Anastasios Lymperopoulos *Editor*

The Cardiovascular Adrenergic System

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Preface

Heart disease is the most lethal human condition in the Western world. The cardiovascular system is one of the most complex biological systems in mammals and is under constant and tight regulation by various endogenous hormones and neurotransmitters. The adrenergic system is, arguably, among those entities that regulate the heart and vessels, the most studied to date. Although not theoretically essential for life, like its cholinergic (or parasympathetic) counterpart is, the adrenergic (or sympathetic) branch of the autonomic nervous system is still essential for the communication of each mammalian organism with its environment and for the response and survival to stress. The cardiovascular system is probably the most important organ system that needs to be regulated and mobilized by the adrenergic hormones (epinephrine and norepinephrine) in order to orchestrate the individual's systemic response to environmental stimuli or insults. Thus, it comes as no surprise that the cardiovascular system is tightly regulated by the adrenergic nervous system and that, in situations where this cardiovascular adrenergic system goes awry, some cardiovascular morbidity or disease almost always ensues. Another testament for the biological and physiological importance of the cardiovascular adrenergic system in medicine and physiology is the fact that a recent Nobel Prize (the 2012 Nobel Prize in Chemistry) was awarded to the identification and crystallization of some of the receptors the cardiovascular adrenergic system depends on to exert its effects (and which are extensively discussed throughout this book), i.e. the G protein-coupled beta-adrenergic receptors.

The present book is an attempt to provide a state-of-the-art, up-to-date overview of the cardiovascular adrenergic system and its roles in heart and vessels' physiology, disease and therapy. It is accordingly structured into two major sections focusing on the interplay between the cardiovascular and adrenergic systems, immediately following an introductory chapter on general biological considerations of the adrenergic nervous system in mammals. The first of these sections comprises chapters describing the adrenergic receptors and their effects in each cardiovascular cell type separately, one by one (cardiac myocytes, cardiac fibroblasts, vascular endothelial cells, vascular smooth muscle cells). The second section consists of chapters that describe and discuss the roles of the cardiovascular adrenergic system in certain diseases/conditions, such as cardiovascular metabolism, aging, the interface between

the central/autonomic nervous systems and the cardiovascular system, as well as in certain cardiovascular therapeutic modalities, such as physical exercise and cellular (cardiac stem cell) therapies. Throughout these chapters, the authors spotlight future avenues for research in basic pathophysiology and in cardiovascular therapy/prevention, in addition to thorough overviews of the current literature pertaining to the adrenergic system and its biological effects.

The comprehensive overview of the effects of the adrenergic hormones and their receptors across the cardiovascular system provided within this book is expected to assist the reader in comprehending the importance of taking into account the role of the adrenergic system in cardiovascular pathologies and also to address questions and unresolved issues regarding the treatment of those cardiovascular pathologies.

Finally, the editor would like to express his sincere appreciation to all the contributors for their dedicated collaboration in this project. I also wish to thank Ms. Susan Westendorf for her competent and patient support, which was instrumental in editing this book.

I sincerely hope this book will enable readers to connect biomedical and clinical knowledge from the field of cardiology with the basic biological principles and concepts of the molecular physiology and pharmacology of the adrenergic nervous system, thereby encouraging future discoveries and developments of new strategies and agents for combating cardiovascular disease and for promoting heart health.

Fort Lauderdale, USA Anastasios Lymperopoulos

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Part I
Introduction/General Considerations

Chapter 1
Introduction/General Considerations

Anastasios Lymperopoulos

The adrenergic nervous system (ANS) exerts numerous effects on the cardiovascular system, including increase in cardiac contractility (positive inotropy), heart rate acceleration (positive chronotropy), hastened cardiac relaxation (positive lusitropy), and accelerated atrioventricular conduction (positive dromotropy). Most of these effects are mediated by adrenergic receptors (also known as adrenoceptors, ARs), which belong to the guanine nucleotide-binding G-protein-coupled receptor (GPCR) superfamily [1]. GPCRs are heptahelical transmembrane sensors, accounting for approximately 4 % of the entire protein-coding genome, widely considered the most important drug targets in physiology and medicine [2]. These receptors consist of seven membrane-spanning domains, three intra- and three extracellular loops, one extracellular N-terminal domain, and one intracellular C-terminal tail [3].

Anatomy

The cell bodies of the sympathetic preganglionic fibers are in the lateral horns of the spinal segments T1-L2, the so-called thoracolumbar outflow. The preganglionic fibers travel a short distance in the mixed spinal nerve and then branch off as white rami (myelinated) to enter the sympathetic ganglia. These are mainly arranged in two paravertebral chains, which lie anterolateral to the vertebral bodies and extend from the cervical to the sacral region. They are called the *sympathetic ganglionic chains*. The short preganglionic fibers (unlike the long preganglionic fibers of the parasympathetic nervous system) that enter the chain make a synapse with a

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postsynaptic fiber either at the same dermatomal level or at a higher or lower level, and then the longer postganglionic fibers usually return to the adjacent spinal nerve via gray rami (unmyelinated) and are conveyed to the effector organ. At most postganglionic sympathetic endings, the chemical transmitter is norepinephrine (NE, also called noradrenaline), which is present in the presynaptic terminal as well as in the adrenal medulla. In contrast, postganglionic sympathetic fibers in sweat glands release acetylcholine, and this transmission is muscarinic. Some preganglionic fibers do not synapse in the sympathetic chains but terminate in separate cervical or abdominal ganglia or travel in the greater splanchnic nerve and directly synapse with chromaffin cellsof the adrenal medulla. The adrenal medulla is innervated by preganglionic fibers, and therefore, epinephrine (Epi, also known as adrenaline) is released from the gland by stimulation of nicotinic cholinergic receptors, although some NE is also secreted [4]. In contrast to the parasympathetic nervous system, the ANS enables the body to be prepared for fear, flight, or fight. ANS responses include an increase in the heart rate, blood pressure, and cardiac output; a diversion of blood flow from the skin and splanchnic vessels to those supplying skeletal muscle; an increase in pupil size; a bronchiolar dilation; a contraction of sphincters; and metabolic changes such as the mobilization of fat and glycogen.

The pressor effects of adrenal extracts were first demonstrated in 1895 by Oliver and Schäfer. Epi and NE are catecholamines, and both are synthesized from the essential amino acid tyrosine by a series of steps that also includes the production of dopamine, which is another endogenous catecholamine. The terminal branches of the sympathetic postganglionic fibers have varicosities or swellings, giving them the appearance of a string of beads. These swellings form the synaptic contact with the effector organ and are also the site of synthesis and storage of NE. On the arrival of a nerve impulse, NE is released from granules in the presynaptic terminal into the synaptic cleft. The action of NE is terminated by diffusion from the site of action, reuptake back into the presynaptic nerve ending where it is inactivated by the enzyme monoamine oxidase (MAO) on the mitochondrial surface or metabolized locally by the enzyme catechol-*O*-methyl-transferase (COMT).

The synthesis and storage of catecholamines in the adrenal medulla are similar to those of sympathetic postganglionic nerve endings; however, owing to the presence of an additional enzyme termed *phenylethanolamine N-methyl transferase* (*PNMT*), the majority of NE is converted to Epi. The adrenal medulla responds to nervous impulses in the sympathetic cholinergic preganglionic fibers by transforming the neural impulses into hormonal secretion. In situations involving physical or psychologic stress, much larger quantities are released. Overall, the actions of catecholamines are mediated by specific postsynaptic cell surface receptors (see below).

Receptors Mediating the Effects of the Cardiovascular ANS

Dale and Berger (1910) studied a wide range of synthetic amines related to Epi and termed their physiologic actions *sympathomimetic*. They later discovered that cocaine or chronic denervation reduced the response to ephedrine and tyramine but not to Epi. Thus, it became evident that the differences between amines were not solely quantitative. Raymond Ahlquist in 1948, based on a series of experiments showing different activities of various sympathomimetic drugs, proposed the existence of two different receptors for catecholamines, which he named α- and β-ARs [5]. α-ARs were those with increased affinity for Epi and NE and minimal affinity for isoproterenol, whereas β-ARs were characterized by their high affinity for isoproterenol and Epi and low affinity for NE. The advent of drugs that selectively blocked β-ARs (β-blockers; by Sir James Black) confirmed Ahlquist's suggested division of ARs and pharmacologic experiments that followed suggested the existence of more than one distinct subclass of these receptors. This experimentation and later molecular cloning led to the identification and characterization of three subfamilies of ARs: α_1-, α_2-, and β-ARs [6]. These can further be subdivided into three subtypes each. Two major subfamilies of α-ARs exist: α_1- and α_2-ARs, identified based on their different affinities for a number of α-AR antagonists such as prazosin (selective for α_1-ARs) and yohimbine (selective for α_2-ARs). Through laborious radioligand-binding experiments and, finally, molecular cloning of individual receptor genes, α_1-ARs are now known to comprise three different subtypes, designated α_{1A}, α_{1B}, α_{1D}, whereas α_2-ARs also consist of three different subtypes, designated α_{2A}, α_{2B}, α_{2C} [7–10]. Phenylephrine is the prototypic α_1-AR-selective agonist, whereas clonidine and α-methyldopa are selective α_2-AR agonists. Classically, α_1-ARs couple to $G_{q/11}$ proteins and stimulate phosphoinositide hydrolysis, which results in the contraction of smooth muscle (e.g., vasoconstriction, ureter, and bladder sphincter constriction), whereas α_2-ARs couple to $G_{i/o}$ proteins and inhibit adenylyl cyclase and activate hyperpolarizing K^+currents, actions that can lead to inhibition of neuronal firing and neurotransmitter release in the central nervous system.

Soon after the division of ARs into α-ARs and β-ARs, β-ARs were found to actually comprise at least two different receptor subtypes, designated β_1- and β_2-ARs, and were identified based on their different relative affinities for Epi and NE [11]. β_1-ARs bind to Epi and NE with equal affinity, whereas β_2-ARs have a very high affinity for Epi but minimal affinity for NE. A third β-AR subtype, β_3-AR, originally identified in adipose tissue, displays higher affinity for NE than isoproterenol and minimal affinity for Epi. It is also unresponsive to classic β-blockers that antagonize β_1- and β_2-ARs [12]. Dobutamine binds to β_1-ARs with much higher affinity than to β_2-ARs, while having no affinity for α-ARs. Dopamine and isoproterenol (or isoprenaline) bind to both β_1- and β_2-ARs with equal affinity. In contrast, drugs such as terbutaline, ritodrine, albuterol, metaproterenol, salmeterol, and formoterol are selective β_2-AR agonists.

All three β-ARs are classically defined through their coupling to Gs proteins leading to the activation of adenylyl cyclase (Table 1.1). In cardiac muscle, this results in increased contractility and rate of contraction (positive inotropy and chronotropy) via cyclic adenosine monophosphate (cAMP) accumulation, whereas in smooth muscle, cAMP leads to muscle relaxation (vasodilation, bronchodilation, uterine muscle relaxation; Table 1.1). β_2-ARs and β_3-ARs can also couple to Gi proteins and inhibit adenylyl cyclase under some circumstances, and β_3-ARs have been shown to couple to nitric oxide (NO) synthase activation (Table 1.1; [1, 6]).

Cardiovascular Effects of the ANS (Under Normal Conditions)

Catecholamines are important regulators of peripheral vascular resistance and venous capacitance. α-ARs increase arterial smooth muscle tone, whereas β_2-ARs relax vascular smooth muscle. Skin and splanchnic vessels have mainly α-ARs and, thus, constrict in response to catecholamines, whereas skeletal muscle vessels have both α- and β_2-ARs and can either constrict or dilate, respectively, after catecholamine stimulation (Table 1.1; [1, 6]). Renal, splanchnic, coronary, and cerebral vessels also have dopaminergic D_1 receptors, and they dilate in response to dopamine (the third endogenous catecholamine hormone) activation [13]. This mediates, in large part, the natriuresis (through increase of the resultant renal glomerular filtration rate) and the hypotension usually observed upon systemic administration of low doses of dopamine (the so-called renal dose of dopamine).

Endogenous catecholamines are extremely important in the regulation of cardiac contractile function primarily through their actions via cardiostimulatory β-ARs (Table 1.1; [1, 11, 13–15]). β_1-ARs are the primary subtype in the heart, comprising 75–80 % of total myocardial β-ARs. The remaining cardiac β-ARs are largely made up of β_2-ARs, with a minor component of β_3-ARs found in human myocardium [1]. β-AR activation results in increased pacemaker activity (at the sinoatrial node and in the Purkinje fibers) and conduction velocity at the atrioventricular node, whereas it decreases the refractory period [1]. All these actions lead to positive chronotropy (Table 1.1). Cardiac contractility is enhanced (positive inotropy), and the relaxation is accelerated (positive lusitropy; Table 1.1). The end result is a cardiomyocyte twitch response of increased tension but reduced duration. The intraventricular pressure of the intact heart rises and falls more rapidly, and the ejection time is decreased. These effects are clearer in the absence of reflexes to blood pressure changes. These normal reflexes usually confound the direct effects of catecholamines on the heart, and the resultant net effect on the heart depends on the relative balance between the actions of these reflexes and the direct actions of catecholamines on cardiac muscle [1, 11, 15–16].

Catecholamines are also very important regulators of systemic blood pressure. Their effects on blood pressure are a function of three parameters: (a) their direct effects on the heart; (b) their effects on peripheral vascular resistance; and (c) on

Table 1.1 Cardiovascular properties of the ANS receptors

	α_1 Subtype	α_2 Subtype	β_1 Subtype	β_2 Subtype	β_3 Subtype
Localization in the CVS—effect	VSM—contraction Cardiac muscle—hypertrophy, positive inotropy (mild)	Cardiac adrenergic nerve terminals—inhibition of NE release (α_{2A}, α_{2C}); VSM—contraction (α_{2B}); Adrenal Medulla—inhibition of Epi & NE secretion (α_{2A}, α_{2B}, α_{2C}); Platelets—aggregation (α_{2A})	Cardiac myocytes—positive inotropy, chronotropy, lusitropy	VSM (skeletal muscle vasculature)—relaxation; Cardiac muscle—activation of K^+ uptake (NKA activation)	Cardiac muscle—negative inotropy (NOS activation)
Signal transduction	$G_{q/11}$-PLC, increase of IP_3-DAG	$G_{i/o}$, AC inhibition, K^+ channel activation	G_s, AC activation	G_s, AC activation-$G_{i/o}$, AC inhibition-NKA activation	G_s, AC activation-NOS activation
Cardiovascular response	↑ Vascular resistance (in all beds); ↑ Cardiac contractility (slightly); ↓ Cardiac HR (vagal reflex); ↓ CO; ↑ Mean-systolic-diastolic BP	↑ Vascular resistance (in some beds only); No direct effects on the heart; ↓ BP (centrally mediated inhibition of sympathetic outflow)	↓ Vascular (in skeletal muscle, renal, splanchnic beds) & total peripheral resistance; ↓ Venous tone; ↑ Cardiac contractility-HR-CO; ↓ Mean-diastolic BP	↓ — Systolic BP ↓ — Cardiac contractility	

CVS cardiovascular system, *NE* norepinephrine, *Epi* epinephrine, *NKA* Na^+ , K^+ -ATPase, *NOS* nitric oxide synthase, *AC* adenylyl cyclase, *BP* blood pressure, *CO* cardiac output, *DAG* 2′-diacylglycerol, *HR* heart rate, IP_3 1′, 4′, 5′-inositol trisphosphate, *PLC* phospholipase C, *SM* smooth muscle, *VSM* vascular smooth muscle

the venous return (Table 1.1). A pure α_1-AR agonist like phenylephrine increases peripheral arterial resistance and decreases venous capacitance. Therefore, systemic blood pressure rises, which then evokes a baroreceptor-mediated reflex increase in vagal tone in order to slow the heart rate. Cardiac output usually does not change, despite the reduction of heart rate, owing to the resultant increase in venous return (Frank–Starling effect on cardiac contractility) and to a minor positive inotropic effect of direct cardiac α_1-AR stimulation. These reflex responses are usually undetectable in hypotensive patients, in whom α_1-AR agonists are given to normalize blood pressure.

Specific catecholamine responses follow the previously discussed characteristics. Epi, which activates all ARs, is a very potent vasoconstrictor and cardiac stimulant. It raises systolic blood pressure by increasing the heart rate and force of contraction (cardiac β-AR effect) and peripheral vascular resistance (α_1-AR effect). However, it also dilates some peripheral vessels (mainly skeletal muscle vessels) via stimulation of β_2-ARs; thus, it might actually decrease peripheral resistance and diastolic pressure. This skeletal muscle vessel dilation contributes to increased skeletal blood flow during exercise. NE shares the activity of Epi at α-ARs but lacks significant β_2-AR activity. Consequently, it increases peripheral resistance (via α_1-ARs), heart rate, and force of contraction (cardiac β_1-AR effect), thus raising both systolic and diastolic blood pressures. Vagal reflexes usually counteract the positive chronotropic but not inotropic effects of NE. A simple β-AR agonist like isoproterenol markedly increases cardiac output, contractility, and rate of contraction (cardiac β-AR effects) while decreasing peripheral resistance by inducing β_2-AR-mediated peripheral vasodilation. Therefore, the net effect on blood pressure is a fall in diastolic and mean arterial pressures in combination with a slight increase (or no change) in systolic blood pressure [6, 15–16].

The following chapters of this book give a detailed description of the effects of the ANS in the cardiovascular system, in both health and disease, in each individual cell type or organ of the circulation (myocardium, vascular endothelium, vascular smooth muscle), followed by discussions of the modulatory effects of the ANS in specific aspects of cardiovascular physiology and pathophysiology, as well as in the interplay between the circulatory and certain other physiological or cellular systems (e.g., neuroendocrine system, stem cells).

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Part II
The Adrenergic System of Individual Cardiovascular Cell Types

Chapter 2
The Adrenergic System of the Myocardium

Grazia Daniela Femminella, Claudio de Lucia, Gennaro Pagano, Klara Komici, Alessandro Cannavo, Vincenzo Barrese, Nicola Ferrara and Giuseppe Rengo

Cardiovascular homeostasis is guaranteed by the interplay of several neurohormonal systems in the body. Among them, the adrenergic (or sympathetic) nervous system (ANS) has a crucial role both in physiological and pathological conditions.

The major consequences of ANS stimulation on cardiovascular function can be summarized in the so-called fight or flight response, which manifests with heart rate acceleration (positive chronotropy), increased cardiac contractility (positive inotropy), reduction of venous capacitance, increased myocardial relaxation (positive lusitropy), acceleration of atrioventricular conduction (positive dromotropy), and constriction of resistance vessels. The mediators of these effects are the catecholamines epinephrine (Epi), or adrenaline, mainly released in the circulation by the adrenal medulla, and norepinephrine (NE), or noradrenaline, released by sympathetic nerve terminals. The effects of the ANS are counterbalanced by the parasympathetic (cholinergic) nervous system, which, through the vagal nerve terminals, is mainly responsible for heart rate reduction (negative chronotropy), with minimal inotropic effects [1].

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The pivotal role of ANS on cardiovascular function regulation is further emphasized by the fact that it constitutes one of the major therapeutic targets in cardiovascular diseases. Indeed, β-adrenergic receptor (β-AR) blockers are a cornerstone in the pharmacological treatment of myocardial ischemia, heart failure (HF), and hypertension [2].

In this chapter, we discuss the effects of ANS stimulation on cardiovascular function in both physiological and pathophysiological conditions, with a particular attention to the numerous evidence derived from preclinical and clinical studies on HF, a cardiovascular disease characterized by ANS hyperactivity.

The Adrenergic Receptors

The ARs belong to the superfamily of G-protein-coupled receptors (GPCRs) or seven transmembrane-spanning domain receptors (7TMRs), with an extracellular N-terminal region and an intracellular C-terminus. Their physiological agonists are the neurotransmitters NE and Epi.

To date, a total of three types and nine subtypes of ARs have been identified and classified into α1-AR (α1A, α1B, α1D), α2-AR (α2A, α2B, α2C), and β-AR (β1, β2, β3). All ARs primarily signal through heterotrimeric G proteins [3].

β-ARs display peculiar tissue distribution and pharmacological properties: β1 is the "cardiac" receptor, while β2 is expressed predominantly in smooth muscle cells, and β3 in the adipose tissue [2]. Recently, the existence of another subtype, the β4, has been postulated, but it is likely that the β4 represents a novel functional status of the β1 receptor [4].

Although β1-AR is the predominantly expressed AR subtype in the normal heart, accounting for 75–80 % of total β-ARs, cardiac cells express also β2 (15–18 %) and, to a lesser extent, β3 (2–3 % of total cardiac β-ARs) [5]. The most important function of cardiac -ARs is the regulation of heart rate and myocardial contractility in response to catecholamines. Indeed, β1-AR and, to a lesser extent, β2-AR stimulation increases cardiac contractility, heart rate, and rate of relaxation [6]. As for β3-ARs, recent data indicate that their stimulation has opposite effects compared with β1-ARs and β2-ARs, resulting in the negative inotropic effect [7].

Human heart also expresses α1-ARs, although at significantly lower levels (20 %) compared with β-ARs. It has been shown that the most predominant subtype in human cardiomyocytes is the α1A; however, the α1B is present in the left and right ventricles of both failing and nonfailing human myocardium. Little is known about the 1D-AR, instead, and its potential role in regulating cardiac contractility. Moreover, it is not clear whether α1-AR subtypes might have a differential expression in the various parts of the heart (endocardium and epicardium). However, the role of α1-AR in the regulation of blood flow by inducing vasoconstriction of the smooth muscle cells in arterial walls is well recognized [8].

Among the α2-AR subtypes, α2B-ARs are known to be present in vascular smooth muscle cells where they mediate vasoconstriction, while α2A-ARs act

as presynaptic inhibitory autoreceptors in the central nervous system, and their stimulation is able to lower systemic blood pressure [9]. Also α2C-AR subtype is a presynaptic receptor regulating NE release from cardiac sympathetic nerve terminals [10].

Several pharmacogenomic studies have demonstrated that ARs present different polymorphic forms in humans, and some of them might affect therapeutic response to AR-modulating drugs (as β-blockers). Briefly, in the human β1-AR gene at least 12 single nucleotide polymorphisms (SNPs) have been described, but only two of them are actually clinically relevant. The first one is Ser49Gly, occurring in the N-terminus region, where it can be involved in receptor downregulation, as well as in intracellular trafficking. The second one is the Arg389Gly in the intracellular C-terminus, with the Arg389 variant showing higher activation both in basal conditions and after agonist stimulation [11].

In the β2-AR protein, three major variants have been identified: Arg or Gly16, Gln or Glu27, and Ile164. Among them, SNPs in positions 16 and 27 are more common, while Ile164 variant is very rare, and its clinical relevance is restricted to a small number of patients. Arg/Gly16 and Gln/Glu27 polymorphisms instead affect receptor downregulation after agonist stimulation [12].

In addition to that, an in-frame deletion of 12 nucleotides, leading to a loss of four aminoacids (Gly-Ala-Gly-Pro), has been described within the α2C-AR protein and associated with increased response to catecholamine stimulation [13].

Cardiac Adrenergic Signaling

As mentioned above, ARs belong to the superfamily of GPCRs; thus, their agonist-induced stimulation catalyzes the exchange of guanosine triphosphate (GTP) for guanosine diphosphate (GDP) on the Gα subunit of heterotrimeric G proteins, resulting in the dissociation of the heterotrimer into Gα and Gβγ subunits, which can activate downstream intracellular pathways [14]. In cardiac myocytes, the stimulation of β1-ARs and β2-ARs results in the activation of stimulatory G (Gs) proteins. Gs signaling, in turn, stimulates adenylate cyclase (AC), which converts adenosine triphosphate (ATP) to the second messenger adenosine 3′,5′-monophosphate or cyclic AMP (cAMP), which binds and activates protein kinase A (PKA). PKA can, in turn, phosphorylate and activate several substrates, including L-type calcium channels and phospholamban, a calcium ATPase regulator localized on the sarcoplasmic reticulum, ultimately stimulating the increase of free intracellular Ca^{2+}, the regulator of cardiac contractility. The stimulation of β-ARs in the heart affects not only myocardial contractility but also other cellular functions such as gene transcription and cell growth, mainly through the activation of the mitogenic-activated protein kinase (MAPK) [15].

Persistent β1-AR stimulation can also induce the PKA-independent activation of the calmodulin-dependent kinase II, inducing cardiomyocyte hypertrophy [16].

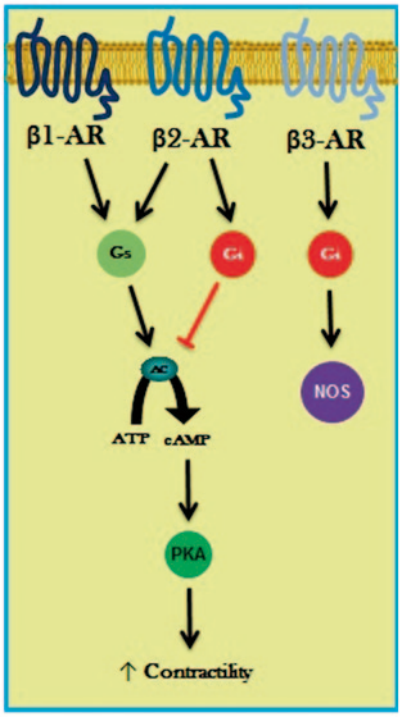

Fig. 2.1 β-adrenergic receptor (*AR*) signaling in cardiac myocytes. The stimulation of β1-ARs and β2-ARs results in the activation of stimulatory G (*Gs*) proteins. Gs signaling, in turn, stimulates adenylate cyclase (*AC*), which converts adenosine triphosphate (*ATP*) to the second messenger cyclic adenosine monophosphate (*cAMP*), binding and activating protein kinase A (*PKA*). PKA can, in turn, phosphorylate and activate several substrates, including L-type calcium channels and phospholamban, ultimately stimulating the increase of free intracellular Ca^{2+}, the regulator of cardiac contractility. β2-ARs also couple to the inhibitory G (*Gi*) protein and may switch its coupling from Gs to Gi proteins. Differently, β3-AR signaling is associated with nitric oxide release via nitric oxide synthase (*NOS*) activation

The effects of catecholamines on β2-ARs are different compared with those mediated by β1-ARs, since β2-ARs also couple to the inhibitory G (Gi) protein. In particular, it has been demonstrated that β2-AR-Gi coupling has a cardioprotective effect, while β1-ARs is proapoptotic [17]. However, the model of β2-AR dual coupling (Gi and Gs proteins) is not well clarified yet. It is likely that β2-AR–Gi signaling compartmentalizes the β2-AR-Gs–c-AMP signaling [18]. Also, β2-AR phosphorylation by different kinases (such as PKA) may switch receptor coupling from Gs to Gi proteins [15].

In preclinical studies, β2-AR stimulation and adenoviral-mediated β2-AR overexpression have protective effects in the heart, with improved cardiac function and reduced apoptosis. In contrast, hyperstimulation or overexpression of β1-AR has detrimental effects in the heart [19–21].

Differently, β3-AR signaling is associated with nitric oxide (NO) release via nitric oxide synthase (NOS) activation (Fig. 2.1) [3].

The α1-ARs, instead, couple to the subfamily of Gq heterotrimeric proteins, which activate phospholipase C (PLC)-β. PLC-β catalyzes the formation of the second messengers inositol trisphosphate (IP3) [1, 4, 5]and 2-diacylglycerol (DAG) from the phospholipid phosphatidylinositol-bisphosphate (PIP2). IP3 stimulates the release of Ca^{2+} from sarcoplasmic reticulum, while DAG activates protein kinase C (PKC), both contributing to increased myocardial contraction and vasoconstriction

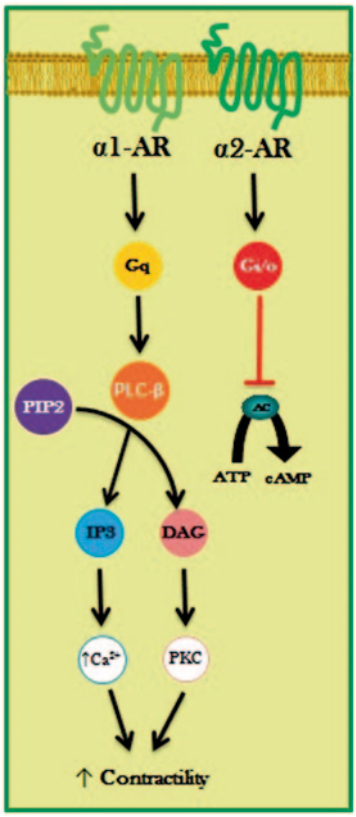

Fig. 2.2 α-adrenergic receptor (*AR*) signaling in cardiac myocytes. The α1-ARs couple to the subfamily of Gq heterotrimeric proteins, which activate phospholipase C-β (*PLC-β*) that catalyzes the formation of the second messengers inositol trisphosphate (*IP3*) and 2-diacylglycerol (*DAG*) from phosphatidylinositol-bisphosphate (*PIP2*). *IP3* stimulates the release of Ca^{2+} from sarcoplasmic reticulum, while *DAG* activates protein kinase C (*PKC*), both contributing to increased myocardial contractility. The α2-ARs are coupled to the Gi/o family members of the G proteins; thus, its activation inhibits the effector enzyme adenylate cyclase (*AC*). *ATP* adenosine triphosphate, *cAMP* cyclic adenosine monophosphate

[1]. Finally, α2-ARs are coupled to the Gi/o family members of the G proteins; thus, its activation inhibits the effector enzyme AC (Fig. 2.2) [22].

Both α2- and β-ARs, like other GPCRs, are subject to complex regulatory mechanisms to protect the receptor from both acute and chronic stimulation, a process termed desensitization. In general, GPCR desensitization involves the following events: (1) receptor phosphorylation and uncoupling from G proteins, (2) internalization of membrane-bound receptors, and (3) downregulation through reduced receptor synthesis or increased degradation of internalized receptors [14].

The desensitization process is orchestrated by three families of proteins: second-messenger-dependent PKs, GRKs, and arrestins. Initially, GRKs (G-protein-coupled receptors kinases) recognize and phosphorylate agonist-bound receptors, and then they promote the association of cytosolic cofactor proteins called arrestins, which target GPCRs for endocytosis and activate G-protein-independent signaling [23].

The GRKs are a family of cytosolic serine/threonine kinases consisting of seven isoforms with structural and functional similarities. They are classified into three subfamilies: (1) the rhodopsin kinase GRK1 and visual pigment kinase GRK7, (2) the β-AR kinases (or GRK2 and GRK3); and (3) the GRK4 group (GRK4–6).

Across mammalian species, GRK2 and GRK5 are the most important members of the GRK family due to their ubiquitous expression; in fact, they are particularly abundant in neuronal tissues and in the heart [24, 25].

GRK2 was first identified as β-AR kinase-1 (β-ARK-1); it is the most expressed GRK subtype in the heart. As a consequence of agonist stimulation, Gβγ subunits interact with GRK2, thus mediating its translocation to the plasma membrane where it initiates receptor desensitization. The importance of GRK2 relies also on the fact that in the last decades, much evidence has suggested a key role for GRK2 in the development of myocardial dysfunction [26, 27].

Cardiac Adrenergic System in Heart Failure

HF is a chronic clinical syndrome characterized by reduced pumping capacity of the myocardium (systolic HF), which can develop in response to several cardiac insults [28]. Regardless of the initial cause, the failing heart usually ends up in a vicious cycle of progressive functional decline, with the increased activity of neurohormonal systems aiming to compensate for the reduced blood pressure and cardiac output [1, 29]. In congestive HF, both the activities of the sympathetic nervous system and the renin-angiotensin system (RAS) are increased [30, 31]. In the long term, however, myocardial exposure to high levels of circulating catecholamines and angiotensin increases cardiac workload ultimately leading to maladaptive cardiac remodeling and myocyte death [32, 33].

In HF, ANS hyperactivity is highlighted by increased plasma NE levels, central sympathetic outflow, and NE plasma spillover from activated sympathetic nerve fibers. It has been demonstrated that in patients with HF, cardiac NE spillover is increased by 50-fold, similar to levels found in healthy subjects during maximal exercise [34]. It has also been observed that patients with HF present decreased neuronal density, which can be assessed with 123I-labeled meta-iodobenzylguanidine (MIBG) imaging. MIBG is an analogue of NE, sharing the same uptake, storage, and release processes. As a result, scintigraphic images obtained with MIBG depict the status of catecholamine storage at the level of the myocardial sympathetic presynaptic fibers [35]. Recent MIBG studies have also shown that diabetic patients with HF have lower cardiac sympathetic activity than nondiabetic patients with HF [36].

The ANS hyperactivity observed in HF can also be ascribed to abnormalities in cardiovascular reflexes. In patients with HF, the arterial baroreceptor reflex is largely suppressed, while the sympathoexcitatory reflexes, including the cardiac sympathetic afferent reflex and the arterial chemoreceptor reflex, are augmented [37].

Moreover, other neurohormonal mechanisms, through their interaction with the ANS, can contribute to the progression of cardiac dysfunction in HF. For instance, it has been demonstrated that angiotensin-II can initiate a feedback mechanism leading to the increased sympathetic outflow through the upregulation of the angiotensin-II type 1 receptor and NO inhibition [1].

From the molecular point of view, typical alterations have been described in failing cardiomyocytes, mainly affecting the adrenergic signaling pathway.

The principal features are the decrease in β1-AR density and mRNA levels, uncoupling of β1-AR from Gs and impaired compartmentalization of cAMP/PKA signaling [38, 39]. As previously mentioned, β-AR desensitization and downregulation are predominantly protective mechanisms, which follow the increase in NE plasma levels with consequent receptor overstimulation. β1-AR abnormalities have been attributed to the recruitment of GRK2 to the agonist-bound receptor [40, 41]. In the past decades, it has been demonstrated that an inverse correlation exists between β-AR density and GRK levels in cardiomyocytes [42], and in transgenic mouse models overexpressing the GRK2 inhibitor β-ARKct, myocardial contractility is increased, and HF development is prevented [43]. In 2005, Iaccarino et al. found an inverse correlation between β-ARs expression and GRK2 levels in patients with HF, with lymphocyte levels mirroring myocardial levels. Interestingly, GRK2 levels also correlated with disease severity, suggesting the importance of GRK2 as a potential biomarker for HF [44]. Recently, the data of a prospective study have been published, indicating that in patients with HF undergoing exercise training, GRK2 levels decrease and are also able to predict survival [45].

Further data on the importance of GRK2 in HF came from preclinical studies using gene therapy approaches. A study was performed in which long-term cardiac gene therapy with an adeno-associated vector encoding for -ARKct resulted in sustained improvement of cardiac function, reversal of remodeling, and normalization of the neurohormonal signaling axis in a rodent model of HF [46].

The role of β2-ARs in HF has not been clearly defined yet. In the failing heart, levels of β2-ARs do not change significantly, and studies in transgenic animals have demonstrated that while only fivefold overexpression of β1-AR results in cardiomyopathy, even a 100-fold increase of β2-ARs in the mouse heart does not have any detrimental consequences but, instead, significantly increases cardiac contractile force [20]. This probably needs to be ascribed to the fact that β2-ARs also signal through Gi proteins, which can activate a protective antiapoptotic pathway. Recently, the role of β2-AR has also been studied in a murine postischemic model of HF. Adenoviral-mediated β2-AR overexpression resulted in improved angiogenesis and enhanced coronary reserve and myocardial blood flow in HF mice. This was associated with the activation of the proangiogenic pathway mediated by the vascular endothelial growth factor (VEGF)/protein kinase B (PKB)/endothelial NOS (eNOS) [47, 48].

The role of β3-ARs in HF has not been elucidated. Some evidence suggest that in HF there is an excess of β3-AR signaling, which exerts a negative inotropic effect by increasing NO production and inhibiting calcium transients [49].

α1-ARs, which are involved in cardiomyocyte growth and pathological hypertrophy, may also play a compensatory role in HF in order to preserve cardiac inotropy. In particular, the α1A-subtype has shown to produce prosurvival effects and to protect from maladaptive remodeling both in models of pressure overload and acute myocardial infarction [50].

As for the role of α2-AR in HF, it has been extensively investigated in studies evaluating the contribution of the adrenal gland function in HF, which will be discussed below.

Adrenergic Signaling in Adrenal Gland During Heart Failure

The ANS hyperactivity has been known as a peculiarity during HF [29, 51]. Sympathetic overdrive is strictly implied in the establishment and development of cardiac dysfunction, determines higher risk of arrhythmias, and contributes to worsen the prognosis in patients with HF (increase in plasmatic concentration of catecholamines leads to significant higher mortality) [52].

Indeed, HF is characterized by elevated sympathetic tone, which involves increased levels of circulating and synaptic catecholamines. Cardiac sympathetic nerve activity is significantly augmented in animal models of HF as well as muscle sympathetic nerve activity in patients with HF [53].

Simultaneously, in the adrenal gland, there is an increase in catecholamine output and secretion attested by higher tyrosine hydroxylase levels that is a key enzyme in the production of both NE and Epi.

Chromaffin cells of adrenal medulla are the major source of circulating catecholamines and secrete into the blood 80 % Epi and 20 % NE [54, 55]. The adrenal gland should be considered a specialized sympathetic ganglion with the distinguishing characteristic to excrete its neurohormones directly into the bloodstream [56]. Catecholamines are secreted from chromaffin cells after acetylcholine stimulation of the nicotinic cholinergic receptors; this secretion is regulated by many receptors, among which we mention β2-ARs and α2-ARs that have, respectively, a facilitatory and an inhibitory role [29].

Recently, some studies have shown the crucial inhibitory role of presynaptic α2-AR in peripheral nerve terminals and in adrenal medulla during HF. In particular, α2A- or α2C-AR knockout (KO) mice that underwent HF after transverse aortic constriction manifested an increase in circulating catecholamines and a worst cardiac function compared with control mice [57], while, more interestingly, double α2A/α2C-AR KO mice developed cardiomyopathy at 4 months of age, without any treatment [58]. Moreover, this finding was corroborated by comparable results in human polymorphisms of α2C-AR: α2CDel322–325 in healthy people determines an increase in ANS activity and in plasmatic catecholamine levels during supine rest and an improved pharmacologically induced NE and Epi secretion; besides, the same polymorphisms associated with high HF risk influence the therapeutic effects of the -blocker bucindolol in patients with HF [59–61].

Few years ago, it has been shown that adrenal GRK2 is a physiological regulator of catecholamine production/secretion [62], and its upregulation is fundamental for α2-AR desensitization/downregulation in animal models of HF [55, 63, 64]. In adrenal gland, overexpressed GRK2 phosphorylates α2-ARs determining their

dysfunction and leading to their incapability to inhibit catecholamine production during HF. A gene therapy that inhibits adrenal GRK2 levels (through the peptide β-ARKct) is able to restore α2-AR membrane levels/function and consequently to decrease plasmatic catecholamine levels. Therefore, this sympatholytic therapy contrasts the detrimental cardiac effects of catecholamines and allows re-sensitizing cardiac β-ARs, to decrease left ventricle dilatation and to importantly improve systolic function. Moreover, adrenal GRK2 downregulation has an important role on beneficial sympatholytic effects of β-blockers and exercise training during HF [65, 66].

Hence, since adrenal GRK2 is crucial for α2-AR dysregulation and subsequent increase of circulating catecholamine secretion during HF, its inhibition (through gene therapy, small molecules, or peptides) may represent an innovative sympatholytic therapeutic strategy for HF [67].

Conclusions

Adrenergic system is a key regulator of cardiac activity both in physiologic and in pathological conditions. Indeed, the consequences of sympathetic hyperactivity (and mainly of β-ARs) during HF have been demonstrated to play a fundamental role in the progression of this disease, thus overturning the therapeutic strategies used in the treatment of this and other cardiovascular diseases. Therefore, efforts have been made to further understand adrenergic signaling influencing heart activity, focusing not only on the heart and β-ARs but also on different "players" in the pathogenesis of HF, thus looking at HF as a "systemic" disease involving a dysregulation of various tissues and systems. In this vein, new potential pivotal regulators of sympathetic systems, such as adrenal presynaptic α2-AR or cardiac and adrenal GRK2, have been identified in the last years; such new targets might represent additional, useful tools to increase the therapeutic strategy in the treatment of cardiovascular diseases.

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Chapter 3
The Adrenergic System in Vascular Endothelial Cells

Michele Ciccarelli, Daniela Sorriento and Guido Iaccarino

Introduction

The importance of the endothelium in physiology and pathology relies on its ability to control and modulate the overall vascular functions, including vasculature tone and permeability, thrombosis, hemostasis, and angiogenesis [1–4]. The endothelium is the inner monolayer of the blood vessels but, despite this anatomic simplicity, it represents the largest organ in the body (approximately six tennis courts), and it is able to integrate the overall information coming from the bloodstream and furnishing, in a time- and space-dependent manner, a fine-tuning of the vascular homeostasis by releasing specific factors. For example, endothelial cells (ECs) regulate vascular tone by releasing various relaxing and contracting factors including catecholamines (CAs), nitric oxide (NO), vasoactive peptides, arachidonic acid metabolites, and reactive oxygen species (ROS) [5–8]. Therefore, the endothelium actively regulates vascular tone and permeability, the balance between coagulation and fibrinolysis, the inflammatory activity as well as cell proliferation, and, consequently, normal endothelial function depends on a constant fine tuning and adjustment of opposing forces and effects [9]. The adrenergic system is the major regulator of cardiac and vascular function and this is accomplished also through the activation of a specific receptor localized on the endothelial surface by a local and systemic release of CA. The adrenergic receptors (ARs) are part of a large family of G-protein-coupled receptors (GPCR) and mediate the functional effects of CAs, like epinephrine and norepinephrine. The AR family includes three β(β_1, β_2, β_3), three α1 (α1A, α1B,α1D), and three α_2 (α2A, α2B, α2C) receptor subtypes.

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Vascular β-ARs mediate adrenergic vasorelaxation through direct activation of vascular smooth muscle cells, while it is relatively more recent knowledge that β-ARs, and, in particular β-$_2$AR, are also localized on the endothelium, where they regulate vasorelaxation through NO release and promote neoangiogenesis [10, 11]. Other subtypes are localized on the endothelium such as α_1-AR but, inversely from β-$_2$AR, their activation inhibits neoangiogenesis [12].

These receptors actively participate in the release of nitric oxide (NO) in order to regulate endothelial function [13–15]. NO plays a crucial role in endothelium homeostasis, with important vasodilatory, antithrombotic and anti-atherogenic properties [16]. Following its release, NO diffuses to the subjacent vascular smooth muscle where it elicits vasorelaxation through the activation of soluble guanylyl cyclase (sGC) enzyme, which then catalyzes the formation of cyclic guanosine monophosphate (cGMP) and hence the activation of cGMP-dependent protein kinase [17, 18]. Classically, NO is released from ECs following the activation of the endothelial or type 3 isoform of NO synthase (NOS-3 or eNOS), which is a Ca^{2+}- and calmodulin-dependent enzyme, and hence many endothelium-dependent vasodilators cause NO release via an increase in intracellular Ca^{2+} [3, 19, 20]. However, other pathways have been also identified to act in a Ca^{2+}-independent manner and involve phosphorylation of various eNOS serine residues by a number of protein kinases [21, 22]. This mechanism is particularly evident for the β-$_2$AR signaling, where the activation of eNOS involves specific kinases such as protein kinase A (PKA) and Akt [23].

It is thus not surprising that the alteration of endothelial function is an important player in both development and progression of pathologies and in particular of cardiovascular disease. The impaired ability of the vascular endothelium to stimulate vasodilation is referred to as “endothelial dysfunction” and the major cause is the decreased bioavailability of NO in different conditions which can be due to various mechanisms: reduced eNOS expression, altered NO production, and increased NO catabolism. Endothelial dysfunction plays a key role in the development of cardiovascular disease such as hypertension, type 2 diabetes and heart failure [9, 16, 24]. In particular, adrenergic vasorelaxation has been demonstrated to be impaired in hypertensive patients, probably due to the presence of increased desensitization and impaired signaling of β-AR [25]. ARs on endothelium have for long not been considered functional in the regulation of the vascular tone. On the contrary, it is possible to identify very specific roles for such receptors in several endothelial functions as will be described below.

Adrenergic System and Endothelial NO Release

NO is a small gaseous molecule which modulates crucial functions including blood flow, platelet aggregation, and neural activity [26]. This molecule is synthesized from L-arginine by three isoforms of NO synthases (nNOS, iNOS, and eNOS) and exerts its activity essentially by stimulating sGC to increase the levels of the second

messenger cGMP. Both neurotransmitters and hormones released from the autonomic nervous system cooperate to preserve the balance between vasoconstriction and vasorelaxation and to control cardiac muscle cells' function, and it is now generally accepted that NO exerts a critical role in this context. ECs express, in heart and vessels of a variety of species including humans, eNOS, an isoform that is activated to produce NO in response to stimulation of both adrenergic and muscarinic cholinergic receptors in cardiac myocytes [27]. Many studies demonstrated that vascular endothelial cells also express β-ARs [10, 28, 29], where β-adrenergic effects on the vessels are facilitated. The main mechanism leading to increased eNOS activity in ECs is calcium dependent [30], but phosphorylation at several loci of the NOS proteins has been recognized as an additional pathway to induce both activation and inhibition of eNOS activity [31, 32]. It is now recognized that β-ARs located in the endothelium play an important role in the relaxation response to CAs [33, 34], which appear mainly related to the presence of the β-$_2$AR. Indeed, studies carried out in humans, in umbilical veins in vitro [6] or in the forearm in vivo [35], showed that vasorelaxation to isoproterenol is abolished by the selective β-$_2$AR antagonist ICI-118,551 and remains unchanged in the presence of the β-$_1$AR-selective antagonist CGP-20712, indicating that, as in the vascular smooth muscle cells [36], the endothelial β-ARs are exclusively or (at least) predominantly of the β_2 subtype [6, 35]. The mechanism of eNOS activation following β_2-AR stimulation is known to be Akt dependent. Indeed, the activity of eNOS is both calcium/calmodulin [37] and Akt phosphorylation dependent (at Ser1177) [38, 39]. This latter mechanism is relevant to many signal transduction pathways, since several kinases can phosphorylate this site, including PKA, PKC, calmodulin-dependent protein kinase II (CaMKII), and Akt [40–42]. Indeed, endothelial activators such as vascular endothelial growth factor (VEGF), bradykinin, insulin, acetylcholine, isoproterenol, and fluid shear stress induce NO production through phosphorylation of eNOS at Ser1177 induced by the serine/threonine kinase Akt [40, 41, 43, 44]. This kinase is primarily activated in response to stimulation of transmembrane receptors with intrinsic tyrosine kinase activity or indirectly coupled to tyrosine kinases or to seven-transmembrane GPCRs [45–47]. Therefore, Akt acts as an integrator of different signal transduction pathways converging on eNOS, including endothelial β_2-AR. β_2-AR signals to Akt independently of a G_s-cyclic adenosine monophosphate (cAMP)-dependent intracellular pathway [48] but involves instead a G_i–Src axis (Fig. 3.1). Specifically, it has been demonstrated that PKA-dependent phosphorylation of the third intracellular loop of the β_2-AR increases the affinity of the receptor for G_i proteins [49, 50]. This switch has two consequences: first, it decreases the rate of cAMP generation, since G_i activation inhibits adenylyl cyclase; second, it increases cAMP-independent signaling through G_i, such as activation of the extracellular signal-regulated kinases ERK1/2 and PI3K [51–55]. G_i-coupled receptors have been shown to regulate nonreceptor tyrosine kinases, such as Src, which acts as an intermediate between G_i and other molecules like Ras, PI3K [54, 56], Akt, and thus eNOS. β_3-AR is mainly located on ECs and acts in conjunction with β_1-AR and β_2-AR to mediate relaxation through the activation of NO synthase pathways and subsequent increase in tissue cGMP content and is reduced by endothelium

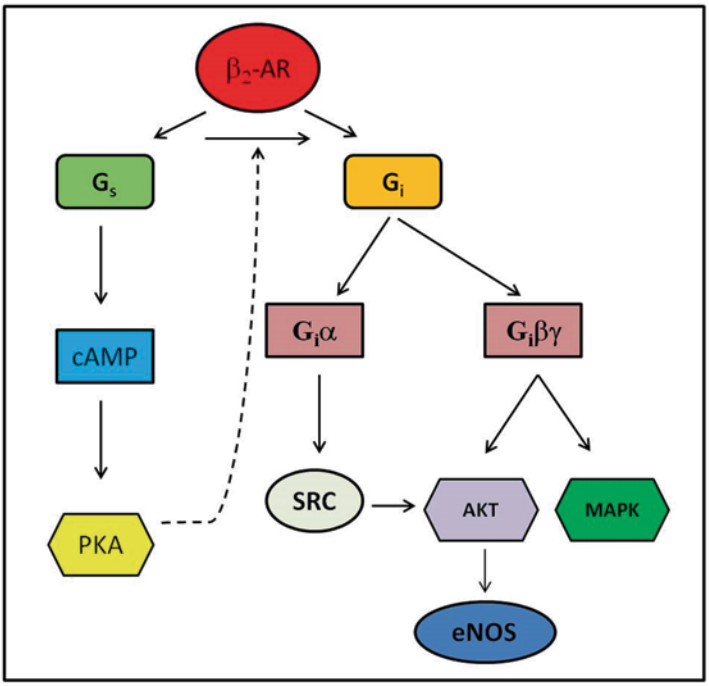

Fig. 3.1 Schematic representation of β_2-AR signaling to eNOS and the role of the G_i–Src–Akt axis. See text for details and molecular acronym definitions. *cAMP* cyclic adenosine monophosphate, *PKA* protein kinase A, *β_2-AR* $\beta_2$adrenergic receptors, *MAPK* mitogen-activated protein kinase, *eNOS* endothelial NO synthase

removal or in the presence of monomethyl-L-arginine, monoacetate (L-NMMA) [57]. This β_3-AR-mediated aortic relaxation seems to be independent of G_i protein stimulation, since the blockade of G_i proteins by pertussis toxin does not modify β_3-AR agonist-induced relaxation. On the contrary, selective blockers of K_{Ca}, K_{ATP}, and K_v channels decreased β_3-AR agonist-induced relaxation. Thus, it appears that this effect results from the activation of several potassium channels (K_{Ca}, K_{ATP}, K_v) [58]. Another intriguing mechanism in eNOS regulation upon adrenergic stimulation involves the G-protein-coupled receptor kinases (GRKs). In order to control their overstimulation, β-ARs are phosphorylated by several kinases to induce receptor desensitization [59, 60]. Both β_1-AR and β_2-AR can be phosphorylated by PKA and by certain GRKs. Because PKA can phosphorylate β-ARs in the absence of agonist, PKA is thought to mediate heterologous (agonist-independent) desensitization without affecting homologous (agonist-dependent) desensitization. In contrast to PKA, GRKs selectively phosphorylate agonist-occupied receptors, primarily on Ser/Thr residues located in their carboxyl-terminal tails. Among the different GRKs, GRK2 has been involved at the level of eNOS regulation. When the receptor is activated by an agonist, GRK2 translocates to the plasma membrane by

means of its interaction with free $G_{\beta\gamma}$ subunits and induces receptor desensitization [61, 62]. Indeed, following GRK2-mediated phosphorylation of β-ARs, β-arrestins are recruited to these receptors and these adaptor molecules block further G protein activation [63] while promoting internalization of the receptors leading to their degradation or resensitization (recycling to the membrane) [64]. In terms of eNOS activation and regulation, GRK2 can interact with eNOS directly or through Akt [65, 66]. In both mechanisms, GRK2 appears to reduce eNOS activity and this is particularly evident in pathophysiological conditions like hypertension [25]. Of note, GRK2 appears also to modulate Akt/eNOS activation by non-GPCR receptors such as the insulin receptor (IR). Indeed, IR is thought to promote eNOS activation by a complex interaction involving β-arrestin2, Akt, and eNOS. Increased levels of GRK2 at the plasma membrane upon insulin stimulation, as observed in ob/ob mice, interfere with this signaling, preventing β-arrestin 2 binding to Akt [67].

There is also evidence for the functional presence of vasorelaxant α_1-AR in the brachial and pulmonary arteries isolated from rabbit and rat, respectively [13, 68]. According to these reports, the pharmacological stimulation of α_1-AR located on ECs, is able to generate NO, whereas the stimulation of α_2-AR releases a relaxing prostanoid [13, 68]. Filippi et al. demonstrated that nanomolar concentrations of phenylephrine, which are devoid of any contractile effect, induced a slight endothelium-dependent vasorelaxation in the rat mesenteric vascular bed through the stimulation of α_{1D}-AR, located on ECs, which act through phospholipase C stimulation, followed by IP_3 generation and NO synthase activation. Conversely, the increase in perfusion pressure induced by micromolar concentrations of phenylephrine is attributable to the stimulation of α_{1A}-AR [69]. α_2-AR agonists cause endothelium-dependent relaxation that is reduced or abolished by inhibitors of L-arginine/NO. The activation of α_2-AR on ECs stimulates the release of NO, an action that would tend to attenuate vasoconstriction produced by the activation of postsynaptic vascular α_1-ARs [19, 20, 70]. The α_2-AR subtype that causes endothelium-dependent relaxation is the $\alpha_{2A/D}$ subtype, despite the prominent presence of α_{2C}-ARs (77 α_{2C} vs. 23 % $\alpha_{2A/D}$) [22]. It appears that this ratio may not be constant, since it varies within the vascular bed. Indeed, Bockman et al. demonstrated that, in the rat mesenteric artery, the α_2-AR is coupled to endothelium-dependent NO-mediated relaxation and is of the $\alpha_{2A/D}$ subtype (appearing in its α_{2D} version) [21]. It has been demonstrated that endothelium-dependent relaxation to α_2-adrenergic agonists is prevented by pertussis toxin [24, 71–75], suggesting the involvement of G_i proteins in the signal transduction from the receptor to the activation of NO synthase [14, 76]. Indeed, α_2-adrenergic agonists cause the activation of G_i proteins in ECs and stimulate NO synthase activity [77, 78]. Surprisingly, cAMP is not involved in the signal transduction pathway of $\alpha_{2A/D}$-AR-mediated NO formation [21]. Indeed, the use of forskolin to oppose α_2-AR-mediated inhibition of cAMP formation in endothelium did not affect the relaxant response to α_2-AR agonists, suggesting that cAMP is not involved in the coupling of α_2-AR to NO. There is a physiological modulation of endothelium-dependent relaxation to α_2-adrenergic agonists. Such relaxation is upregulated by a chronic increase in blood flow [79] or exercise training [80].

Adrenergic System and Angiogenesis

Vascular growth is a complex process involving both angiogenesis (creation of new capillaries) and arteriogenesis (enlargement and remodeling of preexisting collaterals) [81]. More specifically, the term angiogenesis refers to the sprouting, enlargement, or intussusceptions of new endothelialized channels and is tightly associated to ECs proliferation and migration in response to angiogenic stimuli, in particular hypoxia. Arteriogenesis is, instead, a result of growth and positive remodeling of preexisting vessels, forming larger conduits and collateral bridges between arterial networks via recruitment of smooth muscle cells. In particular, angiogenesis has long been known to be a highly ordered multistep molecular process under tight regulation by ECs [82] and closely associated with EC proliferation and migration and to the capability of these cells to modulate the levels of VEGF, the most important cytokine system involved in the formation of new vessels [83]. However, a series of biological, chemical, and hormonal effectors can interfere with this process, including the adrenergic system through activation of the different receptor subtypes with also contrasting effects in promoting or inhibiting angiogenesis. β_2-AR is an endogenous mediator of angiogenesis in vivo, as demonstrated in a rat model of neovascularization and adrenergic activation attributable to hind-limb ischemia and later confirmed by the defective angiogenesis in mice knockout for β_2-AR [11, 12, 84]. Mechanistic studies show that β_2-ARs control specific phenomena involved in angiogenesis such as EC proliferation and stimulating proapoptotic and antiapoptotic pathways. Indeed, β_2-AR exerts a positive effect on EC ERK/MAPK activation by at least two mechanisms: first, stimulation of endothelial β-ARs directly activates ERKs; second, β_2-AR stimulation can induce the release of VEGF, which can also activate ERKs [11, 85].

The α_{1A}- and α_{1B}-AR subtypes, but not the α_{1D} subtype, are expressed in cultured rat aorta ECs. The activation of these α_1-ARs in ECs provide a negative regulation of angiogenesis [12]. Indeed, pharmacological antagonism of α_1-AR in ECs from Wistar-Kyoto rats by doxazosin enhanced, while stimulation of these ARs with phenylephrine, inhibited endothelial mechanisms of angiogenesis such as cell proliferation and DNA synthesis, ERK and retinoblastoma protein (Rb) phosphorylation, cell migration, and tubule formation [12]. A similar phenotype can be observed in vivo, since an increased α_1-AR density in the ischemic hind limb, compared to nonischemic hind limb, suggested an enhanced α_1-AR tone in the ischemic tissue. Treatment with doxazosin did not alter systemic blood pressure but enhanced neoangiogenesis in the ischemic hind limb [12].

ECs and CA Synthesis and Release

ECs are capable of synthesizing and releasing a variety of substances that may exert autocrine, paracrine, or endocrine effects and their function is fine-tuned by several hormones, such as CAs. CAs cause vasoconstriction via α_1- and α_2-ARs and

vasodilation via β_2-ARs in vascular smooth muscle [86]. They are synthesized from the amino acid precursor L-tyrosine. Tyrosine hydroxylase (TH) is the first rate-limiting enzyme in CA synthesis that catalyzes the conversion of tyrosine to L-dihydroxyphenylalanine. The latter is converted to dopamine by L-DOPA decarboxylase (DDC). In turn, dopamine is converted to norepinephrine (NE) by dopamine β-hydroxylase (DBH), and NE is converted to epinephrine (Epi) by phenylethanolamine-*N*-methyl-transferase (PNMT) [87]. Once released, CAs are quickly inactivated by two enzymes that are responsible for their catabolism, catechol-*O*-methyl-transferase (COMT) and monoamine oxidase (MAO) [87]. MAO catalyzes the oxidative deamination of amines, and COMT methylates the meta-hydroxyl group of CAs.

Initially, circulating CAs were thought to be produced exclusively by the adrenal medulla [88]; over the past few years, however, a growing list of peripheral cell types, such as macrophages, lymphocytes, and even cardiac cells, have been shown to be able to synthesize and release CAs on their own [89–94]. Macrophages, for instance, not only respond to CAs but can also produce them, essentially operating on an autocrine feedback loop in which CAs produced and released by the macrophage activate ARs to regulate interleukin (IL)-1β production, which has a key role in inflammatory responses [95]. ECs have also been added to this list. ECs are capable of synthesizing CAs, because they have the complete intracellular machinery for the generation and release of NE and Epi ([96]. In vitro, ECs express all of the enzymes involved in the synthesis of CAs under normal conditions, and this expression is enhanced in response to specific stimuli such as hypoxia. The molecular mechanism that regulates this phenomenon during hypoxia involves activation of PKA/CREB (cAMP response element-binding protein) signaling, which is known to regulate most important endothelial functions, such as eNOS gene transcriptional activation [97]. Indeed, overexpression of both CREB and PKA enhances DBH and PNMT gene expression and CA release, whereas H89, an inhibitor of PKA, exerts the opposite effect, establishing the role of the PKA/CREB pathway in the regulation of CA release in ECs (Fig. 3.2). These results are corroborated by data from an in vivo model of chronic ischemia, where it is demonstrated that TH, DDC, DBH, and PNMT are expressed in the endothelium of the femoral artery after ischemia.

Pathophysiological Implications

Regulation of Vascular Tone and Endothelial Dysfunction

Under physiological conditions, vascular tone is tightly regulated by the adrenergic system, resulting from a balance between α_1-AR-mediated vasoconstriction and β_2-AR-mediated vasodilatation. Abundant evidence indicates that the defective β-AR-dependent vasodilation during pathological conditions, such as hypertension, is related to the reduced NO production and release, part of a complex state of

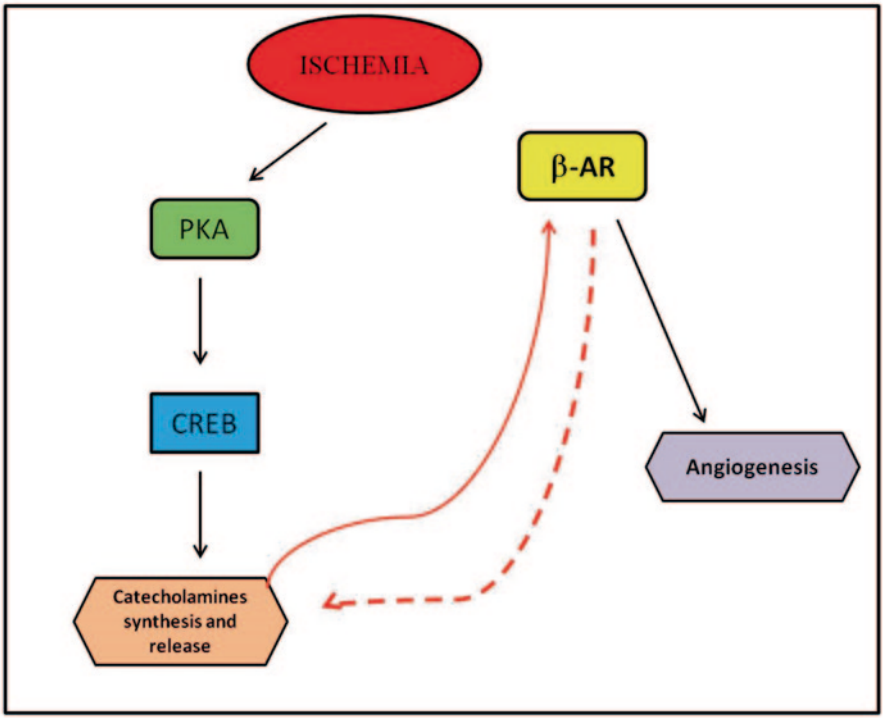

Fig. 3.2 Schematic representation of the regulation of CA synthesis and release in ECs during chronic ischemia. See text for details and definitions of molecular acronyms. *PKA* protein kinase A, *CREB* cAMP response element-binding protein, *β_2-AR* $\beta_2$adrenergic receptors

impaired endothelial function also known as endothelial dysfunction. The link between endothelial dysfunction and vascular diseases is well established [98]. It is known, for instance, that impairment of endothelial function precedes atherosclerosis [7, 9, 16].

As described above, β_2-AR activation at the endothelial level induces the production and release of NO, causing vasodilation [10]. In the hypertensive state, this equilibrium is shifted toward increased vasoconstriction, likely because of a defective vasodilatation in response to β_2-AR stimulation. Indeed, β-AR agonist administration in the human brachial artery induces vasodilatation and, interestingly, this response is attenuated in hypertensive patients [25, 99]. The role of β_2-AR in the vasculature appears to be so critical that genetic variants of this receptor, causing excessive desensitization, may lead to reduced vasodilation [100, 101] and may also promote the occurrence of atherosclerosis [102, 103]. The importance of such a strong molecular interconnection between the adrenergic system and NO release has been also recently demonstrated in several studies with nebivolol conducted in animals and in humans. Nebivolol is a third-generation β-blocker used in the treatment of hypertension which induces vasodilation by increasing NO production. Nebivolol has a distinctive profile among β-blockers, with the greatest selectivity

for cardiac β_1-ARs and the highest β_1 (over β_2) selectivity compared with other β-blockers, and no effect on α-receptors. Its ability to enhance NO release has been observed in the human forearm where its vasodilatative effects were blunted by the eNOS inhibitor, L-NG-nitroarginine methyl ester (L-NAME) [104]. Moreover, when compared to atenolol (another β-blocker), nebivolol showed ability to reverse endothelial dysfunction in hypertensive patients [105]. In addition, nebivolol exerts systemic antioxidative properties and this effect is purported to be an additional factor for increasing NO bioavailability. For example, nebivolol and atenolol similarly reduce blood pressure in hypertensive patients, but oxidative stress markers, such as low-density lipoprotein (LDL) hydroperoxides, 8-isoprostanes, and ox-LDL, were significantly improved only with nebivolol. The mechanism by which nebivolol acts on NO bioavailability is still unclear, with some experimental evidence suggesting activation of β_3-AR [106–108].

GRK2 and Endothelial Dysfunction

As mentioned above, GRKs play significant roles in adrenergic regulation of endothelial function and their influence is particularly evident in the setting of pathological conditions like hypertension and diabetes. For example, GRK2 participates in the determination of animal model of portal hypertension, relying on its physical interaction with Akt [66]. Since Akt is able to activate eNOS, thereby mediating vasodilation, the GRK2-mediated inhibition of Akt has been shown to shift the vascular tone equilibrium toward constriction, in the setting of endothelial dysfunction, due to decreased eNOS activity [66, 109]. Notably, endothelial modulation of the contractile state of vascular smooth muscle has been shown to be impaired in atherosclerosis and in several conditions known to be associated with the premature development of atherosclerosis. Two different reports, both showing that reducing GRK2 activity can reduce the endothelial dysfunction observed in the hypertensive vasculature, support the role of GRK2 in the pathogenesis of arterial hypertension. The first study demonstrated that, in the aged rat, a model of impaired β-AR signaling, chronic exercise may lead to decreased GRK activity in the vasculature, in particular in the endothelium, and that this reduction mirrors an ameliorated vasodilatation [110]. The other report showed the preventive effect of a specific GRK2 inhibitor on vascular dysfunction in diabetic mice, ameliorating Akt/eNOS impairment and improving the translocation of β-arrestin2 to the plasma membrane [111]. Consistent with these data, acute inhibition of GRK2 using the nonselective inhibitor heparin led to diminished desensitization of isoproterenol-dependent vasodilation in normotensive subjects [25]. Interestingly, heparin administration in hypertensive patients was able to restore the impaired β-AR vasodilation, ameliorating local resistance [25]. Remarkably, the correlation between GRK2 levels and hypertension is also present in other conditions characterized by increased blood pressure, including the aforementioned portal hypertension [66] and preeclampsia [112]. In gestational hypertension, the increase in GRK2 in the fetal–placental vas-

culature seems to be compensatory rather than causative of increased blood pressure, in order to balance the excessive vascular tension, since the lack of the protective effect of elevated GRK2 expression negatively affected the outcome of the hypertensive state [66]. Therefore, if increased levels of GRK2 are detrimental for EC function, their excessive reduction is also deleterious for cell survival. GRK2 is needed for embryonic development [113] and, in adult life, cardiac-specific GRK2 deletion alters the cardiac hypertrophic response to chronic β-AR stimulation, leading to an eccentric dilatation of the heart similar to that observed in intermediate–advanced phases of heart failure [114, 115]. This kinase appears also fundamental for EC integrity during adult life. The absence of GRK2 in ECs induces cytokine production and macrophage migration into the vascular media, where they release metalloproteinases (MMPs) [7, 116], such as MMP-2 and MMP-9, corrupting the elastic and the connecting fibers that extend through fenestration of the parallel elastic lamellae. These structures are pivotal in maintaining integrity and mechanical properties of the aortic wall [117] and their disruptions are implicated in disease processes, such as atherosclerosis [118], aneurysm formation [119], and in aging [120]. Early atherescloretic lesions can be indeed observed in mice with specific endothelial deletion of GRK2 which also show defective angiogenesis [7, 121]. It is likely that either elevated or reduced levels of GRK2 affect cellular function and survival and this may appear contradictive to the notion of GRK2 inhibition or downregulation as a therapeutic strategy. A reconciling hypothesis might be that GRK2 interacts with and regulates several substrates through its kinase activity, as well as its regulator of G protein signaling (RGS) and plekstrin homology (PH) domains, rendering it capable of multiple regulatory roles inside the cell [122–124]. Moreover, the most effective therapeutic strategy aiming at counteracting GRK2-mediated receptor desensitization at the plasma membrane level has used the carboxyl-terminal portion of GRK2 (β-ARKct), whose mechanisms in animal models of cardiovascular disease are not completely understood. B-ARKct represents the carboxyl-terminal portion of GRK2, which retains the ability to localize at plasma membrane–residing free Gβγ subunits through its PH domain, but lacks the kinase activity [125, 126]. B-ARKct thus displaces GRK2 from plasma membranes but does not inhibit its kinase activity; thus, GRK2 is theoretically free to move into other cellular compartments like mitochondria where it accomplishes protective roles as promotion of mitogenesis and cellular adenosine triphosphate (ATP) content preservation. Therefore, any potential therapy involving tight modulation of GRK2 activity/levels must take into account the specific role played by GRK2 in the various subcellular compartments.

Angiogenesis in Vascular Diseases and Tumors: Role of the Adrenergic System

Angiogenesis is considered an important feature of a viable endothelium. Its mechanism entails specific, composite, and coordinated sequences [82] of several cellular and molecular processes, intimately regulated by the ECs [127]. Proliferation,

cell migration, and tubule formation by ECs represent the first steps in angiogenesis, leading to the sprouting of immature sinusoids around which a more complex capillary will develop [128]. The connection between angiogenesis and ECs is so close that angiogenesis is now considered to be an aspect of endothelial function and several models of endothelial dysfunction show impaired angiogenesis. As described above, the adrenergic system is pivotal in promoting angiogenesis and this is evident in pathological conditions as chronic ischemia where endogenous CA can activate both endothelial α_1- and β_2-ARs with opposite effects on EC proliferation, migration, and survival. Apart from the potential clinical and therapeutic applications in cardiovascular disease, in particular during chronic ischemia, it is not surprising that these discoveries can be applied also to oncology. The connection between the adrenergic system and tumor progression has been evidenced in stress conditions where the stress derived from social isolation was found to elevate the tumor NE levels in ovarian cancer patients, and its level was correlated with tumor grades and stages [129]. In particular, pancreatic, breast, ovarian, and colorectal cancers have been extensively investigated about the effects of β-ARs in preclinical and clinical settings [130–132]. A study from Thaker and colleagues revealed that chronic stress could elevate tumor NE levels in an orthotropic ovarian cancer in a mouse model and obviously increased tumor burden and aggressiveness of tumor growth [133]. Propranolol, a nonselective β-AR antagonist, completely abolished the effects of chronic stress on tumor growth. In contrast, the β_2-AR-selective agonist terbutaline produced a similar increase in tumor weight just like under chronic stress. Even though the molecular mechanisms are not completely understood, studies in prostate and breast cancer cells also demonstrated that Epi stimulation reduced the sensitivity of cancer cells to apoptosis through β_2-ARs/PKA/inactivation of BAD (proapoptotic protein BCL2-associated death promoter) [134]. Preclinical models further confirmed that stress hormones like Epi promoted prostate carcinogenesis through inhibition of apoptosis and tumor involution mediated by an Epi/β_2-AR/PKA/BAD antiapoptotic signaling pathway [135]. Moreover, Epi and NE can induce proliferation of colorectal cancer cells preferentially through the β_2-AR [132, 136]. Apart from effects on cancer cell proliferation, the adrenergic system can intervene also in angiogenesis, which is known to be essential for tumor growth and metastasis. Several cancer models have shown that Epi and NE can upregulate VEGF and induce tumor angiogenesis and aggressive growth [133, 137–139]. Other angiogenic factors, such as IL-6, IL-8, MMP2, and MMP-9, can be elevated by Epi and NE in a variety of cancer cell lines via β-AR signaling [133, 139, 140]. Therefore, the administration of β-AR antagonists like propranolol could completely abrogate the secretion of these factors and their mediated functions, implying that β-blockers have potential therapeutic value for the management of relevant cancers. This hypothesis is supported by in vivo models of proliferative retinopathies which showed a strong inhibitory role against vascular changes exerted by the blockade of specific β-ARs. In particular, β_2-AR seems to be mostly involved in these responses, and the β_1-/β_2-AR blocker propranolol is highly effective at inhibiting both the increase of VEGF expression caused by a hypoxic insult and the consequent neovascular response. These observations have prompted clinical trials in preterm

infants with retinopathy, where oral administration of propranolol produced positive results in terms of efficacy, although safety problems were also reported [141].

The Latest: ECs Synthesize and Release CAs

As demonstrated in other cellular systems, ECs are capable of producing and releasing CAs. These cells possess the entire enzymatic machinery for synthesis and release and are also capable of metabolizing NE and Epi because they also express COMT and MAO. In addition, it appears that this autocrine feedback catecholaminergic loop of the ECs is a positive feedback one, i.e., it leads to further enhancement of CA synthesis and secretion, because β_2-ARs present in EC membranes can also stimulate CA synthesis via coupling to the classic Gs protein–adenylyl cyclase–cAMP–PKA–CREB signaling pathway [5]. Therefore, the CAs released by the EC can activate the β_2-ARs on its surface and further stimulate their own production. The full pathophysiological implications of this have not yet been elucidated; it appears however that this ability of ECs to produce and release their own CAs serves primarily as a homeostatic, self-protective mechanism to defend the cell against vascular injury/stress and ischemia. Indeed, in mice experiencing hind-limb ischemia secondary to surgical removal of their common femoral artery, expression of the CA biosynthetic enzymes is elevated in the femoral artery and capillary endothelium of the afflicted hind limbs, indicating increased endothelial CA production in response to chronic ischemia in vivo [5]. Thus, ECs appear to be equipped with a "custom-made," ready-to-respond CA synthesis and release apparatus, which enables them to promptly respond to a vascular injury, a stressful insult, or a hypoxic/ischemic emergency by initiating in situ CA-dependent neoangiogenesis [142]. These findings may have important implications for antihypertensive therapy, as well: Given that some of the most useful antihypertensive drugs are β-blockers, and EC-residing β_2-ARs appear instrumental in both CA production and CA-driven angiogenesis by the ECs, it follows that nonsubtype selective β-blockers might be associated with impaired endothelial angiogenesis [142]. Indeed, several lines of evidence indicate that this type of β-blockers might actually be deleterious in heart disease treatment [143]. Thus, β_1-AR-selective agents, especially those that combine vasodilatory action, might be preferable for antihypertensive therapy, at least from the endothelial function standpoint [142].

Conclusions

In the past years, great advances have been made in the study of AR signaling and function in the endothelium thanks to the development of new technologies. Indeed, transgenic mouse models have significantly improved our understanding of the mechanisms of action of specific drugs in vivo. The ability to induce transgene

expression at defined times or in defined tissues is an important tool, as well as the ability to induce or repress the expression of endogenous genes in a developmental or tissue-specific fashion. Indeed, deletion of the genes encoding for AR subtypes has helped identify the specific subtypes which mediate in vivo effects of specific drugs. Thus, the combination of molecular biological, genetic, and pharmacological techniques greatly facilitates our understanding of AR function in vivo, and, in turn, leads to more effective and specific therapeutic treatment in humans. β-ARs, for instance, are already target of therapeutic intervention in many diseases: β-AR stimulation in asthma and obesity or β-AR blockade in hypertension and coronary insufficiency. In conclusion, given the importance of endothelial function in most physiological and pathological conditions, it is clear that the increasing knowledge of the adrenergic system's function in the endothelium is instrumental for future progress in its translation to clinical applications.

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Chapter 4
The Adrenergic System in Vascular Smooth Muscle

James R. Docherty

Introduction

This chapter looks at vascular adrenoceptors, the receptors in blood vessels that mediate the actions of the neurotransmitter noradrenaline and the neurohormone adrenaline.

Definition Adrenoceptors, or adrenergic receptors or adrenoreceptors, can be defined as cell membrane receptors, belonging to the seven transmembrane-spanning G-protein-linked superfamily of receptors, which respond to the physiological agonists noradrenaline and adrenaline by producing a response in the cell, involving a second messenger system or an ion channel linked via the aforementioned G protein. The name adrenoceptor seems more appropriate than noradrenoceptor (named after the neurotransmitter) since, unlike adrenaline, which is a fairly potent activator of all subtypes of adrenoceptor, noradrenaline is a fairly weak activator of the β_2-adrenoceptor. Adrenoceptors have been subdivided pharmacologically by the use of selective ligands, usually receptor antagonists, and have been definitively identified in terms of genes and cloned receptors. Adrenoceptors can be divided into two broad categories, α and β, or more correctly into three major subcategories, α_1, α_2 and β.

In terms of physiological function in vascular smooth muscle, these receptors can be divided into two broad classes. Simply said, α-adrenoceptors mediate vascular smooth muscle contraction and β-adrenoceptors mediate vascular smooth muscle relaxation.

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History of Adrenoceptors

The breakthrough in the classification of adrenoceptors came when Ahlquist [2] described two types of adrenoceptors in studies investigating the actions of six agonists and the antagonistic actions of ergot alkaloid drugs. Previous studies had identified two types of action involving endogenous adrenergic agonists, but this study put adrenoceptor subtypes on a clear pharmacological footing. This delineation into α- and β-adrenoceptors was based on the rank order of potency of the series of agonists, but particularly isoprenaline, adrenaline and noradrenaline. The receptor termed β was mainly inhibitory, except in the heart, and the receptor termed α was mainly excitatory, except in the intestine. In Ahlquist's classification, α-adrenoceptors were receptors present on smooth muscle causing contraction, and the β-adrenoceptors were present in the heart, where they were excitatory, and in smooth muscle where they were inhibitory. This classification only gradually became accepted, but is now seen as the major breakthrough (see Fig. 4.1).

Further studies were hampered by the lack of an antagonist at β-adrenoceptors, until Powell and Slater [111] reported dichloroisoprenaline as the first useful β-blocker. Furchgott [51], using α- and β-adrenoceptor antagonists, confirmed and expanded the results of Ahlquist on intestinal smooth muscle, demonstrating that both α- and β-adrenoceptors were involved in the inhibition.

The Ahlquist classification was refined by the identification of two subtypes of β-adrenoceptor, based on the affinities of adrenaline and noradrenaline, where β_1-adrenoceptors were mainly cardiac and involved in excitation and stimulated by both adrenaline and noradrenaline, and β_2-adrenoceptors were mainly involved in smooth muscle relaxation and the targets of adrenaline [77] (see Fig. 4.1). A third

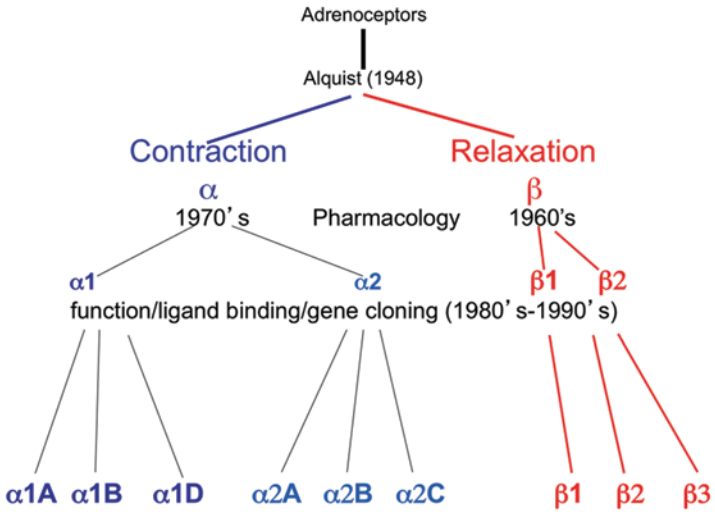

Fig. 4.1 How the subclassification of adrenoceptors has developed since 1948

β-adrenoceptor was later identified [42] and shown to be involved in β-adrenoceptor-mediated relaxations resistant to propranolol and termed the β_3-adrenoceptor [18] (see Fig. 4.1). A fourth β-adrenoceptor termed β_4 and involved in vascular relaxations is now known to be a low-affinity state of the β-adrenoceptor[89].

The next major development in adrenoceptor classification did not occur until 1974, but the detail does not directly concern vascular adrenoceptors. Basically, agonists and antagonists at α-adrenoceptors were found to reduce or increase, respectively, the release of noradrenaline evoked by nerve stimulation from adrenergic nerves, and evidence accumulated to show that these actions were mediated by α-adrenoceptors on the nerve terminals, so-called presynaptic or prejunctional receptors [78, 127].

Once the concept of prejunctional and postjunctional α-adrenoceptors had been accepted, differences between pre- and postjunctional α-adrenoceptors pharmacologically, in terms of the relative potencies of a series of agonists and antagonists, led to the further subclassification of α-adrenoceptors into α_1-postjunctional and α_2-prejunctional. Later, it became clear that α_2-adrenoceptors may also be present postjunctionally, so that this classification was expanded into a purely pharmacological subclassification, independent of location [10] (see Fig. 4.1).

Further refinements in the classification of adrenoceptors have come from the development of new methodologies for the study of receptors. The first of these was the radioligand-binding assay which, beginning in the mid-1980s, began to demonstrate that there were subtypes of α_1-adrenoceptors [61, 97], α_2-adrenoceptors [17] and β-adrenoceptors [42]. However, the relationship between affinity at ligand-binding sites and potency at functional receptors was not always easy to determine [32]. The study of adrenoceptors was revolutionised by the techniques of molecular biology, which definitively defines receptors as gene products. Nine genes for adrenoceptors have now been identified and sequenced (α_{1A}, α_{1B}, α_{1D}; α_{2A}, α_{2B}, α_{2C}; β, β, β) and species orthologues have been identified allowing simplification of classification (human α_{2A} and rat α_{2D} ligand-binding sites are now simply classed as α_{2A}) [18]. Studies of subtypes of receptor have been made easy by transfection of genes into suitable cell lines to produce pure populations of recombinant receptors.

The object of this chapter is to look at the physiological function of subtypes of adrenoceptors in the vascular system, looking at each subtype individually. Figure 4.1 shows the historical developments in our knowledge of the subtypes of adrenoceptor, and the nine established subtypes of adrenoceptor [see 34].

Smooth Muscle α_1-Adrenoceptor Subtypes

α_1-Adrenoceptors were initially subdivided into α_{1A} and α_{1B} subtypes in ligand-binding studies, based on the affinities of a series of ligands, especially WB 4101 and prazosin [97], and based on the ability of the alkylating agent chloroethylclonidine to inactivate the α_{1B} but not the α_{1A} subtype [61].

Molecular cloning techniques revealed initially four subtypes of α_1-adrenoceptor [18]. The α_1B-adrenoceptor subtype was the first to be cloned, from the hamster [26], and this clone was so named because it expressed a protein with the radioligand binding properties of the α_1B-adrenoceptor. Other clones were rat α_{1A} [85], bovine α_{1C} [118] and rat α_{1D} [108]. However, whereas the α_{1C} clone represented the α_{1A}-ligand-binding site, the α_{1A} and α_{1D} clones appeared to represent the same subtype: a novel subtype of α_1-adrenoceptor that was termed to avoid confusion, α_{1D}. These clones have now been renamed to match the functional receptors: α_{1A} (formerly α_{1C}), α_{1B} (formerly α_{1B}) and α_{1D} (formerly α_{1A}/α_{1D}).

Vascular Responses Mediated by α_{1A}-Adrenoceptors

Contractions are reported to be mediated at least partly by α_{1A}-adrenoceptors in a number of tissues including rat mesenteric artery (see Fig. 4.2), rat renal artery (also α_{1D}) [141], rat-tail artery [76, 140] and rabbit ear artery [45]. See also the following section on α_{1D}-adrenoceptors.

α_{1L}-Adrenoceptors

α_1-Adrenoceptors in blood vessels were subdivided based on their affinities for prazosin, WB 4101 and HV 723 into α_{1H} and α_{1L} [100]. α_{1H}-Adrenoceptors had high affinity for prazosin, and appeared to match the α_{1A}, α_{1B}, α_{1D} classification [101], whereas α_{1L} had low affinity for prazosin and did not seem to match the current molecular-cloning-based classifications. Under this classification, and often based on the low potency of prazosin, rabbit aorta, mesenteric and carotid arteries [100],

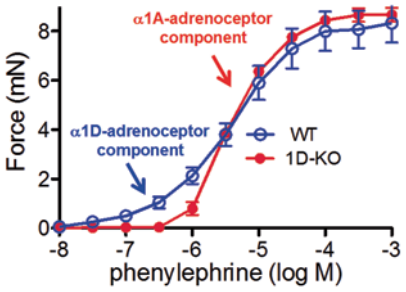

Fig. 4.2 Concentration–response curves for contractions to the α_1-adrenoceptor agonist phenylephrine in small mesenteric artery from wild-type (*open circles*) and α_{1D}-adrenoceptor knockout (KO) mice (*filled circles*). The α_{1D}-adrenoceptor-mediated response is the component found in wild-type but not in α_{1D}-adrenoceptor KO mice. Note that low concentrations of phenylephrine (*and indeed noradrenaline*) selectively activate α_{1D}-adrenoceptors. *WT* wild type. (Taken from Bexis and Docherty, unpublished)

guinea pig aorta, [101], rabbit cutaneous resistance arteries [123] and rat small mesenteric artery [137] were reported to contain α_{1L}-adrenoceptors.

Knockout (KO) of the α_{1A}-adrenoceptor abolishes α_{1L}-adrenoceptor pharmacology. The α_{1A}-adrenoceptor, expressed in the Chinese hamster ovary CHO-K1 cell line, displayed binding properties of the α_{1A}-adrenoceptor, but functionally, in terms of inositol phosphate accumulation, the receptor displayed properties of the α_{1L}-adrenoceptor [49]. Hence, the same receptor showed characteristics of both α_{1A}- and α_{1L}-adrenoceptors: in ligand-binding studies α_{1A}, but in functional studies α_{1L}. However, receptors reported to be α_{1L}-adrenoceptors can be demonstrated under slightly altered conditions to be α_{1A}-adrenoceptors. Hence, in the view of this author, it is likely that the α_{1L}-adrenoceptor is simply the native α_{1A}-adrenoceptor, at which prazosin shows low potency functionally [37].

Vascular Responses Mediated by α_{1B}-Adrenoceptors

The α_{1B}-adrenoceptor has been somewhat a mystery receptor in vascular studies. Contractions are reported to be mediated at least partly by α_{1B}-adrenoceptors in a number of tissues: rabbit corpus cavernosum [104] and rabbit cutaneous resistance arteries [123]. In studies of blood vessels from adrenoceptor KO mice, it was found that the major subtypes involved in contractions are α_{1A} and α_{1D}, but that the α_{1B}-adrenoceptor plays a minor but clearly defined role in contractions in aorta, carotid, mesenteric and tail arteries [28]. For instance, contractions in rat-tail artery develop more slowly in α_{1B}-adrenoceptor KO mice [28], so that subtle differences can be revealed following receptor KO. In the mouse mesenteric vasculature, α_{1B}-adrenoceptor KO resulted in diminished nerve but not agonist-induced vasoconstriction at 10 Hz but not 2 or 4 Hz [136].

In rat aorta, both α_{1A}- and a_{1B}-adrenoceptors are involved in trophic effects [149], and in mouse femoral arteries, employing gene knockout technology, it has been demonstrated that both α_{1A}- and α_{1B}-adrenoceptors are required for neo-intimal formation (no single knockout was effective) [68].

Vascular Responses Mediated by α_{1D}-Adrenoceptors

BMY 7378 is a selective antagonist at α_{1D}-adrenoceptors [58] and has been useful in identifying responses mediated by α_{1D}-adrenoceptors. Contractions are reported to be mediated at least partly by α_{1D}-adrenoceptors in a number of tissues including: rat aorta [1, 69, 110], rat iliac artery [110], rat mesenteric artery and pulmonary artery [69], rat renal artery (also α_{1A}) [141], rat carotid artery, mesenteric artery, aorta [140] and rabbit aorta (also possibly α_{1A}) [45]. In contrast, some studies of rat mesenteric artery did not report α_{1D}-adrenoceptor involvement in contractions [110]. The α_{1D}-adrenoceptor is reported to be constitutively active in the rat aorta, meaning that a response can occur in the absence of an agonist, and antagonists

such as prazosin behave not simply as competitive antagonists but also as inverse agonists to inhibit that constitutive activity [6, 57].

Vascular Responses Mediated by Multiple Subtypes of the α_1-Adrenoceptor

Clearly, contractions in a number of tissues are mediated by more than one subtype of the α_1-adrenoceptor, and currently available subtype selective antagonists are often not selective enough to tease out clearly which receptors are present. In the perfused rat kidney, both α_{1A}- and α_{1D}-adrenoceptors mediate vasoconstriction [5], and in the external carotid circulation, the use of selective antagonists suggests that responses involve α_{1A}, α_{1D}, α_{2A} and possibly α_{2C} [143a]. Unlike the rat aorta, where the predominant receptor is α_{1D}, contractions of the mouse aorta clearly involve α_{1A}- in addition to α_{1D}-adrenoceptors [146]. Receptor KO studies in mice can help. In α_{1D}-adrenoceptor KO mice, there was a reduced vasoconstrictor response to both NA and nerve stimulation in femoral arteries [148], demonstrating the role of this receptor in neurotransmission (see later). In mouse mesenteric arteries, there is evidence from antagonist studies for the involvement of both α_{1A}- and α_{1D}-adrenoceptors in contractions [146], although the predominant α_1-adrenoceptor is the α_{1A}-adrenoceptor.

The role of the α_{1A}- and α_{1D}-adrenoceptor in contractile responses in a number of blood vessels may best be illustrated by Fig. 4.2. Figure 4.2 shows concentration–response curves for contractions produced by the α_1-adrenoceptor agonist phenylephrine in small mesenteric artery from wild-type (WT) and α_{1D}-adrenoceptor KO mice. KO of the α_{1D}-adrenoceptor changes the response from a relatively shallow concentration–response curve to a steep curve. The α_{1D}-adrenoceptor-mediated response is the component found in WT but not in α_{1D}-adrenoceptor KO mice. Note that low concentrations of phenylephrine (and indeed noradrenaline) selectively activate α_{1D}-adrenoceptors (Fig. 4.2). The high-affinity receptor is an α_{1D}-adrenoceptor. This is also true for noradrenaline: Noradrenaline has high potency at the α_{1D}-adrenoceptor and lower potency at the α_{1A}-adrenoceptor, so that knockout of the α_{1D}-adrenoceptor removes the high-potency component of the response to noradrenaline. The high affinity of this receptor for noradrenaline makes it the most likely candidate for the receptor in the neuroeffector region involved in the control of neurotransmission, and indeed blood pressure is reduced by deletion of this receptor (see below).

The suggestion that noradrenaline has higher potency at α_{1D}-adrenoceptors is confirmed in many pharmacological studies. Indeed, noradrenaline has been reported to have high potency (aorta: pEC50 of 8.15) [24] in tissues with a high level of α_{1D}-adrenoceptor messenger RNA (mRNA; aorta: 70–80 %; [89a]) and low potency (pEC50 of 6.32) [126] in the small mesenteric artery which had a high level of α_{1A}-adrenoceptor mRNA (75 %; [89a]).

In mouse mesenteric arteries, where, as discussed above, the predominant receptor is α_{1A}-adrenoceptor (see Fig. 4.2), prazosin potency (pK_B, −log *M*) was 8.8 in WT mice, but 9.6 in α_{1B}-/α_{1D}-adrenoceptor KO mice [91]. However, prazosin pK_B was 10.3 in α_{1A}-/α_{1B}-adrenoceptor KO mice [92]. In another study, prazosin pA_2 was 9.92 in WT and 9.83 in α_1B KO, but 9.30 in α_{1D} KO and 9.43 in α_{1B}/α_{1D} KO [67]. These studies seem to agree that prazosin may be more potent in the mouse than in the rat, but there is no conclusive evidence that its potency is decreased by α_{1D} KO.

Physiological Blood Pressure Responses Mediated by α_1-Adrenoceptors

Piascik et al. (1995) [110] reported that the α_{1A}-adrenoceptor subtype plays a role in the tonic maintenance of blood pressure in the conscious rat, whereas the α_{1B}-adrenoceptor subtype participates in the response to exogenous agonists. In receptor KO mice, α_{1A}-adrenoceptor KO significantly reduced resting blood pressure in only two from four studies, and indeed a combined α_{1A}-/α_{1B}-adrenoceptor KO failed to affect basal blood pressure in two studies (see Table 4.1), α_{1B}-adrenoceptor KO did

Table 4.1 Baseline blood pressure in wild-type and α_1- and α_2-adrenoceptor KO mice

(a) α_1-Adrenoceptor KO mice					
WT	α_{1A} KO	α_{1B} KO	α_{1D} KO	Notes and double KO	Reference
114	104*			Male	[115]
111	102	99	93*	Female	[115]
99			99*	1B/1D KO: 92*	[67]
109					[131]
111				Male 1A/1B KO: 112	[105]
111				Female 1A/1B KO: 111	[105]
120		111			[136]
105	102	104	95*	1A/1B KO: 104	[68]
(b) α_2-Adrenoceptor KO mice					
WT	α_{2A} KO	α_{2B} KO	α_{2C} KO		Reference
120 (est)		120 (est)			[84]
119		118			[21]
128	131				[4]
111	118*				[102]
91			97	2A/2C KO: 114*	[56]
146	155*				[150]

KO knockout

not affect resting blood pressure, but α_{1D}-adrenoceptor KOs significantly reduced resting blood pressure in three from three studies (Table 4.1). In KO mice lacking the α_{1B}-adrenoceptor subtype, there was no effect on basal blood pressure, but pressor responses to phenylephrine were significantly blunted [21]. However, overexpression of α_{1B}-adrenoceptors results in hypotension and cardiac hypertrophy, suggesting that effects seen in α_{1B}-adrenoceptor KO mice may not relate to direct blood pressure actions of α_{1B}-adrenoceptors (otherwise, overexpression would result in increased blood pressure) [151]. In the pithed rat, a component of the vasopressor nerve response, previously identified as α_2-adrenoceptor mediated based on the effects of the α_2-adrenoceptor antagonist yohimbine, has been reclassified as α_{1D}-adrenoceptor mediated [35]. Yohimbine was found to have unexpected α_{1D}-adrenoceptor potency. Hence, in the pithed rat, pressor nerve responses may involve both α_{1A}- and α_{1D}-adrenoceptors [35].

These results may suggest that α_{1A}- and α_{1D}-adrenoceptors and perhaps α_{1B}-adrenoceptors are involved in blood pressure control. However, the clearest result in terms of basal blood pressure is with the α_{1D}-adrenoceptor KO mouse: Blood pressure has been consistently reported to be significantly lowered in α_{1D} KO mice (see Table 4.1).

Smooth Muscle α_2-Adrenoceptor Subtypes

α_2-Adrenoceptors have been subdivided into three subtypes, α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors, based on ligand-binding and molecular cloning studies [18], and the α_{2D}-adrenoceptor initially found in the rat is a species orthologue of the human α_{2A}-adrenoceptor, so that this subtype nomenclature has been dropped. The important features of α_2-adrenoceptor subtypes are shown in Table 4.2.

Table 4.2 Summary of vascular α_2-adrenoceptor subtype characteristics

Receptor subtype	2A	2B	2C
Functional responses (vascular)	Vascular contraction: minor role?	Vascular contraction	Vascular contraction: human saphenous v
Functional responses (blood pressure)	Central control of blood pressure: central hypotension, peripheral pressor	Control of blood pressure: peripheral pressor	Minor??
Affinity of NA?			
Selective agonists			
Non-selective antagonist	Yohimbine	Yohimbine	Yohimbine
Selective antagonists	BRL44408	?	?
G protein	G_i	G_i	G_i
Second messenger	Inhibit AC	Inhibit AC	Inhibit AC

Postjunctional α_2-Adrenoceptors In Vivo

Following the identification of prejunctional α_2-adrenoceptors, evidence was presented, employing α_2-adrenoceptors antagonists and especially yohimbine, that α_2-adrenoceptors may be present on vascular smooth muscle and to mediate vasoconstrictor responses, in terms of actions both of exogenous agonists and of endogenous neurotransmitters [39, 40, 41]. More recently, the subtypes of α_2-adrenoceptor involved in these pressor responses to exogenous agonists were investigated and it was found that the predominant receptor was α_{2A}-adrenoceptor [54]. However, it has since been established that the component of the vasopressor nerve responses in the pithed rat found to be blocked by yohimbine are actually α_{1D}-adrenoceptor mediated [35]. Fortunately, there is indeed an α_{2A}-adrenoceptor-mediated component to pressor responses to exogenous agonists in the pithed rat [36].

Physiological Blood Pressure Responses Mediated by α_2-Adrenoceptors

The effects of α_2-adrenoceptor receptor KOs on resting blood pressure have been examined: The only change in resting blood pressure produced by α_2-adrenoceptor KO is an increase in blood pressure in α_{2A}-adrenoceptor KO mice (see Table 4.1). The major effect of KO of the α_{2A}-adrenoceptors is abolition of a central hypotensive action [102]. As has been elegantly shown using KO mice, α_{2A}-adrenoceptors are involved in the central hypotensive actions of α_2-adrenoceptor agonists and hence the biphasic transient pressor and prolonged depressor response to the α_2-adrenoceptor agonist UK14,304 in WT mice was altered to a transient (but more prolonged) pressor response in α_{2A}-adrenoceptor KO mice [87, 88]. Hence, the crucial α_2-adrenoceptor in terms of blood pressure control is the α_{2A}-adrenoceptor, involved presumably in tonic central control of blood pressure (although a component due to peripheral effect at prejunctional α_{2A}-adrenoceptors on sympathetic nerves innervating blood vessels cannot be ruled out).

There were no obvious haemodynamic effects produced by disruption of the α_{2C}-adrenoceptor [84]. However, the biggest mystery surrounds the α_{2B}-adrenoceptor. Knockout of the α_{2B}-adrenoceptor abolished the early transient pressor response to the α_2-adrenoceptor agonist dexmedetomidine and prolonged the depressor response suggesting that the early pressor response is α_{2B}-adrenoceptor mediated [84]. These effects were admittedly obtained with a single dose of α_2-adrenoceptor agonist. These blood pressure effects are systemic cardiovascular responses and do not allow for subtle effects on different vascular beds, nor do they prove categorically that the α_{2A}-adrenoceptor subtype has purely central actions or that the α_{2B}-adrenoceptor subtype is the only mediator of pressor responses. It is possible that α_{2A}-adrenoceptors mediate both central hypotension and peripheral hypertension, but the central actions dominate in conscious animals.

The receptor knockout studies would suggest that the α_{2B}-adrenoceptor and not the α_{2A}-adrenoceptor is involved in peripheral pressor responses or at least that the α_{2B}-adrenoceptor is the predominant receptor involved under the experimental conditions and with the chosen agonist. How do these findings on blood pressure in KO mice relate to the above studies in the pithed rat in which pressor responses were mediated by α_{2A}-adrenoceptors (see above)? The pithed rat results do not rule out the involvement of α_{2B}-adrenoceptors in pressor responses in pithed rats, but suggest that α_{2A}-adrenoceptors are dominant. The identification of α_{2A}-adrenoceptors relies on BRL 44408, but no reliable selective α_{2B}-adrenoceptor antagonist is available. The lack of really good selective α_{2B}- or indeed α_{2C}-adrenoceptor antagonists hinders pharmacological studies. We need more selective antagonists to answer these questions.

Hence, in general, it seems that the major contributors to exogenous-agonist-induced pressor responses in vitro and to exogenous-agonist-induced contractions in vitro are α_{1A}-, α_{1D}- and α_{2A} (or α_{2B})-adrenoceptors. However, effects of exogenous agonists, while of importance in terms of pharmacological actions of drugs, may tell us little about what receptors are important in the physiological control of the vasculature. Hence, KO studies can be of use in clarification of this point.

α_2-Adrenoceptors in Isolated Tissues

In terms of postjunctional α_2-adrenoceptors in isolated tissues, there is relatively little evidence available as to subtypes mediating vascular contractions, given the relatively few isolated tissue preparations in which these receptors can be demonstrated. The human saphenous vein is a preparation in which it appears that the dominant adrenoceptors mediating contractions are α_2-adrenoceptors. In studies from this laboratory of side branches of the human saphenous vein, there appears to be a homogeneous population of α_2-adrenoceptors with no evidence for α_1-adrenoceptors: Yohimbine was more potent than prazosin; both yohimbine and prazosin produce parallel shifts in the noradrenaline concentration–response curve [see 122]; other α_1-adrenoceptor antagonists such as HV 723 [100] had relatively low potency; the α_1-adrenoceptor selective agonist phenylephrine had low potency; and the α_2-adrenoceptor selective agonist oxymetazoline had high potency [55]. Other studies of the human saphenous vein have reported that contractions are mediated predominantly by α_2-adrenoceptors [99, 128], or by α_1- and α_2-adrenoceptors [43, 114]. However, it is important to note that α_2-adrenoceptors identified employing yohimbine at a time before the functional identification of the α_{1D}-adrenoceptor must be treated with caution until selective α_{1D}-adrenoceptor antagonists are also examined: Yohimbine has actions at α_{1D}-adrenoceptors in concentrations only five- to tenfold less than those at which it has actions at α_2-adrenoceptors. Hence, some of these results have been reconsidered in terms of possible α_{1D}-adrenoceptor involvement: In the human saphenous vein, responses to exogenous noradrenaline have been shown to be α_{2C}- [55] and not α_{1D}-adrenoceptor mediated [24].

In the porcine palmer lateral vein and common digital artery, the low potency of prazosin suggests the presence of α_{2A}-adrenoceptors [12], and α_{2A}-adrenoceptors have also been reported in dog saphenous vein [86]. In the pig ciliary artery, the high potency of BRL44408 suggests the involvement of α_{2A}-adrenoceptors in contractions [143]. In a number of tissues, α_2-adrenoceptors contribute to a predominantly α_1-adrenoceptor-mediated response. In rat cremaster arterioles and venules, a component of the contractile response, based on the potency of BRL 44408, seems to be mediated by α_{2A}-adrenoceptors [83].

Initially, it was suggested that vascular α_2-adrenoceptors may be predominantly extrasynaptic and mediate responses to circulating catecholamines [79], but it was later found that contractions to nerve stimulation in the human saphenous vein are α_2-adrenoceptor mediated, based on high potency of yohimbine and low potency of prazosin [38]. The low potency of prazosin rules out an important role for α_{1D}-adrenoceptors in this response.

Smooth Muscle β-Adrenoceptor Subtypes

Although, in the initial classification by Lands et al. [77], it was the β_2-adrenoceptor that mediated vascular relaxations, it was not long before evidence that both β_1- and β_2-adrenoceptors were involved in vascular relaxations was obtained [106]. Soon after, β_2- [30] and then β_1-adrenoceptors [50] were cloned. A third β-adrenoceptor was identified [42] and shown to be involved in β-adrenoceptor-mediated relaxations relatively resistant to propranolol, and termed the β_3-adrenoceptor [18]. The important features of β-adrenoceptor subtypes are shown in Table 4.3. Information on selective agonists and antagonists is listed in Table 4.3 [7].

β-adrenoceptor-mediated dilatation can involve a number of possible actions:

a. Direct vascular smooth muscle relaxation due to activation of adenylate cyclase and increase in cyclic adenosine monophosphate (cAMP) production. cAMP

Table 4.3 Summary of vascular β-adrenoceptor subtype characteristics

Receptor subtype	β_1	β_2	β_3
Functional responses (vascular)	Relaxation. Neurotransmission target?	Widespread relaxation. Target of adrenaline	Relaxation
Affinity of NA?	High	Low	Low
Selective agonists	Xamoterol	Formoterol	? BRL37344
Non-selective antagonist	Propranolol	Propranolol	propranolol (low potency)
Selective antagonists	atenolol, CGP 20712A	ICI 118551	SR59230A
G protein	G_s	G_s	G_s
Second messenger	Stimulate AC	Stimulate AC	Stimulate AC

activates protein kinase A (PKA) which causes protein phosphorylation linked to vascular relaxation.

b. Endothelium-dependent relaxation involving nitric oxide production and stimulation of guanylate cyclase and increase in cyclic guanosine monophosphate (cGMP).
c. Hyperpolarization of endothelium or smooth muscle cells.

In a comparison of rat mesenteric artery and aorta, it was found that all three β-adrenoceptors were present in both vessels, but in mesenteric artery vasodilatation was mediated by β_1-adrenoceptors on vascular smooth muscle via cAMP production (but with additional β_3-adrenoceptors on endothelium and smooth muscle but perhaps not involved in relaxation), and in aorta was mediated by β_2-adrenoceptors on both the endothelium and smooth muscle and by β_3-adrenoceptors on the endothelium [48a].

In rat mesenteric artery, β-adrenoceptor (mainly β_1) activation produced dilatation, but hyperpolarization was found to be required for vasodilatation to spread along the artery to areas not exposed to β-adrenoceptor stimulation [53]. In cerebral arteries, β_1-adrenoceptor-mediated relaxation also partly involves hyperpolarization [74]. β-Adrenoceptors activate G_s to stimulate an increase in cAMP and one consequence is activation of K_{ATP} channels [112]. Even if the hyperpolarization is not essential for relaxation, this hyperpolarization can spread through the endothelial or smooth muscle cells in either direction. An upstream spread of relaxation could be important physiologically in mediating the increase in local tissue blood flow produced by hormones and neurotransmitters or perhaps even local mediators [53]. Otherwise, a local dilatation in a segment of vessel is unlikely to have any major effect of tissue blood flow unless the whole vessel dilates (a localized dilatation in a short segment of vessel will have little effect on resistance to flow). cAMP-independent effects of β-adrenoceptors, including those involving K^+ channels, seem to involve G_s [130].

The use of knockout technology has demonstrated, in mouse mesenteric artery, that β-adrenoceptor-mediated relaxations were mainly β_1-adrenoceptor mediated, with some β_3- and possibly β_2-adrenoceptors, but all were endothelium dependent involving nitric oxide [3].

β_1-Adrenoceptors

β_1-Adrenoceptors are the major receptors involved in cardiac stimulation. Like all β-adrenoceptors, the main signalling pathway is by activation of the enzyme adenylate cyclase, resulting in increased levels of the second messenger cAMP (see Fig. 4.3). However, β_1-adrenoceptors are also involved in vascular relaxations.

Although β_2-adrenoceptors seem to be the main mediators of vascular relaxation, β_1-adrenoceptors predominate in specialized sites. In the dog hepatic artery, β_1-adrenoceptors are reported to mediate relaxation [121]. In rat mesenteric arteries,

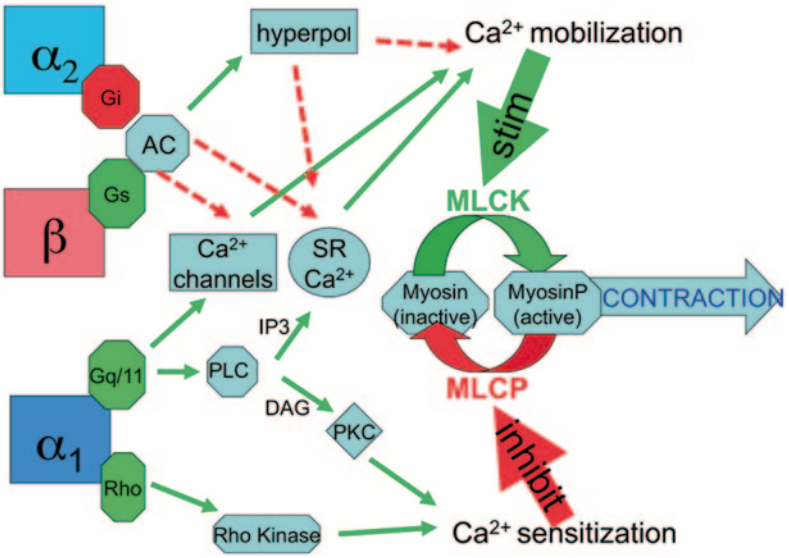

Fig. 4.3 A simplified schematic diagram of actions of α_1-, α_2- and β-adrenoceptors to modulate contractions in vascular smooth muscle. α_1-Adrenoceptor (α_1) activation can result in contract mainly via $G_{q/11}$, but also via Rho, to increase Ca^{2+} mobilization from stores or via Ca^{2+} entry, and to increase Ca^{2+} sensitization. β–Adrenoceptor (β) activation, via increased cAMP levels and K^+ channels, decreases Ca^{2+} mobilization. α_2-Adrenoceptor (α_2) activation acts at least partly by inhibiting cAMP production. For simplicity, some pathways are omitted. Pathways of endothelium-mediated relaxation are not shown, for clarity. *AC* adenylate cyclase, *DAG* diacylglycerol, *IP3* inositol triphosphate, G_i, $G_{q/11}$, G_s G proteins, *hyperpol* membrane hyperpolarization, *MLCK,* myosin light-chain kinase, *MLCP* myosin light-chain phosphatase, *PKC* protein kinase C, *PLC* phospholipase C, *SR* sarcoplasmic reticulum, *stim* stimulate

β_1, but not β_2 or β_3, are reported to mediate relaxations [15]. In the coronary artery, both β_1- and β_2-adrenoceptors are reported to be involved in relaxations [52, 95], and β-adrenoceptor-mediated relaxations have also been reported [29].

β_2-Adrenoceptors

β_2-Adrenoceptors were the archetypal receptors mediating relaxation of both vascular smooth muscle and non-vascular smooth muscle. β_2-Adrenoceptors are generally thought to be non-innervated receptors, far from nerve endings, acting as targets for circulating adrenaline since noradrenaline is a poor agonist at these receptors. In the vascular system, β_2-adrenoceptors are the main mediators of adrenergic relaxation, acting to stimulate the enzyme adenylate cyclase and increase cAMP production, although all β-receptor subtypes mediate relaxation. β-Adrenoceptor-mediated relaxation of the vascular system can be direct by actions on the smooth muscle or indirect by causing release of nitric oxide from the endothelium.

β_3-Adrenoceptors

The β_3 is a controversial β-adrenoceptor, with difficulties in positive identification due to lack of truly selective antagonist drugs. β_2-Adrenoceptors, like β_2- and even β_1-adrenoceptors, mediate smooth muscle relaxation of vascular and non-vascular smooth muscle.

Physiological Blood Pressures Responses Mediated by β-Adrenoceptors

Since this review is concerned only with vascular responses and thus vascular β-adrenoceptors, falls in resting blood pressure produced by combined knockout of β_1- and β_2-adrenoceptors [73] are largely related to cardiac actions. Studies of β_2-adrenoceptor KO mice found no change in resting blood pressure as compared to WT [22].

α-Adrenoceptor-Mediated Relaxation

Although α-adrenoceptors mediate direct contraction of vascular smooth muscle, it is possible for α-adrenoceptors to mediate relaxation through release of nitric oxide from the vascular endothelium. α_{1D}-Adrenoceptors are reported to mediate endothelium-dependent relaxation in the rat mesenteric bed [48]. α_2-Adrenoceptors are also present on vascular endothelial cells to mediate relaxations [47, 90], and adrenoceptor KO studies have confirmed the identification as α_{2A}-adrenoceptors in mouse aorta [119, 138].

Cellular Responses Mediated by Adrenoceptors in Smooth Muscle

All adrenoceptors are G-protein-coupled receptors. G-protein-mediated signalling can be divided into a number of components: rapid second messenger or ion channel response and slower β-arrestin-mediated responses including desensitization and receptor internalization [72]. Hence, G-protein-coupled receptors can not only produce rapid cellular responses via second messengers or ion channels but also regulate cell proliferation and receptor function. Given the complexity of G-protein-mediated signalling, it is not within the scope of this review to discuss this topic in detail, but rather to give a simple overview of signalling as it relates to vascular α- and β-adrenoceptors.

Vascular contractions and relaxations can be simplified to positive or negative effects on the following (see Fig. 4.3):

a. Depolarization-mediated calcium entry through L-type or T-type calcium channels and resulting in increased intracellular Ca^{2+}.
b. Direct activation of Ca^{2+} channels to cause calcium entry and increased intracellular Ca^{2+}.
c. Release of Ca^{2+} from intracellular stores and increased intracellular Ca^{2+}.
d. Ca^{2+} binding to calmodulin and the binding of Ca^{2+}/calmodulin to myosin light-chain kinase (MLCK) which phosphorylates the myosin light chain to allow myosin to cross-bridge to actin and cause contraction.
e. Sensitization of the contractile apparatus to Ca^{2+}. Once contraction has been initiated by increased intracellular Ca^{2+}, contraction is stopped by dephosphorylating the myosin light chain by myosin light-chain phosphatase (MLCP). Hence, modulation of the activity of MLCP changes the sensitivity to Ca^{2+}, by altering the response to a given level of Ca^{2+}.

α_1-Adrenoceptor Second Messenger Systems

α_1-adrenoceptors are coupled to second messenger systems via G proteins, predominantly to pertussis-toxin-insensitive G proteins of the $G_{q/11}$ family to phospholipase C [93, 145], and this seems to be the major pathway of responses. Activation of the receptor causes binding of the G protein and guanosine diphosphate (GDP) release. The G protein is freed from the receptor–G-protein complex by guanosine triphosphate (GTP) and splits into α and βγ subunits, both of which can have actions at effector molecules including enzymes and ion channels [81]. The G protein activation by α_1-adrenoceptor subtypes can produce responses via phospholipase C stimulation leading to formation of inositol triphosphate and diacylglycerol. Diacylglycerol stimulates protein kinase C and inositol triphosphate acts on the inositol triphosphate receptor involved in calcium signalling: The net result is increased entry of extracellular Ca^{2+} and/or release from Ca^{2+} stores [93, 145]. Phasic contractions can be caused by release of Ca^{2+} from ryanodine-sensitive stores and tonic contractions by activation of protein kinase C by diacylglycerol and influx of Ca^{2+} via nifedipine-sensitive stores [16]. α_1-Adrenoceptors have also been reported to signal through pertussis-toxin-sensitive G proteins, the G_i or G_o family linked to phospholipase A_2 [44], or to ERK1/2 activation [124].

α_1-Adrenoceptor activation causes phospholipase A_2 stimulation and arachidonic acid release in the mammalian COS cell line [109], causes arachidonic acid release by phospholipase D activation in rat-tail artery [59] and rat fibroblasts [116] and can lead to cAMP production [109, 116]. Arachidonic acid release and reactive oxygen species generation can cause smooth muscle proliferation [103]. In rat-tail artery, α_1-adrenoceptor-mediated calcium sensitization is due mainly to the activation, via the small GTP-binding protein RhoA, of Rho kinase [98], which phosphorylates myosin light-chain phosphatase, causing inhibition of its function [125] (see Fig. 4.3). β-arrestin-2 has been shown to bind to the α_{1A}-adrenoceptor and may be involved in regulation of the receptor [64].

α_2-Adrenoceptor-Mediated Second Messenger Systems

α_2-Adrenoceptors negatively couple to adenylate cyclase via a pertussis-sensitive G_i protein to decrease vascular smooth muscle cAMP levels, although the overall importance of this mechanism of action in producing vascular contractions remains to be clarified. In rabbit corpus cavernosum, a component of the α-adrenoceptor-mediated contraction is reported to be mediated by α_2-adrenoceptors, and α_2-adrenoceptor agonists inhibit forskolin-stimulated cAMP accumulation [60]. α_2-Adrenoceptor-mediated vasopressor responses are blocked by pertussis toxin [13, 31], suggesting that a G_i or G_o protein is involved in the response. However, in the porcine palmer lateral vein, Wright et al. [144] found that contractions evoked by α_2-adrenoceptor agonists only involved reduction in cAMP when cAMP levels were elevated by agents that stimulate adenylate cyclase. In human subcutaneous resistance vessels, pertussis-toxin-sensitive contractions to α_2-adrenoceptor agonists were found to involve an influx of Ca^{2+} [107]. α_2-Adrenoceptors may also be linked to phospholipase A_2 [107].

ß-Adrenoceptor-Mediated Second Messenger Systems

β-Adrenoceptors, all three subtypes, positively couple to adenylate cyclase via the G_s protein to increase vascular smooth muscle cAMP levels [130]. The increased cAMP levels result in activation of PKA with several possible net actions resulting in relaxation:

a. Inhibition of Ca^{2+}-calmodulin
b. Inhibition of MLCK
c. Increased reuptake of Ca^{2+} in sarcoplasmic reticulum
d. Release of endothelium-dependent mediators such as NO which acts to stimulate guanylate cyclase in vascular smooth muscle cells

Other effects may be dependent or independent of cAMP but involving G_s, including (e) membrane hyperpolarization via K^+ channels. The net effect is to decrease calcium mobilization or decrease Ca^{2+} sensitization (see Fig. 4.3).

Adrenoceptor-Subtype-Selective Drugs

Selectivity between families of adrenoceptor can be achieved by using prazosin as an α_1-adrenoceptor antagonist, yohimbine as an α_2-adrenoceptor antagonist and propranolol as a β-adrenoceptor antagonist (although propranolol has relatively low potency at β_3-adrenoceptors). Prazosin and propranolol show high selectivity (3×10^{-9} M to 3×10^{-8} M) for their respective subtypes but higher concentrations of prazosin (above 10^{-7} M) will block α_2-adrenoceptors, and higher concentrations of

Table 4.4 Summary of vascular α_1-adrenoceptor subtype characteristics

Receptor subtype	1A	1B	1D
Functional responses (vascular)	Vascular contraction: rat mesenteric a (major)	Minor role in contraction: rat-tail a (minor). regulatory?	Vascular contraction: Rat aorta (major); rat mesenteric a. (minor)
Functional responses (blood pressure)	Role in control of blood pressure? Contraction to exogenous agonists	Regulatory?	Control of blood pressure. Neurotransmission?
Nerve-mediated responses			Major role?
Affinity of NA	Low		High
Selective agonists	A61603		
Non-selective antagonist	Prazosin	Prazosin	Prazosin
Selective antagonists	RS 100329	?	BMY 7378
Sensitivity to other agents		More sensitive to CEC?	
G protein	$G_{q/11}$	$G_{q/11}$	$G_{q/11}$
Second messenger	IP3, DAG	IP3, DAG	IP3, DAG

DAG diacylglycerol, *IP3* inositol triphosphate

propranolol (above 3×10^{-6} M) will block α_1-adrenoceptors. Yohimbine has only limited selectivity and has some potency at α_{1D}-adrenoceptors.

α_1-Adrenoceptor-subtype-selective drugs are shown in Table 4.4. A number of selective α_{1A}-adrenoceptor antagonists are available including RS100329. Probably the most useful selective α_{1D}-adrenoceptor antagonist available is BMY 7378 [58]. Currently, there is no ideal antagonist for α_{1B}-adrenoceptors. Risperidone was found to show selectivity for α_{1B}-adrenoceptors as an antagonist in ligand-binding studies but this selectivity has been questioned in functional studies [33] (see Docherty 2005).

Among agonists, noradrenaline has higher potency at α_{1D}- than α_{1A}- adrenoceptors (see Docherty 2005). A61603 is a potent α_{1A}-adrenoceptor-selective agonist, reported to be 200 times more potent than noradrenaline [75].

α_2-Adrenoceptor-subtype-selective drugs are shown in Table 4.2. BRL 44408 is a potent selective antagonist at α_{2A}-adrenoceptors, in both ligand-binding and functional studies [55, 147]. A number of antagonists show low potency at α_{2A}-adrenoceptors, including prazosin and ARC239 [65]. Drugs selective for α_{2B}- and α_{2C}-adrenoceptors are less well established in terms of selectivity.

β-Adrenoceptor-subtype-selective drugs are shown in Table 4.3 [7]. A number of clinically useful β-blockers are β_1 selective such as atenolol, and other selective agents include CGP 20712A. The β_2-selective antagonist widely used is ICI 118551. For β_3-adrenoceptors, SR59230A is widely used but its selectivity is not clear, and development of more selective drugs is warranted [142]. For all β-adrenoceptor antagonists, there may be a problem of α_1-adrenoceptor antagonism [14, 82].

Neurotransmission in Vascular Smooth Muscle

The adrenoceptors mediate most of the actions of the sympathetic nervous system (a small number of effects are mediated by cholinergic receptors, and cotransmitters may be involved in many responses, but those are not the subject of this chapter). Adrenoceptors are widely distributed in vascular smooth muscle cells. In mouse mesenteric and carotid arteries, most or all smooth muscle cells seemed to show α_1-adrenoceptors in WT, but in α_{1B}-/$_{1D}$-adrenoceptor KO mice some cells did not show α_{1A}-adrenoceptor binding [91]. Hence, not all vascular smooth cells express α_{1A}-adrenoceptors (or indeed β-adrenoceptors), suggesting that blood vessel response to neurotransmitter or hormone may involve smooth muscle cell to smooth muscle cell communication of the signal [27]. The dominance of α_{1D} over α_{1A} may be dependent on the degree of innervation: The lesser the innervation the more number of α_{1D}-adrenoceptors [91] (cf. rat aorta that lacks a functional innervation, in which the α_{1D}-adrenoceptor is dominant). Some receptors are localized in the neuro-effector junction, whereas others may be spread along the wall of the blood vessel and less likely to be targets of the neurotransmitter except in special circumstances, whereas other receptors are in situations where they are not likely to be affected by nerve-released neurotransmitter. In the latter category are the β_2-adrenoceptors in vascular smooth muscle, at which the neurotransmitter noradrenaline has low affinity. However, it is clear that the most important adrenoceptors physiologically must be those situated in the neuro-effector junction as targets for the neurotransmitter noradrenaline, and secondary to this those β-adrenoceptors that are largely targets for the hormone adrenaline, remembering that plasma levels of adrenaline are higher than those of noradrenaline.

What is the main mediator of sympathetic-nerve-mediated vasoconstriction? Studies of systolic blood pressure (SBP) in conscious WT and adrenoceptor KO mice can help to answer this question. α_{1B}-Adrenoceptor KO had no effect on basal blood pressure (Table 4.1), α_{1A}-adrenoceptor KOs had inconsistent effects (Table 4.1), but α_{1D}-adrenoceptor KOs significantly reduced blood pressure in three from three studies (Table 4.1). Further studies may be necessary, but the results are consistent with α_{1D}-adrenoceptors being the major receptors involved in sympathetic-nerve-mediated vasoconstriction.

The Physiological Importance of the α_{1d}-Adrenoceptor

Is the α_{1D}-adrenoceptor the most important α-adrenoceptor in the vascular system, because it may be the main mediator of nervous control of the circulation, determining peripheral resistance and tissue blood flow? The evidence for the importance of the α_{1D}-adrenceptor can be summarized as follows.

In some blood vessels, all smooth muscle cells contain α_{1D}, but only some have α_{1A}-adrenoceptors [91, 92].

The proportion of α_{1D}-adrenoceptors increases with denervation in rat vas deferens (admittedly a non-vascular study), and tissues without innervation (e.g. rat

aorta) have predominantly α_{1D}-adrenoceptors. Indeed, denervation supersensitivity or supersensitivity to noradrenaline transporter blockers may be due to spreading of α_{1D}-adrenoceptors from the synaptic area (or increased access for exogenous agonists to the receptors due to transporter block). α_{1D}-Adrenoceptors have also been shown to be involved in reserpine-induced supersensitivity in rat-tail artery [129].

α_{1D}-Adrenoceptors are involved in neurotransmission in the pithed rat [35] and in non-vascular tissues such as rat vas deferens [37].

In receptor KO mice, α_{1D}-adrenoceptor KO produces a consistent fall in basal blood pressure (see Table 4.1).

Control of Blood Flow to Vascular Beds

Sympathetic control of the vasculature is largely in terms of α-adrenoceptor-mediated vasoconstriction in response to the neurotransmitter noradrenaline and to a lesser extent vascular relaxation in response mainly to the hormone adrenaline from the adrenal medulla. The arterioles are the main determinants of peripheral resistance and of tissue blood flow. Widespread arteriolar constriction raises blood pressure by increasing peripheral resistance, but dilatation of some arteriolar vascular beds and constriction of others divert blood through preferred vascular beds with little change in peripheral resistance and blood pressure. In terms of nervous control, these vessels constrict in response to sympathetic activation and dilate in response to removal of sympathetic tone. We can consider a number of circulations to demonstrate the factors determining control of blood flow.

Skeletal Muscle Circulation

In exercise, blood is diverted to exercising skeletal muscle by the following mechanisms:

1. Dilatation of arterioles to skeletal muscle due to:
    a. Withdrawal of sympathetic vasoconstrictor tone
    b. Relaxation in response to circulating adrenaline
    c. Relaxation in response to metabolites produced by exercising muscle
2. Sympathetic-nervous-system-mediated constriction of arterioles to other vascular beds, particularly splanchnic and skin circulations (skin later usually dilates in exercise to cool body) to divert a larger proportion of the cardiac output to skeletal muscle.

Constriction of other vascular beds and dilatation of the skeletal muscle vascular beds means that a greater proportion of the cardiac output reaches the exercising muscle (think of the analogy of water flow in a pipe split into a number of outflows controlled by taps: If there were two taps, closing one diverts all the flow through the other).

Coronary Circulation

The heart muscle extracts 75 % of the oxygen reaching it in the coronary arteries at the basal resting cardiac output. Hence, increases in oxygen consumption can only be achieved by increases in coronary blood flow, and this is mediated by local metabolites. The role of a sympathetic innervation in the coronary circulation is therefore of secondary importance, and as a result α-adrenoceptor-mediated vasoconstriction is minor, and indeed β_1-adrenoceptors are present (see the β-adrenoceptor section). At rest, there is probably no ongoing α-adrenoceptor-mediated vasoconstriction, as α-adrenoceptor antagonists do not increase coronary blood flow [70]. However, α_1-adrenoceptor vasoconstriction is reported to occur in microvessels (>100 micron), and β-adrenoceptor-mediated vasodilatation in small arterioles [46]. This anatomical localization may play a role in maintaining flow to the deeper layers of the left ventricle, the area most vulnerable to ischaemia during systole, when the contracting left ventricle restricts its own blood flow. The α_1-adrenoceptor vasoconstriction may reduce retrograde coronary flow caused by systolic ventricular contraction and so help maintain blood flow to the deeper regions of the left ventricle [96].

However, the presence of α_1-adrenoceptors can lead to enhanced vasoconstriction in pathophysiological situations such as metabolic syndrome, and this may also be linked to diminished β-adrenoceptor-mediated dilatation [11]. In coronary atherosclerosis, damage to the vascular endothelium may also be a factor in the shift of the balance towards vasoconstriction [120]. Atherosclerosis augments α-adrenoceptor vasoconstriction in terms of diminished coronary flow in patients [9], and impairs β_2-adrenoceptor-mediated dilatation [8]. There is some evidence using adrenoceptor agonists for the involvement of α_2-adrenoceptors in coronary constriction in man [9, 70].

In coronary arteries, there is a demonstrable sympathetic innervation, and relaxations are reported to be mediated by both β_1- and β_2-adrenoceptors [52, 95]. In human coronary arteries, relaxations to isoprenaline (non-selective) and BRL37344 (β_3-selective) were reported to be mediated by nitric oxide but insensitive to β_1/β_2-adrenoceptor antagonism by nadolol, and blocked by the β_3-adrenoceptor antagonist bupranolol [29]. β_1-Adrenoceptor-mediated vasodilatation is reported to occur predominantly in conduit coronary arteries, and these receptors may be the predominant target of nerve-released neurotransmitter [71]. Although β-adrenoceptor antagonists may reduce coronary blood flow, this may be due to decreasing cardiac work and so decreasing myocardial oxygen demand rather than block of a vasodilator tone [66]. If all three β-adrenoceptors are present in the coronary circulation, the question is which receptor is the most important. Logically, it should be the β_1-adrenoceptor at which noradrenaline has high potency, allowing for possibly crucial-nerve-mediated relaxations. This remains to be fully confirmed.

Cerebral Circulation

The cerebral circulation is an extremely interesting circulation that has a very high degree of autoregulation in which cerebral blood flow is controlled by the cerebral circulation itself. The total cerebral blood flow is held constant despite large changes in systemic blood pressure (50–150 mmHg) [80], although the regional blood flow within the cerebral circulation will vary depending on metabolic demands. Brain pH and metabolites largely achieve this autoregulation and the role of the sympathetic innervation is thought to be relatively minor. Sympathetic vasoconstriction, particularly of larger arteries, play a role in preventing increases in cerebral blood flow caused by high systemic pressures and by hypercapnia [134].

Splanchnic Circulation

The splanchnic circulation is rather unusual in two respects. Splanchnic blood flow can be greatly reduced by sympathetic stimulation by up to 80% [133], shifting blood to the skeletal muscle vasculature in situations of "fight or flight". This action is presumably predominantly due to α_1-adrenoceptor-mediated vasoconstriction of the arterioles and of the veins and is probably largely α_{1D}-adrenoceptor mediated, based on the logic of which receptors are possible targets for neurotransmitters. A comparison of mesenteric and splanchnic flow in anaesthetized rats showed that, while α_1-adrenoceptor agonists caused constriction in both circulations, α_1-adrenoceptor block caused dilatation only in the femoral [63], suggesting that sympathetic control of the mesenteric circulation is less important in overall control except as an override to close it down. However, rather unusually, and perhaps because of this sympathetic dominance, the splanchnic circulation has its own hormones that control its own blood flow, an autohormonal regulation. The major secretory hormones of digestion, gastrin, cholecystokinin and secretin, released when food is ingested to aid in the production of the acid, enzymes and secretions of digestion, also mediate increased splanchnic blood flow. This is a functional hyperaemia in which the hormones of digestion, released into the bloodstream, travel in the veins to the heart and return in the splanchnic arteries to dilate the arterioles and increase blood flow to aid digestion (local actions within the gastrointestinal tract may also contribute). Not surprisingly, the major inhibitory hormone of digestion somatostatin has an inhibitory effect on splanchnic blood flow, reducing splanchnic inflow as an inhibitory modulator on the vasodilator hormones. Hence, the splanchnic circulation, not blessed with the full benefits of autoregulation due to the powerful override by the sympathetic nervous system, has a novel method of controlling its own blood flow for digestion: autohormonal regulation.

Sexual Function

The interest in treating erectile dysfunction has led to the development of vasodilator drugs such as sildenafil. However, the sympathetic nervous system plays

a major role in the control of penile erection and indeed clitoral erection. Hence, since α_1-adrenoceptors are involved in the inhibitory control of penile blood flow, it is not surprising that α_1-adrenoceptor antagonists such as prazosin can be used to treat erectile dysfunction, particularly in patients with lower urinary tract symptoms (LUTS) [19]. Presumably as a result of sympathetic arousal in the fight-or-flight reaction, sexual arousal is powerfully blocked by α_1-adrenoceptor activation.

It has been known for many years that yohimbine has aphrodisiac properties to treat erectile dysfunction, with suggested mechanisms of action ranging from central nervous to peripheral systems. In rat corpus cavernosum, yohimbine antagonized the vasoconstrictor actions of phenylephrine [117]. This may suggest that the actions of yohimbine in this situation are not at α_2-adrenoceptors but at α_1-adrenoceptors, and as previously discussed these receptors may be neuronal α_{1D}-adrenoceptors, at which yohimbine has moderate potency. Indeed, Mizusawa et al. [94] found that α_{1D}-adrenoceptors were involved in preventing penile erection. Traish et al. [135] showed that the predominant adrenoceptors in human corpus cavernosum in terms of protein expression were α_{1A}- and α_{1D}-adrenoceptors. Gupta et al. [60] showed that contractions of rabbit corpus cavernosum were antagonized by the α_2-adrenoceptor antagonist rauwolscine but also by prazosin, and the high potency of prazosin (pA2 9.08) indicative possibly of α_{1D}-adrenoceptors, but the actions of rauwolscine suggestive of α_{2A}-adrenoceptors in addition. Rho kinase has been shown to be involved in Ca^{2+} entry into rat penile arteries [139].

Although corpus cavernosum may be controlled mainly by α_1-adrenoceptors, contractions of the cavernous artery may involve both α_1- and α_2-adrenoceptors and α_2-adrenoceptors may also be involved in nerve-stimulation-evoked contractions [62]. Penile veins are also reported to be constricted by α_1- and α_2-adrenoceptor stimulation, in both agonist- and nerve-mediated studies [113].

β_2-Adrenoceptors have been reported to be involved in relaxation of the human corpus cavernosum [25], but in the rabbit this does not seem to involve β_1- or β_2-adrenoceptors, suggesting an atypical or β_3-adrenoceptor action [132], and a β_3-adrenoceptor-mediated relaxation has also been reported in man [23]. The cAMP pathway, in addition to the cGMP pathway, is involved in enhanced clitoral blood flow in the rat [20], although there is no information on whether β-adrenoceptors may be involved.

Conclusions

The characteristics of α_1-, α_2- and β-adrenoceptor subtypes in vascular smooth muscle are summarized in Tables 4.2, 4.3 and 4.4. The effects of receptor KO on basal blood pressure in mice are shown in Table 4.1. A simplified scheme of second messenger systems involved in adrenoceptor-mediated vascular contractions and relaxations is shown in Fig. 4.3. Although a great deal is known about the function of adrenoceptor subtypes, a number of major questions remain to be answered:

1. The receptor subtype(s) of major importance in sympathetic nervous control of the vasculature is still unclear. It is the opinion of this author that the α_{1D}-adrenoceptor is the most important subtype involved in neurotransmission.
2. The second messenger systems involved in a number of adrenoceptor-mediated responses have not been fully established.
3. The lack of selective antagonists for a number of adrenoceptor subtypes hinders progress in identifying the physiological role of several adrenoceptor subtypes.
4. The therapeutic potential of the development of receptor subtype selective agonists and antagonists.

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Part III
The Adrenergic System in Cardiovascular Physiology and Therapy

Chapter 5
The Cardiovascular Adrenergic System and Physical Exercise

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Abbreviations

Akt	Protein kinase B
EC	Excitation–contraction
GRK2	G-protein-coupled kinase-2
HCN4	Hyperpolarization-activated cyclic nucleotide-gated channel 4
HF	Heart failure
I_{Ca}	L-type Ca^{2+}channels
NCX	Sarcolemmal Na^{+}/Ca^{2+}exchanger
NFAT	Calcineurin/nuclear factor of activated T cell
PI3K	Phosphatidylinositide-3-kinase
PKA	Protein kinase A
PLB	Phospholamban
RyR	Ryanodine receptors
SERCA	SR Ca^{2+}ATPase
SR	Sarcoplasmic reticulum
T-tubule	Transverse tubule
β-AR	β-Adrenergic receptor

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Introduction

The relationship between physical inactivity and disease is known since antiquity. In ancient Greece, Hippocrates has stated that diet deficiency or reduced physical activity would lead to body sickness [1]. This statement still holds true in contemporary period, especially considering the exponential growth of physical inactivity after the industrial revolution. Indeed, physical inactivity was identified as the fourth leading risk factor for all-cause global death and prevalence of chronic diseases, such as heart and renal failure, cancer, diabetes, and hypertension [2, 3]. Of interest, the prevalence of physical inactivity is also high in adolescents (80.3 % of 13–15-years-olds do not achieve 60 min of moderate-to-vigorous physical activity per day by combining information of 105 countries) [4], which calls for a better surveillance of physical activity levels in both adult and adolescent populations, as well as, the implementation of effective exercise programs for the prevention and treatment of chronic diseases. In fact, physical exercise has been considered an effective therapy to chronic diseases [5, 6]. For this reason, scientific societies have included in their guidelines, recommendations for daily physical activity and proper prescription of exercise [7].

The beneficial effects of chronic physical exercise are due to training adaptations that occur in several organ systems. In fact, these adaptations occur to reduce the homeostatic disturbance caused by chronic physical exercise. Consequently, the organism develops resistance to homeostasis imbalance, reducing both fatigue and installation of diseases [8]. The autonomic nervous system plays a crucial role in integrating chronic physical exercise adjustments by modulating the sympathetic and parasympathetic outflows [9].

In this chapter, the contribution of adrenergic system for the cardiovascular adaptations to acute (a single bout of exercise) and chronic (training) physical exercises is reviewed. Considering that aerobic exercises (endurance) are the main exercise type with sufficient scientific evidence to improve cardiovascular health [10, 11], we will focus on this type of exercise throughout this chapter.

Cardiac Responses to Aerobic Exercise in Health and Disease: Role of Autonomic Nervous System

The autonomic nervous system is known to modulate the cardiovascular function to aerobic exercise by increasing heart rate and cardiac contractility, accelerating cardiac relaxation and atrioventricular conduction, and controlling the vascular tonus [12]. Altogether, these responses increase cardiac performance to exercise, preparing the body for the “fight or flight response” by activating the sympathetic nerve activity and reducing parasympathetic outflow via neural responses influenced by both central and peripheral mechanisms [13].

Centrally mediated cardiovascular adjustments, known as central command, are regulated by the motor cortex, the same brain region responsible for motor unit recruitment. Motor cortex stimulates muscle contraction at the same time it sends signals to cardiovascular control areas at the medulla oblongata [14, 15], thus mediating the autonomic responses required to modify cardiac parameters during aerobic exercise. Additionally, it is proposed that the central command regulates sympathetic nerve activity in an exercise intensity-dependent manner [12, 16].

Early experiments demonstrated that heart rate and ventilation rapidly increase upon involuntary skeletal muscle contraction induced by electrical stimulation [17]. Additionally, seminal works by Alam and Smirk [18, 19] have shown that blood pressure and heart rate were maintained after exercise if blood flow to working muscles was occluded, and that these variables fell promptly when occlusion was removed. Once these responses did not involve cortical stimuli, it was suggested that reflexes within the muscles were able to mediate the cardiorespiratory response to exercise, in a mechanism referred to as exercise pressor reflex [12, 15, 20–22]. In fact, different types of sensory neurons (I–IV) innervate skeletal muscles, and afferent fiber types III and IV are specially related to exercise pressor reflex [21, 22]. Afferent type III fibers are highly excitable upon mechanical stress, acting as mechanoreceptors and the first to contribute to exercise pressor reflex. In turn, afferent type IV fibers increase their firing rate in a linear relationship with the accumulation of metabolic products of muscle contraction, making them metaboreceptors [12, 23–25].

Cardiac Responses to Aerobic Exercise in Health

Heart Rate Responses to Aerobic Exercise

At the onset of exercise, integrated responses of central command and mechanoreceptors in skeletal muscle lead to a vagal withdrawal, which accelerates heart rate [26–29]. After this initial stage, skeletal muscle metabolites' accumulation activates metaboreceptors promoting further increase in heart rate by sympathetic activation during exercise [12, 26–29]. Indeed, increased sympathetic outflow and cardiac β-adrenergic receptor (β-AR) activation are the main mechanisms involved in aerobic exercise tachycardia when the heart rate is more than 100 beats/min [30]. Moreover, during vigorous exercise, parasympathetic activity further declines and sympathetic outflow increases, resulting in a modest or virtually nonexistent vagal modulation of heart rate [31].

One of the main effects of aerobic exercise training is the reduced exercise tachycardia to the same absolute workload during a submaximal exercise. The main mechanism underlying reduced exercise tachycardia in trained individuals is the reduction in both vagal withdrawal and sympathetic intensification to the same absolute workload, as compared with untrained individuals [27, 29, 32–35]. Accordingly, aerobic exercise-trained individuals display reduced sympathetic activity

for any given submaximal workload, compared with sedentary controls [30]. This autonomic adaptation is of particular interest taking into consideration that maximal exercise tachycardia is achieved in a higher exercise workload, which further leads to higher maximal cardiac output and oxygen uptake in trained than untrained individuals.

Another striking adaptation to aerobic exercise training is the resting bradycardia reaching levels as low as 30 beats/min in endurance athletes [36–38]. Accumulated evidences suggest that resting bradycardia after aerobic exercise training is induced by both autonomic and nonautonomic mechanisms [28, 30, 35, 36, 39–41]. The main nonautonomic mechanism involved in resting bradycardia is a reduction in intrinsic heart rate after aerobic exercise training, which has been shown in both animal [28, 40, 42] and human [36, 39, 41, 43] studies. Recently, D'Souza and coworkers [42] have demonstrated a widespread remodeling of pacemaker ion channels with a reduction in hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4) expression and its corresponding current, I_f, in isolated sinus node of exercise-trained mice.

The main methods used to study autonomic control of heart rate include the evaluation of heart rate variability (time and frequency domain) and the cardiac autonomic blockade of β-AR (sympathetic) and muscarinic (vagal) receptors. Studies conducted with both methods have suggested an increased vagal-mediated resting bradycardia induced by aerobic exercise training of proper duration and intensity [30, 44, 45]. However, a reduced cardiac adrenergic tonus leads to resting bradycardia in exercise-trained hypertensive and heart failure (HF) animals, which display cardiac dysautonomia associated with sympathetic hyperactivity [46–48]. These findings highlight the homeostatic role of aerobic exercise training in reducing cardiac sympathetic hyperactivity. It is worth mentioning that sport modality influences the resting bradycardia level and its mechanisms of control in professional athletes. In fact, Azevedo and coworkers [36] have demonstrated that resting bradycardia (evaluated by both cardiac autonomic blockades as heart rate variability) is mainly dependent on an increased cardiac vagal tonus in runners, while cyclists' display of resting bradycardia is associated with a reduced intrinsic heart rate combined with eccentric and concentric hypertrophy. Indeed, the cardiac autonomic control of resting heart rate in athletes may change according to the training season. In fact, Iellamo and coworkers [49] have demonstrated a cardiac conversion from vagal to sympathetic predominance in professional rowers preceding the World Championship.

Cardiac Contractility and Relaxation Responses to Aerobic Exercise

Even though heart rate response to exercise is modulated by sympathetic/vagal balance, cardiac contractility and relaxation response to aerobic exercise are controlled solely by the sympathetic nervous system. This occurs since cardiac ventricles, responsible for contraction, receive almost exclusively adrenergic fiber innervations, whereas the cholinergic system fibers run with the vagus nerve subendocardially,

reaching mainly the atrial myocardium with minimal connections to the ventricular myocardium [13].

Regarding cellular mechanisms underlying the modulation of cardiac function during aerobic exercise, β-AR activation by norepinephrine and epinephrine plays a key role in increased cardiac contractility during an acute bout of aerobic exercise. The increased cardiac contractility is due to β-AR modulation of major Ca^{2+}cycling proteins involved in excitation–contraction coupling, such as L-type Ca^{2+}channels, ryanodine receptors (RyR), and the sarcoplasmic reticulum Ca^{2+}-ATPase regulator, phospholamban (Fig. 5.1a). During a single bout of exercise, the sympathetic activation mediated by β-AR-adenylyl cyclase-cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) signaling pathway leads to the phosphorylation of Ca^{2+}cycling proteins, increasing the intracellular Ca^{2+}transient and contraction amplitudes, which accelerates their kinetics during exercise [50]. Likewise, Ca^{2+}reuptake by sarcoplasmic reticulum Ca^{2+}-ATPase is accelerated by increased β-AR-mediated PKA phosphorylation of phospholamban, which results in a faster cardiac relaxation time during aerobic exercise [51].

Besides the autonomic balance of the cardiovascular system during a single bout of exercise, the aerobic exercise training is also able to modulate the density and responsiveness of β-AR in the heart. In general, cardiac β-AR density has been shown to decline with aerobic exercise training. However, data on such effects of exercise training are controversial in the literature [52, 53]. Taking into consideration the β-AR subtypes, studies have proposed a downregulation in either β_1-AR or β_2-AR subtypes after training [54–57]. This was suggested to be a compensatory adaptation in a tissue exposed to high concentrations of catecholamines during exercise training sessions [58]. Despite this response, compelling evidences from the literature suggest that aerobic exercise training increases cardiac contractility in animal models [59, 60] and humans [61]. In fact, increased adenylyl cyclase activity and myocardium responsiveness to β-AR agonists have been observed in trained rats regardless of training-induced alterations in cardiac structure [59]. Indeed, aerobic exercise training increases cardiac inotropy by phosphorylating key Ca^{2+}handling proteins, such as the RyR and phospholamban [62, 63] (Fig. 5.1a). In addition, an increased myofilament Ca^{2+} sensitivity and enhanced pH regulation are observed in isolated cardiac myocytes from exercise-trained rats [59, 60].

Cardiac Responses to Aerobic Exercise in HF

HF is a common endpoint of cardiovascular diseases, and the development of end-stage HF involves a continuous interaction between myocardial dysfunction and hyperactivation of neurohumoral systems, including adrenergic system. At first, this response is compensatory and may cause myocardium hypertrophy in response to increased cardiac work. However, sustained neurohumoral hyperactivity is toxic and deleterious as cardiac dysfunction persists [64]. Indeed, adrenergic system hyperactivation leads to worsening of HF, which is associated with altered cardiac

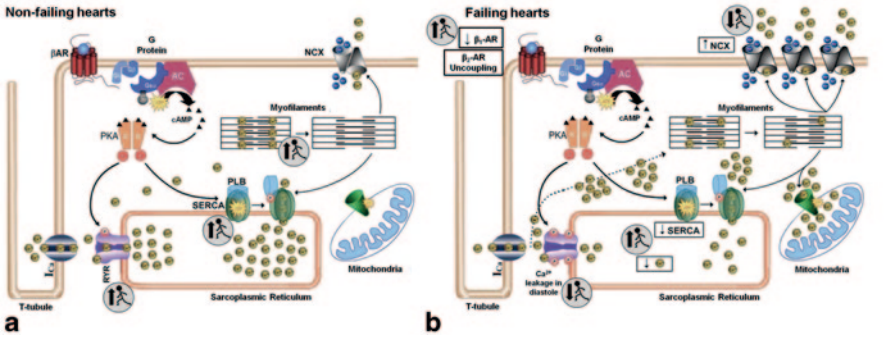

Fig. 5.1 Excitation–contraction (EC) coupling in nonfailing **a** and failing **b** hearts. Adapted from Brum, PC et al. 2006, "Neurohumoral activation in heart failure: the role of adrenergic receptors", Annals of the Brazilian Academy of Sciences 78(3): 485–503. **a** In nonfailing hearts during systole, EC coupling involves depolarization of the transverse tubule (T-tubule), which activates voltage-gated L-type Ca^{2+}channels (I_{Ca}) in the plasma membrane. Ca^{2+}influx via I_{Ca} triggers Ca^{2+}release from the sarcoplasmic reticulum (SR) via ryanodine receptors (RYR). During diastole, intracellular $Ca^{2+}$is pumped out of the cytoplasm by the SR Ca^{2+}ATPase (SERCA), which is regulated by phospholamban (PLB). In addition, $Ca^{2+}$is extruded from the cell by the sarcolemmal Na^{+}/Ca^{2+}exchanger (NCX). The β-adrenergic receptor (β-AR) activation increases EC-coupling gain during systole and diastole through protein kinase A (PKA) phosphorylation of I_{Ca}, RYR, and PLB. The filled black arrows indicate the effects of aerobic exercise training. **b** In failing hearts, EC coupling is altered. RYR are hyperphosphorylated by PKA, which leads to greater sensitivity to Ca^{2+}-induced Ca^{2+}release at low and moderate cytoplasmic Ca^{2+}concentrations. The long-term effect of PKA hyperphosphorylation of RYR is an increased open probability at low intracellular Ca^{2+}concentrations, consistent with Ca^{2+}leakage during diastole. In addition, SERCA is downregulated, while NCX is upregulated in failing hearts, which contributes to depletion of SR Ca^{2+}stores. Aerobic exercise training attenuates abnormal Ca^{2+}handling, rescuing cardiac β_1-AR to normal control levels and reducing β_2-AR uncoupling. It also improves Ca^{2+}reuptake by SERCA, reduces sarcolemmal Ca^{2+}extrusion by NCX, and decreases Ca^{2+}leakage in diastole

adrenergic system components [65, 66], impaired Ca^{2+}handling [46, 48, 67, 68], and pathological cardiac remodeling [69, 70].

In failing hearts, there are abnormalities at multiple levels in the cardiac adrenergic signaling (Fig. 5.1b). β_1-AR, the most expressed AR in the heart, is downregulated in HF with reduced responsiveness regardless of cardiomyopathy etiology [65, 71, 72]. In contrast, β_2-AR levels remain unchanged in most studies [71, 72]. Additionally, the remaining cardiac β_1- and β_2-AR are desensitized mostly due to G-protein-coupled kinase-2 (GRK2) [72, 73], a kinase that phosphorylates and uncouples β-ARs. Increased GRK2-induced β-AR desensitization is supported by findings demonstrating that its inhibition reverses the pathological cardiac remodeling and improves cardiac function [72, 73]. The role of β_3-AR in HF has not been elucidated yet, but it has been demonstrated that β_3-AR signaling is increased in failing hearts [71].

Considering that adrenergic signaling in cardiac myocytes is tightly coupled to regulation of Ca^{2+}transients [74], desensitization of cardiac β-AR signaling pathway will lead to an abnormal Ca^{2+}homeostasis [46, 48]. In fact, altered expression

and function of major Ca^{2+}-regulating proteins have been described in severe HF models [75]. Reduced expression of sarcoplasmic reticulum Ca^{2+}-ATPase and L-type Ca^{2+}channel paralleled by increased Ca^{2+}leaking by RyR and sarcolemmal Ca^{2+}extrusion by Na^{+}/Ca^{2+}exchanger (NCX) is accounted for the reduced Ca^{2+}transient and depletion of sarcoplasmic reticulum Ca^{2+}content in HF [67] (Fig. 5.1b).

Cardiac remodeling associated with fibrosis and cardiac myocyte death is also a landmark of adrenergic system hyperactivity in HF, and the use of drugs that block neurohumoral hyperactivation, such as β-blockers and losartan (angiotensin II receptor antagonist), leads to an anticardiac remodeling associated with reduced mortality and improved cardiac function [68, 69, 76, 77].

Accumulated evidences have shown that aerobic exercise training is an efficient nonpharmacological strategy for HF therapy. Aerobic exercise training in HF patients improves quality of life, which is paralleled by an improved clinical symptom and a reduced hospitalization rate [78–80]. Additionally, beneficial effects of aerobic exercise training in HF are associated with a better autonomic control of the cardiovascular system [81, 82].

One of the first studies showing an improved autonomic balance after aerobic exercise training in HF individuals was published in 1992 by Coats and coworkers [83]. In fact, a landmark of aerobic exercise training in HF is a reduction in sympathetic hyperactivity [72, 84], which is paralleled by an improved cardiac Ca^{2+}handling [46, 48, 82, 85] and anticardiac remodeling [69, 82].

One of the mechanisms underlying the improved cardiac function by aerobic exercise training is related to attenuation of adrenergic signaling dysfunction in failing hearts associated with a rescue of cardiac β_1-AR to a normal control level accompanied by increased cAMP and reduced GRK2 levels [86, 87]. The improved cardiac adrenergic signaling by aerobic exercise training in HF improves, at least in part, Ca^{2+}homeostasis. In fact, in a model of sympathetic hyperactivity-induced HF model in mice, Rolim and coworkers [46] have demonstrated that aerobic exercise training improved the net balance of cardiac Ca^{2+}-handling protein expression represented by improved Ca^{2+}reuptake by sarcoplasmic reticulum Ca^{2+}-ATPase and reduced sarcolemmal Ca^{2+}extrusion by NCX. These results have been corroborated by other studies in different models of HF [59, 88]. The main effects of aerobic exercise training on intracellular Ca^{2+}handling proteins in HF are depicted in Fig. 5.1b.

Besides being an efficient therapy for HF, aerobic exercise training is considered an important strategy for the prevention of cardiovascular disease. Aerobic exercise training prior to HF development confers an important cardioprotector effect, attenuating cardiac dysfunction and abnormal Ca^{2+}handling [48, 89].

Regarding the impact of aerobic exercise training on cardiac remodeling induced by HF, reverse cardiac remodeling with a shift from pathological to physiological cardiac remodeling has been observed in exercise-trained animal models of HF [69, 90].

Despite structural similarities between physiological and pathological cardiac remodeling [90], studies have demonstrated that distinct molecular pathways are involved in each form of cardiac hypertrophy [60, 91–93]. While calcineurin/nuclear factor of activated T cell (NFAT) signaling pathway is one of the major players

in pathological cardiac remodeling [60, 93], physiological cardiac remodeling is mainly associated with phosphatidylinositide-3-kinase/protein kinase B (PI3K/Akt) signaling cascade [91, 92]. In this sense, aerobic exercise training in animal models of HF has been implicated in both deactivation of pathological and activation of physiological pathways involved in cardiac remodeling. Thus, deactivaction of cardiac calcineurin/NFAT signaling pathway by aerobic exercise training has been observed in both sympathetic hyperactivity- and hypertrophic cardiomyopathy-induced HF in mice [69, 94]. Even though disruption of the AKT1 gene abrogates exercise training-induced physiological cardiac hypertrophy, PI3K/Akt pathways seem to play a subtle role in the cardiac antiremodeling effect induced by aerobic exercise training in HF [69, 95].

In summary, aerobic exercise training effectively attenuates the impaired cardiac adrenergic signaling and Ca^{2+}handling, and has a cardiac antiremodeling effect in failing hearts, which ultimately leads to an improved cardiac function in HF.

Circulatory Adjustments to Aerobic Exercise in Health and Disease: Role of Autonomic Nervous System

During exercise, circulatory adjustments are necessary to meet the metabolic demand of active skeletal muscle, and neural control of sympathetic nerve activity plays a major role in ensuring the efficacy of these adjustments [96], since parasympathetic vascular innervation is scarce [97]. In this sense, several neural mechanisms, working in concert, regulate sympathetic nerve activity to active (exercising muscle) and inactive (nonexercising muscle) vascular beds through complex interactions [98, 99]. Local mediators are also involved in this regulation [100].

It is worth to highlight that proper sympathetic nerve regulation to active and inactive vascular beds is of critical importance, since impaired vascular response to aerobic exercise in cardiovascular diseases (e.g., HF) is considered a main mechanism involved in exercise intolerance [101]. Moreover, reduced skeletal muscle blood flow is considered an independent predictor of mortality in patients with HF [102] (Fig. 5.2). In the following section, we will discuss the mechanisms (central and reflexes) that control the sympathetic activity and vascular conductance during exercise in normal subjects and in patients with HF.

Mechanisms Involved in Circulatory Responses to Aerobic Exercise

During exercise, a redistribution of cardiac output occurs to match the high metabolic need imposed by contracting muscle. In this sense, the regulation of sympathetic nervous system to different vascular beds during exercise is crucial to maintain and reset vascular conductance according to blood flow necessity, as well as,

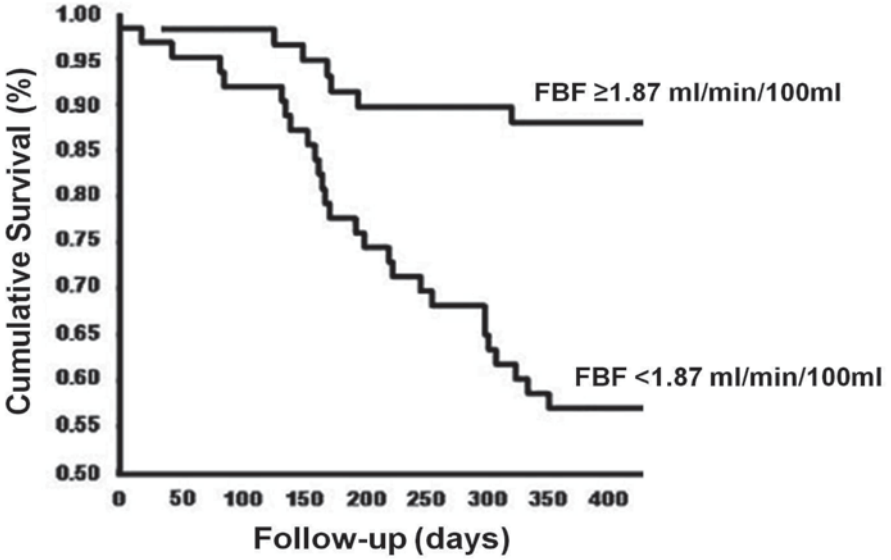

Fig. 5.2 Kaplan–Meier analysis of the cumulative rates of survival in patients with heart failure stratified in two groups on the basis of forearm blood flow (FBF, ml/min/100 ml). Survival rate was significantly lower in patients with forearm blood flow < 1.87 ml/min/100 ml ($p = 0.002$). Adapted from Barreto, ACP et al., 2009, "Increased muscle sympathetic nerve activity predicts mortality in heart failure patients," International Journal of Cardiology 135(3): 302–307

to preserve blood pressure and the perfusion of vital organs (e.g., brain and heart). The net result of this response is the increased vascular conductance and blood flow to active vascular beds while it decreases to inactive ones (e.g., liver, stomach, and skin) [103].

Activation of sympathetic nerve system during exercise is regulated by central command and reflex arising from skeletal muscle (exercise pressor reflex elicited by mechanoreceptors and metaboreceptors) or from aortic arch/carotid arteries (baroreceptors) [97, 103, 104].

At the onset of exercise, sympathoexcitation by central command increases its activity and induces the constriction of visceral and nonexercising muscle arterioles, metarterioles, and small and large veins. Besides its role in redirecting blood flow in favor of exercising muscles and central organs, sympathetic-mediated visceral vasoconstriction plays an important role in sustaining arterial pressure and maintaining an appropriate perfusion of vital organs [97, 103, 105]. Therefore, vasoconstriction of peripheral vessels is essential to the practice of exercise and it seems to be related to exercise intensity [106]. In exercising muscles, central command contributes to sympathoactivation only near maximal effort, which occurs mainly to counteract an exaggerated vasodilation, preventing blood pressure reduction and hypoperfusion, and consequently, a drop in muscle oxygen supply [22, 97, 107, 108].

Both central command and exercise pressor reflex exert an important role in resetting arterial baroreflex to higher blood pressure levels during exercise, which may further contribute to sympathoexcitation and simultaneous increase in heart rate and blood pressure during exercise [96, 104, 109, 110]. The resetting of arterial baroreflex operating point is paralleled by preserved baroreflex sensitivity during exercise [110–113], which seems to be very important to prevent excessive increase in blood pressure during exercise.

Concerning the cardiovascular response to exercise pressor reflex, metaboreflex elicited by afferent fiber type IV seems to have an important role in regulating sympathetic outflow to exercising and nonexercising vascular beds [97, 114–116]. Sympathoexcitation induced by metaboreflex was primarily thought to operate in moderate-to-high-intensity aerobic exercise when metabolites accumulate in a muscle undergoing contraction. However, metaboreflex is also elicited by a reduction in intracellular pH, which provides evidence for its activation even in mild exercise with minimal accumulation of muscle metabolites [24, 117–119]. Of interest, both sympathoexcitation [24, 117, 119] and increase in arterial blood pressure [118] display a significant inverse correlation with intracellular pH drop during exercise. Therefore, these findings give support for metaboreflex activation under inadequate blood flow to assure an appropriated blood supply to muscles' and metabolites' washout. Accordingly, metaboreflex seems to correct any mismatch between muscle blood flow and metabolism overriding central command.

As aforementioned, the main role of mechanoreflex elicited by afferent fibers type III is related to a cardiac vagal withdrawal contributing to exercise tachycardia at the onset of exercise [12]. Even though mechanoreflex contribution for sympathetic activation during muscle contraction has been demonstrated in animals [25, 120], it is difficult to isolate its contribution for sympathoexcitation in humans [12]. This is mainly due to the fact that subpopulations of afferent type III fibers are polymodal, being sensitized by either metabolites or mechanical stimulus [23, 121]. Indeed, it has been demonstrated that muscle mechanoreceptor is sensitized by metabolites [122].

Even though central and reflex activation of sympathetic nerves leads to vasoconstrictor stimulus in active and inactive vascular beds, an increased vascular conductance is observed in exercising muscles. This is due to local vasodilator mechanisms (metabolic vasodilation, shear-stress induced vasodilation, muscular pump, etc), which surpass the vasoconstrictor stimulus matching the high metabolic demand in exercising muscles. Indeed, a reduced responsiveness of active vascular beds to vasoconstrictor stimulus has long been reported [107, 123, 124]. In fact, Remensnyder and coworkers [123] coined the term "functional sympatholysis" to describe this phenomenon. The mechanisms proposed to explain functional sympatholysis include either prejunctional inhibition of noradrenaline release or postjunctional inhibition of noradrenaline binding to adrenergic receptors, which is mediated by metabolites produced in contracting muscles [97]. However, the precise mechanism underlying functional sympatholysis, or even its existence, is currently under debate [125, 126].

Circulatory Adjustments to Acute and Chronic Exercise in Heart Failure

Sympathoexcitation is a well-documented feature of chronic HF, leading to an increased vasoconstrictor tone and reduced blood flow to muscles and other organs. For instance, a consequence of reduced renal blood supply is an increased renin secretion and inappropriate salt and water retention. In fact, HF patients display lower renal cortical and skeletal muscle vascular conductance than healthy individuals [127]. These responses are of clinical importance since impaired exercise-mediated vasodilation by increased circulating noradrenaline is considered independent predictors of mortality in HF patients [102, 128]. Indeed, reduced skeletal muscle blood flow is a main mechanism involved in exercise intolerance in HF [101, 129].

As aforementioned, increased sympathetic activity to exercising vascular beds is offset by local mechanisms, such as increased vasodilation induced by metabolic byproducts. However, sympathetic hyperactivity observed in HF patients changes the balance between vasodilation and vasoconstriction in favor of the former. In fact, the reflex forearm vasodilatory response to handgrip contraction is restored under intra-arterial α-adrenergic receptor blockade by phentolamine in HF patients without affecting arterial pressure [130]. In contrast, reflex vasodilatory forearm blood flow in response to mental stress is not affected by either intra-arterial infusion of acetylcholine or L-arginine [131].

The beneficial effects of aerobic exercise training in HF include a significant reduction in sympathetic hyperactivity [48, 76, 84, 132], as illustrated in Fig. 5.3.

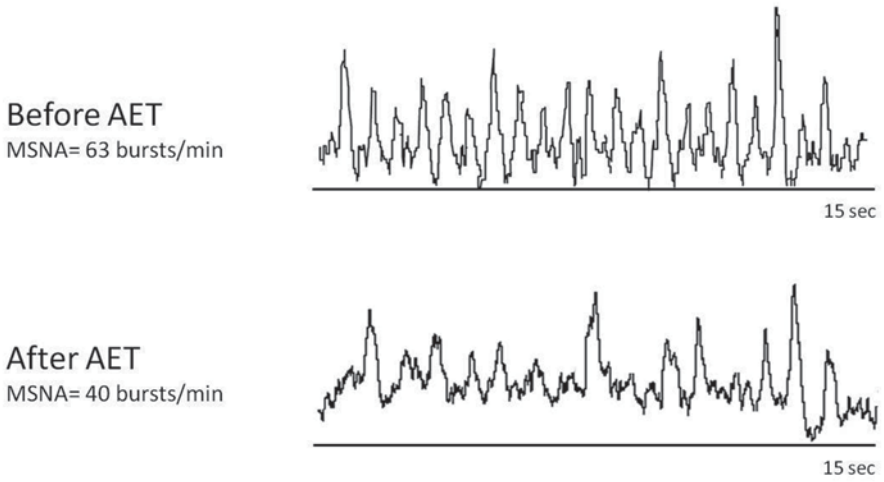

Fig. 5.3 Muscle sympathetic nerve activity (MSNA) of a 58-year-old female heart failure patient (hypertensive cardiomyopathy) with a 36% ejection fraction before and after 4 months of aerobic exercise training (AET). MSNA was obtained by direct recording of muscle sympathetic discharge by microneurography. Note that AET dramatically reduced sympathetic discharge. Data are from Cardiovascular Rehabilitation and Exercise Physiology Laboratory, Heart Institute, Medical School, Universidad de São Paulo

In fact, Coats and coworkers [83] were the first to demonstrate that aerobic exercise training reduced by 16% whole-body radiolabeled norepinephrine spillover in HF patients parallel by an improved cardiac autonomic balance. This is particularly interesting since one of the main pharmacological therapies for HF is the use of β-AR blockade. Therefore, HF therapies that decrease adrenergic hyperactivity should combine aerobic exercise training to optimize the benefits on the cardiovascular system. In this context, we have demonstrated an additional reduction in muscular sympathetic nerve activity by aerobic exercise training in HF patients optimized with carvedilol, a third-generation β-blocker (β_1- and β_2-blockade) with an α-blockade and vasodilatory effect [133]. Additionally, while β-blocker therapy has no impact on exercise capacity in humans or animals with HF [77, 134], a β-blocker combined with exercise training improves exercise capacity [48, 68, 133]. Indeed, it is important to highlight that reduction in sympathetic hyperactivity by exercise training in HF patients is associated with a better clinical outcome [84, 133], which occurs independently of HF patient age [135] or gender [136].

Potential mechanisms underlying reduced sympathetic nerve activity by aerobic exercise training involve the afferent autonomic control of sympathetic nerve activity coordinated by arterial baroreceptors, mechanoreceptors, and metaboreceptors [112, 113, 132, 137, 138].

Exaggerated sympathetic activation by central command is observed in HF. In fact, Koba and coworkers [129] demonstrated that renal and lumbar sympathetic nerve responses to mesencephalic locomotor region stimulation were exaggerated in myocardial infarcted animals. Likewise, increased exercise pressor reflex has been observed in HF [20, 138]. Mechanoreflex overactivity is involved in exaggerated exercise pressor reflex in HF, while the role played by metaboreceptor is still under debate, since some studies suggest a blunted metaboreflex function in HF [139–143] and others demonstrate a metaboreflex overactivation in HF [138, 144, 145]. Of interest, aerobic exercise training attenuates the overactive exercise pressor reflex in HF related mainly through reduced mechanoreflex evidenced by studies conducted in either animals or humans [138, 139].

Increased sympathetic nerve activity in HF is also associated with reduced baroreflex sensitivity in animals [146, 147] and humans [148, 149]. This is of particular interest since impaired baroreflex sensitivity is associated with a poor prognosis in HF patients [150–152]. Regarding the effect of aerobic exercise training on baroreflex function, our group demonstrated that aerobic exercise training reduced renal sympathetic nerve activity associated with an increased arterial baroreceptor afferent sensitivity in control rats [113, 137, 153]. Interestingly, this knowledge was extended to HF by Liu and coworkers [132] who observed reestablished arterial baroreflex control to renal sympathetic nerve activity by aerobic exercise training in a rabbit model of pacing-induced HF. In addition, Rondon and coworkers [154] also demonstrated that improved baroreflex control of renal sympathetic nerve activity in myocardial infarcted rats was associated with increased aortic depressor nerve sensitivity. Importantly, aerobic exercise training improved arterial baroreflex sensitivity in HF patients [155, 156]. The clinical relevance of these findings was firstly demonstrated by La Rovere and coworkers [152], who observed that exercise

training-induced increase in baroreflex sensitivity was able to predict improved prognosis in myocardial infarction patients.

In summary, aerobic exercise training reduces sympathetic hyperactivity in HF, which is associated with an improved outcome. The mechanisms underlying this response involve central and reflex adjustments of adrenergic system that regulate cardiovascular function.

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Chapter 6
The Adrenergic System in Cardiovascular Metabolism and Aging

Gaetano Santulli

The adrenergic (sympathetic) nervous system exerts numerous effects on the cardiovascular system, including increase in cardiac contractility (positive inotropy), heart rate acceleration (positive chronotropy), hastened cardiac relaxation (positive lusitropy), and accelerated atrioventricular conduction (positive dromotropy). Most of these effects are mediated by adrenergic receptors(also known as adrenoceptors, ARs), which belong to the guanine nucleotide-binding G protein-coupled receptor (GPCR) superfamily [111]. GPCRs are heptahelical trans-membrane sensors, accounting for approximately 4% of the entire protein-coding genome, widely considered the most important drug targets in physiology and medicine [47, 84, 86, 97, 98, 129, 145]. These receptors consist of seven membrane-spanning domains, three intra- and three extracellular loops, one extracellular N-terminal domain, and one intracellular C-terminal tail [140]. GPCR signaling is terminated by phosphorylation of the intracellular domains of the receptor by the family of G-protein-coupled receptor kinases (GRKs) [75, 123, 134, 214]. GRK-mediated phosphorylation increases the affinity of GPCRs for the arrestin class of proteins, which uncouples the phosphorylated receptor from the G-protein and subsequently targets the receptor for internalization [76, 112, 128, 172]. Downregulation of GPCRs has been shown to reduce the functional activity of classical signaling paradigms up to 80% [69].

Adrenergic Receptors: A Quick Update

Employing a series of pharmacologic agonists, Raymond Ahlquist was the first to describe two types of adrenoceptors based on the rank order of potency of some agonists [1]. The receptor termed αwas essentially excitatory, except in the in-

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testine, and the receptor termed β was mainly inhibitory, except in the heart. In this first classification [1], α-ARs were receptors present on smooth muscle, i.e. post-junctional receptors. These were later classified as post-junctional α_1ARs, in contrast with pre-junctional α_2ARs. Later, when evidence accumulated for α_2ARs located post-junctionally, such a merely anatomical classification was refined into a pharmacological sub-classification, independent of location [45]. In particular, phenylephrine is considered a selective agonist of αAR whereas isoproterenol is considered a non-selective agonist for βAR [119]. The α_1AR subfamily (a G_q coupled receptor) consists of three highly homologous subtypes, including α_1A-, α_1B- and α_1d-AR [26, 93]. The α_2AR subfamily (a G_i coupled receptor) comprises three subtypes: α_2A-, α_2B- and α_2C-AR [103, 113]. Some species other than humans express a fourth α_2D-AR as well [147].

On the other side, there are three receptor subtypes in βAR family: β_1AR is found at its highest levels in the heart [106, 197], β_2AR is distributed extensively throughout the body [82, 152], and β_3AR is mainly expressed in the white and brown adipose tissue [10, 32, 61]. All three βARs couple primarily to $G\alpha_s$ and subsequent cAMP-related pathways, but under certain conditions can also couple to $G\alpha_i$ [209, 213]. Importantly, various studies have shown that β_2AR signaling can also occur via G-protein independent mechanisms [68, 118, 157, 185, 186]. These paradigms of signaling can be observed in the same cell type based on the functional state of the cell [83, 89, 111]. Hence, a plethora of conditions can modify the response to GPCR stimulus, including chronic stimulation, acidosis, cell hypoxia and aging.

Adrenergic System and Cardiovascular Metabolism

AR activation leads to substantial metabolic responses, including increased lipolysis with subsequent elevated plasmatic levels of free fatty acids (FFA), and increased gluconeogenesis, in order to conserve glucose and to shift fuel metabolism of the muscle towards FFA oxidation [28, 148]. ARs are classically the receptors involved in the ‘fight or flight’ reaction, the physiological response that occurs following a perceived harmful event, attack, or threat to survival [53, 80]. The parasympathetic (cholinergic) nervous system is instead mainly involved in the so-called ‘rest and digest’ response. Sympathetic activation in the cardiovascular systemtranslates into release of the two catecholamines that mediate its effects, i.e., noradrenaline (norepinephrine) and adrenaline (epinephrine) [115, 141, 201]. The active secretion of the adrenal medulla contains approximately 80% adrenaline and 20% noradrenaline, but this proportion is reversed in the sympathetic nerves, which contain predominantly noradrenaline [151]. The effects of adrenaline and noradrenaline are generally similar, albeit they differ from each other in certain of their actions. In particular, noradrenaline constricts almost all blood vessels, while adrenaline causes constriction in many networks of minute blood vessels but dilates the vessels in the skeletal muscles and the liver [41, 141]. Both sympathomimetic agents

increase heart rate and myocardial contractility, thereby augmenting cardiac output and eventually the blood pressure [115, 141]. These hormones also have important metabolic actions. Indeed, adrenaline stimulates the breakdown of glycogen to glucose in the liver, which results in the raising of the level of blood sugar. Both agents increase the level of circulating FFA [108, 188]. Actually, the extra amount of glucose and FFA might be used by the organism as fuel in times of stress or danger where increased alertness or exertion is required [19, 24, 124].

Sympathetic activation causes α_1-AR-mediated vasoconstriction in less vital vascular beds, particularly splanchnic and skin, to divert blood to skeletal muscle in exercise. ARs activation also mobilizes blood from the reservoir in the large veins (the capacitance vessels), again largely involving α_1- and α_2- ARs [45, 103]. These acute physiological responses, typical of the stress conditions, are detrimental when they become chronic [6, 125]. Indeed, a common feature of many pathological conditions involving over-activation of the adrenergic system is the development of important metabolic alterations, including insulin resistance, impaired glucose and lipid metabolism and mitochondrial dysfunction [20, 70]. These patterns are somehow involved with a variably extent among different disorders. However, they are generally correlated to the level of activation of the adrenergic system [28, 111, 158].

Heart failure represents a classical hallmark in the study of metabolic alterations linked to the neuroadrenergic system. Indeed, cardiac contraction is indispensable for providing oxygen and nutrients to the body. Therefore the cardiac muscle has high metabolic demands, among the highest in the body [5, 168]. Moreover, with minimal ATP reserves and complete ATP turnover approximately every ten seconds, the heart heavily depends on a continuous energy supply [8, 37, 107]. Providentially, the heart possesses a metabolic flexibility that allows maintaining its function during stressful conditions. The adult cardiac muscle generates ATP almost exclusively via oxidative phosphorylation by using different metabolic substrates [42, 189]. In particular, in healthy state the major pathway for cardiac ATP production is FFA oxidation whereas the relative contribution of glucose increases during stress or injury [102, 136, 203]. Impairment in cardiac energy metabolism represents thereby a serious risk factor for the development of cardiac disease [5, 56]. Indeed, under pathological conditions, the heart exhibits a severe malfunction of different metabolic pathways, including the tricarboxylic acid cycle and β-oxidation [73]. Such a metabolic remodeling is characterized by a lower oxidative capacity, contractile dysfunction, and insulin resistance [33, 54, 92].

Intriguingly, in both heart failure and type 2 diabetes mellitus (T2DM) circulating insulin levels are chronically elevated, leading to persistent stimulation of insulin receptors [23, 132, 162, 166]. The myocardium has been shown to retain its insulin sensitivity in terms of ability to activate insulin-related signaling cascades in T2DM [54, 208]. Such increase in insulin signaling in the heart promotes FFA uptake and subsequent lipotoxicity [5, 63]. Furthermore, hyperactive insulin signaling has been also shown to accelerate adverse left ventricular remodeling [171, 176]. Recently, insulin has been demonstrated to directly impair adrenergic signaling pathways for contractile function via an insulin receptor/β_2AR signaling complex [54], providing

a potential novel mechanism for cardiac dysfunction associated with hyperinsulinemia in diabetic cardiomyopathy and heart failure.

The onset of T2DM has been associated with increased central sympathetic outflow, altered norepinephrine disposition, and blunted sympathetic responsiveness to carbohydrates [183]. Consistent with these data, insulin resistance has been shown to highly correlate with neuroadrenergic function [77, 99, 100, 101, 109]. Then, an initial prevalence of sympathetic over parasympathetic activity might be responsible for an increased metabolic state, accompanied by increased insulin sensitivity [109, 179]. As in different hormone-regulated pathways, this state is subsequently followed by a reduction in βAR metabolic responsiveness, with a diminished basal metabolic rate and an increased tendency toward anabolic processes, mirrored by insulin resistance and reduced ability to dissipate energy, i.e., weight gain, particularly at the visceral level [139, 184]. This aspect could then lead to a positive feedback loop, in which insulin resistance further stimulates sympathetic activity, thereby worsening insulin resistance itself [182]. Of note, both baseline sympathetic drive and nutritional sympathetic responsiveness have been shown to be important prognostic biological markers for dietary weight loss outcome in obese subjects with metabolic syndrome [116, 183].

To date, several therapeutic approaches targeting glucose or FFA metabolism have been suggested to modulate metabolic pathways in the failing heart, in order to eventually improve cardiac function and metabolic elasticity [3, 43, 64, 74].

Regarding the effect of adrenergic system on metabolism, several investigators have demonstrated that a sustained βAR stimulation induces insulin resistance [30, 40, 57, 133, 146, 195]. In this context, β_2AR and β_3AR seems to have a key role in regulating, not exclusively, glucose and FFA homeostasis, respectively. Indeed, whereas β_2AR acts on both pancreatic β-cell hormone secretion and peripheral glucose metabolism [15, 39, 157] β_3AR is more involved in the modulation of FFA metabolism [17, 95]. In particular, acute treatment of myocytes or skeletal muscle with β_2AR agonists induces a significant increase in glucose uptake, comparable to insulin stimulation [15, 126]. Equally important, cardiac-specific overexpression of β_3AR has been shown to inhibit the hypertrophic response to neurohormonal stimulation both in vivo and in vitro, via a NOS-mediated mechanism [10].

Grks Regulate Cell Metabolism

Beyond their widely acknowledged effects on GPCR regulation, GRKs have been identified as pleiotropic proteins involved in the regulation of innumerable cellular functions through the phosphorylation of cytosolic substrates or, in a phosphorylation-independent manner, via protein-protein interaction [59, 143, 195]. Most recently, new functions for these kinases have been reported in different disorders including inflammatory disease and cancer [38, 49, 52, 60, 135, 202].

In particular, landmark studies demonstrated that insulin induces up-regulation of GRK2, which in turn inhibits insulin signaling and glucose extraction [30, 159]. This

observation places GRK2 at the center of the stage as a potential actor in the pathophysiology of insulin resistance [117, 190]. Also, several conditions associated to insulin resistance including hypertensionand diabetes are characterized by elevated GRK2 levels [4, 159]. *In vitro*, insulin has been shown to induce an increase in GRK2 levels, causing GRK2–IRS1 association in a time-dependent manner [30, 159], alongside with phosphorylation of IRS1 in serine/threonine and inhibition of IRS1 tyrosine phosphorylation eventually leading to inhibition of insulin receptor signaling. If GRK2 up-regulation is associated with insulin resistance, its inhibition has been proposed as beneficial in different animal models. For instance chronic treatment of spontaneously hypertensive rats with an inhibitor of GRK2 kinase activity, Ant-124, ameliorates glucose homeostasis and IRS1 tyrosine phosphorylation, and is accompanied by a reduction of blood pressure levels [30, 159]. Inhibitors of GRK2 that prevent its binding to the substrate have been also shown to correct impaired glucose homeostasis in various animal models of diabetes and/or obesity, including Psammomys obesus gerbils, Zucker diabetic fatty (ZDF) rats, and *db/db* mice [2].

In the heart, GRK2 inhibition obtained via deletion of GRK2 gene or transgenic expression of a truncated mutant which prevents GRK2 localization on the membrane has been shown to be beneficial for the failing heart, preventing the derangement of insulin signaling and significantly delaying the reduction of glucose uptake, eventually preserving myocardial function [27]. In line with these data, lymphocyte GRK2 levels have been demonstrated to significantly increase in patients with myocardial infarction and are associated with worse systolic and diastolic function (Santulli et al. 2011b). Besides, early revascularization and β-blocker therapy influenced GRK2 levels. Two years after myocardial infarction patients with higher GRK2 levels at admission had worse systolic function and cardiac remodeling (Santulli et al. 2011b), implying that GRK2 levels may reflect hemodynamic impairment and might have a prognostic value in post-ischemic heart failure.

Intriguingly, emerging evidence indicates that GRKs exert different effects within the cell depending on the cell type, localization, stimuli, and pathophysiological context [58, 198, 202]. In this sense, Iaccarino and colleagues were the first to demonstrate a mitochondrial localization for GRK2 in immunogold experiments [55]. These studies establish a functional role for GRK2 in organelle biogenesis and ATP production both *in vitro*, in human fibroblasts, and in vivo, in muscles in which GRK2 had been deleted using a *Cre/flox* recombinant technique [55, 158]. GRK2 localization in mitochondria was later confirmed by Koch et al. [22], opening a new field of investigation with intriguing hypotheses and potential therapeutic opportunities.

The Aging Process in the Cardiovascular System

Aging causes evident changes in the cardiovascular system [50, 90, 151; 207] that most likely reflect perturbations of biological and biochemical adaptive mechanisms [25, 170]. These aspects have been confirmed both in men and women [96].

In the heart, noteworthy alterations in ventricular filling and relaxation have been described with aging, including a prolongation of isovolumetric relaxation time, a reversal of the early and late mitral inflow velocities (E/A ratio), a modification of the dynamic longitudinal wall relaxation, and diastolic suction (propagation velocity of early mitral inflow) [137, 158].

The incidence of cardiac disorders as heart failure, atrial arrhythmias, and left ventricular hypertrophy increases dramatically with age [150]. The elderly appear to be particularly predisposed to the development of heart failure. Such a diagnosis is undeniably the leading cause of hospitalizations in people > 65 years of age [144, 150]. Atrial fibrillation is detected in approximately 3–4% of healthy volunteers over age 60 years without clinical coronary artery disease. Such a rate is 10-fold higher than in the general adult population [155]. Overall prevalence of AF has been quantified to be 17.8% in people aged 85 years and above [12, 150]. Of course, development of a rapid irregular pattern of electrical activity may have negative consequences for hearts that are relatively stiff and relax slowly [35, 48, 150, 156]. Prevalence of cardiac hypertrophy increases with rising blood pressure and body mass index [94, 176]. Cross-sectional studies in normotensive subjects indicate that left ventricular wall thickness increases progressively with age. Furthermore, cardiomyocyte enlargement was observed at autopsy in aged subjects without cardiovascular disease, in whom overall cardiac mass was not increased [131].

At the vascular level, several studies in humans have clearly demonstrated an age-associated increase in intimal thickening [91, 199] (the intimal-media thickness increases 2- to 3-fold between 20 and 90 years of age) accompanied by both luminal dilatation and a reduction in distensibility or compliance, resulting in an increase in vessel stiffness. Pulse wave velocity, a noninvasive index of vascular stiffening, increases with age and has been associated to structural alterations in the media, including calcification, increased collagen, reduced elastin content, and elastin fractures [14, 87, 120, 158]. A potential explanation might be that vascular stiffness is governed not only by the structural changes within the matrix, as noted above, but also by endothelial regulation of smooth muscle tone [11, 174, 187]. In particular, endothelial aberrations have been demonstrated to occur in early stages in hypertension, diabetes, and atherosclerosis [29, 77; 154, 157].

Involvement of Adrenergic System in the Aging Process

Several experimental findings indicate an age-associated decrease in catecholamine-responsiveness in the elderly. In particular, an age-associated decrease in βAR sensitivity and density has been shown in the myocardium and has been mainly attributed to down-regulation and impaired coupling of βAR to adenylate cyclase [91, 104]. The age-linked decline in cardiac βAR response seems to be primarily due to a down-regulation of β_1ARs, as reported in aged explanted human hearts [205]. Further, a reduction in the sensitivity of βARs, measured by isoproterenol-

induced changes in the catecholamine stimulated adenylate cyclase activity in the myocardium [130] and in pulse rate and blood pressure [196], has been reported.

The compartmentalization of βARs may also partake in the decreased βAR responsiveness observed with aging: whilst β_1ARs are widely distributed on the plasma membrane, β_2ARs are usually located in the transverse tubules, invaginations of the plasma membrane containing several proteins that couple membrane depolarization (excitation) to calcium-mediated myofilament shortening (contraction) [152, 210]. Henceforward, the peculiar localization of β_2AR in cardiac cells leads to the generation of spatially restricted cAMP production, affecting Ca^{2+}-dependent proteins that control the contraction of myofilaments [212]. A disrupted localization of β_2AR has been described in chronically failing cardiomyocytes, with substantial functional consequences [114, 127]. Importantly, conditions presenting a depressed cardiac function elicit activity from the sympathetic nervous systemthat ultimately increases cardiac output and diverts blood flow to critical organs. The principal actors of this system are the catecholamines, which release is strictly controlled by the GPCR system, relating the adrenal gland and the heart [151]. Numerous studies have confirmed that β_2AR is definitively involved in the regulation of catecholamines secretion by the adrenal gland[21, 34, 51]. Of note, young people are more responsive than elderly subjects to isoproterenol-induced increase in blood flow in the brachial artery [79, 194]. Such features are similar to what seen in patients with failing hearts. Ergo, most of the modifications that occur in the sympathetic nervous system with aging, including decreased βAR responsiveness, increased circulating catecholamines, and hyposensitivity to adrenergic stress [164, 193, 211] Pan et al. 1986) are also common in patients with heart failure [149, 163]. The current literature exploring the relationship between aging and βARs, summarizing the major findings of research on aging in several organs expressing βARs has been recently reviewed [152].

In the vessels, both the medial (vascular smooth muscle cells, VSMC) and the intimal (endothelial cells, EC) layers finely regulate vascular tone [26, 142, 161] (Santulli et al. 2011a) and both VSMC and EC express β_2AR [62, 78, 138, 177]. Therefore, such a receptor is expected to play a pivotal role in the reduced vasoreactivity observed in elderly [18, 67, 204]. In this sense, the age-associated decline in β_2AR function and subsequent cAMP generation [36, 165, 167, 181] represents a common factor to hypertension, atherosclerosis, vascular insufficiency and orthostatic hypotension, all conditions with significant morbidity and mortality [148, 154, 162, 175, 200, 206]. The increased incidence of atherosclerosis and restenosis in aged people may also rely on the age-associated deterioration in βAR-mediated cAMP production, since cAMP may inhibit VSMC proliferation (Begum et al. 2011). An age-dependent impaired αAR responsiveness in healthy humans has also been reported [46, 72, 96, 173], with potential implications for reduced muscle blood flow and augmented blood pressure during exercise, and might contribute to exercise intolerance in ageing humans [44, 46, 158].

Molecular Mechanisms Underlying the Age-related Decline in βAR Function

Several factors (structural and neurohormonal) participate in the modulation of arterial blood pressure, which is determined by a fine equilibrium between peripheral resistances and cardiac output. The sympathetic nervous systemplays a crucial role in the regulation of vascular tonedue to its ability to control at the same time both these parameters [71, 110, 121, 191]. Indeed, systemically circulating or locally released [178] sympathetic catecholamineslead to the activation of two main classes of ARs: α_1AR and β_2AR, causing vasoconstriction and vasodilatation, respectively [26, 78; 160]. With aging, this equilibrium is progressively shifted toward increased vasoconstriction, most likely because of a defective vasodilatation in response to βAR stimulation. Indeed, βAR agonist administration in the human brachial artery induces vasodilatation and, interestingly, this response appears to be attenuated in hypertensive patients [79, 180]. The role of β_2AR in the vasculature appears to be so critical that genetic variants of this receptor [105], causing excessive desensitization, may lead to reduced vasodilation [16, 31, 85] and also promote the occurrence of atherosclerosis [7, 88].

Increased basal levels of circulating catecholamines have been observed with advancing age [216], paralleled by a marked decrease in the number of high-affinity βARs [66]. These data suggest that age-related alterations may be due to βAR desensitization rather than loss of βAR density [169]. βAR affinity for the ligand is dependent upon GPCR phosphorylation, which in turn is in the domain of GRKs [79] (Santulli et al. 2011b). In particular, both GRK2 expression and activity have been shown to increase in vascular tissue with aging [160]. Similarly, a generalized impairment of βAR-mediated vasorelaxation has been shown both in animal models of hypertension[13, 77] and in human hypertensive subjects [79] and such alteration has been related to the increased GRK2 abundance and activity [160]. Indeed, the transgenic overexpression of GRK2 in the vasculature leads to impaired βAR signaling and vasodilatative response, causing a hypertensive phenotype in mice. This aspect has been confirmed in hypertensive patients, where GRK2 expression correlates with blood pressure as well as impaired βAR-mediated adenylate cyclase activity [65]. Most recently, common genetic variants of the β_2AR affecting its translational efficiency have been associated with human longevity [215].

Age-associated decrease in βAR-mediated relaxation has been attributed to different mechanisms, including a decreased receptor density, a less efficient coupling to adenylate cyclase, an impaired generation of cyclic AMP, or an attenuation of protein kinase a (PKA) activation [152]. Variations in cyclooxygenase expression and vasoactive prostanoid levels have also been explored in this sense [81]. However currently there is not a single cellular or molecular factor that can fully explain the decline in βAR function observed in elderly. Nevertheless, the etiology seems to be most likely associated with age-associated alterations in the ability of βAR to respond to agonists at the cellular level. A fundamental understanding of why βAR-mediated vasodilatation is impaired with age will provide new insights and

innovative strategies for the management of the multiple clinical disorders that affect older people [77, 78, 122, 153, 154, 167, 192].

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Chapter 7
The Neuroendocrine Adrenergic System and Cardiovascular Function

Lutz Hein

Introduction

The function of the cardiovascular system is under tight control of the autonomic nervous system to allow for the adaption of cardiac output and blood pressure during rest, physical activity, and stress. A balance of sympathetic and parasympathetic autonomic activity is important to allow this adaptation of the cardiovascular system. In this context, the adrenergic system is not only essential to control physiological functions of cells and organs in the body but also plays an important role in disease pathophysiology. The "sympathetic system" refers to the activating branch of the peripheral autonomic nervous system. Thus, the 'sympathetic system' represents one part of the adrenergic system in the body, which includes all neurons and cells in the body which synthesize or respond to adrenaline or noradrenaline.

Enhanced adrenergic activity has been identified in cardiovascular disease including hypertension [1, 2] and heart failure ([3, 4]; Fig. 7.1). Great advances have made in the diagnosis and treatment of cardiovascular disease resulting in a significant decrease of mortality rates of acute coronary syndromes, hypertension arrhythmias, and other cardiovascular disease [5]. However, despite these advances, the incidence of chronic heart failure is rising [6]. Chronic heart failure is one of the leading causes of morbidity and mortality [5, 6]. In chronic heart failure with impaired systolic function, several neuroendocrine mechanisms including the sympathetic and renin–angiotensin systems are activated to compensate for the loss of cardiac function (Fig. 7.1). While these mechanisms are helpful to maintain cardiac function in the short term, they contribute to the progression of heart failure in the long term [3]. Pharmacological blockade of enhanced adrenergic signaling by

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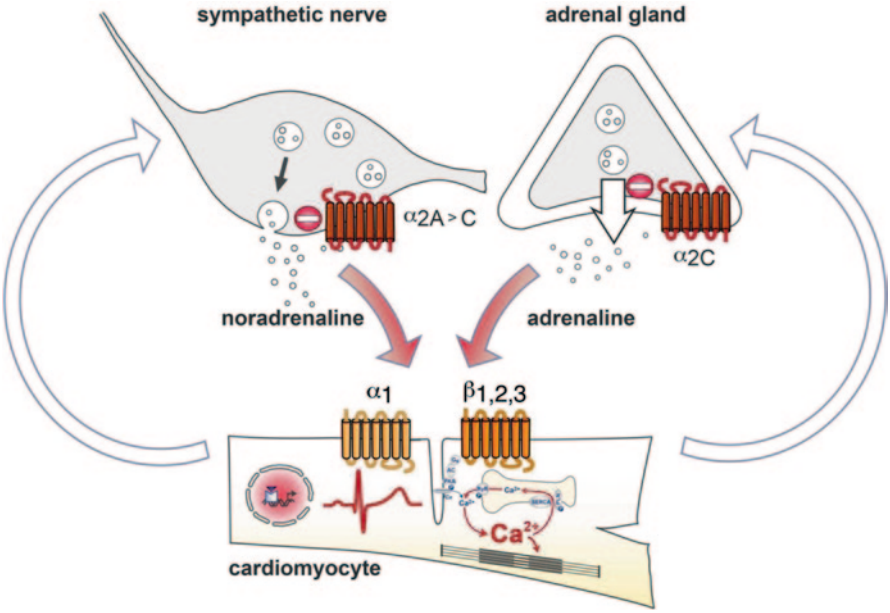

Fig. 7.1 Interaction between the sympathetic nervous system, the adrenal gland, and the cardiovascular system in chronic heart failure. Under physiological conditions, sympathetic nerves innervating the heart release noradrenaline to modulate cardiac function via α_1- and β-adrenoceptors. Adrenaline reaching the heart as a hormone is secreted from the adrenal gland. α_2-adrenoceptors operate as inhibitory feedback receptors to control maximal noradrenaline and adrenaline secretion. In heart failure, feedback loops activate the central sympathetic system to increase the sympathetic outflow to the heart

β-adrenoceptor antagonists exerts a beneficial long-term effect, reducing morbidity and mortality of patients with chronic heart failure [7–9]. The purpose of the present review is to summarize recent advances in the understanding of the pathophysiology of the neuroendocrine adrenergic system in cardiovascular disease with a main focus on chronic heart failure.

The Autonomic Nervous System

In general, the peripheral autonomic nervous system can be divided into several groups: (1) the sympathetic nervous system, (2) the parasympathetic nervous system, and (3) the enteric nervous system [10]. In addition to efferent nerves, the cardiovascular system is also innervated by sensory neurons.

Sympathetic Nervous System

Sympathetic pathways are composed of two types of neurons, termed pre- and postganglionic sympathetic neurons. The bodies of primary, preganglionic sympathetic neurons are located in the intermediolateral column of the thoracic and lumbar regions of the spinal cord. Their axons leave the central nervous system (CNS) to enter the paravertebral chains on both sides of the vertebral column s10]. In sympathetic ganglia within the paravertebral chains, primary sympathetic neurons connect with the secondary, postganglionic sympathetic neurons. In addition to the paravertebral ganglia, postganglionic sympathetic neurons are also located in prevertebral ganglia which are located in close vicinity of the aorta and its main abdominal branches [10].

Postganglionic sympathetic neurons innervate most tissues and organs of the body as a dense plexus of sympathetic axons. In contrast to many neurons in the CNS, sympathetic axons do not connect with their target cells as nerve terminals at a single synapse. Sympathetic (as well as parasympathetic) axons have several branches and neurotransmitter vesicles are stored in multiple varicosities along the axon to form a "pearl and chain" architecture ([11]; Fig. 7.1). Sympathetic varicosities and target cells form a specialized neuromuscular junction at the ultrastructural level [12].

The adrenal gland is an essential part of the sympathetic system, as chromaffin cells in the medulla resemble postganglionic sympathetic neurons lacking an axon [13]. Sympathetic neurons synthesize and release noradrenaline (norepinephrine) as their main neurotransmitter (Fig. 7.2). Chromaffin cells express an additional enzyme, phenylethanolamine *N*-methyltransferase (PNMT), which converts 90% of the synthesized noradrenaline into adrenaline ([13]; Fig. 7.2). Thus, adrenaline reaches its target cells and organs as a hormone via the circulation whereas noradrenaline derives from sympathetic nerves and also—to a smaller degree—from the adrenal gland.

Pre- and postganglionic sympathetic neurons contain additional co-transmitters, mostly neuropeptides as well as adenosine triphosphate (ATP) [13]. Immunohistochemical studies have revealed that sympathetic pathways which innervate particular tissues or organs contain distinct combinations of co-transmitters [13]. This "chemical coding" provides a cellular basis for specific activation of discrete tissues and organs by specific sympathetic pathways and fibers [14]. In the heart, sympathetic activation increases heart rate (chronotropic effect), conduction velocity (dromotropic), speed of contraction (inotropic), and relaxation (lusitropic). In the vascular system, sympathetic activation is linked with both vasoconstriction as well as vasodilatation to control blood pressure, peripheral resistance, and organ perfusion.

The molecular components involved in catecholamine synthesis, storage, release, and action have been unraveled in detail ([15, 16]; Fig. 7.2). After sequential synthesis of noradrenaline (in sympathetic neurons) or adrenaline in (adrenal chromaffin) cells, catecholamines are stored in neurotransmitter vesicles. In postganglionic sympathetic fibers, neurotransmitter vesicles are accumulated in varicosities,

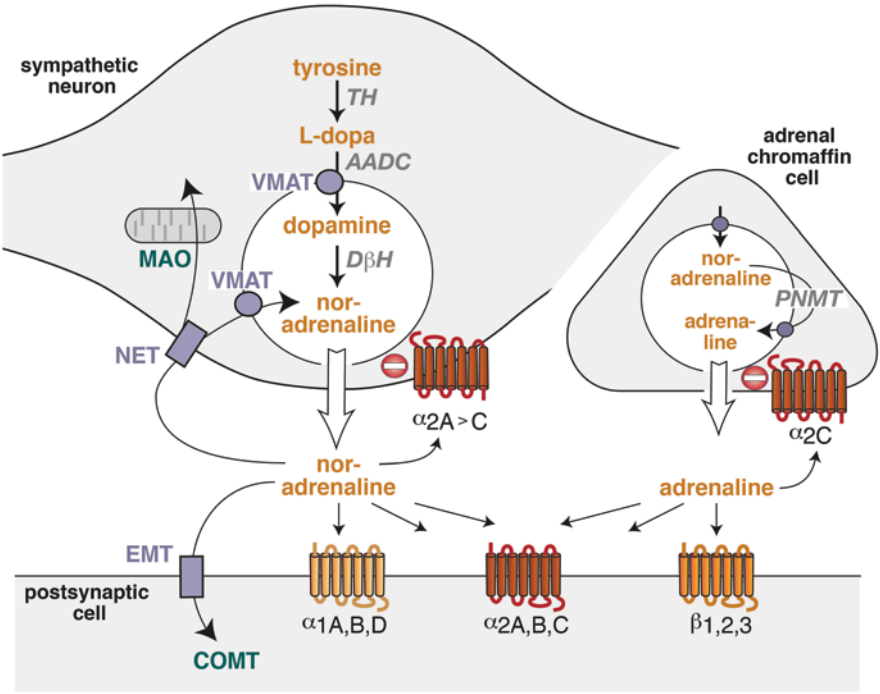

Fig. 7.2 Functional organization of the adrenergic system in sympathetic nerves and in the adrenal gland. In sympathetic nerves, noradrenaline is synthesized from the precursors tyrosine, L-dopa, dopamine and it is stored in synaptic vesicles. In the adrenal gland, noradrenaline is converted into adrenaline. Both catecholamines activate different G-protein-coupled receptors which are located in the post- or presynaptic membranes. Pre- and postsynaptic transporters mediate cellular or vesicular uptake of catecholamine (symbols in *light blue*), and enzymes (*green*) metabolize catecholamines. $\alpha_{1A,B,D}$, $\alpha_{2A,B,C}$, $\beta_{1,2,3}$ adrenoceptor subtypes, *AADC* aromatic amino acid decarboxylase, *COMT* catechol-*O*-methyltransferase, *DβH* dopamine β-hydroxylase, *EMT* extraneuronal catecholamine transporter, *MAO* monoamine oxidase, *NET* noradrenaline transporter, *TH* tyrosine hydroxylase, *VMAT* vesicular monoamine transporter, *PNMT* phenylethanolamine *N*-methyltransferase

from which the transmitter is secreted upon arrival of an action potential. Once noradrenaline has reached the extracellular space of the heart, it may activate surrounding adrenoceptors (Fig. 7.2).

The cellular functions of adrenaline and noradrenaline are mediated by G-protein-coupled receptors which are termed "adrenoceptors" and are located in the plasma membrane of many different cell types. Based on their homology, physiological function, and pharmacological profiles, these adrenoceptors were grouped into α- and β-adrenoceptor subtypes [17]. Until now, nine adrenoceptors have been identified by molecular cloning: three α_1-adrenoceptors (α_{1A}, α_{1B}, α_{1D}), three α_2-adrenoceptors (α_{2A}, α_{2B}, α_{2C}), and three β-adrenoceptors (β_1, β_2, β_3) [17]. For the elucidation of the structure, function, and regulation of adrenoceptors, the Nobel prize in chemistry was awarded to Dr. Robert J. Lefkowitz and Dr. Brian Kobilka in 2012 [18, 19].

Adrenoceptors have been identified in postsynaptic membranes as well as in the presynaptic membrane of sympathetic (and many other) neurons [20]. Presynaptic receptors may be separated into two groups, depending on whether they are activated by their neuron's own transmitter ("autoreceptors") or by other neurotransmitters or signals derived from other cells ("heteroreceptors") [16]. Among the sympathetic autoreceptors, α_2-adrenoceptors are inhibitory receptors while β-adrenoceptors stimulate noradrenaline release. Presynaptic α_2-adrenoceptors are important components of a regulatory feedback which limits the maximal amount of noradrenaline release (Fig. 7.2). In sympathetic neurons, α_2-adrenoceptors are activated by extracellular noradrenaline and inhibit further transmitter exocytosis [20]. Mouse models lacking individual α_2-adrenoceptor subtypes have been important tools to identify the receptor subtype and its function within this feedback loop. In sympathetic neurons, α_{2A}- and α_{2C}-receptors function together as presynaptic inhibitors ([21]; Fig. 7.2). Both receptor subtypes inhibited neurotransmitter release in several neuron types [22–24] and differed in their functional properties: α_{2A}-adrenoceptors inhibited noradrenaline secretion at higher action potential frequencies than α_{2C}-receptors [21]. This finding was consistent with longer persisting activation of intracellular signaling after agonist removal for α_{2C}-receptors than for α_{2A}-receptors [25]. Thus, due to a higher affinity for noradrenaline, α_{2C}-adrenoceptors were suggested to act as feedback inhibitors at low levels of sympathetic activity and α_{2A}-receptors as inhibitors at higher or maximal levels of sympathetic activity [21]. A similar feedback control mechanism has also been described for the adrenal gland (see Sect. 5 below).

Parasympathetic Nervous System

Similar to the sympathetic system, also the parasympathetic part is composed of two neuron types which are connected to each other in series [10]. However, in contrast to the sympathetic system, parasympathetic ganglia are located close to or even within its target organs. Preganglionic parasympathetic neurons responsible for cardiac control originate in the dorsal motor nucleus and the nucleus ambigous and leave the CNS via the vagal nerve. In cardiac ganglia, preganglionic neurons innervate the postganglionic parasympathetic neurons. Axons from these cells innervate several regions of the heart with particular dense levels of innervation in sinus and atrioventricular nodes and in the atria [10]. The major neurotransmitter of the parasympathetic system is acetylcholine which may be released together with additional neuropeptides and ATP as co-transmitters [10]. Some parasympathetic nerves synthesize nitric oxide which may act like a neurotransmitter, although it is not stored in neurons. All ganglionic transmissions in both branches of the autonomic nervous system are mediated by acetylcholine which activates nicotinic receptors of postganglionic neurons. Recent histological studies indicate that not only atria but also cardiac ventricles are innervated by parasympathetic nerves. In particular, the ventricular endocardium and epicardium contain fine networks of parasympathetic fibers [26]. Parasympathetic activation decreases heart rate and

atrioventricular conduction with minimal or no effects on cardiac contractility (for discussion, see [27]).

Sympathetic and parasympathetic systems interact functionally by their opposing effects on postsynaptic cells of the cardiovascular system. In addition, they also interact on the presynaptic side by presynaptic receptors (“heteroreceptors”) which modulate the neurotransmitter release of the other branch of the autonomic system [16]. Thus, noradrenaline alters parasympathetic acetylcholine release and acetylcholine acts at sympathetic nerves to control noradrenaline secretion.

Development of the Cardiac Sympathetic Nervous System

The heart is not homogeneously innervated by autonomic nerves and the development of the pattern of innervation during the prenatal period has been studies in several species [28]. The highest density of sympathetic nerves has been observed in the subepicardium and in the central conduction system [29–31]. All autonomic nerves which innervate the heart originate from neural crest cells during embryonic development [28]. The neural crest not only is the source for sympathetic and parasympathetic neurons but also gives rise to cardiac sensory neurons. In the adult organism, these cardiac sensory neurons are located in the dorsal root ganglia and are part of afferent cardiovascular reflex pathways. They also mediate pain sensation in response to cardiac ischemia [32].

During embryonic development, growth and migration of autonomic nerves from the neural crest to the heart and other target tissues are controlled by a number of neurotrophic factors [28]. Nerve growth factor (NGF) has been identified as one of the key factors for sympathetic growth during development and disease (Fig. 7.3). The final degree of sympathetic innervation of the heart correlates with the level of expression of NGF in the target tissue. During development, NGF is expressed in several regions of the CNS, but mature cardiomyocytes also express high levels of NGF protein [33, 34]. This finding was further supported by studies in mice lacking or overexpressing NGF or its receptor TrkA (tropomyosin related receptor kinase). Ablation of NGF expression as well as deletion of the TrkA gene in mice led to severe impairment of sympathetic ganglion development soon after birth [35, 36]. In contrast, overexpression of NGF under control of a cardiac myocyte-specific promoter led to sympathetic hyperinnervation and increased tissue norepinephrine levels in the heart [37]. NGF plays a similar trophic role during the development of the cardiac sensory system. Thus, cardiac NGF is essential to prevent apoptosis of sympathetic neurons and to guide innervation of the heart and other sympathetic target tissues and organs ([38]; Fig. 7.3).

Several factors which control cardiac expression of NGF have been identified [28]. Endothelin-1 (ET-1) and noradrenaline play opposing roles in the regulation of cardiomyocyte NGF expression (Fig. 7.3). ET-1 induces NGF expression in isolated cardiomyocytes in vitro and in the heart in vitro [33]. In ET-1-deficient mice, cardiac

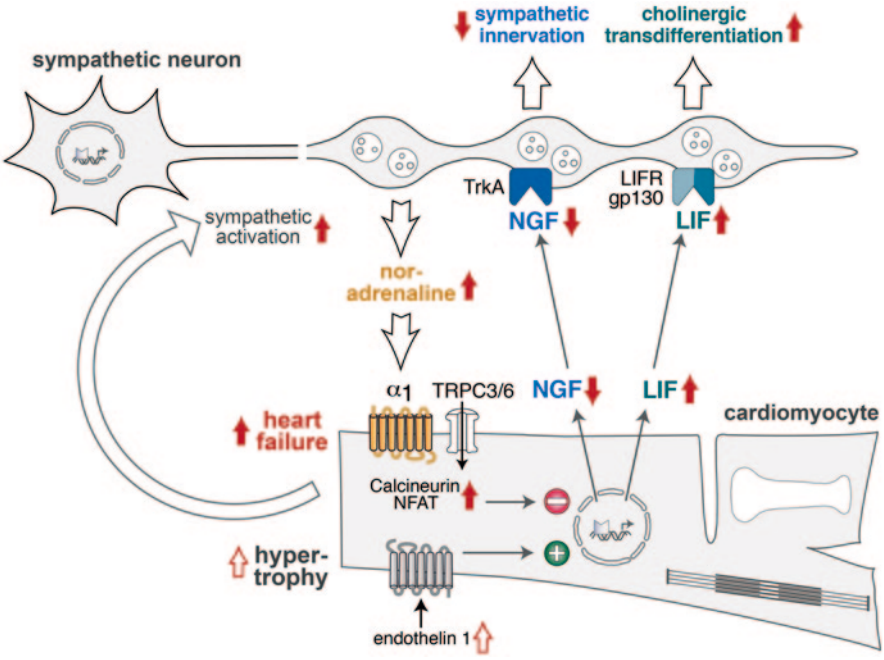

Fig. 7.3 Interactions between sympathetic nerves and cardiomyocytes in cardiac hypertrophy and failure. During development, endothelin-1 activates transcription and release of nerve growth factor (NGF) from cardiomyocytes. NGF activates neuronal TrkA receptors to facilitate sympathetic neuron outgrowth and thus determines sympathetic innervation density of the heart. In the failing heart, increased noradrenaline and Ca^{2+}influx through transient receptor-operated channels (TRPC3/6) activate cardiomyocyte calcineurin/NFAT signaling to inhibit NGF expression and reduce sympathetic innervation. Enhanced LIF expression in the failing heart stimulates sympathetic neurons to express cholinergic genes ("cholinergic transdifferentiation"). *LIF* leukemia inhibitory factor, *LIFR/gp130* LIF receptors, *NFAT* nuclear factor of activated T cells, *TrkA* tropomyosin-related kinase receptor

NGF expression and sympathetic innervation of the heart were significantly diminished [33]. Stellate ganglia from ET-1-deficient mice showed loss of sympathetic neurons due to apoptosis and these phenotypes could be rescued by cardiac-specific overexpression of NGF [33]. Mechanical stretch and activation of α_1-adrenoceptors by noradrenaline were identified as repressive factors for cardiac NGF [39]. Downstream of α_1-adrenoceptors, calcineurin/nuclear factor of activated T cells (NFAT) activation decreased cardiomyocyte expression of NGF [39]. Semaphorin 3a was identified as a second factor which inhibits cardiac sympathetic innervation during the development [40].

In contrast to the sympathetic system, less information exists about the prenatal development of cardiac parasympathetic nerves. Glial cell line-derived neurotrophic factor (GDNF) plays an important neurotrophic role in guiding parasympathetic innervation of the mouse heart via its receptors GFRα2 and Ret [41].

Sympathetic Nerves in Heart Disease

In chronic heart failure, the structure and function of the sympathetic system are altered. Reduced cardiac performance leads to a long-term, persistent sympathetic activation. Clinically, tachycardia has long been known as a symptom of heart failure, reflecting increased stimulation of the sinus node by noradrenaline released from cardiac sympathetic nerves [42]. Patients with heart failure showed elevated circulating and urinary levels of catecholamines [3, 43]. At the same time, the myocardial noradrenaline content was lower in failing hearts as compared with non-failing human hearts [43, 44]. These findings are consistent in both structural and functional alterations of the cardiac sympathetic system in chronic heart failure.

Functional Alterations in Heart Failure

In human and experimental heart failures, sympathetic outflow to the heart, kidney, and vasculature are increased ([27]; Fig. 7.1). Functionally, increased sympathetic activity aims to maintain blood pressure and cardiac output by stimulating cardiac contractility and inducing vasoconstriction. On the venous side, vasoconstriction enhances cardiac preload and on the arterial side it elevates peripheral resistance thus supporting blood pressure. By its action on renal proximal tubular cells, noradrenaline may, similar to angiotensin II, facilitate sodium and water retention. A comprehensive analysis of cardiac noradrenaline kinetics in normal subjects and in patients with heart failure identified the processes which are altered in heart failure [45]. Increased adrenergic drive to the failing heart resulted in increased cardiac release of noradrenaline and was accompanied by decreased efficiency of the presynaptic uptake mechanisms (Fig. 7.3). Thus, spillover of noradrenaline from the cardiac interstitial space into the blood stream was significantly enhanced in patients with heart failure [45]. Remarkably, elevated circulating concentrations of noradrenaline correlated with increased mortality in heart failure [3].

Chronic exposure to high levels of noradrenaline and enhanced β-adrenergic signaling are toxic to the heart [46–48]. On the postsynaptic side, chronic adrenergic activation leads to cardiomyocyte hypertrophy and interstitial fibrosis and several other changes which are summarized by the term “cardiac remodeling” [6, 49]. Several molecular events are combined to desensitize cardiac β-adrenergic signaling in response to chronic stimulation [19, 50]. Desensitization occurs at several levels, including downregulation of β_1-adrenoceptors in cardiomyocytes, uncoupling of β_1- and β_2-receptors from their G proteins by phosphorylation of receptors and arrestin binding, and by redistribution of β_2-receptors in the plasmamembrane [51, 52]. Altogether, these changes lead to a decrease in the inotropic reserve of the heart after chronic adrenergic stimulation.

In comparison with the sympathetic alterations, less is known about the parasympathetic system in heart failure [53]. Parasympathetic outflow to the heart may be reduced, thus contributing to increased heart rate and decreased heart rate variability in patients with heart failure [54].

Heart Failure with Preserved Ejection Fraction

Half of all patients with chronic heart failure have a preserved left ventricular ejection fraction (HF-pEF), but prognosis of these patients may be similar to patients with failure and reduced ejection fraction. Several cardiac and extracardiac factors have been suggested as key factors in the pathogenesis of the disease [55, 56]. However, only limited data are available concerning the activity of neurohumoral systems in HF-pEF. In a small study, patients with HF-pEF had significantly elevated plasma noradrenaline levels compared with control subjects [57]. However, additional studies suggest that stable patients with HF-pEF have lover levels of sympathetic activation than patients with reduced left ventricular function. HF-pEF patients may show similar rises in sympathetic activity during phases of acute decompensation. Thus, the role of neuroendocrine activation in heart failure with preserved ejection fraction remains unclear at present [56].

Alterations in Afferent Autonomic Reflexes and in the CNS

The mechanisms and pathways linking cardiac dysfunction with activation of central autonomic neuronal pathways have been studied intensively (for recent reviews, see [58, 59]). The prevailing model suggests that sympathetic activation is initiated by left ventricular systolic dysfunction which activates cardiovascular baroreceptors. Central reflex pathways which are activated in response to the baroreflex elicit a generalized increased in central sympathetic outflow [58]. However, impaired cardiac performance is by itself not sufficient to account for the elevated levels of sympathetic activation. A spectrum of afferent nerves relays signals from the heart, lung, and vascular system to the CNS. The precise mechanisms of sympathetic augmentation which increase adrenergic activity to higher levels and longer periods than required for maintenance of cardiovascular function are only partially understood [58]. Indeed, several lines of evidence suggest a more selective rather than generalized autonomic activation in heart failure. In early cardiac dysfunction, selective enhancement of cardiac noradrenaline release which precedes the elevation of total body noradrenaline spillover which is characteristic for advanced-stage heart failure [60]. Arterial and cardiopulmonary baroreflexes were found to be altered in human heart failure, thus contributing to enhanced sympathetic stimulation (for review, see [59]). Several alterations in the central circuitry involved in these reflexes, including enhanced angiotensin II signaling in circumventricular organs, have been uncovered [61, 62].

Structural Changes of Sympathetic Nerves

Cardiac disease alters sympathetic innervation of the heart [31, 63–65]. After myocardial infarction, sympathetic nerves degenerate within and distal to the site of ischemia but may regenerate after infarct healing. However, reinnervation of the

heart after injury may be heterogeneous and has been suggested to play a key role in arrhythmia development [66]. In failing hearts, sympathetic innervation decreases leading to depletion of cardiac noradrenaline content [67]. In diabetes mellitus, cardiac NGF expression was found to be reduced and was associated with a decreased sensory innervation of the heart [68, 69].

In addition to its central role during the development of cardiac sympathetic innervation, NGF may also be important for structural alterations of the sympathetic system in the failing heart ([70–72]; Fig. 7.3). In experimental hypertrophy and heart failure induced by chronic noradrenaline infusion, cardiac expression of NGF was suppressed by the activation of calcineurin/NFAT signaling due to elevated intracellular Ca^{2+} (Fig. 7.3). Activation of transient receptor potential channels TRPC3/6 was shown to be important for initiating this pathway [39, 73, 74]. As a consequence of NGF repression, cardiac sympathetic innervation density was significantly reduced (Fig. 7.3). In a rat model of heart failure, the lack of NGF could be rescued by injection of NGF into stellate ganglia thus leading to improved cardiac noradrenaline reuptake and improved cardiac performance [75].

Several experimental data suggest that sympathetic neurons may alter their phenotype in chronic heart failure. In failing hearts, sympathetic ganglia express genes which are typical for cholinergic or immature neurons [76]. Among the cytokines which are elevated in heart failure, leukemia inhibitory factor (LIF) and cardiotrophin-1 led to transdifferentiation of sympathetic neurons to acquire cholinergic activity via activation of gp130 signaling ([77]; Fig. 7.3). Kimura et al. called this phenomenon “functional denervation due to rejuvenation” [28]. At present, it remains unclear whether transdifferentiation of sympathetic nerves to a parasympathetic phenotype is adaptive or maladaptive in heart failure. Mice lacking gp130 signaling in sympathetic neurons did not show signs of transdifferentiation and had a higher mortality rate in a hypoxia-induced heart failure model than wild-type mice [77]. This finding indicates that transdifferentiation of sympathetic neurons may be protective.

Adrenal Gland in Physiology and Heart Failure

Similar to sympathetic neurons, α_{2C}-adrenoceptors operate as feedback regulators of catecholamine release from adrenal chromaffin cells ([50, 78]; Figs. 7.1 and 7.2). Interestingly, basal feedback control of catecholamine release from adrenal medulla or sympathetic nerves was mediated by different α_2-adrenoceptor subtypes [50]. While mice lacking α_{2A}-receptors showed enhanced plasma noradrenaline levels, α_{2C}-deficient mice displayed twofold elevated circulating adrenaline concentrations [50]. At present, it remains unclear which α_2-adrenoceptor subtype(s) are essential for adrenal feedback control in different species. While α_{2C} predominates in mouse chromaffin cells [50], α_{2A} is the major subtype in rat adrenal medulla [78]. Both receptor subtypes have also been identified in the human adrenal [79].

Several experimental models of heart failure have demonstrated functional and structural alterations in the adrenal gland (Fig. 7.4). Most notably, hypertrophy of the adrenal gland has been observed in rats after experimental myocardial infarction and in mice with chronic cardiac pressure overload or transgenic expression of calsequestrin [78, 80]. Interestingly, adrenal weight showed a significant linear correlation with cardiac weight increase after transverse aortic constriction in mice [80]. Furthermore, desensitization of adrenal α_{2C}-adrenoceptors by G-protein-coupled receptor kinase 2 (GRK2) led to an impaired adrenal feedback control and thus elevated catecholamine release in heart failure (Fig. 7.4). Preganglionic cholinergic innervation of the adrenal gland was found to be essential for adrenal hypertrophy, upregulation of GRK2, and increased catecholamine synthesis during cardiac pressure overload ([80]; Fig. 7.4). In addition, acetylcholine stimulated adrenal catecholamine synthesis by induction of PNMT expression via a nicotinic receptor—Ca^{2+}/calmodulin-dependent pathway ([80]; Fig. 7.4).

The essential role of GRK2 upregulation in α_2-adrenoceptor desensitization and enhanced adrenal catecholamine release has been demonstrated by the adrenal-specific overexpression or ablation of GRK2 [81, 82]. In these models, treatment with the β-adrenoceptor antagonist bisoprolol reduced heart failure-related cardiac remodeling and reduced adrenal GRK upregulation and lowered plasma catecholamine levels [83]. In addition to GRK2, β-arrestins, which bind to and desensitize phosphorylated G-protein-coupled receptors [19], contribute to adrenal dysregulation of catecholamine secretion. Ablation of β1-arrestin enhanced survival and

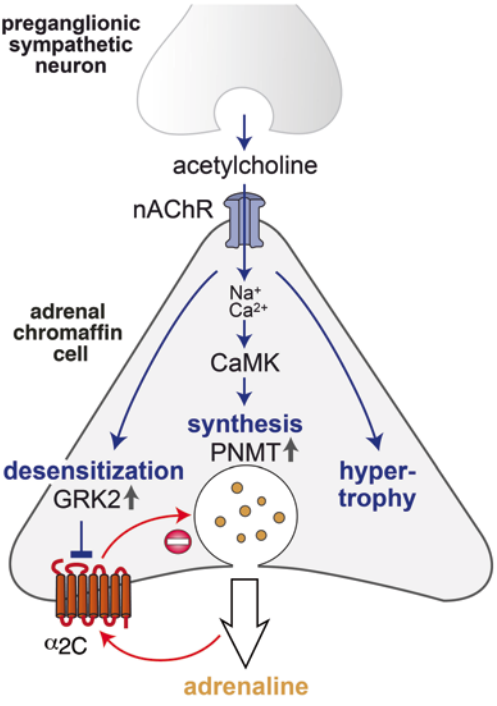

Fig. 7.4 Control of adrenal chromaffin cell function by preganglionic nerves. Preganglionic sympathetic nerves innervating the adrenal medulla release acetylcholine to activate nicotinic acetylcholine receptors (nAChR). Cholinergic activation induces desensitization of α_2-adrenoceptors via GRK2 upregulation, increased catecholamine synthesis, and chromaffin cell hypertrophy. *CaMK* Ca^{2+}/calmodulin-dependent protein kinase, *GRK2* G-protein-coupled receptor kinase 2, *PNMT* phenylethanolamine *N*-methyltransferase

decreased infarct size and adverse cardiac remodeling after experimental myocardial infarction [84]. This protective effect of β1-arrestion ablation could be assigned to both cardiac and adrenal effects [84]. Loss of adrenal β1-arrestin improved feedback control of adrenaline release and thus lowered circulating catecholamine levels [84]. However, GRK2 and β1-arrestin not only modulate adrenal catecholamine secretion but also contribute to elevated aldosterone secretion from the adrenal cortex in heart failure [84–86]. Thus, normalization of adrenal α_2-adrenoceptor signaling by local inhibition of GRK2 or β-arrestin may represent an interesting therapeutic strategy for chronic heart failure [87].

Conclusions

The adrenergic system adapts its structure and function to altered demand during development, physiological, and pathophysiological events at multiple levels. Recent insight into the molecular mechanisms regulating noradrenaline and adrenaline release has uncovered detailed mechanisms of desensitization of feedback mechanisms in heart failure. GRK2 and β-arrestin interactions with sympathetic and adrenal α_2-adrenoceptors may provide novel therapeutic avenues to restore normal feedback control in heart disease. Future studies will be important to determine the efficacy and safety of small molecules specifically targeting these mechanisms. Unexpectedly, structural and phenotypic changes of the sympathetic system have only recently been discovered. In heart disease, sympathetic innervation density decreases due to ischemic damage of sympathetic nerves after myocardial infarction or due to reduced synthesis of cardiomyocyte-derived NGF. In addition, several alterations which alter the phenotype of sympathetic nerves toward fetal or cholinergic properties have been uncovered. Further studies will be essential to determine the precise mechanisms of functional hyperactivity and structural reduction of sympathetic innervation of the cardiovascular system. A detailed molecular understanding of these alterations is expected to advance novel therapeutic concepts for chronic heart failure and other cardiovascular diseases.

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Chapter 8
The Adrenergic System and Stem Cell-Mediated Myocardial Repair

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Adrenergic Nervous System and Heart Function

The adrenergic signaling system (ANS) is an integral part of cardiac physiology [1]. Myocardial homeostasis is achieved by controlled regulation of contractility through the balanced interplay of β-adrenergic receptor (β-AR) expression and catecholamine levels. β-ARs belong to the G-protein-coupled receptor (GPCR) superfamily [2]. Catecholamines such as dopamine (DA), norepinephrine (NE), and epinephrine (EPI) are neurotransmitters and hormones within the ANS that regulate a variety of essential functions in the body. Upon catecholamine binding to specific ARs, a variety of second messengers trigger a complex series of cellular functions. Their immediate activities range from regulating the metabolism and ion channel activity to the neurotransmitter synthesis and release [3, 4]. In addition to controlling of cardiac function, adrenergic signaling is important for myocyte development and growth, programmed cell death, but also the response to injury and during the embryonic development. During stress and/or injury, the sympathetic nervous system is activated, leading to the release of catecholamines, followed by the uncoupling and desensitization of the β-ARs in the heart [1]. Pathologically increased and sustained adrenergic overdrive can lead to augmented receptor desensitization, ultimately resulting in uncontrolled death of cardiac tissue and activation of unfavorable compensatory mechanisms. Interestingly, the contribution of catecholamine-based signaling is essential throughout the entire life span, including both pre- and

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postnatal development and even prior to the initiation of nervous system control [5, 6]. The magnitude of this system seems corroborated following the discovery of intrinsic cardiac adrenergic (ICA) cells in the heart tissue of rat, mouse, and human [7–9]. Initially, Huang et al. 1996 [9] discovered this subset of cardiac cells which contained all enzymes required for catecholamine synthesis and was suggested to be their additional source independently from ANS. These catecholamine-synthesizing ICA cells are present in fetal hearts long before neuronal adrenergic innervations occur and have been shown to have important functions in the embryonic heart development and neonatal cardiac β-AR functionality [10]. ICA cells are credited with the stimulation of pacemaking activity before the appearance of a functional ANS. Moreover, it has been shown that catecholamines are involved in embryonic development in vivo [11–13]. Thus, the existence of different primary sources of catecholamine biosynthesis between embryos and adults is apparent [14].

Stem Cells

A novel therapeutic approach for patients suffering from heart and cardiovascular pathologies, where cardiomyocytes (CMs) are damaged or lost, involves the potential use of stem cells. They are on the forefront of attractive cell-based therapeutic candidates based on their notable capacity for proliferation, self-renewal, and differentiation into various organ-specific cell types [15]. Theoretically, the use of stem cells promises an almost unlimited supply of specific adult cell types, including those that once differentiated into viable CMs could replace damaged myocardium. Stem cells are subclassified to be totipotent, pluripotent, and multipotent, based on their potential to differentiate into one or more specific types of mature cells. They can be isolated during all developmental stages from embryonic and fetal to adult, and can originate from nearly every tissue (hematopoietic, mesenchymal, skeletal, neural, etc.). Potentially relevant stem cell types for clinical repair and regeneration applications include the following:

Embryonic Stem Cells

Embryonic stem cells (ESCs) are totipotent, primitive cells derived from the blastocyst of the early embryo. They have the capacity to maintain an undifferentiated state indefinitely. These cells are highly proliferative both in vitro and in vivo and are capable of differentiating into all specialized lineages including endothelial cells, smooth muscle cells, and CMs. They can also release pro-angiogenic factors [16].

Adult Stem Cells

Adult stem cells are undifferentiated cells found in differentiated tissues such as bone marrow (BM), skeletal muscle, lung, liver, brain, heart, etc. They retain the potential for self-renewal and can differentiate into organ-specific tissues [16].

BM-Derived Stem Cells

BM-derived stem cells (BMSCs) are hematopoietic stem cells (HSCs), which can differentiate into all types of mature blood cells, and into endothelial progenitor cells (EPCs), which in turn can form new blood vessels and differentiate into mesenchymal stem cells (MSCs) [16, 17].

Mesenchymal Stem Cells

MSCs are multipotent stem cells that can be found in many adult tissues, especially in the BM. They have the capability of trans-differentiation into a range of lineages including: adipocytes, chondrocytes, osteoblasts, and myocytes. They can be mobilized and upon release into circulation can be sequestered to an injury site and possibly contribute to healing [16].

Endothelial Progenitor Cells

EPCs are circulating cells that participate in the process of new blood vessel formation and vascular repair. Including a rare subset of cells, endothelial colony forming cells (ECFCs) have the ability to form lumenized capillary-like tubes in vitro and form stable human blood vessels [18].

Organ-Specific/Cardiac Stem Cells

In recent years, heart-specific stem cells identified by the antigen markers cKit and Sca1 have been identified. Their presence, albeit infrequent, suggests that the heart has a degree of regenerative potential [19].

Recent research efforts indicate that catecholamines from neural and nonneural cell origins might have the potential to affect the characteristics of stem cells. Studies of signaling pathways activated by catecholamines suggest their direct involvement in the determination of stem cell mobility, proliferative state, and their ultimate fate during homeostasis and under stress conditions [20]. Despite the limited potential of the adult myocardium to renew and repair itself, adrenergic stimulation appears to influence cardiac progenitor cell (CPC)-based regenerative capabilities.

Role of the Adrenergic System in Cardiac Development and Stem Cell Function

Numerous mouse and rat studies performed during embryonic development indicate the presence of catecholamines and their precursor enzymes in developing myocardium, a discovery that is associated with the early pacemaking and conduction functions [7]. Abnormal embryonic heart development has been detected following the exposure to β-blockers [21]. Homozygous β1-AR null mutant mice are prenatally lethal due to defective cardiac development [22]. Likewise, impaired sympathetic signaling in early postnatal development was associated with decreased synthesis of cardiac DNA and impaired cardiac growth in older animals [23, 24].

As both the disruption and over-induction of β-AR-mediated signaling has been described as detrimental to early cardiac development, further investigation showed that β-ARs were also involved in the regulation of neonatal CMs proliferation [25]. An additional receptor function has also been described as a differentiation factor for mouse MSCs. It has been shown that β-AR signaling has an impact on cardiac differentiation. Increased β-AR signaling enhanced, and signaling inhibition reduced the efficiency of cardiac differentiation of ESCs. Major findings illustrated that the β-AR agonist isoprotrenerol stimulated cardiac differentiation of ESCs by a mechanism that involved both the extracellular-signal-regulated kinase (ERK) and p38 signaling pathways. Both β1-ARs and β2-ARs are present at different stages of cardiac differentiation at both mRNA and protein levels, albeit at significantly different levels. Interestingly, the expression of β1-AR increased only gradually, reaching a peak at postnatal day 14, and remained at a high level until day 21 [25]. β2-AR on the other hand was expressed at a high level even before differentiation, and showed no obvious change after cardiac differentiation induction. These results suggest that β2-AR might be the predominant subtype during early development, while β1-AR might be the predominant subtype required for the late stage of cardiac differentiation and its expression might be tightly regulated upon cardiac differentiation. Additionally, it has been postulated that there is an inverse relationship between stem cell proliferation and differentiation [25].

Of the three known βAR subtypes, CMs express β1 and β2, with β1 playing a pivotal role in mediating heart rate, changes in contractility, increased cell motion, and contractile velocity [26, 27]. Studies have shown that CMs can be differentiated from most uncommitted stem cell types in vitro; however, the most promising were ES cells [28].

Catecholamines Affect Proliferation and Mobilization of HSCs

Cell motility is a hallmark of HSC and hematopoietic progenitor cell (HPC) function and is an essential component of continuous homeostasis even during stress-induced emergency situations. It has been postulated that adrenergic

signaling is essential for the chemokine-controlled homing of progenitor cells [29]. As neurotransmitters are involved in modulating the chemotactic activity of HPCs and contribute to their recirculation among the BM, blood, and other organs, adrenergic signaling increases their migration potential. Increased membrane type-1 matrix metalloproteinase (MT1-MMP) expression and MMP-2 activity after neurotransmitter stimulation suggests that neuronal regulation may serve as an additional way in which the egress, recruitment, and mobilization capacities of progenitor cells are directly regulated [30]. The hypothesis that catecholamines are acting directly on the HSCs and their precursors, as well as on circulating cells, is supported by the finding that in the developing embryo, the β2-AR is actively expressed in both endothelial cells and blood cells [31].

Catecholamines Influence Mesenchymal Stem Cell Fate and Function

MSCs are multipotent stem cells found in numerous adult tissues. Most can be isolated from the adult BM. They are capable of differentiation into various lineage cells such as osteoblasts, adipocytes, chondrocytes, and myocytes. Moreover, MSCs can be mobilized from connective tissue into the blood stream and circulation from where they are directed to sites of injury in order to contribute to regeneration processes. Their commitment and differentiation potential are controlled by complex signaling cascades mediated by cytokines and catecholamines. Recent research efforts indicate that catecholamines released from both neural and other cells affect their function and properties [32]. In a recent report, the nervous system was suggested to be crucially involved in the regulation of MSC's range of functions [33]. Migration, proliferation, and trans-differentiation are described to be under the control of the ANS [33]. It has been reported that CM-like cells can be differentiated from BM-derived MSC in vitro [34]. Within 1 day after differentiation, these cells express functional β1-AR and β2-AR, demonstrating remarkable functional response in agreement with the timing of neonatal rat CMs [35]. An interesting aspect of the influence of the adrenergic signaling on the fate of progenitor cell is the fact that MSC could potentially differentiate into several distinct cell lines. It has been suggested that MSC fate could be regulated by regulatory transcription factors and microRNA (miRNA) downstream of β-adrenergic signaling [36]. Catecholamine function is credited with regulating skeletal muscle regeneration and increased muscle mass [37, 38]; however, despite the in vitro ability for MSCs to be induced into CM-like cells, the direct effect of catecholamines in vivo has not been identified.

β2-AR Stimulation Enhances Angiogenic Characteristics of Endothelial Progenitor Cells

Just as during the development and in the EPCs, the EPCs of postnatal mice express a functional β2-AR. Stimulation of this receptor by means of catecholamines causes the EPCs to proliferate and enhances their migration [39]. The induction of the signaling pathway enhances their angiogenic ability both in vitro and in vivo. Additionally, the finding that β2-AR overexpression in EPCs results in increased proliferation and angiogenic differentiation in vitro not only makes them an interesting target for laboratory studies but also increases the likelihood of them being expanded and developed into potential therapeutic tools [39].

Current research supports the model in which BM-derived EPCs contribute to the repair and regeneration of the injured endothelium [40]. Catecholamines modulate the postischemic neovascularization by inducing migration of EPCs and promoting their egress from the BM niche. Interestingly, following isolation from peripheral blood, two distinct types of EPCs have been identified in the in vitro cultures. Early EPCs represent the alternatively activated macrophages (M2 cells) which have been described to promote repair through the release of anti-inflammatory paracrine factors. Late EPCs on the other hand are progenitors in the process of trans-differentiating into endothelial cells, which allows for their contribution to vascular repair, as they incorporate into the sites of vascular damage [40]. An additional functional quality of EPCs has been discovered recently, which promotes a mechanism to rescue stressed or damaged endothelial cells. They have the capacity of initiating intercellular communication through "tunneling nanotubes" with which they can transfer lysosomes and mitochondria to stressed cells [41]. Despite higher rate of egress into the blood stream following catecholamine stimulation, chronically hypertensive patients have been diagnosed with EPC dysfunction [42, 43]. This appears to be due of AngII-mediated increase of reinin–angiotensin system (RAS) activity which leads to increased oxidative stress and EPC senescence [44].

Role of the Adrenergic System in Cardiac Progenitor Cells

One of the most exciting and controversial aspects of the study of regenerative capacity of a developed myocardium is the discovery of CPCs [45–47]. The extent to which CPCs contribute to the turnover of adult CMs is an active field for debate. Discovered more than a decade ago, this small population of cKit-positive cardiac residing stem cells has been postulated to contribute to the functional repair of the injured myocardium and to the regeneration of lost CMs [45]. Further investigation of this cell population revealed similarities to the stages of neonatal CM development. During the ANS-driven phase of extensive cell proliferation in the postnatal hearts, the neonatal CMs express almost exclusively the β2-AR. However, following maturation, the AR expression profile of adult CMs shifts predominantly to

β1-AR. At this point, an extended increase of catecholamine levels becomes maladaptive and cardiotoxic. Recent findings demonstrate that adrenergic stimulation has a positive impact on CPC survival and proliferation [48, 49]. Before full lineage commitment, the immature CPCs have been shown to express almost exclusively the β2-AR. β1-AR expression was induced only subsequent to their lineage commitment [48]. Furthermore, the importance of the temporal activity balance of the two ARs is demonstrated. With the inhibition of β1-AR expression and activation of β2-AR, CPCs continue to survive and proliferate following catecholamine stimulation, thus they can achieve an increased pool of immature progenitors. On the other hand, the acquisition of β1-AR expression correlated with cardiac lineage commitment and predisposition to catecholamine-induced cell death [48].

Limited adult cardiac regenerative response to pathologic injury is thus postulated to be caused by elevated β1-AR-mediated sensitivity to apoptotic signaling in response to increased catecholoamine presence. Taking into account their location in the myocardium which is in close proximity to the site of injury and their capacity to differentiate into functioning CMs, CPCs pose a great potential resource to contribute to myocyte replacement [46].

Translational Approaches

As stem cell therapies for many chronic diseases are being evaluated and tested, the importance of stem cell research becomes increasingly more important. Despite controversies and misconceptions, it is critically imperative to understand stem cell physiology and its relationship with the pathways in an already functioning biological system. In this chapter, the known impact of β-adrenergic signaling on the cardiovascular stem cells was investigated. Although still in its infancy, the current body of research clearly indicates the crucial impact of β-adrenergic signaling not only on the healthy and pathological processes in the heart but also on the different types of stem cells with the potential to aid the recovery.

It is important to emphasize how the duration and timing of adrenergic signaling affects the physiological processes of stem cells. On one hand, increased catecholamine signaling reduces cells adhesion, allowing for a better migration and egress of stem cells from their niches of origin and homing to the site of injury. In instances where ESCs are transplanted into infarcted myocardium, exposure of patients to β-blocker medications may prove counterproductive, since it might obstruct or decrease the efficiency of cardiac differentiation. Thus, the experimental evidence suggests that during the early stage of stem cell-based cell replacement therapy, patients should not be treated with β-AR blockers. Additionally, during prolonged traumatic injury, patients have been diagnosed with BM dysfunction leading to anemia and other complications. During chronic injury being cardiac or otherwise, the impact of constant catecholamine release leads to sustained stimulation and excessive mobilization of HPCs. The continued mobilization is likely to be the cause of overuse and exhaustion of the progenitor cell pool, thus leading to

BM dysfunction. In these instances, the treatment with β-blockers might help limit mobilization, preserve BM function, and aid recovery [50]. Further studies will help in understanding the exact pathways involved in ANS and its role in modulating stem cell characteristics.

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