H]rauwolscine within the same study (Urano et al. 2004). However, [^{3}H]CGP 12177 in the concentrations used labels β_1- and β_2- but not β_3-AR (Niclauß et al. 2006), implying that these experiments do not allow conclusions to be made on the presence of β_3-AR protein.

Positive immunostaining for β_3-AR protein has been reported in human pancreas with an exclusive localization in the islets and co-localization with insulin (Perfetti et al. 2001). However, these data are difficult to interpret because the antibody being used has not been thoroughly validated for target specificity, and many β_3-AR antibodies lack target specificity when tested under stringent conditions (Cernecka et al. 2014).

The species differences in adrenoceptor subtype expression between humans and rodents are in agreement with more recent evidence from transcriptomic studies: only three G protein-coupled receptors were among the top-10 expressed receptors in both species, including Gpr56, GLP1R, and Ffar1 (Amisten et al. 2017). The other seven differed between mice and humans; moreover, the top-10 expressed receptors even differed between mouse strains. Taken together, these data urge caution in the translation of rodent data to humans.

2.3.1 α_2-AR Signaling in the Pancreas

The prototypical signaling pathway of α_2-AR involves the activation of G proteins of the $G_{i/o}$ family that leads to inhibition of adenylyl cyclase and the modulation of the

activity of various ion channels. Therefore, these signaling pathways and the involvement of $G_{i/o}$ proteins in responses to α_2-AR agonists have been tested in various studies. Inhibition of cAMP formation was observed in RINm5F cells, a cell line derived from rat pancreatic β cells (Chen and Hsu 1994). The secretion of insulin induced by high glucose concentrations or by antidiabetic drugs of the sulphonylurea type such as glyburide involves reduced K^+ permeability of the β-cell plasma membrane mediated by the closure of ATP-sensitive K^+ channels and cellular hyperpolarization (Chan 1993). Some sulphonylurea drugs have direct AR binding properties on top of their inhibition of ATP-sensitive K^+ channels; however, this is unrelated to their direct ion channel effects as glyburide preferentially inhibits α_2-AR, whereas tolbutamide preferentially inhibits β-AR (Cherksey and Altszuler 1984). Therefore, various studies have explored how AR stimulation affects K^+ channel activity and intracellular Ca^{2+} concentrations and which G proteins are involved in such regulation.

The α_2-AR antagonists clonidine and SL 840418[1] inhibited [^{86}]Rb efflux from mouse pancreatic islets induced by 3 mM glucose or by diazoxide, both being surrogates for inhibition of K^+ channel activity, and directly inhibited ATP-sensitive K^+ channels in patch-clamp experiments with β cells; however, these effects were not mimicked by adrenaline, indicating that they occur independent of α_2-AR (Jonas et al. 1994; Plant et al. 1991). Accordingly, α_2-AR agonists having or lacking an imidazoline moiety caused hyperpolarization of mouse insulinoma 6 cells, also known as MIN 6 cells (Scheltdorf and Mest 2002).

Alterations of the K^+ channel permeability leads to changes in intracellular Ca^{2+} concentrations. Experiments in rat pancreatic islets found that elevation of intracellular Ca^{2+} level inhibits the effect of the α_2-AR agonist clonidine on insulin release (Laychock and Bilgin 1989). In transformed hamster β cells (HIT cells), adrenaline and clonidine attenuated elevations of free intracellular Ca^{2+} elicited by depolarization using high extracellular K^+ or by the Ca^{2+} channel opener Bay K 8644 but not that elicited by carbachol (largely stemming from mobilization of Ca^{2+} from intracellular stores); ^{86}Rb efflux was not affected by adrenaline (Hsu et al. 1991a). These findings were mimicked by somatostatin, a hormone also acting via G_i proteins (Hsu et al. 1991b). Direct evidence from patch-clamp experiments demonstrated inhibition of voltage operated Ca^{2+} channels in RINm5F cells (Schmidt et al. 1991).

The modulation of ion channel activity and/or inhibition of insulin release by α_2-AR agonists was repeatedly shown to be blocked by inactivation of $G_{i/o}$ proteins by pertussis toxin in various β cell-derived cell lines including rat RINm5F cells (Chen and Hsu 1994; Schmidt et al. 1991), mouse MIN 6 cells (Scheltdorf and Mest 2002), and hamster HIT cells (Hsu et al. 1991a). Indirect evidence for the involvement of such G proteins comes from rat pancreas where interventions such as exercise

[1] A listing of all adrenoceptor ligands mentioned in this chapter and their primary molecular target is provided in Table 1.

Table 1 Listing of adrenoceptor (AR) ligands mentioned in this chapter

Ligand	Receptor
α-Methyl-noradrenaline	α_2-AR agonist
Atenolol	β_1-AR antagonist
Bisoprolol	β_1-AR antagonist
Brimonidine (UK 14304)	α_2-AR agonist
BRL 26830	β_3-AR agonist
BRL 28410	β_3-AR agonist
BRL 37344	β_3-AR agonist
Buspirone	Serotonin 5-HT_{1A} receptor agonist
CGP 12177	Nonselective β-AR antagonist
CL 316243	β_3-AR agonist
Clonidine	α_2-AR agonist
ICI 118551	β_2-AR antagonist
Idazoxan	α_2-AR antagonist
Isoprenaline	Nonselective β-AR agonist
L 659,066	α_2-AR antagonist
Midaglizole	α_2-AR antagonist
Mirabegron	β_3-AR agonist
MK 467	α_2-AR antagonist
Moxonidine	α_2-AR agonist
Nadolol	Nonselective β-AR antagonist
Phentolamine	Nonselective α-AR antagonist
Prazosin	α_1-AR antagonist
Propranolol	Nonselective β-AR antagonist
Rauwolscine	α_2-AR antagonist
Rilmenidine	α_2-AR agonist
Salbutamol	β_2-AR agonist
SL 840418	α_2-AR antagonist
Solabegron	β_3-AR agonist
Yohimbine	α_2-AR antagonist

concomitantly regulated the α_2-AR inhibition of insulin release and the expression of G_{i2} protein (Urano et al. 2004). Experiments in α_{2A}- and of α_{2C}-AR knock-out mice suggest that the two subtypes use distinct signaling mechanisms to inhibit insulin release with inhibition of cAMP accumulation and hyperpolarization of β cells with α_{2A}-AR but not with α_{2C}-AR (Peterhoff et al. 2003). Experiments with conditional expression of the S1 unit of pertussis toxin in murine pancreatic β cells also impair α_2-AR effects on insulin release (Regard et al. 2007). In conclusion, in rodents, α_2-AR in β cells inhibit cAMP formation, open certain K^+ channels to cause hyperpolarization, and inhibit voltage-dependent Ca^{2+} channels via pertussis toxin-sensitive G proteins.

3 Modulation of Insulin Release by α-Adrenoceptors

The sympathetic modulation of insulin release is considered to play a physiological role during stress and exercise but may also have a role in the pathogenesis of type 2 diabetes (T2DM) (Chan 1993; Fagerholm et al. 2011; Liggett 2009; Ruffolo et al. 1991). The prevailing effect of catecholamines on insulin secretion is inhibition mediated by α_2-AR on the plasma membrane of β cells in pancreatic islets. Thus, α_2-AR agonists reduce, and antagonists increase, circulating insulin levels in rats, mice, and humans (Chan 1993; Laychock 1990; Ruffolo et al. 1991).

In agreement with the limited or absent expression of α_1-AR in the pancreas (see above), no effect on insulin release was reported for the α_1-AR antagonist prazosin in isolated rat pancreatic islets (Ostenson et al. 1989). Nonetheless, prazosin, whether administered acutely or chronically, was reported to increase plasma glucose and insulin concentrations in rats on a high sucrose diet (Fajardo and Deshaies 1996), that may represent an indirect effect. Based on the overall lack of evidence for an involvement of α_1-AR in the modulation of endocrine function of the pancreas, the following section focuses on data related to α_2-AR.

3.1 Cell Line Data

Experiments in cell lines can provide profound mechanistic insights since modifying variables such as innervation and blood supply are eliminated. However, they may have limited predictive value for the more complex in vivo situations. Work related to adrenergic modulation of insulin release was largely performed in β cell lines derived from rat (e.g., RINm5F or INS-1 cells), mouse (e.g., MIN 6 cells), or hamster (e.g., HIT cells). An inhibition of insulin release was reported for α_2-AR agonists such as medetomidine in rat RINm5F cells (Chen and Hsu 1994), agonists such as adrenaline or imidazolines in murine MIN 6 cells (Scheltdorf and Mest 2002), or adrenaline or clonidine in hamster HIT cells (Hsu et al. 1991a). Of note, inhibition was observed regardless of whether insulin release was promoted by the phosphodiesterase inhibitor isobutyl methyl xanthine (Chen and Hsu 1994), depolarization (Hsu et al. 1991a), or in unstimulated cells (Scheltdorf and Mest 2002). Interestingly, α_2-AR agonists such as clonidine inhibited not only insulin release but also that of neuropeptide Y in INS-1 cells (Waeber et al. 1993). Taken together, these data establish that inhibition of insulin release by α_2-AR agonists at least partly represents a direct effect on the β cells at least in rodents. The overall pathways of α_2-AR-mediated regulation of insulin release from the β cells are summarized in Fig. 3.

3.2 Animal Data

The effects α_2-AR agonists on insulin release have been investigated in rodents both in isolated pancreatic islets and in vivo with the latter condition being the most complex setting and typically determining insulin concentrations in plasma and not

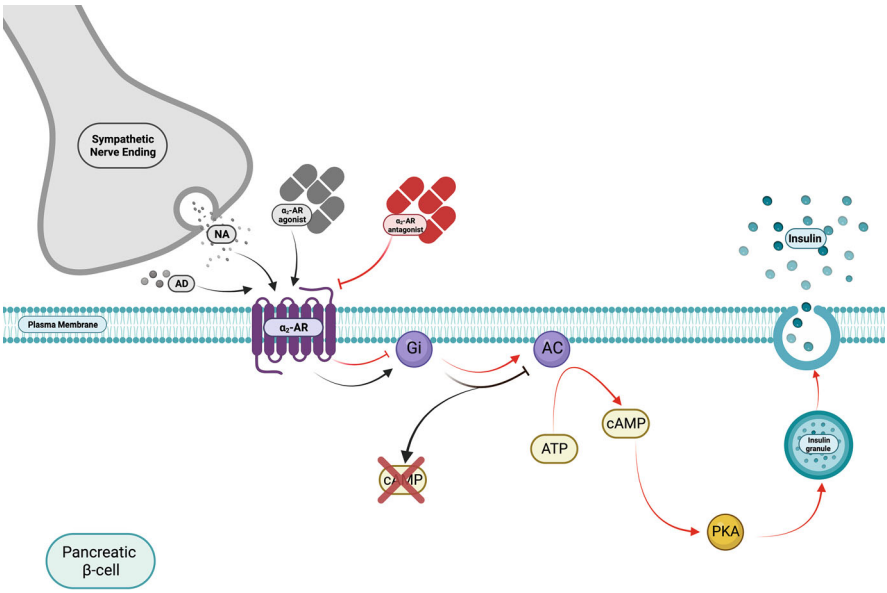


Fig. 3 The effect of α_2-AR ligands on insulin release in pancreatic β cells. Activation of α_2-AR by neuronal noradrenaline (NA), circulating adrenaline (AD), or xenobiotic agonists (black arrows) will activate G_i that in part will inhibit adenylyl cyclase (AC) activity to result in a reduction in cyclic AMP (cAMP) levels and consequently reduction in protein kinase A (PKA)-mediated exocytosis of the insulin granule. On the other hand, inhibition of α_2-AR by an antagonist (red arrows), will inhibit the G_i response and hence will improve PKA mediated insulin release. Note that this schematic drawing is largely based on rodent data, and it remains unclear which parts also occur in humans

insulin release. In rat isolated pancreatic islets, several α-agonists and the endogenous hormones namely clonidine (Laychock and Bilgin 1989), adrenaline (Chan et al. 1994), noradrenaline and clonidine (Urano et al. 2004), or brimonidine (also known as UK 14304) (Ostenson et al. 1989) inhibit glucose-induced insulin release, an effect blocked by α_2-AR but not by α_1-AR antagonists. Another α-agonist Moxonidine also inhibited insulin release from rat islets, but this was only partly sensitive to α_2-AR antagonists (Tsoli et al. 1995). Conversely, phentolamine (but not idazoxan) enhanced glucose-induced insulin release (Ostenson et al. 1989). Enhanced insulin release was also reported for the α_2-AR antagonist SL 840418 in mouse islets (Jonas et al. 1994). This provides additional evidence for a direct effect independent of innervation or blood supply. This is important since α_2-AR have prejunctional inhibitory effects on transmitter release (Starke 1987) and are expressed in adipocytes that also play a role in the control of energy homeostasis (Saulnier-Blache et al. 1992). It is possible that α_2-AR in the brain also contribute to the regulation of insulin release and glucose homeostasis (Hiyoshi et al. 1995).

Clonidine, UK14304, and adrenaline also inhibit glucose-induced insulin release in hamster islets (Lacombe et al. 1993).

Several α_2-AR agonists induce early hyperglycemia accompanied by reduced circulating insulin levels in vivo in lean and obese spontaneously hypertensive rats with this effect fading during treatment for 3 weeks (Velliquette and Ernsberger 2003). Only limited evidence for α_2-AR agonist and antagonist has been presented in non-rodent species, but their effect in dogs apparently is similar to that in rats and mice (Kallio-Kujala et al. 2018).

The order of potency of various antagonists in counteracting the effect of brimonidine in isolated pancreas suggests that inhibition of insulin secretion occurs via the α_{2A}-AR in mice (Angel et al. 1990) and rats (Niddam et al. 1990). Additional experiments conducted in knock-out mice found that neither knock-out of α_{2A}- nor α_{2C}-AR markedly altered inhibition of insulin release in mice, but a double knock-out abolished it (Peterhoff et al. 2003). Others have reported that the effect of brimonidine (Hu et al. 2005) or the α_2-partial agonist dexmedetomidine (Fagerholm et al. 2004) on glucose-induced insulin release was abolished in α_{2A}-AR knock-out mice. Moreover, α_2-AR binding was abolished in the knock-out mice of the latter study, indirectly indicating that expression at the protein level is largely accounted for by the α_{2A}-AR. Interestingly, basal glucose levels were lower in α_{2A}-AR knock-out mice, indicating inhibition by endogenous catecholamines is tonically active in vivo.

However, the in vivo regulation of insulin release by α_2-AR antagonists may be more complex: The intracerebroventricular administration of neostigmine in rats increased plasma levels of catecholamines and glucose but not insulin (Hiyoshi et al. 1995), apparently reflecting that endogenous adrenaline had inhibited insulin release despite elevated glucose. Pretreatment with systemically administered phentolamine increased insulin levels under those conditions, an effect reduced by concomitant the muscarinic antagonist atropine (both drugs not affecting plasma catecholamine levels). Under basal conditions, i.e., in the absence of neostigmine, a range of α-AR antagonists exhibited a complex picture that was not matched by any known α-AR subfamily or subtype and may reflect effects on imidazoline binding sites. Generally, specific imidazoline recognition sites have been proposed and many α_2-AR ligands with an imidazoline structure additionally bind to and possibly act via imidazoline recognition sites (Michel and Ernsberger 1992). While it is now clear that the I_2 binding site is part of the enzyme monoaminoxidase (apparently without functional effects), the identity of the I_1 site remains disputed. While the role of α_2-AR in the regulation of insulin release is undisputed, some data indicate that ligands of an imidazoline site may have additional, perhaps qualitatively different effects (Chan 1993; Scheltdorf and Mest 2002; Schulz and Hasselblatt 1989; Tsoli et al. 1995; Velliquette and Ernsberger 2003). Due to the poorly elucidated identity of such sites, these effects are not discussed here.

3.3 Human Data

Early human studies reported that a 1 h infusion of the α-AR-antagonist phentolamine failed to alter serum insulin concentrations in controls but considerably increased it in diabetic patients (delta 3 ± 2 and 14 ± 9 μU/ml, respectively). However, phentolamine raised serum glucose to a greater extent in control as compared to diabetic subjects following an i.v. glucose pulse of 20 g (Robertson et al. 1976). Acute administration of selective α_2-AR antagonists also failed to increase serum or plasma insulin levels in humans, including compounds from various chemical classes such as L 659,066 (Schäfers et al. 1992), SL 840418 (Berlin et al. 1994), MK 467 (Sciberras et al. 1994), and yohimbine (Schäfers et al. 1999). A lack of effect was also reported following a 5-day administration of buspirone, an agonist at 5-HT_{1A} serotonin receptors that also has considerable α_2-AR antagonist properties (Berlin et al. 1995). However, MK 467 dose-dependently increased insulin levels upon exercise, possibly reflecting antagonism of increased catecholamine release during exercise (Sciberras et al. 1994). Conversely, the α_2-AR agonist α-methyl-noradrenaline dose-dependently increased blood glucose concentration but had little effect on insulin. While the α_1-antagonist prazosin and β-antagonist propranolol did not substantially modify this response, the α_2-antagonist yohimbine reduced glucose concentration and enhanced the insulin response (Schäfers et al. 1999). In contrast, studies with α_1-AR antagonists failed to detect consistent effects on insulin levels or glucose tolerance (Khoury and Kaplan 1991).

3.4 Conclusion on α-AR Based on Pharmacological Approaches

In summary, there is overwhelming evidence that α_2-AR agonists inhibit, and α_2-AR antagonists enhance insulin secretion. However, enhancement of insulin release in vitro, i.e., under conditions of presumed absence of endogenous sympathetic tone, by only some α_2-AR antagonists led to the proposal that this effect may at least partly be unrelated to α_2-AR antagonist activity (Chan 1993). Direct effects on ATP-sensitive K^+ channel activity and/or on imidazoline recognition sites have been proposed as alternative/additional mechanisms (Chan 1993). However, the alternative explanation that this reflects different degrees of inverse agonism (Michel et al. 2020) has not been ruled out.

3.5 Genetic Evidence

Genetic evidence for the role of α_2-AR subtypes in the regulation of insulin release comes from genetically modified animals (mostly mice) and from studies on naturally occurring AR variants in human populations. Various groups generated mice lacking one or more α_2-AR subtypes in the 1990s. The inhibition of insulin release in freshly isolated and cultured pancreatic islets was not altered in α_{2A}-AR and even enhanced in α_{2C}-AR knock-out mice relative to wild-type animals (80%, 100% and

83% inhibition, respectively (Peterhoff et al. 2003). However, the inhibitory effect of adrenaline was abolished in $\alpha_{2A/2C}$-AR double knock-out mice. In contrast, others reported that the inhibition of glucose-stimulated insulin release in isolated islets by the agonist brimonidine was abolished in cells isolated from α_{2A}-AR knock-out mice (Hu et al. 2005). In vivo experiments in α_{2A}-AR knock-out mice found that fasting glucose levels were lower in the knock-out as compared to wild-type animals of both sexes but concomitantly, plasma insulin levels were higher (Savontaus et al. 2008). These strain differences were not affected by administration of propranolol or atropine. While glucose-stimulated insulin secretion was not increased in the knock-out mice, glucose tolerance was improved. The genetic removal of α_{2A}-AR caused major differences in the proteomic profiles of isolated islets (Hu et al. 2005).

Indirect data in rats support conclusions on the role of α_{2A}-AR in the regulation of glucose metabolism. In Goto-Kakizaki rats, a hereditary model of T2DM, a genomic locus was identified as being linked to impaired insulin granule docking at the plasma membrane and reduced exocytosis from β cells. The locus includes the Adra2a gene encoding the α_{2A}-AR receptor, that was markedly over-expressed in diabetic rats (Rosengren et al. 2010). Insulin secretion was rescued by pharmacological inhibition with yohimbine or silencing of α_{2A}-AR expression.

A single nucleotide polymorphism in the human ADRA2A gene (rs553668, located in the 3′ untranslated region of the gene) has been identified as associated with overexpression of the receptor (Rosengren et al. 2010). Insulin secretion in response to a glucose challenge was reduced in isolated islets from homozygous and heterozygous carriers of the risk allele, an effect counteracted by yohimbine (Rosengren et al. 2010). In follow-up from the same group, 50 T2DM patients carrying the ADRA2A gene variant or the wild-type allele entered a randomized study involving administration of placebo or 10 or 20 mg of yohimbine during three separate visits (Tang et al. 2014). The primary endpoint was insulin secretion at 30 min during an oral glucose tolerance test. Those with the risk variant displayed a 25% lower insulin response. Administration of 20 mg yohimbine to patients with the risk allele normalized the insulin response to levels seen in patients with the wild-type allele in the absence of yohimbine.

A 12Glu9 polymorphism in the human ADRA2B gene with both alleles similarly present was associated with an increased risk to develop T2DM in a Finnish population (Siitonen et al. 2004). Homozygous carriers of the 9Glu allele had the lowest insulin response in a glucose tolerance test. However, these data are difficult to understand based on the limited to non-existent expression of α_{2B}-AR in the human pancreas (see above) and await confirmation.

3.6 Regulation

Similar to many other GPCR, the expression of α_2-AR at the mRNA and/or protein level may undergo regulation by various factors. One of them is homologous desensitization by prolonged exposure to an agonist. A study in isolated rat islets found that incubation with the α_2-AR agonist brimonidine desensitized inhibition of

insulin release by noradrenaline (Chan et al. 1994). Repeated exercise training, implying repeated exposure to elevated catecholamines, did not change α_2-AR protein expression in rat islets but abolished inhibition of insulin release by noradrenaline (Urano et al. 2004), possibly reflecting desensitization due to a decrease in G_i protein expression, that is required for signal transduction by α_2-AR.

The expression of α_{2A}-AR protein in HIT-T15 cells, a model of pancreatic β cells, was markedly increased by exposure to various glucocorticoids and aldosterone, but not affected by sex steroids, increases in glucose exposure, or exposure to insulin or phorbol ester (Hamamdzic et al. 1995). Mice with targeted overexpression of the rat glucocorticoid receptor in β cells exhibited impaired glucose tolerance at 3 months that progressed to diabetes at 12–15 months (Davani et al. 2004). This was not attributable to morphological changes or increased apoptosis in the pancreas but to augmented inhibition of insulin secretion by α_2-AR. This was accompanied by an increased α_2-AR expression at the protein level as assessed by [^{3}H]UK 14304 binding. Furthermore, treatment of islets from transgenic mice with the antagonist benextramine restored insulin release to wild-type levels.

While this section mostly focuses on regulation of insulin release by α_2-AR agonists and antagonists, the opposite, regulation of α_2-AR expression and function by insulin, may also occur. Evidence for this largely comes from cells and tissues outside the pancreas. For instance, exposure to insulin downregulated α_{2A}-AR expression in colonic HT-29 cells (Devedjian et al. 1991) or human platelets (Kahn and Sinha 1992) but in contrast, no regulation of α_{2C}-AR expression was observed in the hepatic HepG2 cell lines (Schaak et al. 2000). Similarly, one group suggests that insulin exposure attenuates vasoconstriction mediated by α_2-AR but not that to α_1-AR agonists (Lembo et al. 1994; Lembo et al. 1997a; Lembo et al. 1996). This was blocked by the NO synthase inhibitor L-NMMA, indicating that the target may be the α_2-AR in the endothelium. The same group reported that insulin enhanced responses to UK14304 in rat aorta (Lembo et al. 1997b).

Based on the proposed role of α_2-AR in the development and possible treatment of T2DM (Fagerholm et al. 2011; Liggett 2009), particularly in carriers of a risk allele of α_{2A}-AR (Gribble 2010), it has been examined how α_2-AR and responsiveness are regulated in diabetes. An early human study comparing 12 diabetic and 44 non-diabetic subjects found that infusion of phentolamine increased circulating insulin levels by more than 50% in diabetics but not in non-diabetic subjects (Robertson et al. 1976). The insulin response to acute glucose load was augmented fivefold in diabetics but not in controls, a response not affected by concomitant treatment with propranolol. These findings may at least partly be explained by greater plasma catecholamine levels in response to a glucose load in the diabetic group. Animal studies have attempted to explore this in more depth. In pancreatic islets from rats with neonatal exposure to streptozotocin, a T2DM model, brimonidine was about 10-folds more potent for inhibition of insulin release compared to control animals. Given that baseline insulin levels were lower, an almost complete inhibition was achieved in the diabetic, but not in the control islets (Ostenson et al. 1989). Others using the same model in vivo found that basal plasma insulin was similar in both models and increased by the α_2-AR antagonist SL 840418

in diabetic but not in control rats (Angel et al. 1996). The peak insulin response to an acute glucose load was greater in control and in antagonist-treated diabetic than in diabetic rats. Within the same study, insulin levels were below the detection limit in a rat model of T1DM, in which β cells had been destroyed by streptozotocin. Under these conditions, the α_2-AR antagonist had no effect and did not improve glucose tolerance, further supporting that it acted directly on the pancreatic β cells.

4 Modulation of Insulin Release by β-Adrenoceptors

Following early studies reporting enhanced insulin secretion upon administration of a β_2-AR agonist in human but not in rat pancreatic islets (Lacey et al. 1990), several studies have explored these effects in more detail. Early studies in this field have been reviewed (Haffner and Kendall 1992).

4.1 Cell Line Data

The agonists BRL 37344 and CL 316243 concentration- and time-dependently enhanced insulin release in the rat insulinoma cell line RIN 1040-38, but concentrations of BRL 37344 exceeding 1 nM had less effect (Perfetti et al. 2001). The latter most likely represents off-target effects because BRL 37344 can act not only on β_2-AR but also on various other targets other than β_3-AR (Vrydag and Michel 2007). When RIN 1040-38 cells were transfected with the wild-type human β_3-AR and its Arg64 variant, the stimulation of insulin release was enhanced with the wild-type but not with the Arg64 variant (Perfetti et al. 2001). The prevalence of this polymorphisms varies a lot between ethnicities but its functional role remains controversial (Michel 2023).

4.2 Animal Data

One group has systematically explored the role of β-AR in the regulation of insulin release in mice. In the initial study, BRL 26830 increased insulin levels within 5 min in fasted mice, followed by a decrease of glucose and an increase of glucagon levels after 30 min (Yoshida 1992; Yoshida et al. 1991a). This was markedly inhibited by a high dose of the β-antagonist propranolol (50–100 mg/kg), partly by the β_2-antagonist ICI 118551, but not at all by metoprolol (a nonselective β-antagonist or a β-antagonist with less than 10-fold β_1-selectivity) in doses up to 100 mg/kg; however, findings with these high doses are difficult to assign to a specific AR subtype. Interestingly, BRL 26830 did not affect insulin, glucagon, or glucose in diabetic mice in which pancreatic β cells had been destroyed by streptozotocin. This data indicates that insulin release from murine β cells may be promoted by a combination of β_2- and β_3-AR. However, the findings could also be explained by BRL 26830 primarily acting on skeletal muscle (Sato et al. 2014). In a separate study, treatment

with BRL 26830 for 2 weeks resulted in exaggerated insulin responses during an acute intraperitoneal glucose loading test (Yoshida et al. 1991b).

BRL 26830 increased plasma insulin concentrations in fasted rats and improved glucose disposal after a glucose load in non-diabetic rats and mice (Cawthorne et al. 1984). CL 316243 also increased plasma insulin levels in rats (Atef et al. 1996). BRL 26830 also increased blood insulin in dogs (Yoshida 1992).

While the inhibitory effects of α_2-AR agonists on insulin release are largely mediated by a direct effect on β cells (see above), it remains unclear whether the stimulatory effects of β-AR agonists also are mainly direct. Neither BRL 26830 nor its congener BRL 28410 (0.1–1 μM) stimulated insulin release from isolated rat islets in the presence of 2.8 or 5.6 mM glucose (Yoshida 1992). On the other hand, the increase in plasma insulin in rats upon administration of the agonist CL 316243 was accompanied by an increased blood flow in the pancreatic islets, but not in the overall pancreas. A high dose of the β-antagonist bupranolol (a general β-AR agonist also blocking β_3-AR) inhibited this, whereas nadolol (not blocking β_3-AR) did not, providing circumstantial evidence for a possible involvement of β_3-AR (Atef et al. 1996). While vasodilation is typically attributed to β_2-AR, β_3-AR can also contribute in some blood vessels (Guimaraes and Moura 2001). Therefore, it was proposed that enhanced insulin release, at least in rats, is not only a direct effect on the β cells but also involves a component secondary to vasodilatation (Atef et al. 1996). Another proposal for an indirect component is that, at least in mice, that β_3-AR agonists can enhance insulin release secondary to lipolysis and release of free fatty acids (Heine et al. 2018). In the dog study, BRL 26830 concomitantly increased insulin and glucagon levels and those of free fatty acids, but did not change those of blood glucose (Yoshida 1992); this also argues for an indirect effect with the primary anatomical target being adipose tissue. However, experiments in in situ perfused mouse pancreas found that CL 316243 concentration-dependently stimulated insulin secretion (Yoshida et al. 1994). Very high concentrations of propranolol or ICI 118551 (0.2 mM) partly inhibited this, whereas metoprolol had no effect. BRL 26830 had no effect on insulin levels in mice in which β cells had been destroyed by treatment with streptozotocin (Yoshida 1992; Yoshida et al. 1991a). The effects of β_3-AR agonists on insulin release in a rat insulinoma cell line (Perfetti et al. 2001) also argue in favor of a direct effect on the β cells. Whether these at least partly contradicting findings represent genuine species differences or are examples of poor data robustness remains to be determined.

Taken together, these data indicate that β-AR agonists promote insulin release from the pancreas of rats and mice. Based upon a single study (Yoshida 1992), a similar situation may exist in dogs. These effects appear to be largely mediated by β_3-AR, but a β_2-AR component cannot be excluded. Whether the enhancement of insulin release by β-AR agonists in vivo is a direct effect on β cells or, at least partly, occurs secondary to those on adipose tissue, skeletal muscle, and/or blood vessels remains unclear (Fig. 4).

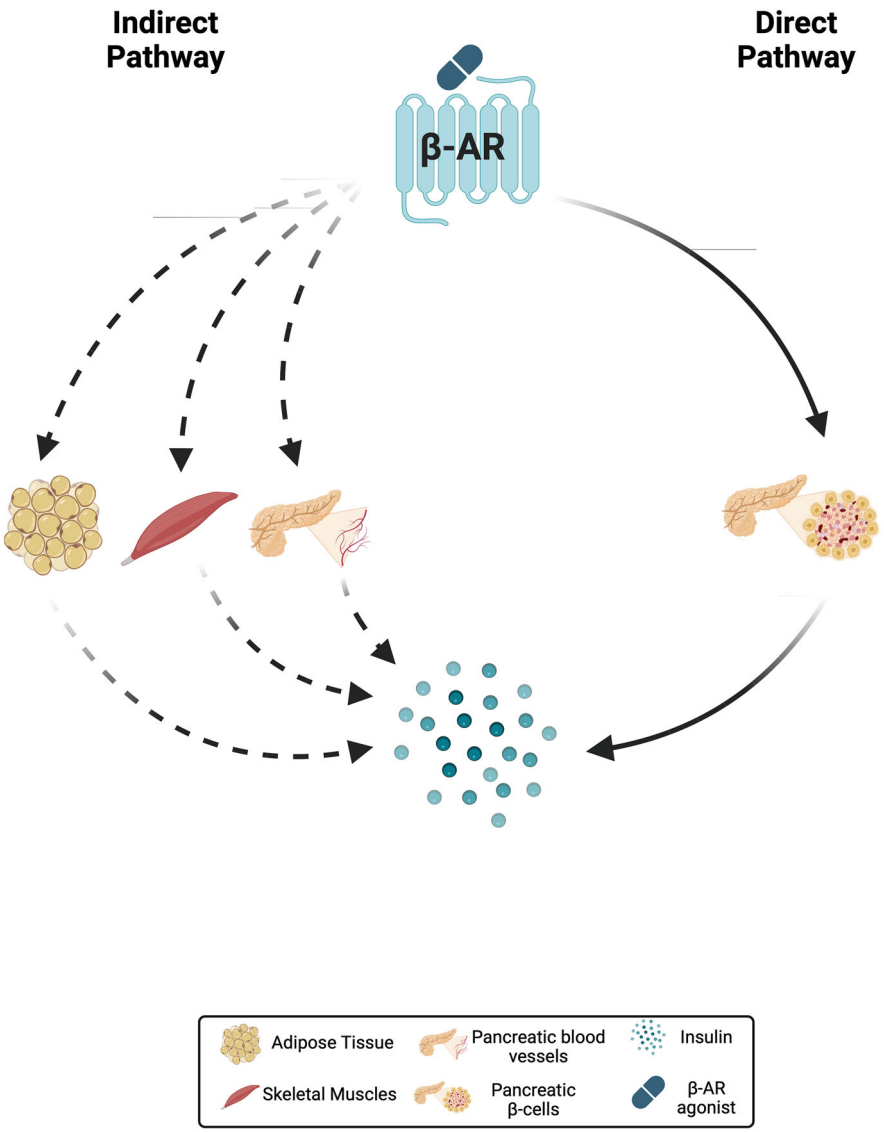


Fig. 4 Schematic representation of β-adrenoceptor stimulated insulin release. To which degree this occurs indirectly by effects on adipose tissue, skeletal muscle, pancreatic blood vessels, or β cells in the islets of Langerhans, remains unclear. Moreover, the receptor subtype being involved may differ between species; for details see text

4.3 Human Data

Early work on the roles of β_2-AR in the regulation of insulin levels in humans has been reviewed (Haffner and Kendall 1992). An infusion of the β_2-agonist terbutaline increased plasma insulin and glucose levels, whereas injection of the β_1-AR selective

partial agonist xamoterol decreased insulin but not glucose levels (Haffner et al. 1993), further supporting a role for β_2-AR. A group from Dundee conducted multiple studies on a possible role of β-AR subtypes in the regulation of human metabolism. Their initial study infused isoprenaline at doses of 0.5–3.0 μg/min in the presence of placebo, atenolol (25 mg), or nadolol (5, 20, and 80 mg) (Wheeldon et al. 1993). While isoprenaline caused thermogenesis and increased plasma glucose and insulin, this was blocked only partly even by the highest dose of nadolol, implying that it involved multiple β-AR subtypes possibly including β_3-AR. A follow-up study administered a single oral dose of BRL 35135 (8 mg; a prodrug rapidly converted to BRL 37344 upon oral administration) in comparison to the β_2-agonist salbutamol (8 mg) or placebo after pretreatment with placebo, bisoprolol (5 mg) or nadolol (20 mg) (Wheeldon et al. 1994). Although BRL 35135 is a β_3-AR agonist, the observed increase in serum glucose and insulin was fully blocked by nadolol, implying that little involvement of β_3-AR. In both studies, the concomitant increase in glucose and insulin (and concomitant inhibition thereof by antagonists) indicates that the increased insulin may not reflect a direct effect on the pancreas but more likely occurred secondary to the increase in glucose levels. This conclusion is further supported by a study in which a high (supra-therapeutic) dose of mirabegron (100 mg q.d. for 4 weeks) did not alter insulin responses in a glucose tolerance test (O'Mara et al. 2020). Taken together these limited data demonstrate increases in plasma insulin by administration of β-AR agonists. These effects appear to be largely mediated by β_2-AR, but a minor component of β_3-AR cannot be excluded. This contrasts findings in rats and mice. Perhaps more importantly, it remains unclear how much of this response reflects a direct effect on pancreatic β cells, and how much occurs indirectly secondary to an increase in glucose levels.

4.4 Genetic Evidence

A comprehensive study in mice employing both transgenic overexpression and knock-out of β_3-AR found that acute administration of the agonist CL 316243 increased insulin levels by 50–100-fold in control mice, along with a doubling of energy expenditure and a reduction of food intake (Grujic et al. 1997). These responses were abolished in the β_3-AR knock-out mice, but transgenic expression of the receptor in white or brown adipose tissues rescued the phenotype, indicating that the major increase in insulin occurs not by directly promoting release from β cells but rather indirectly by effects on adipose tissue.

Later experiments by another group found that male β_2-AR knock-out mice exhibited a reduced glucose-induced insulin release from isolated pancreatic islets, whereas adenovirus-mediated gene transfer rescued insulin release (Santulli et al. 2012), supporting the idea of a direct effect on the β cells. Others observed that pancreas-specific deletion of the β_2-AR causes impaired insulin secretion and glucose tolerance, but this was observed only in female and not in male mice (Ceasrine et al. 2018). In additional experiments, this phenotype was recapitulated when the β_2-AR was deleted from the β cells or in neonatal, but not adult mice. This was

apparently related to changes in the production of VEGF in female mice resulting in hypervascularization during development disrupting insulin production and exocytosis and inhibiting the VEGF receptor rescued the metabolic phenotype. Thus, these experiments support a direct effect on the β cells but nonetheless an indirect mechanism secondary to vascularization.

4.5 Regulation

One study reported that aged (20-month-old) mice exhibited impaired insulin secretion and glucose tolerance along with reduced β_2-AR mRNA and protein expression as compared to 6-months-old animals (Santulli et al. 2012). However, most studies on the regulation of β-adrenergic regulation have been performed in diabetic patients and animal models of diabetes.

In mice, an early study found that BRL 26830 given for 2–6 weeks reduced fasting insulin concentration and improved glucose tolerance in ob/ob mice (obese mouse model lacking leptin) (Cawthorne et al. 1984). The authors also examined db/db mice that are characterized by hyperinsulinemia from 10 days to 4–5 months age, followed by a decline and pancreatic islet disruption. When administered to young db/db mice, BRL 26830 further enhanced plasma insulin levels (control 48, db/db 116, db/db plus BRL 26830 279 μU/ml). However, pancreatic insulin content was reduced in db/db mice and restored upon treatment with the agonist (3.8 vs. 0.6 vs. 3.9 mU/mg), indicating that increased circulating insulin with BRL 26830 did not come at the expense of depleting the β cells and rather from restoring their ability to express insulin. However, others found that treatment of young db/db mice with three selective β_3-AR agonists for 14 days, including solabegron that has been tested clinically in overactive bladder patients (Ohlstein et al. 2012), dose-dependently reduced plasma insulin concentrations (Uehling et al. 2006).

KK-Ay mice are mostly used as a model of mild hepatic steatosis and inhibit an impaired glucose tolerance but not overt diabetes. In this model, BRL 26830 further augmented the already larger insulin response to a glucose load (Yoshida et al. 1991b). The same investigators reported that BRL 26830 did not affect insulin, glucagon, or glucose in diabetic mice in which pancreatic β cells had been destroyed by streptozotocin (Yoshida 1992), providing evidence that the β_3-AR agonist may have acted directly on the β cells.

Studies in humans are limited, but one group has studied the effect of insulin on β-AR function using human forearm blood flow in vivo as the model. Infusion of insulin enhanced vasodilation responses to isoprenaline in the absence of systemic hemodynamic effects by either agent (Lembo et al. 1997a; Lembo et al. 1996). While this points to cross-regulation between insulin and β-AR, it remains unclear whether this also occurs in the pancreas.

5 Modulation of Glucagon Release: α- and β-Adrenoceptor Involvement

Compared to the large number of studies on insulin release, modulation of glucagon release by AR ligands remains an under-investigated area. The α_2-AR antagonist SL 840418 did not alter plasma glucagon levels in doses where it increased insulin levels (Berlin et al. 1994). Another study in lean and in obese spontaneously hypertensive rats also found limited effects of α_2-AR ligands on circulating glucagon, but the α_2-agonists moxonidine and rilmenidine reduced glucagon and increased insulin, apparently via an imidazoline site in both strains (Velliquette and Ernsberger 2003). Similarly, neither the agonist dexmedetomidine nor the antagonist MK-467 affected plasma glucagon levels in dogs in doses that affected insulin levels (Kallio-Kujala et al. 2018). On the other hand, plasma glucagon levels were elevated in α_{2A}-AR knock-out mice (Savontaus et al. 2008). Conversely, glucagon partly reversed the inhibitory effects of clonidine on insulin release in rat pancreatic islets (Laychock and Bilgin 1989).

BRL 26830 increased glucagon levels in mice 30 min after administration (Yoshida 1992; Yoshida et al. 1991a). However, this occurred later than the increase in plasma insulin observed as early as 5 min after administration and rather coincided with a decrease in glucose levels, indicating that the effect on glucagon may have occurred secondary to hypoglycemia. Similarly, BRL 26830 also increased blood glucagon levels in dogs within the same report. The pancreatic glucagon content after acute insulin administration was similar in wild-type and β_2-AR knock-out mice (Santulli et al. 2012).

6 Conclusions

AR play an important role in the regulation of pancreatic insulin release. Due to inhibitory effects of α_2-AR, the net effect of the α_2-AR stimulation typically is an inhibition of insulin secretion (Chan 1993; Ruffolo et al. 1991). There appears to be endogenous tone as shown by an increased insulin secretion in healthy and diabetic humans and animals in response to α_2-AR antagonists (Chan 1993; Ruffolo et al. 1991). Of note, most of the available evidence comes from rodents, and the limited human data indicate that they may not be representative, at least related to the role of β-AR and their subtypes. Based on animal studies and on an association of α_{2A}-AR polymorphism with an elevated risk for diabetes, increased α_{2A}-AR signaling in the pancreatic β cells has been proposed as a risk factor for the development of T2DM and the α_{2A}-AR was proposed as a drug target for the treatment thereof (Fagerholm et al. 2011; Liggett 2009). While some development programs such as midaglizole have targeted α_2-AR for the treatment of T2DM (Chan 1993; Ruffolo et al. 1991), none have resulted in an approved medicine for this condition. Therefore, the regulation of endocrine pancreatic function is physiologically interesting but may be of limited therapeutic relevance.

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References

Alemzadeh R, Tushaus KM (2004) Modulation of adipoinsular axis in prediabetic Zucker diabetic fatty rats by diazoxide. Endocrinology 145:5476–5484

Amisten S, Atanes P, Hawkes R, Ruz-Maldonado I, Liu B, Parandeh F, Zhao M, Huang GC, Salehi A, Persaud SJ (2017) A comparative analysis of human and mouse islet G-protein coupled receptor expression. Sci Rep 7:46600

Angel I, Niddam R, Langer SZ (1990) Involvement of alpha-2 adrenergic receptor subtypes in hyperglycemia. J Pharmacol Exp Ther 254:877–882

Angel I, Burcelin R, Prouteau M, Girard J, Langer SZ (1996) Normalization of insulin secretion by a selective α_2-adrenoceptor antagonist restores GLUT-4 glucose transporter expression in adipose tissue of type II diabetic rats. Endocrinology 137:2022–2027

Atef N, Lafontan M, Double A, Helary C, Ktorza A, Penicaud L (1996) A specific ß$_3$-adrenoceptor agonist induces pancreatic islet blood flow and insulin secretion in rats. Eur J Pharmacol 298: 287–292

Barlow TE, Greenwell JR, Harper AA, Scratcherd T (1974) The influence of the splanchnic nerves on the external secretion, blood flow and electrical conductance of the cat pancreas. J Physiol 236:421–433

Berkowitz DE, Price DT, Bello EA, Page SO, Schwinn DA (1994) Localization of messenger RNA for three distinct α_2-adrenergic receptor subtypes in human tissues. Evidence for species heterogeneity and implications for human pharmacology. Anesthesiology 81:1235–1244

Berlin I, Rosenzweig P, Chalon S, Fuseau E, Landault C, Cesslin F, Blacker C, Puech AJ (1994) Reduction of hyperglycemia after oral glucose load by the new α_2-adrenergic receptor antagonist SL 84.0418 in healthy subjects. Clin Pharmacol Ther 55:338–345

Berlin I, Chalon S, Payan C, Schöllnhammer G, Cesselin F, Varoquaux O, Puech AJ (1995) Evaluation of the α_2-adrenoceptor blocking properties of buspirone and ipsapirone in healthy subjects. Relationship with the plasma concentration of the common metabolite 1-(2-pyrimidinyl)-piperazine. Br J Clin Pharmacol 39:243–249

Bockman DE (1993) Anatomy of the pancreas. The exocrine pancreas: biology, pathobiology and disease. Raven Press, New York, pp 1–8

Cade JE, Hanison J (2017) The pancreas. Anaesth Intensive Care Med 18:527–531

Cawthorne MA, Carroll MJ, Levy AL, Lister CA, Sennitt MV, Smith SA, Young P (1984) Effects of novel beta-adrenoceptor agonists on carbhohydrate metabolism: relevance for the treatment of non-insulin-dependent diabetes. Int J Obes (Lond) 8(Suppl. 1):93–102

Ceasrine AM, Lin EE, Lumelsky DN, Iyer R, Kuruvilla R (2018) Adrb2 controls glucose homeostasis by developmental regulation of pancreatic islet vasculature. eLife 7

Cernecka H, Pradidarcheep W, Lamers WH, Schmidt M, Michel MC (2014) Rat ß$_3$-adrenoceptor protein expression: antibody validation and distribution in rat gastrointestinal and urogenital tissues. Naunyn Schmiedebergs Arch Pharmacol 387:1117–1127

Chan SLF (1993) Role of α_2-adrenoceptors and imidazoline-binding sites in the control of insulin secretion. Clin Sci 85:671–677

Chan SLF, Brown CA, Scarpello KE, Morgan NG (1994) The imidazoline site involved in control of insulin secretion: characteristics that distinguish it from I_1- and I_2 sites. Br J Pharmacol 112: 1065–1070

Chan SL, Perrett CW, Morgan NG (1997) Differential expression of alpha 2-adrenoceptor subtypes in purified rat pancreatic islet A- and B-cells. Cell Signal 9:71–78

Chen TH, Hsu WH (1994) Inhibition of insulin release by a formamidine pesticide amitraz and its metabolites in a rat beta-cell line: an action mediated by alpha-2 adrenoceptors, a GTP-binding protein and a decrease in cyclic AMP. J Pharmacol Exp Ther 271:1240–1245

Cherksey B, Altszuler N (1984) Tolbutamide and glyburide differ in effectiveness to displace alpha- and beta-adrenergic radioligands in pancreatic islet cells and membranes. Diabetes 33:499–503

Davani B, Portwood N, Bryzgalova G, Reimer MK, Heiden T, Ostenson CG, Okret S, Ahren B, Efendic S, Khan A (2004) Aged transgenic mice with increased glucocorticoid sensitivity in pancreatic beta-cells develop diabetes. Diabetes 53(Suppl 1):S51–S59

Devedjian JC, Fargues M, Denis-Pouxviel C, Daviaud D, Prats H, Paris H (1991) Regulation of the α_{2A}-adrenergic receptor in the HT29 cell line. Effects of insulin and growth factors. J Biol Chem 266:14359–14366

Dwaib HS, Michel MC (2023) Is the $ß_3$-adrenoceptor a valid target for the treatment of obesity and/or type 2 diabetes? Biomol Ther 13:1714

Eason MG, Liggett SB (1993) Human α_2-adrenergic receptor subtype distribution: widespread and subtype-selective expression of α_2C10, α_2C4, and α_2C2 mRNA in multiple tissues. Mol Pharmacol 44:70–75

Fagerholm V, Grönroos T, Marjamäki P, Viljanen T, Scheinin M, Haaparanta M (2004) Altered glucose homeostasis in alpha2A-adrenoceptor knockout mice. Eur J Pharmacol 505:243–252

Fagerholm V, Haaparanta M, Scheinin M (2011) α2-adrenoceptor regulation of blood glucose homeostasis. Basic Clin Pharmacol Toxicol 108:365–370

Fajardo N, Deshaies Y (1996) Glucose tolerance and postprandial glucose and insulin kinetics in rats with short- and long-term α_1-adrenergic blockade. J Cardiovasc Pharmacol 28:402–408

Gribble FM (2010) Alpha2A-adrenergic receptors and type 2 diabetes. N Engl J Med 362:361–362

Grujic D, Susulic VS, Harper ME, Himms-Hagen J, Cunningham BA, Corkey BE, Lowell BB (1997) ß3-adrenergic receptors on white and brown adipocytes mediate ß3-selective agonist-induced effects on energy expenditure, insulin secretion, and food intake. A study using transgenic and gene knockout mice. J Biol Chem 272:17686–17693

Guimaraes S, Moura D (2001) Vascular adrenoceptors: an update. Pharmacol Rev 53:319–356

Haffner CA, Kendall MJ (1992) Metabolic effects of beta 2-agonists. J Clin Pharm Ther 17:155–164

Haffner CA, Kendall MJ, Maxwell S, Hughes B (1993) The lipolytic effect of $ß_1$- and $ß_2$-adrenoceptor activation in healthy human volunteers. Br J Clin Pharmacol 35:35–39

Hamamdzic D, Duzic E, Sherlock JD, Lanier SM (1995) Regulation of α_2-adrenergic receptor expression and signaling in pancreatic ß-cells. Am J Physiol 269:E162–E171

Heine M, Fischer AW, Schlein C, Jung C, Straub LG, Gottschling K, Mangels N, Yuan Y, Nilsson SK, Liebscher G, Chen O, Schreiber R, Zechner R, Scheja L, Heeren J (2018) Lipolysis triggers a systemic insulin response essential for efficient energy replenishment of activated brown adipose tissue in mice. Cell Metab 28:644–655.e644

Hiyoshi Y, Miura H, Uemura K, Endo H, Ozawa K, Maeda N, Tamagawa T, Iguchi A (1995) Effects of imidazoline antagonists of α_2-adrenoceptors on endogenous adrenaline-induced inhibition of insulin release. Eur J Pharmacol 294:117–123

Hsu WH, Xiang H, Rajan AS, Boyd AE III (1991a) Activation of α_2-adrenergic receptors decreases Ca^{2+} influx to inhibit insulin secretion in a hamster ß-cell line: an action mediated by a guanosine triphosphate-binding protein. Endocrinology 128:958–964

Hsu WH, Xiang HD, Rajan AS, Kunze DL, Boyd AE 3rd (1991b) Somatostatin inhibits insulin secretion by a G-protein-mediated decrease in Ca^{2+} entry through voltage-dependent Ca^{2+} channels in the beta cell. J Biol Chem 266:837–843

Hu X, Friedman D, Hill S, Caprioli R, Nicholson W, Powers AC, Hunter L, Limbird LE (2005) Proteomic exploration of pancreatic islets in mice null for the alpha2A adrenergic receptor. J Mol Endocrinol 35:73–88

Jonas JC, Plant TD, Angel I, Langer SZ, Henquin JC (1994) In vitro stimulation of insulin release by SL 84.0418, a new α_2-adrenoceptor antagonist. Eur J Pharmacol 254:27–33

Kahn NN, Sinha AK (1992) Down regulation of α_2 adrenergic receptor number in platelets by insulin. Biochim Biophys Acta 1134:292–296

Kallio-Kujala IJ, Bennett RC, Raekallio MR, Yatkin E, Meierjohann A, Savontaus E, Scheinin M, Spillmann T, Vainio OM (2018) Effects of dexmedetomidine and MK-467 on plasma glucose, insulin and glucagon in a glibenclamide-induced canine hypoglycaemia model. Vet J 242:33–38

Khoury AF, Kaplan NM (1991) α-Blocker therapy of hypertension. An unfulfilled promise. JAMA 266:394–398

Lacey RJ, Berrow NS, London NJ, Lake SP, James RF, Scarpello JH, Morgan NG (1990) Differential effects of beta-adrenergic agonists on insulin secretion from pancreatic islets isolated from rat and man. J Mol Endocrinol 5:49–54

Lacey RJ, Chan SLF, Cable HC, James RFL, Perrett CW, Scarpello JHB, Morgan NG (1996) Expression of α_2- and ß-adrenoceptor subtypes in human islets of Langerhans. J Endocrinol 148: 531–543

Lacombe C, Viallard V, Paris H (1993) Identification of α2-adrenoceptors and of non-adrenergic idazoxan binding sites in pancreatic islets from young and adult hamsters. Int J Biochem 25: 1077–1083

Lammert E, Lammert E, Zeeb M, Zeeb M (2014) Metabolism of human diseases: organ physiology and pathophysiology. Springer

Laychock SG (1990) Glucose metabolism, second messengers and insulin secretion. Life Sci 47: 2307–2316

Laychock SG, Bilgin S (1989) Calcium mobilization, prostaglandin E_2 and α_2-adrenoceptor modulation of glucose utilization and insulin secretion in pancreatic islets. Biochem Pharmacol 38:2511–2520

Lembo G, Iaccarino G, Rendina V, Volpe M, Trimarco B (1994) Insulin blunts sympathetic vasoconstriction through the α_2-adrenergic pathway in humans. Hypertension 24:429–438

Lembo G, Iaccarino G, Vecchione C, Rendina V, Parrella L, Trimarco B (1996) Insulin modulation of ß-adrenergic vasodilator pathway in human forearm. Circulation 93:1403–1410

Lembo G, Iaccarino G, Vecchione C, Barbato E, Izzo R, Fontana D, Trimarco B (1997a) Insulin modulation of an endothelial nitric oxide component present in the α_2- and ß-adrenergic response in human forearm. J Clin Invest 100:2007–2014

Lembo G, Iaccarino G, Vecchione C, Barbato E, Morisco C, Monti F, Parrella L, Trimarco B (1997b) Insulin enhances endothelial α_2-adrenergic vasorelaxation by a pertussis toxin mechanism. Hypertension 30:1128–1134

Liggett SB (2009) alpha2A-adrenergic receptors in the genetics, pathogenesis, and treatment of type 2 diabetes. Sci Transl Med 1:12ps15

Mahadevan V (2019) Anatomy of the pancreas and spleen. Surgery (Oxford) 37:297–301

Michel MC (2023) Are β3-adrenoceptor gene polymorphisms relevant for urology? NeurourolUrodyn 42:33–39

Michel MC, Ernsberger P (1992) Keeping an eye on the I site: imidazoline-preferring receptors. Trends Pharmacol Sci 13:369–370

Michel MC, Michel-Reher MB, Hein P (2020) A systematic review of inverse agonism at adrenoceptor subtypes. Cells 9:1923

Nagain C, Chariot J, Roze C (1995) Differential effects of peptide YY, neuropeptide Y, and σ ligands on neurally stimulated external pancreatic secretion in the rat. Pancreas 10:123–130

Niclauß N, Michel-Reher MB, Alewijnse AE, Michel MC (2006) Comparison of three radioligands for the labelling of human ß-adrenoceptor subtypes. Naunyn Schmiedebergs Arch Pharmacol 374:99–105

Niddam R, Angel I, Bidet S, Langer SZ (1990) Pharmacological characterization of alpha-2-adrenergic receptor subtype involved in the release of insulin from isolated rat panreatic islets. J Pharmacol Exp Ther 254:883–887

Ohlstein EH, von Keitz A, Michel MC (2012) A multicenter, double-blind, randomized, placebo controlled trial of the ß 3 -adrenoceptor agonist solabegron for overactive bladder. Eur Urol 62: 834–840

O'Mara AE, Johnson JW, Linderman JD, Brychta RJ, McGehee S, Fletcher LA, Fink YA, Kapuria D, Cassimatis TM, Kelsey N, Cero C, Abdul-Sater Z, Piccinini F, Baskin AS, Leitner BP, Cai H, Millo CM, Dieckmann W, Walter M, Javitt NB, Rotman Y, Walter PJ, Ader M, Bergman RN, Herscovitch P, Chen KY, Cypess AM (2020) Chronic mirabegron treatment increases human brown fat, HDL cholesterol, and insulin sensitivity. J Clin Invest 130:2209–2219

Ostenson CG, Cattaneo AG, Doxey JC, Efendic S (1989) Alpha-adrenoceptors and insulin release from pancreatic islets of normal and diabetic rats. Am J Physiol 257:E439–E443

Pandol S (2011) The exocrine pancreas

Perfetti R, Hui H, Chamie K, Binder S, Seibert M, McLenithan J, Silver K, Walston JD (2001) Pancreatic ß-cells expressing the Arg64 variant of the $ß_3$-adrenergic receptor exhibit abnormal insulin secretory activity. J Mol Endocrinol 27:133–144

Peterhoff M, Sieg A, Brede M, Chao CM, Hein L, Ullrich S (2003) Inhibition of insulin secretion via distinct signaling pathways in α_2-adrenoceptor knockout mice. Eur J Endocrinol 149:343–350

Plant TD, Jonas JC, Henquin JC (1991) Clonidine inhibits ATP-sensitive K^+ channels in mouse pancreatic ß-cells. Br J Pharmacol 104:385–390

Porte D Jr (1967) A receptor mechanism for the inhibition of insulin release by epinephrine in man. J Clin Invest 46:86–94

Price DT, Lefkowitz RJ, Caron MG, Berkowitz D, Schwinn DA (1994) Localization of mRNA for three distinct α_1-adrenergic receptor subtypes in human tissues: implications for human α-adrenergic physiology. Mol Pharmacol 45:171–175

Regard JB, Kataoka H, Cano DA, Camerer E, Yin L, Zheng YW, Scanlan TS, Hebrok M, Coughlin SR (2007) Probing cell type-specific functions of Gi in vivo identifies GPCR regulators of insulin secretion. J Clin Invest 117:4034–4043

Robertson RP, Halter JB, Porte D Jr (1976) A role for alpha-adrenergic receptors in abnormal insulin secretion in diabetes mellitus. J Clin Invest 57:791–795

Rosengren AH, Jokubka R, Tojjar D, Granhall C, Hansson O, Li DQ, Nagaraj V, Reinbothe TM, Tuncel J, Eliasson L, Groop L, Rorsman P, Salehi A, Lyssenko V, Luthman H, Renström E (2010) Overexpression of alpha2A-adrenergic receptors contributes to type 2 diabetes. Science 327:217–220

Ruffolo RR Jr, Nichols AJ, Hieble JP (1991) Metabolic regulation by α_1- and α_2-adrenoceptors. Life Sci 49:171–183

Santulli G, Lombardi A, Sorriento D, Anastasio A, Del Giudice C, Formisano P, Béguinot F, Trimarco B, Miele C, Iaccarino G (2012) Age-related impairment in insulin release: the essential role of β_2-adrenergic receptor. Diabetes 61:692–701

Sato M, Dehvari N, Oberg AI, Dallner OS, Sandström AL, Olsen JM, Csikasz RI, Summers RJ, Hutchinson DS, Bengtsson T (2014) Improving type 2 diabetes through a distinct adrenergic signaling pathway involving mTORC2 that mediates glucose uptake in skeletal muscle. Diabetes 63:4115–4129

Saulnier-Blache JS, Bouloumie A, Valet P, Devedjian JC, Lafontan M (1992) Androgenic regulation of adipocyte α2-adrenoceptor expression in male and female Syrian hamsters: proposed transcriptional mechanism. Endocrinology 130:316–327

Savontaus E, Fagerholm V, Rahkonen O, Scheinin M (2008) Reduced blood glucose levels, increased insulin levels and improved glucose tolerance in alpha2A-adrenoceptor knockout mice. Eur J Pharmacol 578:359–364

Schaak S, Cayla C, Lymperopoulos A, Flordellis C, Cussac D, Denis C, Paris H (2000) Transcriptional down-regulation of the human α2C-adrenergic receptor by cAMP. Mol Pharmacol 58: 821–827

Schäfers RF, Elliott HL, Howie CA, Reid JL (1992) A preliminary, clinical pharmacological assessment of L-659,066, a novel α_2-adrenoceptor antagonist. Br J Clin Pharmacol 34:521–526

Schäfers RF, Nürnberger J, Herrmann B, Wenzel RR, Philipp T, Michel MC (1999) Adrenoceptors mediating the cardiovascular and metabolic effects of α-methylnoradrenaline in man. J Pharmacol Exp Ther 289:918–925

Scheltdorf M, Mest HJ (2002) Pertussis toxin converts hyperpolarization caused by α_2-adrenoceptor agonists containing an imidazoline moiety into depolarization in MIN 6 cells. Naunyn Schmiedebergs Arch Pharmacol 366:204–208

Schmidt A, Hescheler J, Offermanns S, Spicher K, Hinsch KD, Klinz FJ, Codina J, Birnbaumer L, Gausepohl H, Frank R, Schultz G, Rosenthal W (1991) Involvement of pertussis toxin-sensitive G-proteins in the hormonal inhibition of dihydropyridine-sensitive Ca^{2+} currents in an insulin-secreting cell line (RINm5F). J Biol Chem 266:18025–18033

Schulz A, Hasselblatt A (1989) Dual action of clonidine on insulin release: suppression, but stimulation when α_2-adrenoceptors are blocked. Naunyn Schmiedebergs Arch Pharmacol 340: 712–714

Sciberras DG, Reed JW, Elliott C, Blain PG, Goldberg MR (1994) The effects of a peripherally selective α_2-adrenoceptor antagonist, MK-467, on the metabolic and cardiovascular response to exercise in healthy man. Br J Clin Pharmacol 37:39–44

Scofield MA, Liu F, Abel PW, Jeffries WB (1995) Quantification of steady state expression of mRNA for alpha-1 adrenergic receptor subtypes using reverse transcription and a competitive polymerase chain reaction. J Pharmacol Exp Ther 275:1035–1042

Siitonen N, Lindström J, Eriksson J, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Tuomilehto J, Laakso M, Uusitupa M (2004) Association between a deletion/insertion polymorphism in the α2B-adrenergic receptor gene and insulin secretion and type 2 diabetes. The Finnish diabetes prevention study. Diabetologia 47:1416–1424

Starke K (1987) Presynaptic α-autoreceptors. Rev Physiol Biochem Pharmacol 107:74–146

Talathi SS, Zimmerman R, Young M (2023) Anatomy, abdomen and pelvis, pancreas. In: StatPearls [Internet]. StatPearls Publishing

Tang Y, Axelsson AS, Spegel P, Andersson LE, Mulder H, Groop LC, Renström E, Rosengren AH (2014) Genotype-based treatment of type 2 diabetes with an α_{2A}-adrenergic receptor antagonist. Sci Transl Med 6:257ra139

Thomas RF, Liggett SB (1993) Lack of $ß_3$-adrenergic receptor mRNA expression in adipose and other metabolic tissues in the adult human. Mol Pharmacol 43:343–348

Tsoli E, Chan SLF, Morgan NG (1995) The imidazoline I_1 receptor agonist, moxonidine, inhibits insulin secretion from isolated rat islets of Langerhans. Eur J Pharmacol 284:199–203

Uehling DE, Shearer BG, Donaldson KH, Chao EY, Deaton DN, Adkison KK, Brown KK, Cariello NF, Faison WL, Lancaster ME, Lin J, Hart R, Milliken TO, Paulik MA, Sherman BW, Sugg EE, Cowan C (2006) Biarylaniline phenethanolamines as potent and selective $ß_3$ adrenergic receptor agonists. J Med Chem 49:2758–2771

Uhlen M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson A, Kampf C, Sjöstedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Al-Khalili Szigyarto C, Odeberg J, Djureinovic D, Ottosson Takanen J, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen JJ, Ponten F (2015) Tissue-based map of the human proteome. Science 347:1260419

Urano Y, Sakurai T, Ueda H, Ogasawara J, Sakurai T, Takei M, Izawa T (2004) Desensitization of the inhibitory effect of norepinephrine on insulin secretion from pancreatic islets of exercise-trained rats. Metabolism 53:1424–1432

Varney MJ, Benovic JL (2024) The role of G protein-coupled receptors and receptor kinases in pancreatic ß-cell function and diabetes. Pharmacol Rev 76:267–299

Velliquette RA, Ernsberger P (2003) The role of I_1-imidazoline and α_2-adrenergic receptors in the modulation of glucose metabolism in the spontaneously hypertensive obese rat model of metabolic syndrome X. J Pharmacol Exp Ther 306:646–657

Vrydag W, Michel MC (2007) Tools to study ß$_3$-adrenoceptors. Naunyn Schmiedebergs Arch Pharmacol 374:385–398

Waeber G, Thompson N, Waeber B, Brunner HR, Nicod P, Grouzmann E (1993) Neuropeptide Y expression and regulation in a differentiated rat insulin-secreting cell line. Endocrinology 133: 1061–1067

Wheeldon NM, McDevitt DG, Lipworth BJ (1993) Do beta 3-adrenoceptors mediate metabolic responses to isoprenaline. Q J Med 86:595–600

Wheeldon NM, McDevitt DG, McFarlane LC, Lipworth BJ (1994) ß-Adrenoceptor subtypes mediating the metabolic effects of BRL 35135 in man. Clin Sci 86:331–337

Yoshida T (1992) The antidiabetic ß$_3$-adrenoceptor agonist BRL 26830A works by release of endogenous insulin. Am J Clin Nutr 55:237S–241S

Yoshida T, Hiraoka N, Kondo M (1991a) Effects of a beta 3-adrenoceptor agonist, BRL 26830A, on insulin and glucagon release in mice. Endocrinol Jpn 38:641–646

Yoshida T, Hiraoka N, Yoshioka K, Hasegawa G, Kondo M (1991b) Anti-obesity and anti-diabetic actions of a beta 3-adrenoceptor agonist, BRL 26830A, in yellow KK mice. Endocrinol Jpn 38: 397–403

Yoshida T, Yoshioka K, Hiraoka N, Umekawa T, Sakane N, Kondo N (1994) Effects of CL 316,243, a novel ß$_3$-adrenoceptor agonist, on inuslin secretion in perfused mouse pancreas. Endocr J 41:671–675

Yuan Q, Pan A, Fu Y, Dai Y (2021) Anatomy and physiology of the pancreas. In: Integrative pancreatic intervention therapy. Elsevier, pp 3–21

β-Adrenoceptors in Cancer: Old Players and New Perspectives

Rosario Amato, Martina Lucchesi, Silvia Marracci, Luca Filippi, and Massimo Dal Monte

Contents

Abstract

Distress, or negative stress, is known to considerably increase the incidence of several diseases, including cancer. There is indeed evidence from pre-clinical models that distress causes a catecholaminergic overdrive that, mainly through the activation of β-adrenoceptors (β-ARs), results in cancer cell growth and cancer progression. In addition, clinical studies have evidenced a role of negative stress in cancer progression. Moreover, plenty of data demonstrates that β-blockers have positive effects in reducing the pro-tumorigenic activity of catecholamines, correlating with better outcomes in some type of cancers as evidenced by several clinical trials. Among β-ARs, β2-AR seems to be the main β-AR subtype involved in tumor development and progression. However,

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there are data indicating that also β1-AR and β3-AR may be involved in certain tumors. In this chapter, we will review current knowledge on the role of the three β-AR isoforms in carcinogenesis as well as in cancer growth and progression, with particular emphasis on recent studies that are opening new avenues in the use of β-ARs as therapeutic targets in treating tumors.

Keywords

Cancer cell proliferation · Carcinogenesis · Catecholamines · Dedifferentiation · Immune-tolerance · Stress · Tumor growth · Tumor infiltration · Tumor microenvironment

1 Introduction

From an evolutionary point of view, animals need to develop strategies to face environmental changes that may impact on their lives. In particular, the exposure to a stressful environment triggers homeostatic responses aiming at facing the deriving perturbation. In this respect, it is known that the nature of the stress may influence the nature of the response, with acute stressors mainly inducing positive effects while chronic stressors leading to deleterious outcomes (Jessop 2019). David Livingstone, in 1857, had a direct experience of positive responses to stress: "[. . .] I heard a shout. Starting, and looking half round, I saw the lion just in the act of springing upon me. I was upon a little height; he caught my shoulder as he sprang, and we both came to the ground below together. Growling horribly close to me ear, he shook me as a terrier dog as a rat. The shock produced a stupor similar to that which seems to be felt by a mouse after the first shake of the cat. It caused a sort of dreaminess, in which there was no sense of pain nor feeling of terror, though quite conscious of all that was happening. It was like what patients partially under the influence of chloroform describe, who see all the operation, but feel not the knife. This singular condition was not the result of any mental process. The shake annihilated fear, and allowed no sense of horror in looking round at the beast. This peculiar state is probably produced in all animals killed by the carnivora and, if so, is a merciful provision by our benevolent Creator for lessening the pain of death" (Livingstone 1857). In the case of Dr. Livingstone (we presume, of course), the stressful condition acted on pain receptors, enkephalins and possible additional players that are not part of the present story, which is instead based on adrenoceptors. And, particularly, on the response that adrenoceptors evoke when an individual is exposed to chronic stress conditions, as chronic stress (distress), may induce illness states.

2 Stress and Cancer

The homeostatic response to stressors involves two different, although inter-related, systems: the hypothalamus-pituitary-adrenal axis and the sympathetic nervous system. Perceiving stress results in the activation of these pathways, whose dysregulation is responsible for an increased risk of developing diseases, including cancer (Flaherty et al. 2019). As recently reviewed, preclinical data seem to point on a pro-carcinogenic role of stress hormones, although clinical studies remain inconclusive about this point, suggesting instead a role of stress in cancer progression (Mravec et al. 2020a). Among stress hormones, there is extensive evidence that norepinephrine and epinephrine may modulate both cancer cell biology and the tumor microenvironment, whose strict relationship with cancer cells is paramount in cancer progression (Mravec et al. 2020b). It is of note that catecholamines may modulate tumor cells with opposite effects, giving rise to the recently defined "cancer catecholamine conundrum" (Wackerhage et al. 2022). In fact, it has been suggested that, for instance in the context of exercise, catecholamines may have a positive effect on cancer, possibly linked to the induction of a eustress condition, that is a stress condition having beneficial effects on health. In this respect, mice bearing liver cancer raised in condition of enriched environment, a condition known to produce eustress, showed an increased antitumor immunity and reduced malignant progression with respect to mice raised in standard condition (Liu et al. 2021). Similarly, exercise training in mice reduced the growth of melanoma xenotransplant by about 60% with respect to untrained mice, due to induction of migration and activation of immune cells into the tumor mass (Pedersen et al. 2016). In contrast, chronic distress, such as psychosocial stress, has been associated to tumor development or to tumor progression, as evidenced both in pre-clinical models and in humans (see for Ref. Wackerhage et al. 2022). In particular, many studies indicate that catecholamines stimulate cancer cell growth and cancer progression mainly acting at β-adrenoceptors (β-ARs) (Mravec et al. 2020b). The first evidence indicating a role of β-ARs in tumor growth dates back to the late '80s, when Schuller and Cole showed that human lung adenocarcinoma cells proliferate when stimulated with isoprenaline, an effect blunted by propranolol (Schuller and Cole 1989). After that, plenty of data demonstrated that norepinephrine stimulates the proliferation of different types of cancer cells and induces several hallmarks of cancer, including cell proliferation, cell migration and angiogenesis. In addition, β-blockers reduce the pro-tumorigenic effect of stress hormones, decreasing tumor growth in preclinical models and reducing mortality and recurrence in tumor patients (see for Refs. Mravec et al. 2020a; b, c; Gosain et al. 2020; Dal Monte et al. 2019). However, in clinical trials the use of β-blockers correlates with better outcomes only in specific types of cancer, such as melanoma and ovarian cancer, but not in breast, colorectal or lung cancer (Musselman et al. 2018; Yap et al. 2018). Given that differential β-AR subtype expression is found in cancer cells, and that the activation of these receptors in different cancer types has diverse effects on tumor proliferation, migration, and invasion (Tang et al. 2013), one could speculate that the effectiveness of β-blockers should depend not only on the tumor subtype, but also on the specific β-blocker. In

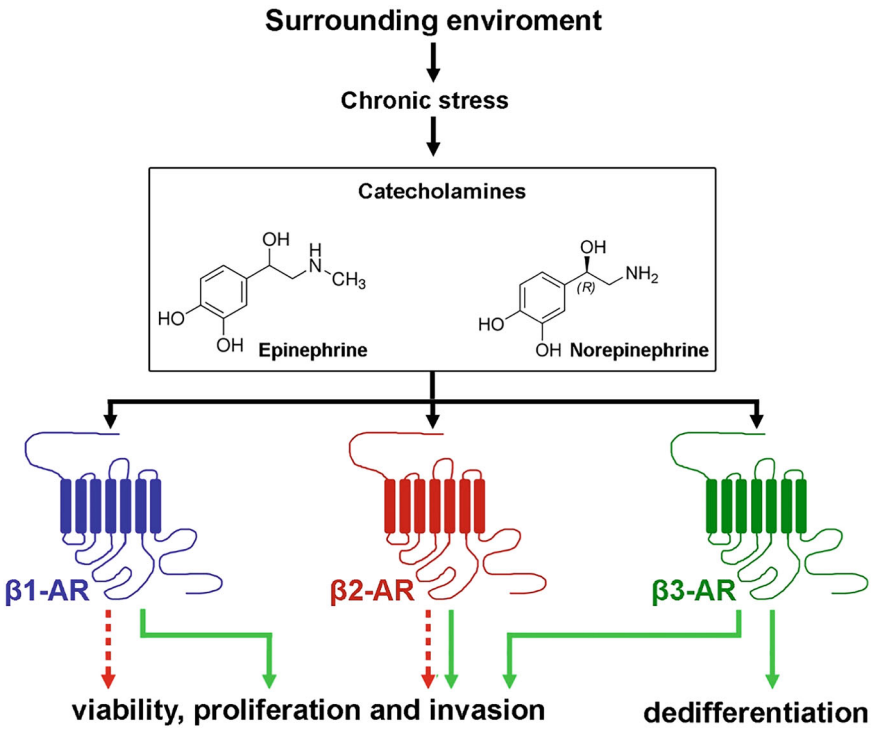

Fig. 1 Effects of stress-induced catecholamine overdrive on β-ARs expressed by cancer cells. The increased levels of epinephrine and/or norepinephrine acting at β1-, β2-, and/or β3-ARs promote tumor cell viability, proliferation, and invasion, also inducing the dedifferentiation of cancer cells (green arrows). There is however some evidence that in specific cancers β1- and β2-AR activation may have protective effects against tumor growth (red dashed arrows)

this context, it is easy to understand the importance of deeper investigations on the usage of specific β-AR antagonists/agonists in order to achieve the best possible outcome with the minimum risk of adverse events. To accomplish this goal, it is crucial to unravel the role of each single β-AR in the examined tumor type. Although β2-AR seems to be the main β-AR involved in tumor development and progression, there are data indicating that also β1-AR and β3-AR may be involved in certain tumors. Therefore, in this article, we review literature data, referring to both pre-clinical and clinical studies, about the involvement of the three β-AR isoforms in cancer. Figure 1 summarizes the effects that catecholamines, acting at the three different β-ARs, exert on tumor cells.

3 β1-ARs

Since most studies rely on the use of β-AR agonists/antagonists that target both the β1 and β2-AR subtypes, it is often difficult to extrapolate the specific role of each subtype in tumor biology over that of β2-ARs. However, evidence has been provided that β1-ARs may play a role in cancer. The potential involvement of β1-ARs in tumor progression was first demonstrated in 1990 when Hough and Chuang showed that β1- and β2-AR mRNAs s were downregulated in C6 rat glioma cells after exposure to the non-selective β-AR agonist isoproterenol, although its effect on protein levels of β1- and β2-ARs was not investigated (Hough and Chuang 1990). In addition, the authors observed that in growing C6 cells β-AR transcripts are downregulated with time of culture and that β-AR downregulation is accompanied by contact inhibition, suggesting a possible role of β-ARs in glioma cell proliferation. In line with this study, Hosoda et al. demonstrated that exposure of C6 cells to isoproterenol caused a biphasic modulation of β1-AR mRNA expression, with transcript levels raised by short-term treatment, and decreased by long-term exposure (Hosoda et al. 1994). In particular, it was shown that β1-AR transcriptional regulation is mediated by cAMP through binding to cAMP responsive elements present in the human and rat β1-AR gene (Collins et al. 1993; Hosoda et al. 1994). In addition, the expression of β1-ARs have been found in human melanoma cell lines and biopsies of benign naevi and melanomas, with a higher expression level in malignant tumors, suggesting that blockade of β1-ARs may represent a target to slow down melanoma progression (Moretti et al. 2013). Moreover, Gao et al. in a clinical cohort study showed that autoantibodies against β1-ARs were higher in de novo multiple myeloma patients than in normal participants, suggesting that β1-AR autoantibodies may be used as predictors to identify multiple myeloma patients (Gao et al. 2018). A recent in silico study concerning functional network analysis has evidenced that atenolol, a commonly used "cardio-selective" β1-AR blocker that in the rat is three- to fourfold more potent on β1-ARs than on β2-ARs (Minneman et al. 1979) and that shows a profile of inverse agonist (Baker et al. 2003; Hopkinson et al. 2000; Michel et al. 2020), may target several signaling pathways involved in pancreatic cancer development, suggesting that atenolol may be repurposed as a novel therapy for this type of cancer (Hermawan et al. 2020).

The specific targeting of β1-ARs has recently proved its efficacy in the treatment of infantile hemangiomas, a benign vascular tumor in which the pharmacologic treatment accelerates the shrink away of the tumor in respect with its natural history. Indeed, even though propranolol is currently the most common treatment for infantile hemangiomas (Pam et al. 2021), atenolol has lately risen interest in this field (Alexopoulos et al. 2018; Bayart et al. 2017). In particular, a recent prospective, multicenter, randomized clinical trial has shown that oral atenolol is equally effective as propranolol in the treatment of problematic infantile hemangiomas. Nevertheless, different from propranolol, atenolol can be administered as a daily therapy and, because of its hydrophilic nature, it is less prone to produce central nervous system-related adverse events compared to the lipophilic propranolol. In addition, it is also less likely to produce bronchial related adverse events than propranolol, suggesting

that oral atenolol may be a valid alternative treatment in infantile hemangioma patients requiring systemic therapy (Ji et al. 2021). However, since atenolol is not so selective towards β1-ARs, it is not clear whether the atenolol-induced regression of infantile hemangioma is a β1-AR-mediated response or, rather, a more general β-AR-mediated phenomenon. Among new chemicals designed to have a more specific targeting of β1-ARs, landiolol hydrochloride is a new generation, ultra-short acting β1-selective antagonist that has been developed in Japan, with a selectivity for β1-ARs 255 times higher than for β2-ARs and whose short half-life (4 min) enables rapid recovery after cessation of administration if side effects occur (Iguchi et al. 1992). Its putative preventive effect against early recurrence after curative surgery for non-small cell lung cancer is currently being evaluated in a phase III, multicenter, randomized trial, which was expected to be completed in May 2023. In this study, landiolol has been continuously infused intravenously at 2.5 μg kg^{-1} min^{-1} for 72 h from just before surgery (Yamamoto et al. 2019). In addition, landiolol hydrochloride has already been proven to be effective in improving relapse-free survival rate, prolonging relapse-free survival and overall survival when administered at low doses during lung resection surgery for lung malignancies, suggesting that targeting β1-ARs with landiolol-based therapies may be an adjuvant to current therapies in combating any resectable cancer (Sakamoto et al. 2019).

Besides most part of the paper investigating the role of β1-ARs in cancer point on a pro-tumorigenic role of their activation, some studies report a possible anti-tumorigenic role of β1-AR agonism For instance, in surgically resected gastric cancer specimens, a negative correlation between β1-AR expression and the number of metastatic lymph nodes has been recently reported, suggesting that a reduced β1-AR expression is associated with an aggressive behavior and that β1-AR activation may inhibit tumor progression in gastric cancer (Bae et al. 2019). A similar role of β1-AR agonism in the tumor microenvironment has also been proposed. For instance, in sub-population of T cells endowed with a potent antitumor activity and expressing β-ARs, the β1-AR antagonist bisoprolol partially reduced their cytotoxicity, suggesting that the cytotoxic activity of these cells at least in part relies on β1-AR signaling (Baker et al. 2020).

Overall, these studies suggest that β1-AR activation by endogenous catecholamines may have tumor-inhibiting or -promoting effects depending on tumor type. What is certain is that, given the encouraging findings coming not only from pre-clinical studies but also from clinical trials, the possible clinical usage of specific β1-AR blockers in the treatment of some cancers deserves to be further investigated. The controversial effects resulting from the activation of β1-ARs expressed by cancer cells and by T cells belonging to the tumor microenvironment are summarized in Fig. 2.

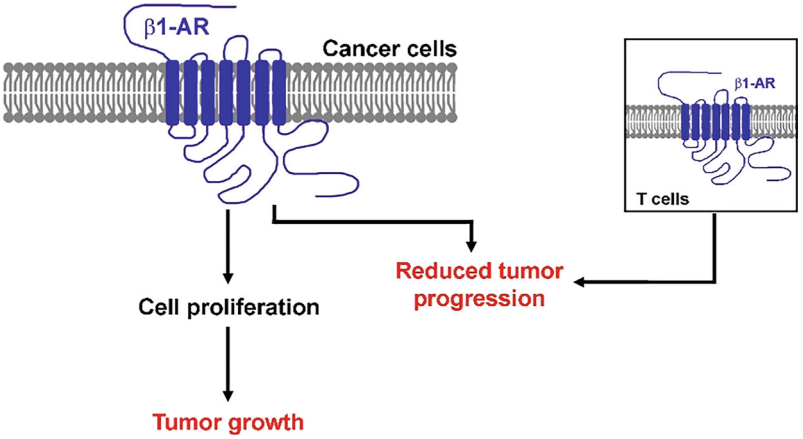

Fig. 2 Schematic diagram depicting the effects of β1-AR activation in cancer cells and in T cells of the tumor microenvironment. The activation of β1-ARs expressed by cancer cells leads to different results in different cancers, ranging from the induction of cell proliferation and tumor growth (as for instance in pancreatic cancer or in lung cancer) to the reduction of tumor progression, which seems to be also reduced by the activation of β1-ARs expressed by T cells of the tumor microenvironment

4 β2-ARs

There are studies highlighting the crucial role that β2-ARs exert in cancer cells. The role of selective and non-selective β-blockers has been studied in many preclinical models of cancer, showing that, in many cases, the capability of non-selective β-blockers in reducing tumor growth and tumor cell migration is replicated by the selective blockade of β2-ARs but not of β1-ARs, thus suggesting a major role of β2-ARs over β1-ARs in tumorigenesis. For instance, in colon carcinoma cells norepinephrine (NE) stimulates cell migration, an effect that is inhibited by propranolol but not by atenolol, suggesting that in these cells the locomotor phenotype is mediated by β2-ARs (Masur et al. 2001). In addition, in prostate carcinoma cells expressing both β1- and β2-ARs, the NE-induced cell migration is abolished by the β2-AR antagonist ICI-118,551 but only partially prevented by atenolol, indicating that in these cells NE acts mainly through β2-AR-activated signaling (Lang et al. 2004). Moreover, in primary cells derived from clear cell renal cell carcinoma β2-AR blockade with either propranolol or ICI-118,151 similarly interferes with two central aspects of cancer progression, that is inflammation and oxidative stress (Albiñana et al. 2022). Furthermore, in triple-negative brain-metastatic breast cells, which are characterized by high expression of β2-ARs and low expression of β1-ARs, proliferation, migration and invasion are stimulated by selective β2-AR agonism and are blunted by propranolol, indicating that the metastatic features of these cells mainly rely on β2-AR activation (Choy et al. 2016). Recently, a fundamental role of β2-ARs in gastric cancer progression and metastasis has been demonstrated both in vitro, in

several gastric cancer cell lines, and in vivo, in nude mice implanted with human gastric cancer cells. In vitro, propranolol and ICI-118,551 decreased NE-induced cancer cell proliferation, invasion and viability, while in vivo they reduced tumor growth and metastasis. On the contrary, atenolol had almost no effect either in vitro or in vivo; in particular, atenolol reduced gastric cancer cell proliferation by about 12% only at 50 μM, a concentration that is not selective. Overall, these finding suggest that pathways downstream β2-AR activation play a major role in progression and metastasis of gastric cancer and indicate that β2-AR blockers may represent a new paradigm in complementing the armamentarium presently used against gastric cancer (Zhang et al. 2019a). Similarly, β2-AR activation seems to be mainly involved in promoting tumorigenesis, proliferation, invasiveness, and angiogenesis in lung cancer (see for Ref. Huang et al. 2018) and in hemangioblastomas from von Hippen-Lindau disease patients (Cuesta et al. 2019).

Besides their expression by tumor cells, β2-ARs also represent the main β-AR subtype expressed by cells of the tumor microenvironment, in particular by immune cells, which are known to be inhibited by catecholamines (Ben-Eliyahu et al. 2000). Catecholamines may indeed decrease the activation of antitumor natural killer cells and the overall T cell response, while they may increase the activity of immunosuppressive cells (see for Ref. Silva et al. 2022). For instance, in human and murine macrophages, catecholamines induce the phenotypic shift towards an M2 state, which characterizes the tumor-associated macrophages, and increase the expression of pro-tumorigenic genes. In these cells, either propranolol or β2-AR silencing equally prevented the effect of catecholamines, suggesting that the phenotypic shift of tumor-associated macrophages that promotes cancer progression may be, at least in part, associated to β2-AR activation (Qin et al. 2015). In addition, myeloid-derived suppressor cells, characterized by an immunosuppressive activity that favors the tumor immune escape, were stimulated by β2-AR activation and inhibited by either β2-AR blockade or β2-AR deletion. The same study also demonstrated that co-injecting breast cancer cells and myeloid-derived suppressor cells in β2-AR knockout mice resulted in a decreased expression of immunosuppressive genes, an increased expression of antitumor cytokines and a reduced tumor growth with respect to wild type mice, suggesting a major role of β2-ARs in promoting the pro-tumorigenic functions of immunosuppressive cells (Mohammadpour et al. 2019). On the other hand, a recent bioinformatics analysis investigating the crosstalk between β2-AR expression and breast cancer-infiltrating immune cells, revealed that β2-AR expression is positively related with T cells endowed with antitumor activity and negatively correlated with T cells endowed with pro-tumorigenic activity. The same study also reported a functional analysis showing an enrichment in pathways related to the activation of the immune system, including those downstream β2-AR-regulated transcription factors, suggesting that β2-AR activation may have promising protective effects in breast cancer and indicating them as a possible target for boosting immunotherapy (Wei et al. 2021). In the same line, a clinical study has shown that a high β2-AR expression may be a favorable prognostic factor in patients with human epidermal growth factor receptor 2 positive breast cancer (Caparica et al. 2020). Overall, this apparent contradiction

about a role that depresses or, on the contrary, stimulates the activity of the immune system suggests that further preclinical as well as controlled trials using selective β2-AR agonists are required.

If in some instances the expression of β2-AR has been proposed as a favorable prognostic factor in breast cancer (Wei et al. 2021; Caparica et al. 2020), there are also studies indicating that this receptor could be considered a marker associated with poor prognosis in other tumors. For instance, a bioinformatic analysis performed on a dataset containing 300 different gastric cancer samples has revealed that β2-ARs are highly expressed in diffuse type gastric cancer, a type associated with an unfavorable prognosis, and that β2-AR expression level is negatively correlated with disease prognosis (Li et al. 2021). In the same line, β2-AR levels have been negatively associated with poor overall survival and/or recurrence-free survival in patients suffering from hepatocellular carcinoma (Chen et al. 2012), oral squamous cell carcinoma (Krishna et al. 2022), pancreatic ductal adenocarcinoma (Gong et al. 2022), colorectal cancer (Ogawa et al. 2020), estrogen receptor-negative breast cancer (Kurozumi et al. 2019) and malignant melanoma (Shimizu et al. 2016), among others. In addition, there is growing evidence that single nucleotide polymorphisms (SNPs) of the *ADRB2* gene, the gene encoding β2-ARs, may be associated to cancer susceptibility, prognosis, and response to medical treatment in patients suffering from some cancers, mainly lung, breast, and pancreatic cancers (see for Ref. Wang and Jiang, 2021). For instance, in the SNP rs1042711, in which the replacement of a Cys residue with and Arg leads to β2-AR downregulation (McGraw et al. 1998), the minor allele C has been found to be associated with an increased risk by about 67% of developing lung cancer (Mei et al. 2019) and with a worse drug response in acute lymphoblastic leukemia, characterized by a statistically significant worse two-year overall survival of about 10% as compared with the major allele T (Pottier et al. 2010). In addition, the SNP rs1042713 has been found to be associated with the increased risk of developing lung adenocarcinoma (by about 42%) and breast cancer (by about 16%) in Chinese populations (Du et al. 2019; Wang et al. 2006), or pancreatic cancer (by about 52%), as evidenced in a population-based case-control study in Minnesota (Zhang et al. 2014). The same SNP has also been associated to progression and metastasis of pancreatic cancer, which is almost doubled than in subjects suffering from pancreatic cancer but not expressing the SNP (Wenjuan et al. 2013). Interestingly, this SNP is associated with an increased expression of β2-AR and with its increased agonist sensitivity (Large et al. 1997; Wenjuan et al. 2013), suggesting a direct role of β2-AR activation by catecholamines in development and progression of some cancers. On the other hand, the GG and AG genotypes of the SNP rs1042713 have been found to be associated to a reduced risk of developing breast cancer in a Chinese population (by about 28%), in a Japanese cohort (by about 33%), and in Hispanic but not in non-Hispanic white women in the southwestern United States (by about 26%) (Connor et al. 2012; Du et al. 2019; Huang et al. 2001). Overall, these data suggest that the possibility to consider β2-AR expression and/or the presence of β2-AR SNPs as a negative or positive prognostic factor may depend on the type of tumor, its progression state and ethnicity. However, most of the studies rely on epidemiological data, therefore

further elucidation of the molecular mechanisms activated downstream the different *ADRB2* haplotypes coming from preclinical investigations is needed to validate β2-ARs as a possible biomarker in cancer.

A novel frontier about the role of β2-ARs in cancer is the possible use of promising combinatory approaches in which β2-AR antagonists, either non-selective or selective, are associated to conventional anticancer therapies to synergize with them and overcome phenomena of drug resistance. For instance, in non-small cell lung cancer, the treatment with the VEGF receptor 2 inhibitor apatinib led to β2-AR upregulation, while activation of the receptor downstream signaling caused the therapeutic resistance to apatinib. However, the treatment of human non-small cell lung cancer cells with a combination of apatinib and either ICI-118,551 or propranolol enhanced cell sensitivity to apatinib, thus increasing its antitumor effect. The same approach has shown that, in nude mice xenografted with human non-small cell lung cancer cells, the combination of apatinib and propranolol greatly enhances the efficacy of apatinib, leading to a reduction of the xenograft volume that is about threefold larger than that following apatinib or propranolol alone (Xu et al. 2022). Propranolol has also been demonstrated to be effective in enhancing the effect of the chemotherapeutic drug Irinotecan in counteracting the growth of colorectal cancer in a syngeneic mouse model (Lin et al. 2023) and in sensitizing human chemotherapy-resistant prostate cancer cells reducing the resistance to docetaxel (Zhang et al. 2023). Similar results have been obtained in human head and neck squamous cell carcinoma cell lines, in which the combined treatment with the mitogen activated protein kinase (MAPK) inhibitor U0126 and ICI-118,551 was more effective than the single treatments in inducing cell death, thus suggesting that the most adopted therapy for this cancer, which relies on MAPK inhibition and often leads to drug resistance, may be complemented by β2-AR antagonists (Mele et al. 2020). These findings suggest that in comparison with traditional monotherapy, the combination with β2-AR blockers may represent a promising therapeutic strategy, by improving the efficacy of classic chemotherapeutics and reducing drug toxicity. However, whether the combinatorial approach with β2-AR blockers and conventional chemotherapeutic agents may be used to enhance the anticancer effects in a wide range of malignancies requires further preclinical studies before translation in the clinics. In the meantime, supported by preclinical findings, the combination of propranolol with the checkpoint inhibitor pembrolizumab has been tested in a phase I clinical trial that demonstrated the safety of the combination and gave preliminary results on the antitumor efficacy in patients with metastatic melanoma (Gandhi et al. 2021). The effects resulting from the activation of β2-ARs expressed by cancer cells and by immune cells belonging to the tumor microenvironment are summarized in Fig. 3.

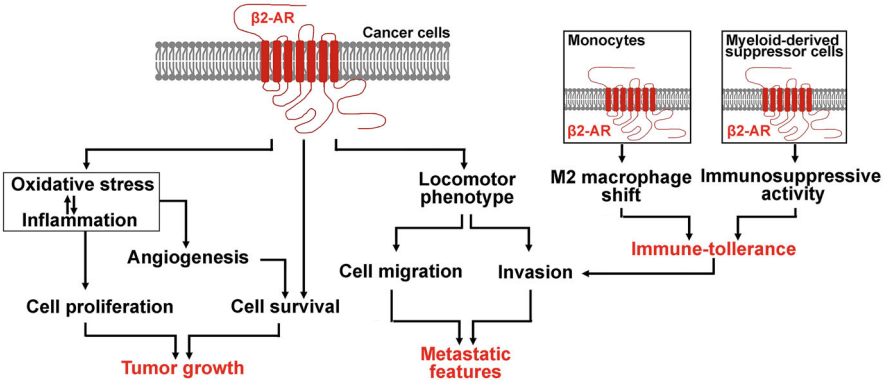

Fig. 3 Schematic diagram depicting the effects of β2-AR activation in cancer cells and in immune cells of the tumor microenvironment. The activation of β2-ARs expressed by cancer cells, through the stimulation of oxidative stress and inflammatory processes, induces cell proliferation and angiogenesis, contributing to cancer cell survival, which is also directly stimulated by activated β2-ARs. Overall, all these processes trigger tumor growth. In addition, β2-AR activation leads to the acquisition of a locomotor phenotype by cancer cells that migrate and spread to distant sites, acquiring metastatic features. Moreover, the activation of β2-ARs expressed by immune cells of the tumor microenvironment participates, by inducing phenomena of immune-tolerance, to cancer cell invasion of surrounding tissues

5 β3-ARs

Although the interest regarding the role of the adrenergic system in the progression of tumors has been focused mainly on β2-ARs, in recent years awareness of a possible involvement of β3-ARs has progressively grown. On the other hand, while the use of beta blockers as co-adjuvant in treating cancer patients gave evidence supporting the role of β2-ARs in several malignancies (Gales et al. 2022), the possible involvement of β3-ARs is mainly based on preclinical results obtained in vitro and animal models.

The first reports concerned the identification of β3-AR mRNA in different tumors including colon cancer (Perrone et al. 2008), vascular tumors (Chisholm et al. 2012), and human leukemia cells (Lamkin et al. 2012). In addition, the Trp64Arg β3-AR polymorphism was associated to an increased susceptibility in developing colon or endometrial cancer by about 1.5–3 times (Babol et al. 2004; Takezaki et al. 2001).

Alongside studies exploring the role of stress and the involvement of the adrenergic system in the progression of human melanoma, in vitro and in vivo experiments demonstrated the presence of β3-ARs in mouse melanoma cells and explored a possible contribution of β3-ARs in melanoma growth and vascularization in a mouse model. This idea arose after demonstrating that β3-ARs were involved in hypoxia-induced vascularization processes (Dal Monte et al. 2013a).

The presence of β3-ARs on the cellular surface, the up-regulation of their expression under hypoxia (a strategy to reproduce the environment of the growing

melanoma in vivo) and their involvement in the induction of VEGF production were demonstrated in mouse melanoma B16F10 cells. The blockade of β3-ARs with SR59230A or L-748,337, or their silencing with selective siRNAs reduced melanoma cell proliferation, induced their apoptosis, and prevented hypoxia-induced VEGF up-regulation. Moreover, in mice bearing mouse melanoma B16F10 cells, the pharmacologic antagonism of β3-ARs with the same drugs reduced melanoma growth and its vascularization thanks to a significant downregulation of VEGF (Dal Monte et al. 2013b). Although SR59230A, the widely used β3-AR antagonist, is not selective for β3-ARs (Vrydag and Michel 2007), the results obtained with the selective antagonist L-748,337 in vivo and with the siRNA approach in vitro point on a specific functional role of β3-ARs in melanoma growth. These effects of SR59230A and L-748,337 were mediated by the inhibition of the expression of the inducible form of nitric oxide synthase and the promotion of apoptosis (Dal Monte et al. 2013b, 2014). These results were confirmed in β1/2-AR knockout mice bearing melanoma, where the treatment based on L-748,337 was again particularly effective in reducing tumor proliferation and vascularization. Interestingly, in this model intratumor level of NE was statistically higher than in controls suggesting a synergy between β3-ARs and catecholamines in melanoma growth (Sereni et al. 2015). β3-AR expression in tumor cells was demonstrated to be a poor prognostic factor also in different human cancers, such as melanoma (Calvani et al. 2015), non-small cell lung carcinoma (Zheng et al. 2020) and in breast cancer (Zhou et al. 2022).

In melanoma, the expression of β3-ARs was demonstrated not only in cancer cells, but also on the membrane of many cells constituting the tumor microenvironment, such as cancer-associated fibroblasts, endothelial progenitor cells, mesenchymal stem cells, and monocytes. In all these human cells β3-ARs were upregulated by hypoxia and, for the first-time, specific functions were attributed to β3-ARs such as the ability to stimulate the NE-mediated recruitment of circulating stromal cell precursors to favor the invasiveness of melanoma cells and to promote cancer stemness. Indeed, in human melanoma cells, a catecholaminergic stimulus increased both the expression of stemness markers, such as CD20 and CD133, and the ability to form melanospheres through the activation of β3-ARs (Calvani et al. 2015).

In a series of subsequent studies, some of the functions of β3-ARs were better elucidated. β3-ARs were demonstrated to be involved in the metabolic rearrangement of human melanoma stem cells by promoting an accelerated glycolysis (Warburg effect), as suggested by the increased glucose uptake and lactate export (Calvani et al. 2018). Interestingly, β3-AR activation with the agonist BRL37344 can promote this metabolic switch by upregulating the expression of some key-enzymes involved in glycolysis such as hexokinase 2, or transmembrane proteins such as monocarboxylate transporter-4, but also by reducing mitochondrial activity through the induction of the specific uncoupling protein 2 (UCP-2), which uncouples the activity of the respiratory chain from ATP synthesis (Calvani et al. 2018). In fact, UCP2 activation by β3-ARs simultaneously induces a significant reduction of ATP synthesis, a decrease of mitochondrial reactive oxygen species (ROS) content, and an increase of lactate production/export in the

microenvironment. Limiting ROS production preserves the cancer cells from oxidative stress that causes cell death (Aggarwal et al. 2019), while the reduction of extracellular pH promotes the disaggregation of surrounding tissues and facilitates the infiltration of the tumor (De la Cruz-López et al. 2019).

A recent study suggested the involvement of β3-ARs in the induction of chemoresistance. In this study performed on human myeloid leukemia cell lines, the exposition of a leukemic doxorubicin-resistant cell line to hypoxia increased at the same time the expression of β3-ARs and the cell chemoresistance. On the other hand, SR59230A reverted such doxorubicin resistance, suggesting that the levels of β3-ARs and chemoresistance were not simply associated but closely related phenomena (Calvani et al. 2020a). Although this preliminary study needs further confirmation, some mechanisms promoting chemoresistance have been suggested: in K562 human myeloid leukemia cells β3-ARs modulate the expression of P-glycoprotein (an efflux protein encoded by the multiple drug resistance gene), UCP-2 levels, and hypoxia-inducible factor-1 (HIF-1) expression (Calvani et al. 2020a), proteins that are actively involved in chemoresistance induction in myeloid neoplasms (Zhang et al. 2019b). Additional mechanisms involved in chemoresistance are likely to be under regulation of β3-ARs. In this respect, it is important to note that NE, through the activation of β3-ARs, increases intracellular concentration of glutathione (Yoshioka et al. 2016), whose major function is the detoxification of xenobiotics in cancer (Traverso et al. 2013).

Considering that cancer relies on a hypoxic immune-tolerant context (Facciabene et al. 2011), the assumption that hypoxic induction of β3-ARs in tumor infiltrating lymphocytes could affect tumor immunoediting was investigated in a syngeneic mouse model of melanoma, with the hypothesis that β3-ARs should be able to promote an immune-tolerance confined to the site of intense proliferation, without systemic immunological effects. The data showed that the treatment with SR59230A or β3-AR silencing reduced tumor growth promoting the switch from an immunosuppressive (rich in regulatory T cells, myeloid-derived suppressor cells, M2 macrophages and N2 neutrophils) to an immunocompetent tumor microenvironment (with higher presence of natural killer cells, CD8 cells, M1 macrophages, and N1 neutrophils), within the tumor mass. These data supported the hypothesis that β3-ARs play a role in the promotion of immune-tolerance of cancer (Calvani et al. 2019).

Considering that β3-AR expression is modulated by oxygen levels and that hypoxia promotes immune-tolerance (Facciabene et al. 2011), our hypothesis is that hypoxia may promote the shift towards a tolerant immunophenotype through the upregulation of β3-ARs, which may be the trick adopted by cancer cells to create an *aura* of immune-tolerance in an immune-competent environment (Calvani et al. 2019). The observation that many of the functions exerted by β3-ARs in tumor models were replicated in embryonic cells (Calvani et al. 2020a) and in placental tissues (Calvani et al. 2020b) suggested the hypothesis that the tumor microenvironment reactivates fetal competences, including local immunosuppression, predominantly through the activation of β3-ARs (Filippi et al. 2022). In essence, β3-ARs, hypoxia and stemness appear to be closely related, as confirmed by the recent

demonstration of the genetic link that binds HIF-1 and β3-ARs (Amato et al. 2022). In the earliest stages of fetal development, the low oxygen tension is necessary to initiate the embryonic stem cell proliferation, and this physiologic hypoxia is strictly associated with high levels of HIF-1 and β3-ARs. At the same time, it is well-known that during embryo development oxygen levels represent the signal to induce tissue differentiation (Fathollahipour et al. 2018; Simon and Keith 2008). The close relationship between oxygen, HIF-1 and β3-ARs suggested that oxygen might regulate embryo differentiation through the modulation of β3-ARs. As pregnancy progresses, the progressively increasing levels of oxygen could induce a gradual down-regulation of β3-ARs during embryogenesis (Fujinaga and Scott 1997) favoring embryonic differentiation. Therefore, in light of this hypothesis, β3-AR antagonism of highly undifferentiated tumors expressing high levels of β3-ARs was hypothesized to be the biological sign able to promote cancer differentiation. However, even considering the different role that β3-ARs exert in adult mice and in humans, the translational perspective of studies performed in preclinical models needs to be further assessed.

In a recent study performed in a syngeneic mouse model of melanoma, SR59230A was able to reduce the expression of cancer stem cell markers and induce a differentiated phenotype of hematopoietic subpopulations and mesenchymal stem cells within the tumor (Calvani et al. 2020c). In detail, the study showed the development of a hematopoietic niche within the tumor mass, following the recruitment of hematopoietic progenitor cells that had already started the differentiation process in the bone marrow. Within the tumor mass it was also possible to demonstrate a process of trans-differentiation from mesenchymal stem cell to pre-adipocytes, which explains the yellowish and greasy tumor appearance. This finding was in line with the effect of the treatment of infantile hemangiomas with propranolol, where β-blockade promoted the adipogenic trans-differentiation of hemangioma stem cells (Ma et al. 2014). A similar effect was demonstrated in the human breast cancer MCF-7 cell line where β3-AR activation prevented the trans-differentiation of MCF-7 cells into adipocyte-like cells (Zhou et al. 2022).

In a study performed in mice bearing murine Neuro2A neuroblastoma cells, treatments with SR59230A or with β3-AR siRNAs inhibited the growth of neuroblastoma and its progression (Bruno et al. 2020). These data were in agreement with a previous study demonstrating the ability of SR59230A and of β3-AR silencing to inhibit neuroblastoma cell proliferation through the suppression of the mTOR pathway (Deng et al. 2019). Experiments performed on human neuroblastoma cells demonstrated that SR59230A reduced the expression of stemness markers, such as the capability to form neurospheres and the levels of the stem cell marker CD34, while it increased neurite formation. Similar results were observed in mice bearing syngeneic neuroblastoma tumor cells, where SR59230A decreased the expression levels of the early neuronal differentiation markers and increased the intermediate and late neuronal differentiation markers (Bruno et al. 2020). More recently, in a murine syngeneic model of neuroblastoma, SR50230A was demonstrated to be effective in reactivating the host immune response in the tumor microenvironment, leading to a decrease in tumor growth through the involvement

of the programmed death 1/programmed death ligand-1 signaling axis. The same study, also showed that in specimens from neuroblastoma patients, the high expression of the *ADRB3* gene is associated with a reduction in event-free survival probability and in overall survival probability in respect to the low expression of the receptor (from 70% to 50% and from 80% to 60%, respectively) (Bruno et al. 2023). In conclusion, these data suggest a strong relationship between the expression of β3-ARs and the undifferentiated state of cancer, and the possibility to promote tumor cell differentiation antagonizing these receptors. This possibility opens very promising therapeutic scenarios because the differentiation grade of tumors is closely correlated with the biology of their malignancies, being the undifferentiated tumors the most aggressive and malignant (Bao et al. 2013). At the same time, these results confirm the role played by β3-ARs in promoting stemness and undifferentiated state, both in embryo and in cancer.

Currently, the antagonism of β3-ARs may represent a new therapeutic approach to counteract the proliferation of cancer, its metabolic shift, chemoresistance, immune-tolerance and to promote its differentiation. The effects resulting from the activation of β3-ARs expressed by cancer cells and by cells belonging to the tumor microenvironment are summarized in Fig. 4.

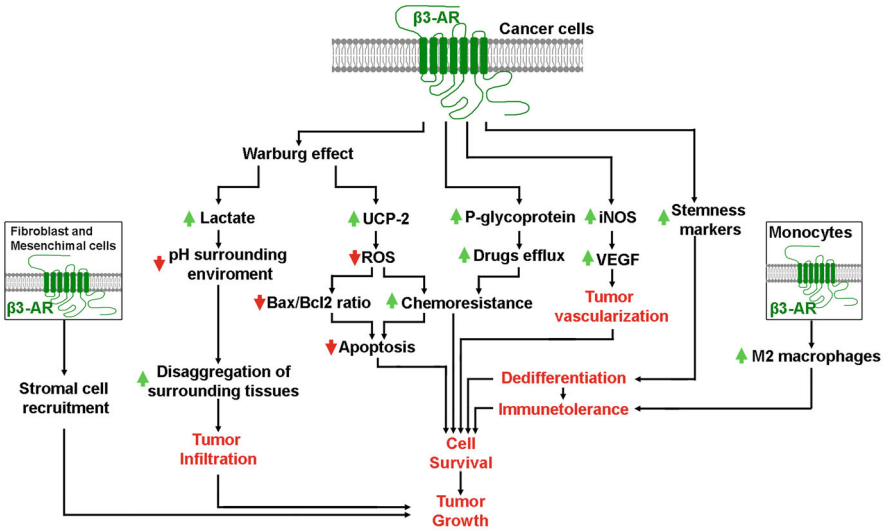

Fig. 4 Schematic diagram depicting the effects of β3-AR activation in cancer cells and in cells of the tumor microenvironment. The activation of β3-ARs expressed by cancer cells, through the induction of Warburg effect leads to the acidification of the surrounding tissue that favors tumor infiltration and growth. Through: (i) the reduction of oxidative stress-dependent apoptosis, which is a consequence of the Warburg shift, (ii) the induction of chemoresistance derived from an increase in the activity of drug efflux pumps, (iii) The activation of angiogenic processes, (iv) the dedifferentiation of cancer cells and (v) the induction of stemness-related immune-tolerance, β3-AR agonism favors cell survival and tumor growth. In addition, also the activation of β3-ARs expressed by cells of the tumor microenvironment participates, directly and indirectly, to tumor growth

6 Conclusions and Future Perspectives

Distress conditions may importantly affect the development of cancer and its progression. In particular, stress-induced catecholamine overdrive stimulates carcinogenesis and tumor growth, as shown by results from pre-clinical and clinical studies indicating that β-ARs expressed by tumor cells and in the tumor microenvironment are the target mediating these effects of epinephrine/norepinephrine. Although β2-ARs have been recognized as the main β-AR subtype involved in the pro-tumorigenic effects of catecholamines, there is growing evidence that also β1- and β3-ARs may have a role in tumor biology, thus indicating the perspective of β-ARs as intriguing targets to fight cancer.

Although some reports indicating that β1-AR activation may have an anticancer potential, these β-AR subtypes seem to have a role in the growth of certain tumors, such as infantile hemangiomas, highlighting the role of β1-AR blockers in the treatment of specific malignancies. However, additional studies are required to better define the potential tumorigenic role of these receptors.

A paramount role of β2-ARs in many tumors has been recognized, and β2-AR blockade has been demonstrated to be effective in counteracting tumor growth in pre-clinical models. In addition, several studies have shown that the previous use of β-AR blockers in tumor patients increases survival and reduces recurrence and metastasis rates. In this respect, several studies have demonstrated that β-AR blockers targeting both β1- and β2-ARs exert their antitumor effects acting mainly at β2-ARs. The finding that β2-ARs are expressed not only by tumor cells but also by cells of the tumor-microenvironment, the possibility that β2-ARs or particular SNPs of these receptors may be recognized as biomarkers of specific tumors, and the evidence that β2-AR blockade may synergize with conventional antitumor drugs in a combinatorial approach to tumor treatment reveal that there may be still unexplored or only partially understood uses of β2-AR-targeting molecules, which may be useful to counteract cancer growth and progression. Then, although further investigations are required to clarify the molecular mechanisms mediating β2-AR blocker effects in different tumors and to assess the importance of a minority of studies, based on bioinformatics, reporting a possible protective role of β2-ARs in some tumors, the use of β2-AR blockers seems to be not so far from moving from the bench to the bedside.

Regarding the less studied β-ARs, β3-ARs, during the last decade they have been demonstrated to be involved in tumor growth to the point that their expression can be considered a poor prognostic factor in specific human cancers such as neuroblastoma. Being expressed by tumor cells, as well as in the tumor microenvironment, blocking these receptors in animal models has been proven to be effective in reducing the growth of melanoma and neuroblastoma, suggesting a potential use of β3-AR blockers in tumor treatment. In this respect, the restricted expression in the human body of β3-ARs with respect to that of β1- and β2-ARs should be of advantage in treating tumor patients since off-target effects of β3-AR blockers may be, in principle, less than those of β1- and β2-AR antagonists. However, it is difficult to imagine the use of β3-AR blockers in tumor patients in a near future,

since the currently available β3-AR blockers have problems of selectivity and specificity and are not marketed for human use. On the other hand, the finding obtained from pre-clinical studies are so encouraging that they may pave the way to future clinical trials essaying the available β3-AR blockers (and, hopefully, newly synthetized ones) as treatment for selected cancers. Of note, the finding that β3-AR activation stimulates tumor cell dedifferentiation, reactivating embryo competences, is opening a new way that may be of importance in studying tumor biology. On the other hand, the fact that β3-AR blockade is effective in hampering tumor growth and that β3-AR activation has an opposite effect, may represent the other side of the coin of the increasing use of β3-AR agonists in the treatment of overactive bladder, the only use for which β3-AR-acting drugs are approved in humans.

7 Antitumor Effect of the Catecholaminergic System Beyond β-ARs: Is There a Role for α2-ARs?

Among ARs, β-ARs are the main subtypes studied for their role in tumor biology. However, some evidence about a role for α-ARs has been produced and, although this role has not been thoroughly examined, the expression level of α-ARs has been linked to poor prognosis in human breast cancer (Powe et al. 2011). Among α-ARs, α2-ARs have been identified for a potential role in regulating the growth of different tumors, although results from different studies seem to be contradictory. In fact, it has been demonstrated that α2-AR agonism with dexmedetomidine or clonidine promotes proliferation and migration in human breast cancer cell lines (Castillo et al. 2017; Vazquez et al. 2006; Xia et al. 2016). In addition, dexmedetomidine treatment results in an increase in tumor growth and metastasis formation in syngeneic mouse models of breast cancer (Lavon et al. 2018; Szpunar et al. 2013), as well as in syngeneic mouse models of lung carcinoma and colon adenocarcinoma (Lavon et al. 2018). On the contrary, the α2-AR agonist UK14,304 inhibits the growth of human cholangiocarcinoma cells (Kanno et al. 2002), while α2-AR agonism with ST91 attenuates tumor growth in a syngeneic mouse model of melanoma (Maccari et al. 2022). A possible explanation of these conflicting results may lie in the models, in the tumors and/or in the drug and in their doses used in the different studies, and points on the need of additional studies in order to obtain definitive data about the pro- or anti-tumorigenic role of α2-AR activation. In this respect, a very recently published paper seems to put a full stop on the matter. Zhu and co-authors (2023) indeed demonstrated that α2-AR agonists (guanabenz, clonidine, and guanfacine) exert an impressive antitumor effect in either syngeneic or allogeneic mouse models of different cancers. The effects of α2-AR agonists were blocked by α2-AR antagonists and were not observed in α2-AR knockout mice, indicating (i) the selectivity of these effects and (ii) that these effects are not exerted on tumor cells but on host cells belonging to the tumor microenvironment. Overall, this work demonstrated that α2-AR agonism acts directly on macrophages that, in turn, would stimulate the adaptive immune response of T lymphocytes. Of note, α2-AR agonists not only strongly reduced tumor growth when used as monotherapy but

were also able to synergize with immune checkpoint blockers leading to a complete tumor rejection in many mice. Finally, the authors showed that in patients suffering from lung adenocarcinoma there is a high statistically significant association between a high expression of α2-ARs and both the progression-free survival and the overall survival, suggesting the translatability of the results of this study to patients. It is obvious that the translational implications of this study need to be carefully verified, and the definition of the doses of α2-AR agonists to be used in humans may be only the starting point of this path. However, the fact that some α2-AR agonists are clinically available, that their safety profile is known and that they have been used for many years in treating hypertension, may accelerate the development of treatments (either mono- or combined therapies) for specific human cancers.

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References

Aggarwal V, Tuli HS, Varol A, Thakral F, Yerer MB, Sak K, Varol M, Jain A, Khan MA, Sethi G (2019) Role of reactive oxygen species in cancer progression: molecular mechanisms and recent advancements. Biomol Ther 9(11):735

Albiñana V, Recio-Poveda L, González-Peramato P, Martinez-Piñeiro L, Botella LM, Cuesta AM (2022) Blockade of β2-adrenergic receptor reduces inflammation and oxidative stress in clear cell renal cell carcinoma. Int J Mol Sci 23(3):1325

Alexopoulos A, Thanopoulou I, Dakoutrou M, Georgiadou E, Chrousos GP, Kakourou T (2018) Atenolol treatment for severe infantile hemangiomas: a single-centre prospective study. J Eur Acad Dermatol Venereol 32(3):e117–e119

Amato R, Pisani F, Laudadio E, Cammalleri M, Lucchesi M, Marracci S, Filippi L, Galeazzi R, Svelto M, Dal Monte M, Bagnoli P (2022) HIF-1-dependent induction of β3 adrenoceptor: evidence from the mouse retina. Cells 11(8):1271

Babol K, Przybylowska K, Lukaszek M, Pertynski T, Blasiak J (2004) An association between the Trp64Arg polymorphism in the beta3-adrenergic receptor gene and endometrial cancer and obesity. J Exp Clin Cancer Res 23(4):669–674

Bae GE, Kim HS, Won KY, Kim GY, Sung JY, Lim SJ (2019) Lower sympathetic nervous system density and β-adrenoreceptor expression are involved in gastric cancer progression. Anticancer Res 39(1):231–236

Baker JL, Hall IP, Hill SJ (2003) Agonist and inverse agonist actions of beta-blockers at the human beta 2-adrenoceptor provide evidence for agonist-directed signaling. Mol Pharmacol 64(6): 1357–1369

Baker FL, Bigley AB, Agha NH, Pedlar CR, O'Connor DP, Bond RA, Bollard CM, Katsanis E, Simpson RJ (2020) Systemic β-adrenergic receptor activation augments the ex vivo expansion and anti-tumor activity of Vγ9Vδ2 T-cells. Front Immunol 10:3082

Bao B, Ahmad A, Azmi AS, Ali S, Sarkar FH (2013) Overview of cancer stem cells (CSCs) and mechanisms of their regulation: implications for cancer therapy. Curr Protoc Pharmacol. Chapter 14:Unit 14.25

Bayart CB, Tamburro JE, Vidimos AT, Wang L, Golden AB (2017) Atenolol versus propranolol for treatment of infantile hemangiomas during the proliferative phase: a retrospective noninferiority study. Pediatr Dermatol 34(4):413–421

Ben-Eliyahu S, Shakhar G, Page GG, Stefanski V, Shakhar K (2000) Suppression of NK cell activity and of resistance to metastasis by stress: a role for adrenal catecholamines and beta-adrenoceptors. Neuroimmunomodulation 8(3):154–164

Bruno G, Cencetti F, Pini A, Tondo A, Cuzzubbo D, Fontani F, Strinna V, Buccoliero AM, Casazza G, Donati C, Filippi L, Bruni P, Favre C, Calvani M (2020) β3-adrenoreceptor blockade reduces tumor growth and increases neuronal differentiation in neuroblastoma via SK2/S1P2 modulation. Oncogene 39(2):368–384

Bruno G, Nastasi N, Subbiani A, Boaretto A, Mannurita SC, Mattei G, Nardini P, Della Bella C, Magi A, Pini A, De Marco E, Tondo A, Favre C, Calvani M (2023) β3-adrenergic receptor on tumor-infiltrating lymphocytes sustains IFN-γ-dependent PD-L1 expression and impairs anti-tumor immunity in neuroblastoma. Cancer Gene Ther 30(6):890–904

Calvani M, Pelon F, Comito G, Taddei ML, Moretti S, Innocenti S, Nassini R, Gerlini G, Borgognoni L, Bambi F, Giannoni E, Filippi L, Chiarugi P (2015) Norepinephrine promotes tumor microenvironment reactivity through β3-adrenoreceptors during melanoma progression. Oncotarget 6(7):4615–4632

Calvani M, Cavallini L, Tondo A, Spinelli V, Ricci L, Pasha A, Bruno G, Buonvicino D, Bigagli E, Vignoli M, Bianchini F, Sartiani L, Lodovici M, Semeraro R, Fontani F, De Logu F, Dal Monte M, Chiarugi P, Favre C, Filippi L (2018) β3-Adrenoreceptors control mitochondrial dormancy in melanoma and embryonic stem cells. Oxidative Med Cell Longev 2018:6816508

Calvani M, Bruno G, Dal Monte M, Nassini R, Fontani F, Casini A, Cavallini L, Becatti M, Bianchini F, De Logu F, Forni G, la Marca G, Calorini L, Bagnoli P, Chiarugi P, Pupi A, Azzari C, Geppetti P, Favre C, Filippi L (2019) β3-Adrenoceptor as a potential immuno-suppressor agent in melanoma. Br J Pharmacol 176(14):2509–2524

Calvani M, Dabraio A, Bruno G, De Gregorio V, Coronnello M, Bogani C, Ciullini S, Marca G, Vignoli M, Chiarugi P, Nardi M, Vannucchi AM, Filippi L, Favre C (2020a) β3-Adrenoreceptor blockade reduces hypoxic myeloid leukemic cells survival and chemoresistance. Int J Mol Sci 21(12):4210

Calvani M, Dabraio A, Subbiani A, Buonvicino D, De Gregorio V, Ciullini Mannurita S, Pini A, Nardini P, Favre C, Filippi L (2020b) β3-adrenoceptors as putative regulator of immune tolerance in cancer and pregnancy. Front Immunol 11:2098

Calvani M, Bruno G, Dabraio A, Subbiani A, Bianchini F, Fontani F, Casazza G, Vignoli M, De Logu F, Frenos S, Filippi L, Favre C (2020c) β3-Adrenoreceptor blockade induces stem cells differentiation in melanoma microenvironment. Int J Mol Sci 21(4):1420

Caparica R, Richard F, Brandão M, Awada A, Sotiriou C, de Azambuja E (2020) Prognostic and predictive impact of Beta-2 adrenergic receptor expression in HER2-positive breast cancer. Clin Breast Cancer 20(3):262–273.e7

Castillo LF, Rivero EM, Goffin V, Lüthy IA (2017) Alpha$_2$-adrenoceptor agonistss trigger prolactin signaling in breast cancer cells. Cell Signal 34:76–85

Chen D, Xing W, Hong J, Wang M, Huang Y, Zhu C, Yuan Y, Zeng W (2012) The beta2-adrenergic receptor is a potential prognostic biomarker for human hepatocellular carcinoma after curative resection. Ann Surg Oncol 19(11):3556–3565

Chisholm KM, Chang KW, Truong MT, Kwok S, West RB, Heerema-McKenney AE (2012) β-Adrenergic receptor expression in vascular tumors. Mod Pathol 25(11):1446–1451

Choy C, Raytis JL, Smith DD, Duenas M, Neman J, Jandial R, Lew MW (2016) Inhibition of β2-adrenergic receptor reduces triple-negative breast cancer brain metastases: the potential benefit of perioperative β-blockade. Oncol Rep 35(6):3135–3142

Collins S, Ostrowski J, Lefkowitz RJ (1993) Cloning and sequence analysis of the human beta 1-adrenergic receptor 5′-flanking promoter region. Biochim Biophys Acta 1172(1–2):171–174

Connor A, Baumgartner RN, Kerber RA, O'Brien E, Rai SN, Wolff RK, Slattery ML, Giuliano AR, Risendal BC, Byers TE, Baumgartner KB (2012) ADRB2 G-G haplotype associated with breast

cancer risk among Hispanic and non-Hispanic white women: interaction with type 2 diabetes and obesity. Cancer Causes Control 23(10):1653–1663

Cuesta AM, Albiñana V, Recio-Poveda L, de Rojas-P I, Gómez V, de Las HK, Aguirre DT, Botella LM (2019) The β2-adrenergic receptor antagonist ICI-118,551 blocks the constitutively activated HIF signalling in hemangioblastomas from von Hippel-Lindau disease. Sci Rep 9: 10062

Dal Monte M, Filippi L, Bagnoli P (2013a) Beta3-adrenergic receptors modulate vascular endothelial growth factor release in response to hypoxia through the nitric oxide pathway in mouse retinal explants. Naunyn Schmiedeberg's Arch Pharmacol 386(4):269–278

Dal Monte M, Casini G, Filippi L, Nicchia GP, Svelto M, Bagnoli P (2013b) Functional involvement of β3-adrenergic receptors in melanoma growth and vascularization. J Mol Med (Berl) 91(12):1407–1419

Dal Monte M, Fornaciari I, Nicchia GP, Svelto M, Casini G, Bagnoli P (2014) β3-adrenergic receptor activity modulates melanoma cell proliferation and survival through nitric oxide signaling. Naunyn Schmiedeberg's Arch Pharmacol 387(6):533–543

Dal Monte M, Calvani M, Cammalleri M, Favre C, Filippi L, Bagnoli P (2019) β-Adrenoceptors as drug targets in melanoma: novel preclinical evidence for a role of β3 -adrenoceptors. Br J Pharmacol 176(14):2496–2508

De la Cruz-López KG, Castro-Muñoz LJ, Reyes-Hernández DO, García-Carrancá A, Manzo-Merino J (2019) Lactate in the regulation of tumor microenvironment and therapeutic approaches. Front Oncol 9:1143

Deng J, Jiang P, Yang T, Huang M, Qi W, Zhou T, Yang Z, Zou Y, Gao G, Yang X (2019) Targeting β3-adrenergic receptor signaling inhibits neuroblastoma cell growth via suppressing the mTOR pathway. Biochem Biophys Res Commun 514(1):295–300

Du Y, Lin Y, Yin K, Zhou L, Jiang Y, Yin W, Lu J (2019) Single nucleotide polymorphisms of let-7-related genes increase susceptibility to breast cancer. Am J Transl Res 11(3):1748–1759

Facciabene A, Peng X, Hagemann IS, Balint K, Barchetti A, Wang LP, Gimotty PA, Gilks CB, Lal P, Zhang L, Coukos G (2011) Tumour hypoxia promotes tolerance and angiogenesis via CCL28 and T(reg) cells. Nature 475(7355):226–230

Fathollahipour S, Patil PS, Leipzig ND (2018) Oxygen regulation in development: lessons from embryogenesis towards tissue engineering. Cells Tissues Organs 205(5–6):350–371

Filippi L, Pini A, Cammalleri M, Bagnoli P, Dal Monte M (2022) β3-adrenoceptor, a novel player in the round-trip from neonatal diseases to cancer: suggestive clues from embryo. Med Res Rev 42(3):1179–1201

Flaherty RL, Falcinelli M, Flint MS (2019) Stress and drug resistance in cancer. Cancer Drug Resist 2(3):773–786

Fujinaga M, Scott JC (1997) Gene expression of catecholamine synthesizing enzymes and beta adrenoceptor subtypes during rat embryogenesis. Neurosci Lett 231(2):108–112

Gales L, Forsea L, Mitrea D, Stefanica I, Stanculescu I, Mitrica R, Georgescu M, Trifanescu O, Anghel R, Serbanescu L (2022) Antidiabetics, Anthelmintics, statins, and beta-blockers as co-adjuvant drugs in cancer therapy. Medicina (Kaunas) 58(9):1239

Gandhi S, Pandey MR, Attwood K, Ji W, Witkiewicz AK, Knudsen ES, Allen C, Tario JD, Wallace PK, Cedeno CD, Levis M, Stack S, Funchain P, Drabick JJ, Bucsek MJ, Puzanov I, Mohammadpour H, Repasky EA, Ernstoff MS (2021) Phase I clinical trial of combination propranolol and pembrolizumab in locally advanced and metastatic melanoma: safety, tolerability, and preliminary evidence of antitumor activity. Clin Cancer Res 27(1):97–95

Gao W, Guo WJ, Hou DY, Yang GZ, Wu Y, Li YC, Leng Y, Tang Y, Xu L, Liu JM, Wang H, Wang X, Zhang J, Zhao WS, Chen WM, Zhang L (2018) Autoantibodies against β1-adrenergic receptor: response to induction therapy with bortezomib-containing regimens for multiple myeloma patients. Leuk Lymphoma 59(3):717–724

Gong C, Hu B, Chen H, Zhu J, Nie J, Hua L, Chen L, Fang Y, Hang C, Lu Y (2022) β2-adrenergic receptor drives the metastasis and invasion of pancreatic ductal adenocarcinoma through activating Cdc42 signaling pathway. J Mol Histol 53(4):645–655

Gosain R, Gage-Bouchard E, Ambrosone C, Repasky E, Gandhi S (2020) Stress reduction strategies in breast cancer: review of pharmacologic and non-pharmacologic based strategies. Semin Immunopathol 42(6):719–734

Hermawan A, Putri H, Utomo RY (2020) Functional network analysis reveals potential repurposing of β-blocker atenolol for pancreatic cancer therapy. Daru 28(2):685–699

Hopkinson HE, Latif ML, Hill SJ (2000) Non-competitive antagonism of beta(2)-agonist-mediated cyclic AMP accumulation by ICI 118551 in BC3H1 cells endogenously expressing constitutively active beta(2)-adrenoceptors. Br J Pharmacol 131(1):124–130

Hosoda K, Feussner GK, Rydelek-Fitzgerald L, Fishman PH, Duman RS (1994) Agonist and cyclic AMP-mediated regulation of beta 1-adrenergic receptor mRNA and gene transcription in rat C6 glioma cells. J Neurochem 63(5):1635–1645

Hough C, Chuang DM (1990) Differential down-regulation of beta 1- and beta 2-adrenergic receptor mRNA in C6 glioma cells. Biochem Biophys Res Commun 170(1):46–52

Huang XE, Hamajima N, Saito T, Matsuo K, Mizutani M, Iwata H, Iwase T, Miura S, Mizuno T, Tokudome S, Tajima K (2001) Possible association of beta2- and beta3-adrenergic receptor gene polymorphisms with susceptibility to breast cancer. Breast Cancer Res 3(4):264–269

Huang Q, Tan Q, Mao K, Yang G, Ma G, Luo P, Wang S, Mei P, Wu F, Xu J, Guo M, Lv Z, Fan J, Zhang S, Wang X, Jin Y (2018) The role of adrenergic receptors in lung cancer. Am J Cancer Res 8(11):2227–2237

Iguchi S, Iwamura H, Nishizaki M, Hayashi A, Senokuchi K, Kobayashi K, Sakaki K, Hachiya K, Ichioka Y, Kawamura M (1992) Development of a highly cardioselective ultra short-acting beta-blocker, ONO-1101. Chem Pharm Bull (Tokyo) 40(6):1462–1469

Jessop DS (2019) The power of positive stress and a research roadmap. Stress 22(5):521–523

Ji Y, Chen S, Yang K, Zhang X, Zhou J, Li L, Xiang B, Qiu T, Dai S, Jiang X, Lu G, Qiu L, Kong F, Zhang Y (2021) Efficacy and safety of propranolol vs atenolol in infants with problematic infantile hemangiomas: a randomized clinical trial. JAMA Otolaryngol Head Neck Surg 147(7): 599–607

Kanno N, Lesage G, Phinizy JL, Glaser S, Francis H, Alpini G (2002) Stimulation of α2-adrenergic receptor inhibits cholangiocarcinoma growth through modulation of Raf-1 and B-Raf activities. Hepatology 35(6):1329–1340

Krishna A, Singh V, Singh N, Singh S, Mohanty SK, Singh R, Kumar V, Singh US, Singh RK (2022) Expression pattern and clinical significance of beta 2-adrenergic receptor in oral squamous cell carcinoma: an emerging prognostic indicator and future therapeutic target. Clin Transl Oncol 24(11):2191–2199

Kurozumi S, Kaira K, Matsumoto H, Hirakata T, Yokobori T, Inoue K, Horiguchi J, Katayama A, Koshi H, Shimizu A, Oyama T, Sloan EK, Kurosumi M, Fujii T, Shirabe K (2019) β2-adrenergic receptor expression is associated with biomarkers of tumor immunity and predicts poor prognosis in estrogen receptor-negative breast cancer. Breast Cancer Res Treat 177(3): 603–610

Lamkin DM, Sloan EK, Patel AJ, Chiang BS, Pimentel MA, Ma JC, Arevalo JM, Morizono K, Cole SW (2012) Chronic stress enhances progression of acute lymphoblastic leukemia via β-adrenergic signaling. Brain Behav Immun 26(4):635–641

Lang K, Drell TL 4th, Lindecke A, Niggemann B, Kaltschmidt C, Zaenker KS, Entschladen F (2004) Induction of a metastatogenic tumor cell type by neurotransmitters and its pharmacological inhibition by established drugs. Int J Cancer 112(2):231–238

Large V, Hellström L, Reynisdottir S, Lönqvist F, Eriksson P, Lannfelt L, Arner P (1997) Human beta-2 adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte beta-2 adrenoceptor function. J Clin Invest 100(12):3005–3013

Lavon H, Matzner P, Benbenishty A, Sorski L, Rossene E, Haldar R, Elbaz E, Cata JP, Gottumukkala V, Ben-Eliyahu S (2018) Dexmedetomidine promotes metastasis in rodent models of breast, lung, and colon cancers. Br J Anaesth 120(1):188–196

Li S, Yu C, Cheng Y, Du F, Wen G (2021) Bioinformatics analysis identifies biomarkers associated with poor prognosis in diffuse type gastric cancer. Mol Med Rep 23(3):193

Lin Y, Liu Y, Gao Z, Jing D, Bi R, Cui X, Cao Q, Zhao Q, Gao R, Su Y, Liu S, Zhao M, Yang Y, Chen A, Dai B, Gao X (2023) Beta-adrenergic receptor blocker propranolol triggers anti-tumor immunity and enhances irinotecan therapy in mice colorectal cancer. Eur J Pharmacol 949: 175718

Liu C, Yang Y, Chen C, Li L, Li J, Wang X, Chu Q, Qiu L, Ba Q, Li X, Wang H (2021) Environmental eustress modulates β-ARs/CCL2 axis to induce anti-tumor immunity and sensitize immunotherapy against liver cancer in mice. Nat Commun 12(1):5725

Livingstone D (1857) Missionary travels and researches in South Africa: including a sketch of sixteen years' residence in the interior of Africa. Jonh Murray Publisher, London

Ma X, Zhao T, Ouyang T, Xin S, Ma Y, Chang M (2014) Propranolol enhanced adipogenesis instead of induction of apoptosis of hemangiomas stem cells. Int J Clin Exp Pathol 7(7): 3809–3817

Maccari S, Buoncervello M, Ascione B, Stati T, Macchia D, Fidanza S, Catalano L, Matarrese P, Gabriele L, Marano G (2022) α-Adrenoceptor stimulation attenuates melanoma growth in mice. Br J Pharmacol 179(7):1371–1383

Masur K, Niggemann B, Zanker KS, Entschladen F (2001) Norepinephrine-induced migration of SW 480 colon carcinoma cells is inhibited by beta-blockers. Cancer Res 61(7):2866–2869

McGraw DW, Forbes SL, Liggett SB (1998) Polymorphisms of the 5′ leader cistron of the human beta2-adrenergic receptor regulate receptor expression. J Clin Invest 102(11):1927–1932

Mei L, Huang C, Wang A, Zhang X (2019) Association between ADRB2, IL33, and IL2RB gene polymorphisms and lung cancer risk in a Chinese Han population. Int Immunopharmacol 77: 105930

Mele L, Del Vecchio V, Marampon F, Regad T, Wagner S, Mosca L, Bimonte S, Giudice A, Liccardo D, Prisco C, Schwerdtfeger M, La Noce M, Tirino V, Caraglia M, Papaccio G, Desiderio V, Barbieri A (2020) β2-AR blockade potentiates MEK1/2 inhibitor effect on HNSCC by regulating the Nrf2-mediated defense mechanism. Cell Death Dis 11(10):850

Michel MC, Michel-Reher MB, Hein P (2020, 1923) A systematic review of inverse agonism at adrenoceptor subtypes. Cells 9(9)

Minneman KP, Hegstrand LR, Molinoff PB (1979) The pharmacological specificity of *Beta*-1 and *Beta*-2 adrenergic recepotors in rat heart and lung *in vitro*. Mol Pharmacol 16(1):21–33

Mohammadpour H, MacDonald CR, Qiao G, Chen M, Dong B, Hylander BL, McCarthy PL, Abrams SI, Repasky EA (2019) β2 adrenergic receptor-mediated signaling regulates the immunosuppressive potential of myeloid-derived suppressor cells. J Clin Invest 129(12): 5537–5552

Moretti S, Massi D, Farini V, Baroni G, Parri M, Innocenti S, Cecchi R, Chiarugi P (2013) β-Adrenoceptors are upregulated in human melanoma and their activation releases pro-tumorigenic cytokines and metalloproteases in melanoma cell lines. Lab Investig 93(3): 279–290

Mravec B, Tibensky M, Horvathova L (2020a) Stress and cancer. Part I: mechanisms mediating the effect of stressors on cancer. J Neuroimmunol 346:577311

Mravec B, Horvathova L, Hunakova L (2020b) Neurobiology of cancer: the role of β-adrenergic receptor signaling in various tumor environments. Int J Mol Sci 21(21):7958

Mravec B, Tibensky M, Horvathova L (2020c) Stress and cancer. Part II: therapeutic implications for oncology. J Neuroimmunol 346:577312

Musselman RP, Bennett S, Li W, Mamdani M, Gomes T, van Walraven C, Boushey R, Al-Obeed O, Al-Omran M, Auer RC (2018) Association between perioperative beta blocker use and cancer survival following surgical resection. Eur J Surg Oncol 44(8):1164–1169

Ogawa H, Kaira K, Motegi Y, Yokobori T, Takada T, Kato R, Osone K, Takahashi R, Suga K, Ozawa N, Katayama C, Oyama T, Shimizu A, Yao T, Asao T, Saeki H, Shirabe K (2020) Prognostic significance of β2-adrenergic receptor expression in patients with surgically resected colorectal cancer. Int J Clin Oncol 25(6):1137–1144

Pam N, Kridin K, Khamaysi Z (2021) Propranolol for infantile hemangioma: evaluating efficacy and predictors of response and rebound growth. Dermatol Ther 34(3):e14936

Pedersen L, Idorn M, Olofsson GH, Lauenborg B, Nookaew I, Hansen RH, Johannesen HH, Becker JC, Pedersen KS, Dethlefsen C, Nielsen J, Gehl J, Pedersen BK, Thor Straten P, Hojman P (2016) Voluntary running suppresses tumor growth through epinephrine- and IL-6-dependent NK cell mobilization and redistribution. Cell Metab 23(3):554–562

Perrone MG, Notarnicola M, Caruso MG, Tutino V, Scilimati A (2008) Upregulation of beta3-adrenergic receptor mRNA in human colon cancer: a preliminary study. Oncology 75(3–4): 224–229

Pottier N, Paugh SW, Ding C, Pei D, Yang W, Das S, Cook EH, Pui CH, Relling MV, Cheok MH, Evans WE (2010) Promoter polymorphisms in the β-2 adrenergic receptor are associated with drug-induced gene expression changes and response in acute lymphoblastic leukemia. Clin Pharmacol Ther 88(6):854–861

Powe DG, Voss MJ, Habashy HO, Zänker KS, Green AR, Ellis IO, Entschladen F (2011) Alpha- and beta-adrenergic receptor (AR) protein expression is associated with poor clinical outcome in breast cancer: an immunohistochemical study. Breast Cancer Res Treat 130(2):457–463

Qin JF, Jin FJ, Li N, Guan HT, Lan L, Ni H, Wang Y (2015) Adrenergic receptor β2 activation by stress promotes breast cancer progression through macrophages M2 polarization in tumor microenvironment. BMB Rep 48(5):295–300

Sakamoto A, Yagi K, Okamura T, Harada T, Usuda J (2019) Perioperative administration of an intravenous beta-blocker landiolol hydrochloride in patients with lung cancer: a Japanese retrospective exploratory clinical study. Sci Rep 9(1):5217

Schuller HM, Cole B (1989) Regulation of cell proliferation by beta-adrenergic receptors in a human lung adenocarcinoma cell line. Carcinogenesis 10(9):1753–1755

Sereni F, Dal Monte M, Filippi L, Bagnoli P (2015) Role of host β1- and β2-adrenergic receptors in a murine model of B16 melanoma: functional involvement of β3-adrenergic receptors. Naunyn Schmiedeberg's Arch Pharmacol 388(12):1317–1331

Shimizu A, Kaira K, Mori K, Kato M, Shimizu K, Yasuda M, Takahashi A, Oyama T, Asao T, Ishikawa O (2016) Prognostic significance of β2-adrenergic receptor expression in malignant melanoma. Tumour Biol 37(5):5971–5978

Silva D, Quintas C, Gonçalves J, Fresco P (2022) Contribution of adrenergic mechanisms for the stress-induced breast cancer carcinogenesis. J Cell Physiol 237(4):2107–2127

Simon MC, Keith B (2008) The role of oxygen availability in embryonic development and stem cell function. Nat Rev Mol Cell Biol 9(4):285–296

Szpunar MJ, Burker KA, Dawes RP, Brown EB, Madden KS (2013) The antidepressant desipramine and α2-adrenergic receptor activation promote breast tumor progression in association with altered collagen structure. Cancer Prev Res (Phila) 6(12):1262–1272

Takezaki T, Hamajima N, Matsuo K, Tanaka R, Hirai T, Kato T, Ohashi K, Tajima K (2001) Association of polymorphisms in the beta-2 and beta-3 adrenoceptor genes with risk of colorectal cancer in Japanese. Int J Clin Oncol 6(3):117–122

Tang J, Li Z, Lu L, Cho CH (2013) β-Adrenergic system, a backstage manipulator regulating tumour progression and drug target in cancer therapy. Semin Cancer Biol 23(6 Pt B):533–542

Traverso N, Ricciarelli R, Nitti M, Marengo B, Furfaro AL, Pronzato MA, Marinari UM, Domenicotti C (2013) Role of glutathione in cancer progression and chemoresistance. Oxidative Med Cell Longev 2013:972913

Vazquez SM, Mladovan AG, Perez C, Bruzzone A, Baldi A, Lüthy IA (2006) Human breast cell lines exhibit functional alpha(2) adrenoceptors. Cancer Chemother Pharmacol 58:50–61

Vrydag W, Michel MC (2007) Tools to study beta3-adrenoceptors. Naunyn Schmiedeberg's Arch Pharmacol 374(5–6):385–398

Wackerhage H, Christensen JF, Ilmer M, von Luettichau I, Renz BW, Schönfelder M (2022) Cancer catecholamine conundrum. Trends Cancer 8(2):110–122

Wang Y, Jiang S (2021) The role of ADRB2 gene polymorphisms in malignancies. Mol Biol Rep 48(3):2741–2749

Wang H, Hao B, Chen X, Zhao N, Cheng G, Jiang Y, Liu Y, Lin C, Tan W, Lu D, Wei Q, Jin L, Lin D, He F (2006) Beta-2 adrenergic receptor gene (ADRB2) polymorphism and risk for lung adenocarcinoma: a case-control study in a Chinese population. Cancer Lett 240(2):297–305

Wei X, Chen L, Yang A, Lv Z, Xiong M, Shan C (2021) ADRB2 is a potential protective gene in breast cancer by regulating tumor immune microenvironment. Transl Cancer Res 10(12): 5280–5294

Wenjuan Y, Yujun L, Ceng Y (2013) Association of single nucleotide polymorphisms of β2-adrenergic receptor gene with clinicopathological features of pancreatic carcinoma. Acta Histochem 115(3):198–203

Xia M, Ji N-N, Duan M-L, Tong J-H, Xu J-G, Zhang Y-M, Wang S-H (2016) Dexmedetomidine regulate the malignancy of breast cancer cells by activating α2-adrenoceptor/ERK signaling pathway. Eur Rev Med Pharmacol Sci 20(16):3500–3506

Xu Y, Wang J, Wang X, Zhou X, Tang J, Jie X, Yang X, Rao X, Xu Y, Xing B, Li Z, Wu G (2022) Targeting ADRB2 enhances sensitivity of non-small cell lung cancer to VEGFR2 tyrosine kinase inhibitors. Cell Death Discov 8(1):36

Yamamoto H, Hamasaki T, Onda K, Nojiri T, Aragaki M, Horie N, Sato N, Hida Y (2019) Landiolol, an ultra-short acting beta-1 blocker, for preventing postoperative lung cancer recurrence: study protocol for a phase III, multicenter randomized trial with two parallel groups of patients. Trials 20(1):715

Yap A, Lopez-Olivo MA, Dubowitz J, Pratt G, Hiller J, Gottumukkala V, Sloan E, Riedel B, Schier R (2018) Effect of beta-blockers on cancer recurrence and survival: a meta-analysis of epidemiological and perioperative studies. Br J Anaesth 121(1):45–57

Yoshioka Y, Kadoi H, Yamamuro A, Ishimaru Y, Maeda S (2016) Noradrenaline increases intracellular glutathione in human astrocytoma U-251 MG cells by inducing glutamate-cysteine ligase protein via β3-adrenoceptor stimulation. Eur J Pharmacol 772:51–61

Zhang J, Dhakal IB, Zhang X, Prizment AE, Anderson KE (2014) Genetic variability in energy balance and pancreatic cancer risk in a population-based case-control study in Minnesota. Pancreas 43(2):281–286

Zhang X, Zhang Y, He Z, Yin K, Li B, Zhang L, Xu Z (2019a) Chronic stress promotes gastric cancer progression and metastasis: an essential role for ADRB2. Cell Death Dis 10(11):788

Zhang J, Gu Y, Chen B (2019b) Mechanisms of drug resistance in acute myeloid leukemia. Onco Targets Ther 12:1937–1945

Zhang M, Chen F, Sun X, Huang Y, Zeng Y, Chen J, Wu S, Xu C (2023) Sympathetic β_2-adrenergic receptor blockade overcomes docetaxel resistance in prostate cancer. Biochem Biophys Res Commun 657:69–79

Zheng M, Zhou Z, Tian X, Xiao D, Hou X, Xie Z, Liang H, Lin S (2020) ADRB3 expression in tumor cells is a poor prognostic factor and promotes proliferation in non-small cell lung carcinoma. Cancer Immunol Immunother 69(11):2345–2355

Zhou Z, Zhan J, Luo Q, Hou X, Wang S, Xiao D, Xie Z, Liang H, Lin S, Zheng M (2022) ADRB3 induces mobilization and inhibits differentiation of both breast cancer cells and myeloid-derived suppressor cells. Cell Death Dis 13(2):141

Zhu J, Naulaerts S, Boudhan L, Martin M, Gatto L, Van den Eynde BJ (2023) Tumor immune rejection triggered by activation of α2-adrenergic receptors. Nature 618(7965):607–615