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Styliani Vlachou
Kevin Wickman *Editors*

Behavioral Neurobiology of $GABA_B$ Receptor Function

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Preface

It has been just over 40 years since Norman G. Bowery discovered and named the $GABA_B$ receptor. It has been 10 years since the last comprehensive book presentation focused on $GABA_B$ receptors.

The main goal of this book is to provide the field with a contemporary and comprehensive perspective on the $GABA_B$ receptor, its physiological relevance, and its therapeutic potential. The volume is organized into introductory and special interest sections presented by experts who study the $GABA_B$ receptor from structural, signaling, pharmacologic, physiological, pathophysiological, and therapeutic perspectives. More specifically, the first chapter of this volume focuses on a brief history and the significance of the $GABA_B$ receptor, followed by a detailed presentation of the structural basis of the $GABA_B$ receptor regulation and signaling in the second chapter. The third chapter offers extensive background and knowledge on the mechanisms and regulation of neuronal $GABA_B$ receptor-dependent signaling, while the chapter "$GABA_B$ Receptor Chemistry and Pharmacology: Agonists, Antagonists and Allosteric Modulators" provides an in-depth understanding of the $GABA_B$ receptor chemistry and pharmacology through the presentation of the development efforts of agonists, antagonists, and allosteric modulators over the last few decades. Starting from the fifth chapter, a particular focus on the role of $GABA_B$ receptors in neuropsychiatric disorders is presented, with the chapter "$GABA_B$ Receptors and Drug Addiction: Psychostimulants and Other Drugs of Abuse" covering the role of $GABA_B$ receptors on addiction, with a focus on psychostimulants. The sixth and seventh chapters further add to the above, by extensively presenting all the preclinical and clinical work, respectively, on the $GABA_B$ receptor as it relates to alcohol use disorder. The eighth chapter provides a detailed account of the role of $GABA_B$ receptors in pain, followed by an extensive presentation of the role of $GABA_B$ receptors on anxiety and mood disorders in the ninth chapter. Finally, the tenth chapter focuses on neurodegeneration and the role $GABA_B$ receptors can play in this setting, and the eleventh and final chapter of this book volume explores the role of $GABA_B$ receptors and cognitive processing in health and disease.

The editors would like to thank all excellent contributors for their participation in this volume. Their expertise and dedication to this research work over decades has formulated an excellent background for the potential use of $GABA_B$ receptors as therapeutic targets for a variety of psychiatric disorders and medical conditions.

Except for the essential contributions to this book volume, this work would not have been possible without the excellent support by Springer and all assistant editors and project managers, specifically Alamelu Damodharan, Gerit Rother, Coral Zhou, Susanne Dathe, and Arunkumar Kathiravan.

Most importantly, we would like to thank Mark A. Geyer, Charles A. Marsden, Bart A. Ellenbroek, and Thomas R.E. Barnes, editors of Current Topics in Behavioral Neurosciences (CTBN) without whom this book would not have taken shape.

I (Styliani Vlachou) would love to dedicate this CTBN book volume to my husband Bruno Di Micco whose incredible support and patience accompanies me in all my academic steps in the last few years and gives me more strength to continue. I cannot mention the extensive support in the form of cuddles by all of our paw babies – four dogs and a cat – in the house while working on this book volume, especially during the COVID-19 pandemic. Further, I would like to thank my co-editor, Kevin Wickman, for his extensive work on our book volume, but mainly for his expert input, especially at crucial time points, and his patience, kindness, and collaborative spirit throughout this process. Although we still have not met in person, it was an absolute pleasure working with you, Kevin. A special thanks and dedication goes out to Mark Geyer, who was never my mentor, but I always wish he was! This work would not have been possible without you.

I (Kevin Wickman) dedicate this volume to my many mentors in science, the exceptional colleagues I have had the great pleasure of collaborating with over the years, and all of the early-career scientists who have worked with me on pursuits that include the $GABA_B$ receptor. I sincerely appreciate the invitation from Styliani Vlachou to contribute as co-editor for this volume. I can sense your positive energy and passion for science across an ocean, and I recognize all of the hard work you put in to making this vision a reality.

The book is meant to appeal to a broad spectrum of biomedical and clinical scientists, to any scholars with an interest in the $GABA_B$ receptor.

The editors hope readers find this work to be thought-provoking, instructive, and informative.

Dublin, Ireland — Styliani Vlachou
Minneapolis, MN, USA — Kevin Wickman
12 October 2021

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A Brief History and the Significance of the $GABA_B$ Receptor

Styliani Vlachou

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Abstract γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain. GABA type B ($GABA_B$) receptors ($GABA_B$Rs) are the only metabotropic G protein-coupled receptors for GABA and can be found distributed not only in the central nervous system, but also in the periphery. This chapter introduces important, fundamental knowledge related to $GABA_B$R function and the various potential therapeutic applications of the development of novel $GABA_B$R-active compounds, as documented through extensive studies presented in subsequent chapters of this Current Topic in Behavioral Neurosciences volume on the role of the neurobiology of $GABA_B$R function. The compounds that have received increased attention in the last few years compared to $GABA_B$R agonists and antagonists – the positive allosteric modulators – exhibit better pharmacological profiles and fewer side effects. As we continue to unveil the mystery of $GABA_B$Rs at the molecular and cellular levels, we further understand the significance of these receptors. Future

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directions should aim for developing highly selective $GABA_BR$ compounds for treating neuropsychiatric disorders and their symptomatology.

Keywords Animal models · Cognition · Drug and alcohol addiction · $GABA_B$ receptors · Humans · Neurochemistry · Neurodegeneration · Neurodevelopmental disorders · Pharmacology · Pharmacotherapy · Treatment

1 Introduction

It is an absolute honor to introduce the content of this book volume on the neurobiology of the metabotropic γ-aminobutyric acid (GABA) type B ($GABA_B$) receptor ($GABA_BR$). I cannot help point out that the goal this chapter aims to achieve is challenging. How can anyone introduce and capture in a few pages the extensive investigations conducted by esteemed colleagues on the neurobiology of $GABA_B$ receptor function since its discovery and description in mammalian tissue by Norman G. Bowery over 40 years ago? This chapter will only present a summary of the overall work on the $GABA_BR$. The chapters to follow in this volume constitute excellent contributions of the state-of-the-art in the $GABA_BR$ research field, as they provide extensive details on the structure, signal transduction, pharmacology, and neurochemistry of $GABA_BRs$, but also on the role of $GABA_BRs$ in a variety of settings, such as drug and alcohol addiction, anxiety and mood disorders, neurodevelopment, neurodegeneration, and cognitive processes in health and disease.

GABA is the primary inhibitory neurotransmitter in the brain and one of the most studied neurotransmitters in the brain over the last 50 years (Smart and Stephenson 2019). The $GABA_BR$ is the only metabotropic G protein-coupled receptor (GPCR) of the three identified and characterized receptors of GABA, and it belongs to Class C of GPCRs. The history of the $GABA_BR$ begins before these receptors were identified and characterized, at the time when Norman G. Bowery was conducting many highly innovative studies on the extrasynaptic and asynaptic GABA systems in an effort to help solve the mystery of their presence in the ganglia and the peripheral nerves, and their significance [as extensively reviewed by (Brown 2018)]. This early work took place in the early 1970s and it proved preparatory to the discovery of the $GABA_BR$.

The pioneering work on the $GABA_BR$ can only but be traced back to Norman G. Bowery and his research team, when in 1979 and 1980, respectively, they described for the first time a receptor that was responsive to GABA. This receptor was activated by β-(4-chlorophenyl)-γ-aminobutyric acid (baclofen), the most extensively studied $GABA_BR$ agonist, but it was not responsive to bicuculline, a light-sensitive competitive antagonist of $GABA_A$ receptors, in mammalian tissue (Bowery et al. 1979, 1980). This response differentiated that newly identified receptor from

the already identified and characterized $GABA_A$ receptor, which was responsive to bicuculline.

Many years went by without any further advances, until extensive work by Klemmens Kaupmann, Wolfgang Froestl, Bernard Bettler and colleagues, as well as the groups of Jones and White and their colleagues, described the molecular structure of the $GABA_BR$, characterized the gene sequence and identified the heteromerization of the $GABA_BR$ into subunits necessary to be assembled together in order to generate a functional $GABA_BR$ (Kaupmann et al. 1997, 1998; Jones et al. 1998; Kaupmann and Bettler 1998; White et al. 1998).

2 $GABA_B$ Receptor Structure, Function, and Distribution

All the experts participating in this volume have made major contributions to the understanding of the neurobiology of the $GABA_BR$. Details of the structure, characteristics, trafficking, and function of the $GABA_BR$ heterodimer can be found in many contributions in this volume, especially in Fritzius et al. (2020), Rose and Wickman (2020), Nieto et al. (2021).

In more recent years, there is a lot more understanding of the $GABA_BR$ at the molecular and functional level (Bettler et al. 2004; Emson 2007; Schwenk et al. 2016; Shaye et al. 2021). $GABA_BRs$ play a crucial role in mediating slow and long-lasting synaptic inhibition through indirect neuronal K^+ and Ca^{2+} channel gating and through effects on other second messenger targets like cAMP. $GABA_BRs$ consist of principal and auxiliary subunits that influence receptor properties in different ways. The principal subunits affect the surface expression and the axonal versus dendritic distribution of these receptors, whereas the auxiliary subunits determine the potency of agonists on the receptor and kinetics of the receptor response to them (Gassmann and Bettler 2012). The two main subunits of the $GABA_BRs$ are $GABA_BR1$ and $GABA_BR2$. In order for the $GABA_BR$ to be active and functional, these subunits need to interact to form a stable heterodimer. Importantly, orthosteric agonists and antagonists bind to $GABA_BR1$, while PAMs bind to the $GABA_BR2$ subunit. Most recent findings show that $GABA_BRs$ are receptor complexes consisting of primary or other subunits, but also linked with and affecting numerous factors and proteins. The homeostatic interaction between all these components plays an important role in $GABA_BR$ function.

Interestingly, $GABA_BRs$ can be found on both presynaptic and postsynaptic membranes. When presynaptic receptors are activated, they inhibit either the release of GABA (in the case of autoreceptors on GABA neuronal terminals) or the release of other neurotransmitters and peptides (in the case of heteroreceptors on other-than-GABA-neurotransmitter neuronal terminals). When postsynaptic receptors are activated, they in turn activate K^+ channels and induce slow inhibitory postsynaptic potentials.

$GABA_BRs$ are widely expressed and distributed in the mammalian central nervous system (CNS) [e.g., (Bowery et al. 1984; Boyes and Bolam 2007; Metz et al.

2011)], with the highest receptor densities occurring in the cerebral cortex, the cerebellum, the interpeduncular nucleus, and the dorsal horn of the spinal horn (Chu et al. 1990). Importantly, $GABA_BRs$ can also be found in other brain areas, such as the hippocampus, the thalamus, the amygdala and the basal ganglia, and outside the CNS (please see below the section "Other conditions outside the CNS" in this chapter). Thus, they are very widely expressed in mammalian tissues. As expected, their distribution is not equal in the different brain areas. However, lower distribution does not mean lower significance of these receptors in that particular area (Bowery 2010).

3 $GABA_B$ Receptor Ligands: Agonists, Antagonists, and Allosteric Modulators

The synthesis of the first exogenous GABA analogue (β-(4-chlorophenyl)-GABA (baclofen) in 1962, followed by the identification of the three GABA receptor types, paved the way for developing numerous $GABA_BR$ agonists, partial agonists, inverse agonists, and antagonists (Malcangio and Bowery 1995). Baclofen has been the most extensively studied compound in both preclinical and clinical studies, and the only $GABA_BR$-selective drug clinically approved for muscle relaxation in humans.

Positive allosteric modulators (PAMs) of $GABA_BR$ were described 20 years ago by Stephan Urwyler and colleagues (Urwyler et al. 2001; Urwyler 2011). Since then, numerous PAMs have been reported in the literature by different research groups and/or pharmaceutical companies. A major difference between $GABA_BR$ agonists and allosteric modulators is that the latter bind to a region of the $GABA_BR$ different from and outside of the ligand-binding (i.e., orthosteric) site. Through this action, they either increase (i.e., in the case of PAMs) or decrease (i.e., in the case of negative allosteric modulators; NAMs) the effects of GABA, without possessing intrinsic agonistic activity, but only by modulating the endogenous GABA release effects on the $GABA_BRs$ (Ong and Kerr 2005; Kniazeff 2020). Thus, they produce fewer and/or less severe side effects compared to the $GABA_BR$ agonists or antagonists, and they activate the $GABA_BR$ without inducing desensitization (Gjoni and Urwyler 2008, 2009; Froestl 2010). Although numerous PAMs of the $GABA_BR$ have been developed over the years, it is only recently that the first NAM was described (Porcu et al. 2021), leading the way toward the development of more NAMs for therapeutic purposes.

It is critical to note that through extensive efforts spanning approximately four decades, there has been only one $GABA_BR$ compound – baclofen – approved for clinical use as a muscle relaxant. The extensive and informative presentation of the history of, as well as the development and characteristics of $GABA_BR$ agonists, partial agonists, inverse agonists, antagonists and allosteric modulators can be found in Nieto et al. (2021) of this volume. This chapter, with other contributions in this

volume, will help shed light on the future directions necessary for developing $GABA_B$R-active compounds as potential therapeutic targets.

4 $GABA_B$ Receptors as Potential Therapeutic Targets

4.1 Drug and Alcohol Use Disorders

$GABA_B$Rs are considered mediators or potential therapeutic targets for many neuropsychiatric disorders, including drug addiction. The story of understanding of the role of $GABA_B$Rs in drug and alcohol use disorders has two main components, one that focuses on the effects of the addictive drugs on $GABA_B$R signaling and the other that focuses on the effects of $GABA_B$R compounds on those of drugs of abuse. Extensive efforts in recent years have showcased the important role of these receptors and their ligands on the effects of drugs of abuse, such as psychostimulants, opioids and alcohol, in both preclinical and clinical studies. Alternatively, drugs of abuse can evoke plasticity of $GABA_B$R-dependent signaling in the brain, with changes marked at various molecular or cellular levels, such as RNA expression, receptor trafficking, G protein coupling, and changes in the actions of effector proteins, and affecting various neurotransmitter systems.

Although dopaminergic agents have been the primary therapeutic targets for drug dependence treatment, in recent years, GABA and glutamate, with their metabotropic $GABA_B$Rs and glutamatergic receptors, have received increased attention as potential "alternative" approaches to dopamine-targeting compounds. More specifically, considering the enhanced dopamine (DA) signaling in the mesocorticolimbic circuitry through molecular and cellular mechanisms by drugs of abuse (McCall et al. 2019), earlier efforts were mainly focused on the blocking and/or inhibitory effects of DA-active compounds on the rewarding and reinforcing effects of drugs of abuse in rodents. Furthermore, nowadays, the focus is not only on a plethora of overlapping but distinct neurotransmitter pathways, including GABA and glutamate systems, but also on further exploring and highlighting key $GABA_B$R-related sex differences, which can potentially affect susceptibility to drugs of abuse (DePoy et al. 2016; DeBaker et al. 2021). The presence of $GABA_B$Rs in different areas of the brain reward circuitry and how drugs of abuse induce alterations in $GABA_B$R signaling is well-presented in Li and Slesinger (2021) of this volume.

Numerous early studies at the preclinical level have shown that baclofen and other $GABA_B$R agonists or mediators, such as GABA metabolism or reuptake inhibitors, reduced the rewarding effects of cocaine, nicotine, morphine, and alcohol in animal models mainly using rats and mice at different phases of drug dependence [e.g., (Brebner et al. 1999; Xi and Stein 1999; Corrigall et al. 2000; Fattore et al. 2002; Paterson et al. 2004, 2005); for thorough reviews, please see Vlachou and Markou (2010); Phillips and Reed (2014); Jacobson et al. (2018), as well as Holtyn and Weerts (2020), Logge et al. (2020), Li and Slesinger (2021) of this volume].

While these effects appeared promising, the side effects of the $GABA_BR$ agonists also presented in some of these studies (e.g., muscle relaxation, sedation, cognitive deficits, fatigue, tolerance development, seizures) indicated that the development of more selective agents with less side effects was necessary for many of these efforts to translate to clinical testing and possible approval for therapeutic purposes.

In more recent years, studies have focused on the effects of PAMs and NAMs of $GABA_BRs$ on drugs of abuse [for thorough reviews, please see Agabio and Colombo (2015); Filip et al. (2015); Maccioni and Colombo (2019)], as these compounds have yielded more promising findings than those of $GABA_BR$ agonists, due to their mechanism of action and thus, fewer side effects. Extensive studies have been conducted on the effects of $GABA_BR$ compounds on the behavioral, cognitive, locomotor, and rewarding effects of psychostimulants in particular; however, studies on other drugs of abuse are still in their infancy.

Importantly, a limited number of studies with $GABA_BR$ knock-out (KO) mice lacking either the $GABA_BR1$ or the $GABA_BR2$ subunit (Vigot et al. 2006) have been conducted in relation to drug addiction. The vast majority of them have focused on the role the two subunits in the different phases of nicotine dependence (e.g., acute administration or withdrawal phase) and some explore their relation to reward and stress [e.g., (Varani et al. 2012, 2014, 2015, 2018; Jacobson et al. 2016)].

At clinical level, after so many years of research, still in 2021 only baclofen, a $GABA_BR$ agonist, is approved for use in humans as a muscle relaxant. Importantly, it was also approved in France for treating alcohol use disorder in 2018, although with controversy (Braillon et al. 2020). Numerous other studies have examined the effects of baclofen on drug dependence in humans over many years and they have not led to clinical approval. With regard to PAMs and their potential use in humans, a study on only one compound, the PAM ADX71441 (developed by Addex Therapeutics), was recently funded by the NIH for clinical studies on cocaine-dependent individuals (Kalinichev et al. 2017). We can only but hope that in the next few years more compounds will proceed to clinical trials and will be approved as safe therapies for substance use disorders.

4.2 Pain and Analgesia

There is extensive evidence that $GABA_BRs$ are involved in the processing of pain signals and the induction of analgesia for chronic pain conditions (Pan et al. 2008; Malcangio 2018). The contribution of $GABA_BRs$ to the generation and modulation of pain signals, their involvement in chronic pain states, and their potential use as targets for developing novel analgesic compounds are discussed in detail by Dietmar Benke in Benke (2020) of this volume.

Briefly, numerous studies at preclinical level have shown that $GABA_BRs$ are present in nociceptors (Enna and McCarson 2006; Hanack et al. 2015), which are sensory neurons that respond to potentially harmful heat, mechanical, or chemical stimuli. Nociceptors can transmit slow or fast signals, depending on whether they are

unmyelinated or myelinated, respectively. Nociceptor synapses can be found in the dorsal horn of the spinal cord where $GABA_BRs$ have also been identified [e.g., (Zhou et al. 2017)]. More specifically, $GABA_BRs$ or their subunits have been identified in both central and peripheral axon endings of nociceptors, as well as in dorsal horn interneurons and projection neurons, in supraspinal areas and in descending pain control pathways. Considering the extensive presence of $GABA_BRs$ in all the loci and pathways mentioned above, researchers have investigated the potential therapeutic effects of $GABA_BR$ compounds in different kinds of chronic pathological pain, such as inflammatory or neuropathic pain [e.g., (Barr et al. 2013; Meuwissen et al. 2020)], and the overall results have suggested that the enhancement of $GABA_BR$ activity can relieve or alleviate pain, although the exact mechanisms with which the nociceptive processes are activated are complex.

4.3 Anxiety and Mood Disorders

Many studies over the last few years have shown that alteration of the function of the $GABA_BR$ can contribute to stress- and anxiety-related conditions and in affective disorders. At the preclinical level, numerous studies have shown that $GABA_BR$ agonists reduce anxiety-like and depressive-like symptomatology in animal models, although contradictory findings also exist (Mombereau et al. 2004; Cryan and Kaupmann 2005; Cryan and Slattery 2010; Jacobson et al. 2018).

As noted above, the side-effect profile of $GABA_BR$ agonists such as baclofen (Agabio et al. 2013) is pronounced, reducing hope for the use of these types of compounds as therapeutics for a number of conditions, including mood disorders. Clinical studies of $GABA_BR$ agonists and their effects in the setting of anxiety disorders are more sparse. However, overall, preclinical and clinical findings, presented in detail in Felice et al. (2020) of this volume suggest a possible therapeutic role of $GABA_BR$ compounds in anxiety and depression, especially when the focus switches to novel PAMs and NAMs, and proteins that affect $GABA_BR$ activity, which have been tested more extensively in recent years.

Importantly, research advancements in diagnostic and therapeutic procedures such as the use of transcranial magnetic stimulation in major and treatment-resistant depression patients (Fatih et al. 2021; Kinjo et al. 2021) have recently shown deficits in $GABA_BR$ neurophysiology (i.e., $GABA_BR$-mediated cortical inhibition) (Premoli et al. 2014a, b, 2017; Veronezi et al. 2016; Lissemore et al. 2018) that is affected by anti-depressant medication. Further advances in the use of innovative techniques are expected to help elucidate the role of $GABA_BR$ in anxiety and affective disorders.

4.4 Neurodegeneration

A considerable role of the $GABA_BR$ has been identified in neurodegeneration in relation to both its neuropathology and symptomatology, and the potential use of $GABA_BR$ compounds as therapeutics. The GABAergic system has been linked with numerous neurodevelopmental and neurodegenerative conditions (Kleppner and Tobin 2001; Smart and Stephenson 2019; Murrell et al. 2020), although the initial focus was on the $GABA_A$ receptors, and their agonists and antagonists [for reviews see, (Solomon et al. 2019; Bhagat et al. 2021; Castellano et al. 2021)].

Several studies have implicated $GABA_BRs$ and their subunits in the pathophysiology of Alzheimer's disease, with changes in the density of expression of the $GABA_BRs$ identified [e.g., (Iwakiri et al. 2005; Pilipenko et al. 2018, 2019; Dinamarca et al. 2019; Martín-Belmonte et al. 2020)]. Further, although the main neuropathological features are diverse among neurodegenerative disorders, $GABA_BRs$ seem to be strongly implicated in the pathophysiology of Parkinson's disease [e.g., (de Groote et al. 1999; Enna et al. 2006; Yang et al. 2021); for a recent review, see Roberts et al. (2021)] and Huntington's disease [e.g., (Allen et al. 2009; Rekik et al. 2011; Kim and Seo 2014; Holley et al. 2019; Barry et al. 2020)]. Extensive work has focused on the role of $GABA_BRs$ on epilepsy, specifically on temporal lobe epilepsy (TLE) (Princivalle et al. 2002, 2003; Chandler et al. 2003; Straessle et al. 2003; Dugladze et al. 2013; Rocha et al. 2015; Sheilabi et al. 2018)] and absence epilepsy [e.g., (Sperk et al. 2004; Han et al. 2013; Jafarian et al. 2021; Pagès et al. 2021)]. $GABA_BR$ antagonists may be of most therapeutic benefit for the treatment of seizures in epilepsy. However, the difficulty lies not only in the selectivity of these agents, which would minimize side effects, but mainly in the route of administration (central or systemic) and the overall pharmacokinetics of $GABA_BR$ compounds.

An extensive presentation of the most recent developments on the role of GABA and $GABA_BRs$ in neurodegeneration, with a particular focus on Alzheimer's and Parkinson's disease and epilepsy is presented in Princivalle (2021) of this volume.

4.5 Cognitive Processes in Neurodevelopment, Health and Disease

The role of $GABA_BRs$ in cognitive processes has been extensively studied from different perspectives. The vast majority of the existing literature focuses on animal models of learning and memory to assess the role of $GABA_BRs$ in these processes. Examples of the animal models used are active and passive avoidance paradigms, the Morris water maze and radial arm maze, many working memory tasks (e.g., T and Y mazes), and the novel-object recognition, and (dis) location tasks (Bowery 2006; Serrats et al. 2017). However, a lot of work has also been conducted on how cognition functions in neurodevelopment and neurodegeneration, and what may be

the potential beneficial or therapeutic role that the $GABA_BR$ can play in cognitive development, decline, or enhancement (Heaney and Kinney 2016).

Examples of neurodevelopmental conditions more extensively studied under the scope of the potential therapeutic role of the $GABA_BR$ include autism spectrum disorders, fragile X syndrome, and Down syndrome; examples of neurodegenerative conditions are Alzheimer's disease, epilepsy, and autoimmune anti-$GABA_B$ encephalitis. Many studies have specifically examined the role of $GABA_BRs$ in cognition, learning, and memory processes in these conditions. Although there are controversial findings in the literature, altogether, results from studies in this area, presented in detail in the last chapter of this volume on the role of the $GABA_B$ receptors and cognitive processing in health and disease (Vlachou 2021), indicate a potential therapeutic role of $GABA_BR$ compounds for treating cognitive dysfunction and learning/memory impairments for some of these conditions, especially in neurodegenerative disorders. However, the ongoing effort to develop more selective $GABA_BR$ compounds with fewer side effects will help further elucidate how alteration of $GABA_BR$ function can help battle cognitive processes symptomatology in health, neurodevelopment, and neurodegeneration.

4.6 Other Conditions Outside the CNS

Considering that the $GABA_BR$ is present not only in the CNS, but also in the periphery and even outside the nervous system, the potential therapeutic use of $GABA_BR$ compounds for symptoms and conditions outside the CNS has been examined over a number of years. Although studies in this direction are not the focus of this volume, the $GABA_BR$ has been investigated as a potential target for the treatment of gastroesophageal reflux disease, chronic abdominal pain, and overactive bladder (Alstermark et al. 2008; Lehmann 2009; Hyland and Cryan 2010; Brozmanová et al. 2013; Scarpellini et al. 2015). The focus on these conditions was based on the findings that the $GABA_BR$ is present in the gastrointestinal tract and plays a role in the regulation of a number of processes including intestinal motility, gastric acid secretion and gastric emptying, and esophageal sphincter relaxation. AZD3355 (Lesogaberan) was a selective peripheral $GABA_BR$ agonist, which was tested for the treatment of gastroesophageal reflux disease in earlier years in animal models or in human patients (Boeckxstaens et al. 2011; Canning et al. 2012; Shaheen et al. 2013), but more recent studies with the PAMs ADX71441 and ADX71943 have shown analgesic effects in a rat model of bladder pain and in the acetic acid-induced writhing test in mice and formalin tests in mice and rats, respectively (Kalinichev et al. 2014; Kannampalli et al. 2017). The efforts in this direction are focused on developing compounds that are peripherally selective and thus show less side effects than centrally active $GABA_BR$ agonists. Thus, peripherally selective allosteric modulators may be the drugs of choice in this direction.

Other efforts try to shed light on the role of $GABA_BRs$ in the treatment of different types of gastrointestinal system-related cancers, such as colorectal or

pancreatic cancer [e.g., (Al-Wadei et al. 2011; Banerjee et al. 2014; Schuller 2017; An et al. 2021; Wang et al. 2021)]. Importantly, in the case of colorectal cancer, it was recently shown that $GABA_BR1$ expression was significantly lower in tumor tissues than those in non-tumor normal tissues, and that those colorectal cancer patients with high $GABA_BR1$ expression lived longer (Wang et al. 2021). In the case of pancreatic cancer, preclinical findings suggest that GABA itself, but not baclofen, may be promising for the treatment of pancreatic cancer in tobacco smokers, as low doses of nicotine within the range of nicotine replacement therapy induce gemcitabine resistance in pancreatic cancer and GABA significantly reverses this effect (Banerjee et al. 2014).

Most interesting findings derive from the recent profiling of $GABA_A$ and $GABA_BR$ expression in the myometrium of the human uterus (Söderhielm et al. 2018) and the significant role of the $GABA_{B1}R$ subunit in embryo implantation and uterine decidualization in preclinical studies in mice (Chen et al. 2021). Studies in the near future will shed light on the role of $GABA_BR$ expression in the female reproductive organs, pregnancy, and embryonic development.

5 Conclusion

Altogether, the most important conclusion deriving from all research conducted over the last four decades is that the clinical promise of the $GABA_BR$ agonists, antagonists, PAMs, and NAMs is extensive (Enna and Bowery 2004). Most of the efforts in that direction focus on PAMs and NAMs in the last few years, due to their improved and more selective pharmacological profile exhibiting more direct effects and less side effects in the various neuropsychiatric and other disorders. To fully understand the significant role of the $GABA_BR$ research findings, one must delve into the following chapters of this volume, which I hope you will find informative, enjoyable and thought provoking.

Ultimately, I want to point out that the expanded "$GABA_BR$ family" consists of esteemed colleagues from around the world, all of whom have significantly contributed to the evolving history of the $GABA_BR$, and many of whom have kindly participated and offered excellent contributions to this volume. In addition to the recent loss of Norman G. Bowery (1944–2016), the "$GABA_BR$ family" has lost two more esteemed members in the last few years – including Wolfgang Froestl (1946–2015), who was a leading scientist for the discovery of most of the early $GABA_BR$ agonists and antagonists, and Athina Markou (1961–2016), who offered major contributions to the role of $GABA_BR$ on drug dependence, specifically on nicotine dependence, reward mechanisms, and depressive-like behavior. The author of the first and last chapter and editor of this volume worked closely with both. I want to dedicate this introductory chapter of the Current Topics in Behavioral Neurosciences volume on the neurobiology of $GABA_BRs$ to Athina Markou, my postdoctoral mentor, who introduced me to the $GABA_BR$ research and "family" and made me love it as much as she did, and to Wolfgang Froestl, who was not only an

excellent chemist and neuroscientist, but also one of the most gentle people I have met in our field.

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Structural Basis of $GABA_B$ Receptor Regulation and Signaling

Thorsten Fritzius, Michal Stawarski, Shin Isogai, and Bernhard Bettler

Contents

Abstract $GABA_B$ receptors (GBRs), the G protein-coupled receptors for the inhibitory neurotransmitter γ-aminobutyric acid (GABA), activate Go/i-type G proteins that regulate adenylyl cyclase, Ca^{2+} channels, and K^+ channels. GBR signaling to enzymes and ion channels influences neuronal activity, plasticity processes, and network activity throughout the brain. GBRs are obligatory heterodimers composed of GB1a or GB1b subunits with a GB2 subunit. Heterodimeric GB1a/2 and GB1b/2 receptors represent functional units that associate in a modular fashion with regulatory, trafficking, and effector proteins to generate receptors with distinct physiological functions. This review summarizes current knowledge on the structure, organization, and functions of multi-protein GBR complexes.

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1 Heterodimers Are the Minimal Functional Receptor Units

The GB1a and GB1b subunit isoforms were cloned in 1997 using a radioligand binding approach (Kaupmann et al. 1997). This showed that GBRs belong to class C GPCRs, which include metabotropic glutamate receptors, Ca^{2+}-sensing receptors, and taste receptors (Kaupmann et al. 1997). As for other class C GPCRs, GB1 subunits contain a large extracellular venus fly trap domain (VFTD), the typical heptahelical transmembrane domain (7TMD), and an intracellular C-terminal domain (Fig. 1). When expressed in heterologous cells, GB1 subunits exhibited ~tenfold lower affinity for GABA than native GBRs (Kaupmann et al. 1997, 1998; White et al. 1998). In addition, GB1 subunits failed to exit the endoplasmic reticulum (Couve et al. 1998) and to efficiently inhibit adenylyl cyclase or activate Kir3 channels (Kaupmann et al. 1997). This showed that GB1 subunits do not form functional receptors by themselves. Soon after cloning of GB1 subunits, cDNA homology searches and yeast-two-hybrid screens identified the sequence-related GB2 subunit (Kaupmann et al. 1998; Kuner et al. 1999; Ng et al. 1999; White et al. 1998). GB2 was again non-functional when expressed in heterologous cells (Galvez et al. 2001). In situ hybridization studies showed that neurons generally co-express GB1 and GB2 transcripts, which indicated that GB1 and GB2 subunits might function together in a heterodimeric receptor. Co-expression of GB1 with GB2 subunits indeed generated receptors with tenfold higher affinity for GABA that also efficiently signaled to neuronal effectors, including adenylyl cyclase, Kir3 channels, and P/Q/N-type Ca^{2+} channels (Kaupmann et al. 1998; Kuner et al. 1999; Ng et al. 1999; White et al. 1998). This finding represented the earliest demonstration of an obligatory heterodimeric G protein-coupled receptor (Marshall et al. 1999). The GB1a and GB1b subunit isoforms derive from the same gene by differential promoter usage and exhibit distinct expression patterns in the central nervous system (Bischoff et al. 1999). Structurally, GB1a differs from GB1b by the presence of two sushi domains in the N-terminal domain (SD1, SD2) (Blein et al. 2004) (Fig. 1). The N-terminal SD1 has an intrinsically disordered structure, while SD2 is more compactly folded. The SDs function as axonal trafficking signals (Biermann et al. 2010) and stabilize the receptor at the cell surface (Hannan et al. 2012). Accordingly, axons predominantly express GB1a/2 receptors, while the somatodendritic compartment expresses GB1b/2 receptors. However, GB1a/2 receptors are also present in the dendrites but excluded from the spine heads, in contrast to GB1b/2 receptors (Biermann et al. 2010; Dinamarca et al. 2019; Vigot et al. 2006).

GBRs have evolved quality control signals that prevent unfolded or unassembled subunits from exiting the endoplasmic reticulum (ER) and the Golgi apparatus. The

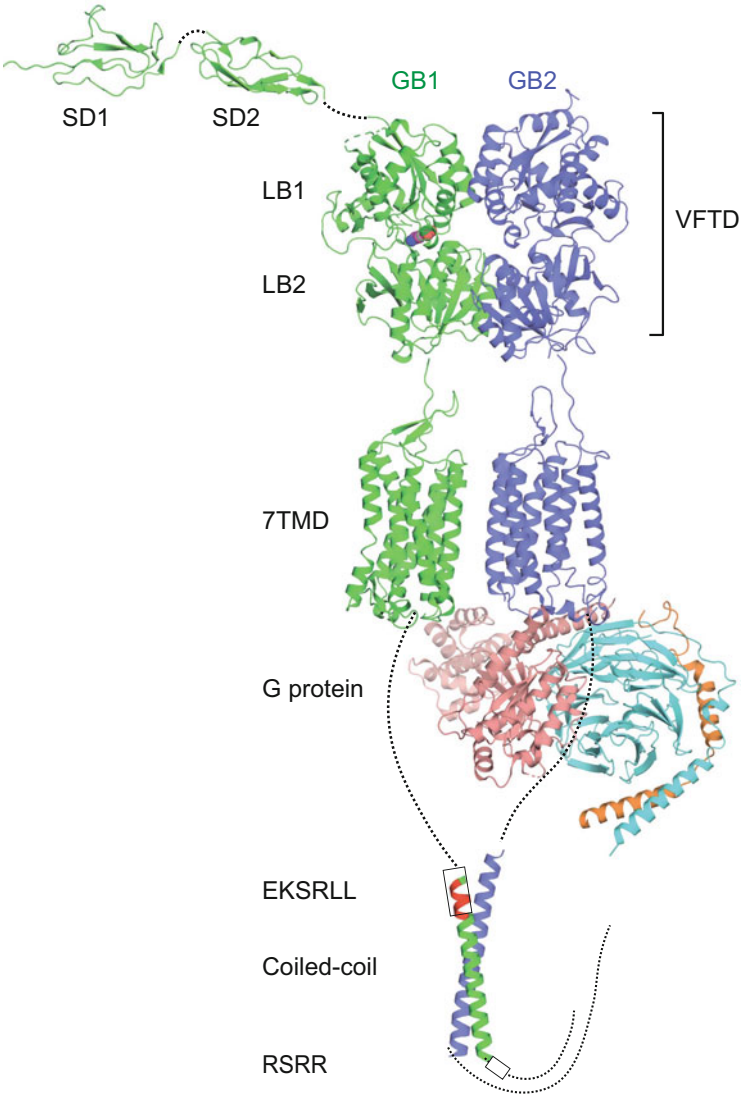

Fig. 1 Structural model of the GBR heterodimer. The model is based on the published structures of SD1 [PDB ID: 6HKC (Rice et al. 2019)], SD2 [1SRZ (Blein et al. 2004)], baclofen-bound VFTDs [4MS4 (Geng et al. 2013)], active and inactive mGlu5 [6N51 and 6N52 (Koehl et al. 2019)], the coiled-coil domain of GBRs [4PAS (Burmakina et al. 2014)] and the heterotrimeric G protein complex [3SN6 (Rasmussen et al. 2011)]. GB1 and GB2 are colored in green and slate, respectively. The active and inactive conformations of the $7TMD_{mGlu5}$ were used in GB1 and GB2, respectively. The boxes in the C-terminal domain of GB1 indicate the retention motifs RSRR and EKSRLL that control heterodimer assembly during biosynthesis

intracellular C-terminal domain of GB1 subunits encodes the ER retention signal RSRR, which is located distal to a coiled-coil heterodimerization domain (Margeta-Mitrovic et al. 2000; Pagano et al. 2001) (Fig. 1). Prenylated rab acceptor family 2 (PRAF2) protein, an ER-resident molecule, binds to the ER retention signal and prevents exit of the GB1 subunit from the ER (Doly et al. 2016). Coiled-coil heteromerization of GB1 with GB2 subunits competitively displaces PRAF2 from its binding motif and enables forward trafficking of the GB1/2 heterodimer in the biosynthetic pathway. The coat protein complex I (COPI) also binds to the ER retention signal and shuttles unassembled GB1 subunits from the cis-Golgi back to the ER (Brock et al. 2005). An additional signal within the coiled-coil domain of GB1, the di-leucine motif EKSRLL (Fig. 1), controls release of receptors from the trans-Golgi network (Restituito et al. 2005). Msec7-1, a guanine-exchange factor protein of the ARF family of GTPases, binds to this di-leucine motif and prevents exit of unassembled GB1 subunits from the Golgi apparatus. Structural data show that heterodimerization of GB1 with GB2 subunits occludes the di-leucine signal and prevents Msec7-1 from binding (Burmakina et al. 2014).

At high cell surface density, GB1/2 heterodimers assemble by random collision into higher-order oligomers of two or more heterodimers (Calebiro et al. 2013; Comps-Agrar et al. 2011; Maurel et al. 2008; Schwenk et al. 2010; Stewart et al. 2018). It appears that in higher-order oligomers GB1 subunits arrange in a line via the opposite sides of their 7TMDs, while GB2 subunits are on the side (Xue et al. 2019). Mutation of either E380 + L382, T410 + E412, or E413 in the VFTD of rat GB1a ($VFTD_{GB1a}$) disrupts the formation of higher-order oligomers (Comps-Agrar et al. 2011; Stewart et al. 2018). Molecular modeling indicates that two G proteins can couple to one GBR tetramer (Xue et al. 2019). Interestingly, however, higher-order oligomerization limits the capacity of GBRs to activate G proteins, presumably because only one of the agonist binding sites in the two neighboring GB1 subunits of a GBR tetramer can be occupied (Stewart et al. 2018). It is unknown whether suppression of G protein signaling in higher-order oligomers is of regulatory significance or not.

2 Signal Transduction in the Receptor Heterodimer

GB1 and GB2 subunits fulfill distinct functions in the receptor heterodimer. Only GB1 contains a GABA binding site (Galvez et al. 1999), whereas GB2 couples to the G protein (Duthey et al. 2002; Havlickova et al. 2002). GB2 additionally allosterically increases GABA affinity at GB1 (Kaupmann et al. 1998; White et al. 1998). After binding of GABA at GB1, multiple allosteric interactions between subunit domains are necessary to activate the G protein at GB2. X-ray structures of the extracellular dimerization interface are now available (Geng et al. 2012, 2013). The $VFTD_{GB1}$/$VFTD_{GB2}$ dimer structure shows that GB1 and GB2 subunits interact sideways, facing opposite directions (Geng et al. 2013) (Fig. 2). Each VFTD has a bi-lobed structure, where lobe 1 (LB1) is positioned more distant from the plasma

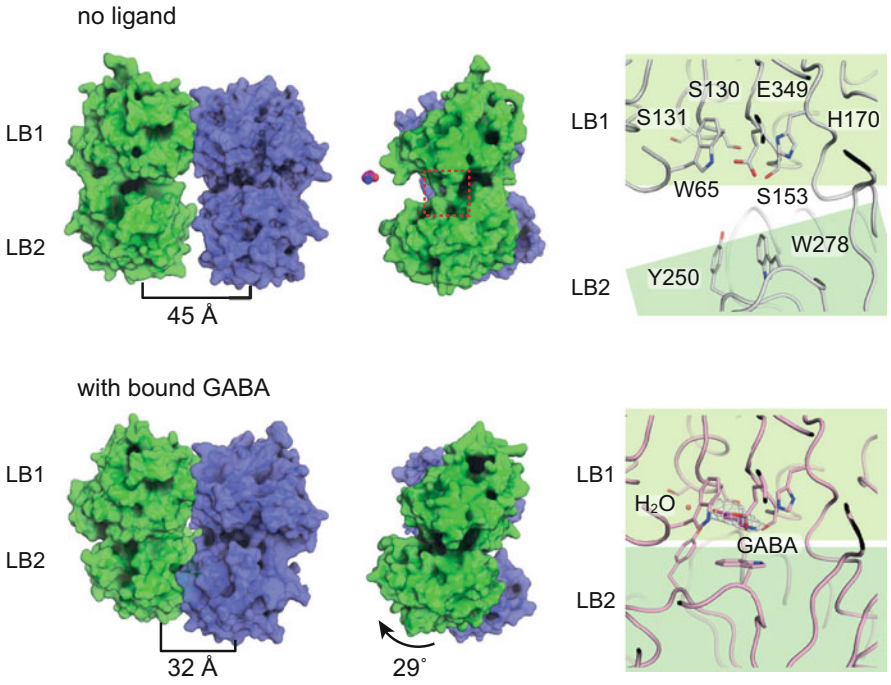

Fig. 2 Conformational changes in VFTDs during receptor activation. Structures of the VFTDs in the absence of a ligand [PDB ID: 4MQE, top] and with GABA bound to $VFTD_{GB1}$ [4MS3, bottom (Geng et al. 2013)] are shown. Upon GABA binding, LB2 of $VFTD_{GB1}$ (green) rotates by 29° and moves toward $VFTD_{GB2}$ (slate) by ~10 Å. In contrast, LB2 of $VFTD_{GB2}$ rotates only by 9° upon activation. Amino acid residues in $VFTD_{GB1}$ involved in the binding of GABA are shown on the right, with numbering of residues according to human GB1b (Geng et al. 2013). Y250, W278, and S131 (water mediated contact) on LB2 interact with GABA and subsequently close the interface between the LB domains. The 2F(o)-F(c) electron density map of GABA is shown as mesh at $\sigma = 1.5$ (right bottom)

membrane than LB2. A peptide hinge connects LB1 and LB2, enabling LB2 to move in relation to LB1. In the heterodimer, $VFTD_{GB1}$ and $VFTD_{GB2}$ bind to each other via LB1. The LB1/LB1 interaction is stabilized by multiple hydrophobic contacts (Y113/Y117 in GB1b; Y118/W149 in GB2, amino acid numbering refers to human sequences in the remainder of the manuscript), salt bridges (R141 in GB1b, D109 in GB2), hydrogen bonds (E138 in GB1b, N110 in GB2), and multiple van der Waals contacts (Geng et al. 2013). The hydrophobic patch is located at the center of the interface and flanked by sites forming hydrogen bonds and water-mediated contacts.

Mutagenesis and X-ray crystallography studies show that agonists and antagonists bind to a large crevice between LB1 and LB2 in $VFTD_{GB1}$ (Galvez et al. 1999; Geng et al. 2013). Agonists and antagonists form multiple interactions with residues in LB1, including hydrogen bonds with S130, S153, H170, and E349, van der Waals contacts with W65, and water-mediated contacts with S131 (GB1b numbering)

(Geng et al. 2013) (Fig. 2). Agonists additionally bind to Y250 and W278 in LB2. The antagonists CGP54626 and SCH50911 also bind to W278 in LB2, which increases antagonist-binding affinity (Geng et al. 2013). The large substituents at either side of antagonists physically prevent $VFTD_{GB1}$ closure, which stabilizes $VFTD_{GB1}$ in an open conformation (Geng et al. 2013). Conversely, agonists induce $VFTD_{GB1}$ closure (Geng et al. 2012; Kniazeff et al. 2004). Mutations that stabilize $VFTD_{GB1}$ closure therefore lead to constitutive activity. Upon agonist binding, LB2 in $VFTD_{GB1}$ rotates 29° about a nearly horizontal axis, bringing LB2 close to LB1 and closing $VFTD_{GB1}$ (Fig. 2). This rotation additionally moves $VFTD_{GB1}$ closer to LB2 of $VFTD_{GB2}$ that remains in a constitutively open conformation stabilized by hydrogen bonds between LB1 and LB2 (Geng et al. 2012). LB2 of $VFTD_{GB2}$ twists by ~9° about a nearly vertical axis, moving it toward LB2 of $VFTD_{GB1}$. As a result, a new LB2/LB2 interface forms that stabilizes $VFTD_{GB1}$ in the closed conformation and increases agonist affinity. The LB2/LB2 interface is essential for receptor activation, as disruption of the interface by insertion of a glycan wedge precludes receptor activation (Rondard et al. 2008). Conversely, a covalent disulfide bridge linking the LB2 lobes locks the receptor in a constitutively active state (Geng et al. 2013). While agonist binding promotes $VFTD_{GB1}$/$VFTD_{GB2}$ interaction, it simultaneously causes a spatial reorientation of $7TMD_{GB1}$ and $7TMD_{GB2}$ that enables activation of the G protein at $7TMD_{GB2}$ (Matsushita et al. 2010; Monnier et al. 2011). Allosteric activation of $7TMD_{GB2}$ occurs in *cis* and in *trans* via $VFTD_{GB2}$ and $7TMD_{GB1}$, respectively (Monnier et al. 2011). Receptor activation disrupts an ionic lock at the intracellular side of $7TMD_{GB2}$ (Binet et al. 2007). The ionic lock is formed by a salt bridge between D688 in TM6 and K574 in TM3, which prevents outward movement of TM6 and stabilizes the inactive closed conformation of the $7TMD_{GB2}$. Disruption of the ionic lock by mutation allosterically increases agonist affinity. Recent studies show that crosslinking of the TM6-TM6 interaction between GB1 and GB2 is sufficient for receptor activation and leads to constitutive activity (Xue et al. 2019). Interestingly, GBRs also exhibit constitutive activity in the absence of agonists (Galvez et al. 2001; Grunewald et al. 2002). This suggests that GBRs exhibit high intrinsic conformational flexibility and spontaneously oscillate between inactive and active states, similar as shown for the isolated VFTDs of metabotropic glutamate receptor 2 (Olofsson et al. 2014).

Almost all known allosteric modulators of GBRs bind to $7TMD_{GB2}$ (Binet et al. 2004; Dupuis et al. 2006; Sun et al. 2016). The only exception is Ca^{2+}, which binds to S269 in LB1 of GB1a (S153 in GB1b) and thereby increases affinity for GABA (Galvez et al. 2000; Wise et al. 1999) (Fig. 2). Positive allosteric modulators (PAMs) at GBRs generally increase agonist potency and efficacy (Urwyler et al. 2005). Some PAMs also have agonistic properties and activate the receptor in the absence of orthosteric agonists, presumably by stabilizing the active conformation of $7TMD_{GB2}$. The negative allosteric modulator (NAM) CLH304 has inverse agonist properties, suppressing basal activity as well as agonist-induced receptor activation, likely by preventing $7TMD_{GB2}$ from reaching the active conformation (Chen et al. 2014; Sun et al. 2016). The binding sites of allosteric modulators in $7TMD_{GB2}$ are unknown. Crystal structures of other class C G protein-coupled receptors suggest

that allosteric modulators enter a cavity located between transmembrane domains 3, 5, 6, and 7 (Christopher et al. 2015; Dore et al. 2014; Gregory et al. 2011; Wu et al. 2014). A GB2 homology model predicts a hydrophobic binding pocket in $7TMD_{GB2}$ and identified potential amino acid residues involved in binding of allosteric modulators (Freyd et al. 2017).

3 Auxiliary KCTD Subunits

Several observations pointed to native GBRs being composed of more than just a GB1 and a GB2 subunit. For example, GBR complexes isolated from brain tissue had molecular masses of 0.6–1.1 MDa, while the heterodimer only accounts for 240 kDa (Schwenk et al. 2010). Moreover, the kinetic properties of native GBR responses varied and differed from those of GB1/2 heterodimers expressed in heterologous cells (Turecek et al. 2014). Quantitative proteomic approaches identified approximately 30 proteins that interact with GB1 or GB2 in the brain (Dinamarca et al. 2019; Schwenk et al. 2010, 2016; Turecek et al. 2014). These proteins provide a molecular basis to explain the functional diversity of native GBRs. Known interactions between components of GBR complexes have been summarized recently (Fritzius and Bettler 2020).

Abundant GBR-interacting proteins are the K^+ channel tetramerization domain (KCTD) proteins KCTD8, KCTD12, KCTD12b, and KCTD16 (herein collectively referred to as the KCTDs) (Schwenk et al. 2010). The KCTDs are part of a larger family of KCTD proteins comprising 26 members with sequence similarity to the cytoplasmic tetramerization (T1, also known as BTB or POZ) domain of voltage-gated K^+ channels (Correale et al. 2013; Zheng et al. 2019). The KCTDs are composed of the N-terminal T1 domain and a H1 domain, with both isolated domains capable of forming oligomers (Correale et al. 2013; Fritzius et al. 2017). KCTD8 and KCTD16 additionally encode a C-terminal H2 domain that scaffolds effector channels and other receptor-associated proteins (see below). Structural studies demonstrate that $T1_{KCTD12}$ and $T1_{KCTD16}$ form homopentamers (Pinkas et al. 2017; Smaldone et al. 2016; Zheng et al. 2019; Zuo et al. 2019) (Figs. 3a and 5). The $T1_{KCTD16}$ pentamer is open, with a gap of 8–16 Å at its narrowest and widest points. Since one $T1_{KCTD16}$ monomer in the pentamer occupies 25 Å, the gap in the pentamer is too small to accommodate a sixth $T1_{KCTD16}$ monomer. Multiple electrostatic interactions and nonpolar associations stabilize adjacent $T1_{KCTD16}$ domains. Most of the conserved amino acid residues are involved in $T1_{KCTD16}$ interactions, supporting that all four KCTDs assemble as pentamers. Co-crystallization of $T1_{KCTD16}$ with a C-terminal GB2 peptide shows that the $T1_{KCTD16}$ pentamer wraps around the peptide (Fig. 3a, b). The GB2 peptide loops inside the central opening of the pentamer, entering and leaving the pentamer at its N-terminal surface. The apex of the GB2 peptide loop forms a short helix that contains the Y903 residue critical for KCTD binding (Correale et al. 2013; Schwenk et al. 2010; Zheng et al. 2019) (Fig. 3a). X-ray crystallography reveals that Y903 is

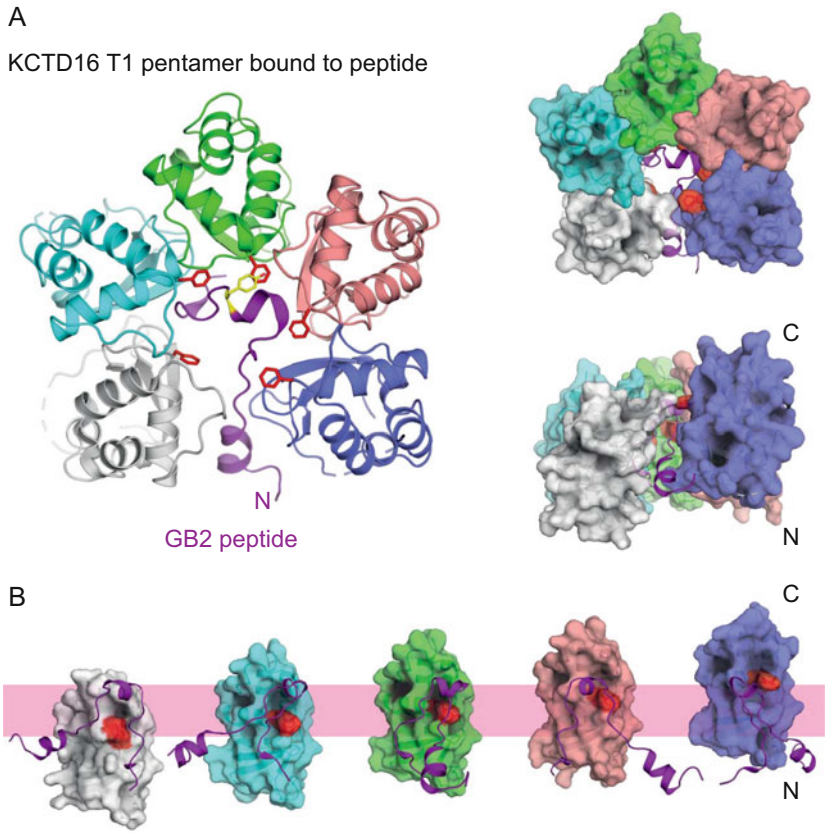

Fig. 3 Binding of the GB2 C-terminus to the $T1_{KCTD16}$ pentamer. (**a**) Structure of a GB2 C-terminal peptide bound to the $T1_{KCTD16}$ pentamer [PDB ID: 6M8R (Zheng et al. 2019)]. The F80 (red) and Y903 (yellow) residues in $T1_{KCTD16}$ and GB2, respectively, are highlighted. $T1_{KCTD16}$ domains form an open pentamer with C6 symmetry. A twist of the ring prevents the sixth subunit from being inserted. Upon binding to the GB2 C-terminal peptide, the open pentamer contracts by roughly 4–5 Å and creates a tight channel for the peptide (right). The orientation of the complex is indicated by the N- and C-terminus of KCTD16. (**b**) The twisted ring structure enables each $T1_{KCTD16}$ subunit to form a distinct binding interface with the peptide. A cross-section of the pentamer shows the interaction of each of the five $T1_{KCTD16}$ domains with the GB2 peptide. The F80 residues (red) and the $T1_{KCTD16}$ domains are aligned vertically according to the position of GB2 peptide

located in the middle of an extensive interaction interface (Zuo et al. 2019). The interaction takes place off center in the central pore of the pentamer, opposite of the gap. In each $T1_{KCTD16}$ domain of the pentamer, the F80 residue protrudes into the central pore. A slight offset due to a tilt of each $T1_{KCTD16}$ monomer forms a spiraling ladder of F80 residues in the inner wall of the pentamer (Fig. 3b). This arrangement allows F80 residues to bind many side chains in a GB2 peptide of 25 amino acid residues (Zheng et al. 2019). Consistent with the X-ray data, the F80A mutation

completely abrogates KCTD16 binding to GBRs (Zuo et al. 2019). Interestingly, binding of GB2 results in a compaction of the $T1_{KCTD16}$ pentamer (Fig. 3a).

Reverse affinity purification experiments using KCTD-specific antibodies revealed that KCTD8, KCTD12, KCTD12b, and KCTD16 not only bind to GB2 but also to the Gβγ subunits of the G protein (Turecek et al. 2014; Zheng et al. 2019). Co-crystallization studies show that each $H1_{KCTD12}$ pentamer binds five Gβγ subunits in a near perfect C5 rotational symmetry (Zheng et al. 2019) (Fig. 4). The five Gβγ molecules form a tightly packed outer ring in which every Gβ subunit directly contacts neighboring Gβ subunits as well as two adjacent H1 domains of the pentamer. $H1_{KCTD12}$ folds into a β sheet made up by five antiparallel β strands (β1-5) interspersed with two short α helices (α1 and α2). The amino acids at and around the loops between β1/β2 as well as β3/α2 bind to an acidic patch at the top of the Gβ propeller (interface I) and a groove between the N-terminal helix and the β propeller of Gβ (interface II) (Fig. 4). In $H1_{KCTD12}$, R232 (contacting interface I on Gβ) and R257 (contacting interface II on Gβ) are particularly important for the interaction, as mutation of either residue completely abolishes Gβγ binding and modulation of G protein signaling by KCTD12. The Gγ subunit is located peripherally and does not interact with the H1 domain. However, Gγ allows anchoring of the complex at the plasma membrane (Figs. 4 and 5). When incubating $KCTD12_{H1}$ with a substoichiometric amount of Gβγ, only full 5/5 complexes and free KCTD12 were observed, with no evidence of partial oligomers (Zheng et al. 2019). This suggests that binding of $H1_{KCTD12}$ to Gβγ is highly cooperative. Supported by 3D reconstructions of electron microscopy images of the full-length KCTD12 protein in complex with Gβγ (Zheng et al. 2019), the picture of a large multi-protein complex emerges, in which KCTDs simultaneously bind via their T1 and H1 domains GB2 and Gβγ subunits, respectively (Fig. 5). Of note, Gβγ binding to the $H1_{KCTD12}$ pentamer partially occludes the Gα binding-site on the surface of Gβγ, indicating that the trimeric G protein does not assemble with KCTD12 into a pentameric complex. This contrasts earlier biochemical findings that support that GBRs and KCTDs form a complex with the heterotrimeric G protein (Turecek et al. 2014). Co-crystallization of the H1 domain with Gβγ may therefore favor a structure that differs from the structure of the full-length KCTD protein assembled with receptor and the heterotrimeric G protein.

Dual binding of the KCTDs to the receptor and the G protein enables KCTDs to regulate the kinetics of receptor signaling (Fritzius et al. 2017; Schwenk et al. 2010; Seddik et al. 2012; Turecek et al. 2014). Pre-assembly of the G protein via the KCTDs at the receptor significantly accelerates G protein signaling, most likely by overcoming slow diffusion-limited association of the G protein with the receptor (Turecek et al. 2014). When studying GBR-mediated K^+ current responses, KCTDs shorten both the rise time and the delay between agonist binding and the onset of K^+ currents. The KCTDs are therefore responsible for the fast kinetics observed with GBR-induced current responses in neurons (Schwenk et al. 2010; Turecek et al. 2014). While all KCTDs accelerate GBR signaling, selectively KCTD12 and KCTD12b induce a rapid desensitization of GBR-mediated K^+ currents (Schwenk et al. 2010; Seddik et al. 2012) through an activity-dependent uncoupling of Gβγ

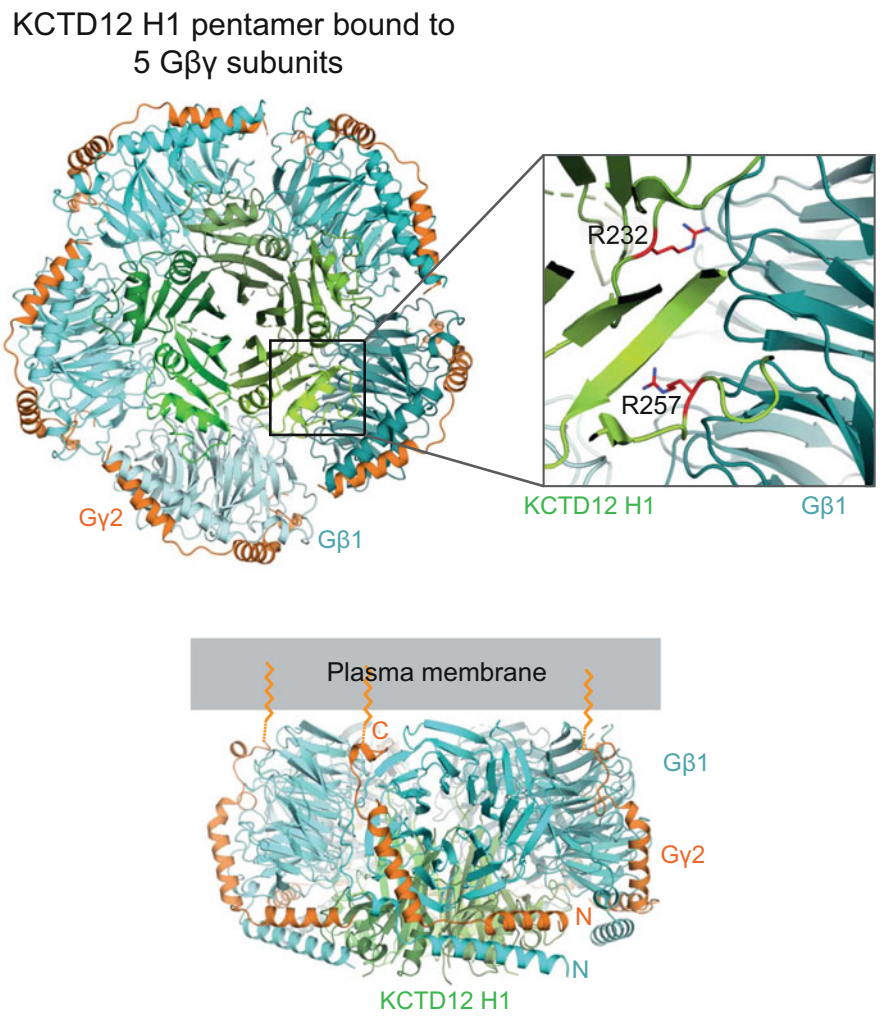

Fig. 4 The $H1_{KCTD12}$/Gβγ complex. Top: $H1_{KCTD12}$ pentamers and five Gβ1γ2 subunits form together a complex with C5 symmetry [PDB ID: 6M8S, (Zheng et al. 2019)]. Each of the five Gβ1γ2 subunits binds two $H1_{KCTD12}$ subunits. The cutout shows R232 (interface I) and R257 (interface II) that are crucial for Gβ1 recognition. Bottom: Due to lipidation of Gβγ subunits, the complex is expected to be tethered to the plasma membrane

from effector channels (Turecek et al. 2014; Zheng et al. 2019). Some neuronal populations simultaneously express multiple KCTDs, raising the possibility that the KCTDs form hetero-oligomers. Indeed, in heterologous cells, KCTD8, KCTD12, and KCTD16 form hetero-oligomers in all possible combinations (Balasco et al. 2019; Fritzius et al. 2017). Association of KCTD12/16 hetero-oligomers with GBRs in hippocampal pyramidal cells confers unique kinetic properties to GBR-induced K^+ currents, showing that hetero-oligomers increase the kinetic repertoire of GBR

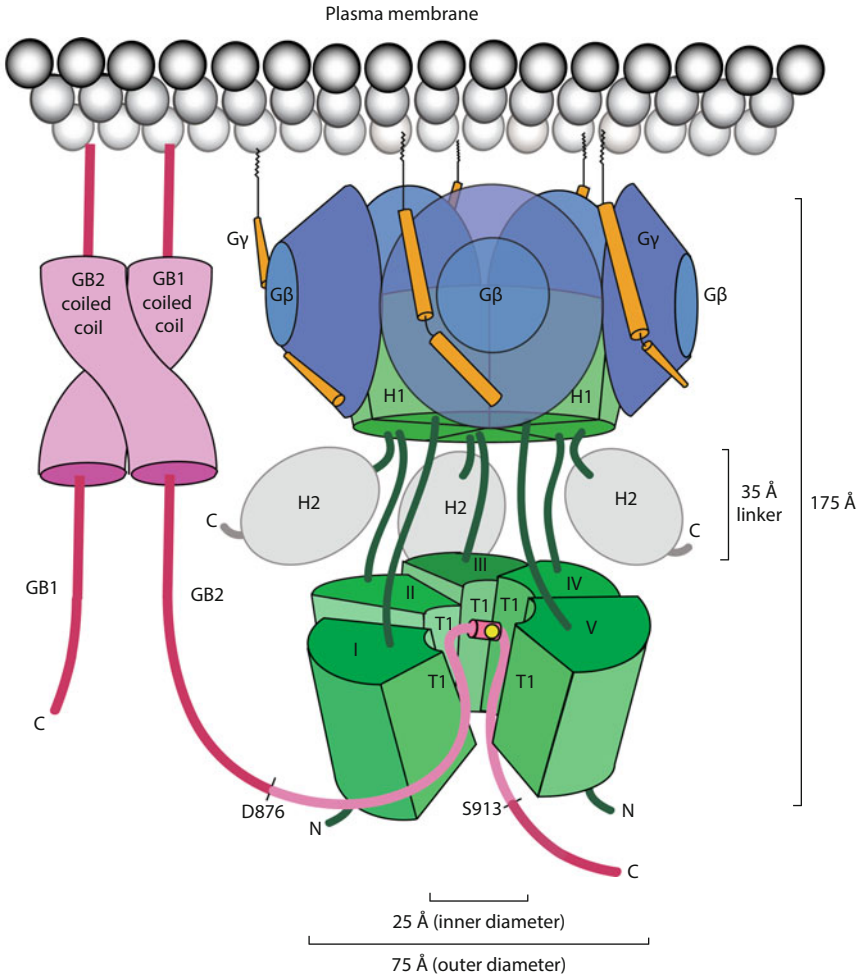

Fig. 5 Scheme of the multi-protein GBR/KCTD12/G protein signaling complex. The intracellular part of the GBR (dark pink), a pentamer of KCTD12 proteins (green), Gβ (blue), and Gγ (orange) subunits are depicted. The N-terminal T1 domain of the KCTDs forms an open pentamer that interacts with the cytoplasmic tail of GB2 (the GB2 peptide, containing the amino acids D888 to S913, co-crystallized with the $T1_{KCTD16}$ is highlighted in bright pink). This part of GB2 loops inside the central opening of the T1 pentamer, entering and leaving it at its N-terminal surface. The amino acid Y903 (yellow circle) at the apex of the GB2 loop is critical for KCTD binding. A slight offset due to a tilt of each $T1_{KCTD16}$ monomer allows the pentamer to bind a large number of amino acid side chains within the cytoplasmic tail of GB2. A short linker (35 Å) connects the N-terminal T1 domain with the C-terminal H1 domain of KCTDs, which binds to the Gβγ heterodimer of the G protein. The scheme depicts the closed $H1_{KCTD12}$ pentamer bound to five copies of Gβγ. Anchoring of the Gγ subunit to the phospholipid bilayer tethers KCTDs to the plasma membrane. The expected location of the C-terminal H2 domain present in KCTD16 and KCTD8 is indicated for three of the KCTDs in the pentamer. Distances are derived from three-dimensional negative-stain electron microscopy reconstructions (Zheng et al. 2019)

signaling (Fritzius et al. 2017). Of note, the KCTDs exert little influence on allosteric and orthosteric binding sites of GBRs (Rajalu et al. 2015).

Reverse-affinity purification experiments support that the KCTDs do not bind to other GPCRs (Schwenk et al. 2016; Turecek et al. 2014). The KCTDs are non-obligatory GBR components, which, however, are expressed by most neurons (and some glial cells) in the vertebrate brain (Metz et al. 2011). Since the KCTDs stably associate with the receptor and control receptor kinetics and surface expression (Ivankova et al. 2013), they should be viewed as auxiliary receptor subunits.

4 SD-Interacting Proteins

Proteomic studies showed that the β-amyloid precursor protein (APP), the adherence junction-associated protein 1 (AJAP-1), and the PILRα-associated neural protein (PIANP) form three distinct complexes with GB1a/2 receptors (Dinamarca et al. 2019; Schwenk et al. 2016). NMR studies identified sequence-related epitopes in the extracellular domains of APP, AJAP-1, and PIANP that bind with nanomolar affinities to the N-terminal SD1 of GB1a, with a rank order of affinities AJAP-1 > PIANP >> APP (Dinamarca et al. 2019). APP is best known as the source of β-amyloid (Aβ) peptides in Alzheimer's disease. Electrophysiological and biochemical experiments showed that binding of APP to GB1a is necessary for vesicular trafficking of GBRs to axon terminals (Dinamarca et al. 2019), consistent with the proposed role of the SDs in axonal trafficking (Biermann et al. 2010). Proteomic data show that APP associates with calsyntenins and c-Jun N-terminal kinase-interacting proteins (JIPs) that link the APP/GBR complex in cargo vesicles to the axonal kinesin-1 motor. Of potential relevance for Alzheimer's disease, complex formation with GBRs stabilizes APP at the cell surface and reduces proteolysis of APP to Aβ (Dinamarca et al. 2019). A related study showed that binding of the soluble form of APP (sAPP) to the SD1 of GB1a inhibits neurotransmitter release, synaptic transmission and spontaneous neuronal activity (Rice et al. 2019). The fact that a GBR antagonist disinhibits sAPP-inhibited neurotransmitter release supports that sAPP acts as a GBR agonist or positive allosteric modulator. However, it was also reported that sAPP has no functional effects on GBR signaling in heterologous cells (Dinamarca et al. 2019). Therefore, additional studies need to confirm sAPP effects on GBR signaling. AJAP-1 and PIANP, the two other proteins binding to the SD of GB1a, do not play a role in axonal trafficking of GBRs (Dinamarca et al. 2019). These proteins localize to adherens junctions that stabilize cell-cell interactions (Winkler et al. 2019; Yamada and Nelson 2007) and may be important for anchoring GB1a/2 receptors at presynaptic sites, either in *cis* or through trans-synaptic interactions. In support of this hypothesis, PIANP knock-out mice exhibit a deficit in GBR-mediated inhibition of glutamate release in the hippocampus (Winkler et al. 2019).

Amyloid-like protein 2 (APLP2) and integral membrane protein 2B (ITM2B) and ITM2C are additional transmembrane proteins that selectively co-purify with GB1a/

2 receptors (Dinamarca et al. 2019; Schwenk et al. 2016). These proteins associate with APP and are secondary interactors of GBRs. GBRs can therefore assemble with multi-protein APP complexes into super-complexes (complexes of complexes).

5 Effector Channels

The best-studied GBR functions in the central nervous system are the gating of voltage-sensitive Ca^{2+} (Ca_v) channels and inwardly rectifying Kir3-type K^+ channels by the Gβγ subunits of the activated G protein (Gassmann and Bettler 2012). GBRs inhibit N- and P/Q-type Ca_v channels, which suppress neurotransmitter release at most synapses in the brain. GBR activation of Kir3 channels hyperpolarizes the membrane, shunts postsynaptic currents in the dendrites, and inhibits neuronal firing. The α1B, α2, δ1, and δ2 subunits of N-type Ca_V channels co-purify with the GB1, GB2, and KCTD16 subunits, supporting that these channels bind to GBRs via KCTD16 (Schwenk et al. 2016). Association of GBR with N-type Ca_V channels directly links the receptor to the presynaptic release machinery. Proteomic work did not support a physical association of native GBRs with Kir3 channels (Schwenk et al. 2016), in contrast to earlier studies in heterologous expression systems (Ciruela et al. 2010; David et al. 2006; Fowler et al. 2007). It is possible that proteomic approaches miss weak interactions of Kir3 channels with GBRs. Alternatively, overexpression of two membrane proteins in heterologous cells may lead to artificial aggregates detected in BRET and immunoprecipitation experiments. Proteomic work additionally identified novel effector channels of GBRs, such as the transient receptor potential vanilloid 1 (TRPV1) (Hanack et al. 2015) and HCN2 channels (Schwenk et al. 2016). Sensitization of TRPV1 channels is central to the initiation of pathological forms of pain. TRPV1 assembles in a complex with GB1 (Hanack et al. 2015). Since agonist activity at GB1 reverts the sensitized state of TRPV1 channels, it may be possible to exploit the TRPV1/GB1 complex for anti-pain therapy. HCN1 and HCN2, like N-type Ca_v channels, appear to associate with GBRs through KCTD16 (Schwenk et al. 2016). HCN channels are widely expressed in the heart and the central nervous system, where they are involved in the generation of rhythmic activity (Biel et al. 2009). GBRs activate HCN currents in dopaminergic neurons of the ventral tegmental area and thereby shorten the duration of inhibitory postsynaptic potentials (Schwenk et al. 2016). The mechanism of GBR-induced HCN channel activation is unknown but may include (1) membrane hyperpolarization via Kir3 channels, (2) allosteric gating of HCN channels by conformational changes in the receptor, and/or (3) dynamic interactions of HCN channels with G protein subunits or second messengers.

6 Additional Receptor-Associated Proteins

Additional components of native GBR signaling complexes are calnexin, reticulocalbin-2, inactive dipeptidyl-peptidases 6/10, 14-3-3 proteins, synaptotagmin-11, and neuroligin-3 (Schwenk et al. 2016). The anatomically and temporally restricted expression of these proteins in the brain limits the set of available receptor constituents in individual cells and further supports a modular GBR architecture. For some of these receptor components binding sites on GB1, GB2, or the KCTDs have been identified (Fritzius and Bettler 2020). Yeast-two-hybrid screens identified several additional proteins that potentially interact with GB1 or GB2 (Pin and Bettler 2016). These proteins may represent low-abundance or transiently interacting GBR components that escaped detection in proteomic approaches.

7 Concluding Remarks

During the past decade, numerous structural and biophysical studies have greatly improved our understanding of the sequence of allosteric events involved in the activation of heterodimeric GBRs. However, the structures of the full-length heterodimeric GBR at atomic resolution in its active and inactive state, with and without bound G protein or allosteric modulators, are still missing. Cryo-electron microscopy appears to be a promising approach to obtain such high-resolution structural information, which is necessary to validate and extend current concepts. The functional relevance of higher-order GBR complexes is still unclear and needs to be addressed in native tissue. The recognition that GBR heterodimers interact with an inventory of ~30 proteins to form a variety of multi-protein complexes with distinct kinetic properties, localizations, and functions represents a departure from earlier concepts based on receptor protomers working in isolation. For some GBR interacting proteins (KCTDs, APP, HCN channels), we have identified functional effects and/or obtained high-resolution structures in association with the receptor. However, we still lack functional and structural information for most of the receptor components identified in proteomic approaches. Furthermore, much effort needs to be devoted to the study of the structural dynamics in GBR complexes during physiological processes. Understanding the structure and function of identified GBR complexes in the brain hopefully will help to identify promising molecular targets for therapeutic intervention.

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Mechanisms and Regulation of Neuronal $GABA_B$ Receptor-Dependent Signaling

Timothy R. Rose and Kevin Wickman

Contents

Abstract γ-Aminobutyric acid B receptors ($GABA_BRs$) are broadly expressed throughout the central nervous system where they play an important role in regulating neuronal excitability and synaptic transmission. $GABA_BRs$ are G protein-coupled receptors that mediate slow and sustained inhibitory actions via modulation of several downstream effector enzymes and ion channels. $GABA_BRs$ are obligate heterodimers that associate with diverse arrays of proteins to form modular

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complexes that carry out distinct physiological functions. $GABA_BR$-dependent signaling is fine-tuned and regulated through a multitude of mechanisms that are relevant to physiological and pathophysiological states. This review summarizes the current knowledge on $GABA_BR$ signal transduction and discusses key factors that influence the strength and sensitivity of $GABA_BR$-dependent signaling in neurons.

Keywords Adenylyl cyclase · GABA · $GABA_B$ receptor · GIRK channel · Phosphorylation · Plasticity · RGS · Voltage-gated Ca^{2+} channel

1 Introduction

γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the adult mammalian brain that crucially regulates the balance of excitation and inhibition that is necessary for proper brain function (McCormick 1989, Wu and Sun 2015). GABA acts on either the ionotropic $GABA_A$ or $GABA_C$ receptors ($GABA_ARs$ or $GABA_CRs$) or metabotropic $GABA_B$ receptors ($GABA_BRs$). $GABA_ARs$ and $GABA_CRs$ are ligand-gated chloride channels that mediate fast synaptic inhibition (Kittler et al. 2002), while $GABA_BRs$ are G protein-coupled receptors (GPCRs) that mediate slow synaptic inhibition throughout the central nervous system (CNS). $GABA_BRs$ were first identified by Dr. Norman Bowery in 1979 and became better understood due to the development of selective agonists (e.g., baclofen) and antagonists, as well as the cloning of the receptor in 1997 (Bowery and Hudson 1979, Bowery and Brown 1997, Kaupmann et al. 1997). Since then, our understanding of $GABA_BR$ structure and function has evolved dramatically. Discovery of the existence and functional relevance of $GABA_BR$ subunit isoforms, interacting proteins, macromolecular complexes, and regulatory mechanisms has revealed the diverse and dynamic nature of $GABA_BR$-dependent signaling throughout the brain. At the same time, dysregulation of $GABA_BR$ activity has become increasingly recognized as a driver of neurological and neuropsychiatric disorders (Gassmann and Bettler 2012, Fritzius and Bettler 2019). The goal of this review is to highlight some of the key proteins involved in mediating and regulating $GABA_BR$-dependent signaling in the brain, with an emphasis on mechanisms that fine-tune $GABA_BR$ activity that are relevant to physiology and disease.

2 $GABA_BR$ Structure

Evidence from biochemical, electrophysiological, and behavioral studies revealed that the predominant native $GABA_BR$ is an obligatory heterodimer composed of the $GABA_{B1}$ (GB1) and $GABA_{B2}$ (GB2) subunits (Fan et al. 2017, Møller et al. 2017).

The fact that most neurons co-express GB1 and GB2 and that both subunits show subcellular colocalization strongly suggested the formation of a complex (Kaupmann et al. 1998a, Charles et al. 2001, Gonchar et al. 2001, Kulik et al. 2003). Functional studies in heterologous systems and subunit-specific knockout mice ($GB1^{-/-}$ & $GB2^{-/-}$) revealed that expression of both subunits is required for electrophysiological and biochemical receptor responses (Schuler et al. 2001, Gassmann et al. 2004, Gassmann and Bettler 2012). Further support for the existence and relevance of the $GABA_BR$ heterodimer stemmed from the striking overlap in behavioral phenotypes between $GB1^{-/-}$ and $GB2^{-/-}$ mice, including spontaneous seizures, hyperalgesia, hyperlocomotion, increased anxiety, a reduced threshold for fear responses, and cognitive impairments (Gassmann et al. 2004, Gassmann and Bettler 2012). Immunoprecipitation experiments provided some of the first direct evidence of complex formation between GB1 and GB2, and later studies confirmed these results using other biochemical assays and ultrastructural techniques (Schwenk et al. 2016, Fan et al. 2017, Frangaj and Fan 2018). Crystal structures of $GABA_BR$ subunit domains have revealed that both GB1 and GB2 contain an extracellular Venus flytrap (VFT) domain, a seven-transmembrane (7TM) domain, and an intracellular carboxyl (C)-terminal domain (Frangaj and Fan 2018).

2.1 Ligand Binding

The VFT domain of GB1 contains the orthosteric binding site for GABA, as well as other agonists and antagonists. GABA interacts with key residues in the GB1 VFT to induce closure of the domain and stabilize its active conformation (Frangaj and Fan 2018). The GABA-binding site of GB1 is well conserved across species, unlike the VFT domain of GB2 (Freyd et al. 2017). Consistent with a lack of genetic conservation, the GB2 VFT cannot bind ligands, and the receptor still functions upon deletion of the domain (Monnier et al. 2011, Møller et al. 2017). However, the GB2 VFT does enhance the agonist affinity for the GB1 VFT through direct interactions that stabilize the agonist-bound state (Galvez et al. 2001, Liu et al. 2004).

2.2 Coupling to G Proteins

The 7TM domain of GB2 facilitates coupling between the receptor and G proteins. The intracellular loops of the GB2 7TM domain are required for functional coupling, as mutations in either the second or third intracellular loops prevent G protein activation (Robbins et al. 2001, Duthey et al. 2002, Havlickova et al. 2002, Binet et al. 2004). While GB1 is not required for G protein coupling, the GB1 7TM domain enhances coupling efficiency (Fan et al. 2017).

2.3 Cell Surface Trafficking

The C-terminal domains of both subunits form a coiled-coil structure that facilitates heterodimerization and surface expression of $GABA_BRs$ (Jiang et al. 2012, Burmakina et al. 2014). When expressed alone in heterologous cells or native neurons, GB1 did not reach the cell surface and was retained in the endoplasmic reticulum (ER) (Couve et al. 1998, Gassmann et al. 2004). Although GB2 did reach the cell surface when expressed alone, it remained non-functional: unable to respond to GABA or the $GABA_BR$ agonist baclofen (Schuler et al. 2001). When co-expressed, GB2 traffics GB1 to the cell surface to generate a receptor capable of high ligand affinity, G protein binding, and effector activation (Galvez et al. 2001).

Two sequences on the C-terminal tail of GB1 prevent exit from the ER (Restituito et al. 2005, Fan et al. 2017). The first is the arginine-based ER retention/retrieval (RSRR) motif, and the second is an upstream di-leucine (LL) motif. Mutations in the RSRR motif enable GB1 to exit the ER and reach the cell surface in the absence of GB2 (Margeta-Mitrovic et al. 2000, Calver et al. 2001, Pagano et al. 2001). Combining RSRR mutations with LL mutations enhanced the exit of GB1 from the ER (Margeta-Mitrovic et al. 2000, Restituito et al. 2005). These findings, alongside the crystal structure of the GB1/GB2 coiled-coil complex (Burmakina et al. 2014), revealed that interactions between the C-termini of GB1 and GB2 mask the ER retention motif to enable trafficking of the receptor to the cell surface. Subsequent investigations would discover that prenylated Rab acceptor 1 domain family, member 2 (PRAF2) sequesters GB1 in the ER to prevent its progression to the Golgi apparatus (Doly et al. 2016). PRAF2 directly interacts with the RSRR and LL motifs of GB1 to prevent ER exit and is competitively displaced from GB1 by GB2. The stoichiometry of PRAF2, GB1, and GB2 concentrations delicately controls surface density of the $GABA_BR$ (Doly et al. 2016). Beyond PRAF2, the C-terminus of GB1 associates with a number of intracellular proteins involved in receptor trafficking and heterodimerization (Table 1).

2.4 Alternative Splicing

The expression of multiple GB1 isoforms contributes to the diverse functions of $GABA_BRs$. There are 14 known isoforms of GB1 (GB1a–n), which can be generated by differential transcription or splicing of the mRNA (Bettler et al. 2004, Jiang et al. 2012, Xu et al. 2014). GB1a and GB1b are the most abundant isoforms in the brain and are the only isoforms that are highly conserved across vertebrate species (Kaupmann et al. 1997, 1998b, Benke et al. 1999, Bettler et al. 2004). Both isoforms have some differences in their spatial and temporal expression patterns in the rodent brain, as well as distinct subcellular localizations (Bettler et al. 2004, Jiang et al. 2012, Kasten et al. 2015, Castelli and Gessa 2016). In general, GB1a is expressed

Table 1 Potential $GABA_BR$-interacting proteins

	Interaction site(s)	*Impact(s) on signaling*	*References*
Core components			
GB1a	GB2	Agonist binding	Gassmann and Bettler (2012), Frangaj and Fan (2018)
GB1b	GB2	Agonist binding	Gassmann and Bettler (2012), Frangaj and Fan (2018)
GB2	GB1a, GB1b, G protein	G protein coupling	Gassmann and Bettler (2012), Frangaj and Fan (2018)
G proteins ($G\alpha_{i/o}$)	GB2 (intracellular loops), KCTD proteins	Mediate $GABA_BR$ signaling through downstream effectors	Schwenk et al. (2016), Fritzius and Bettler (2019)
KCTD proteins	GB2 (C-terminus), G protein ($G\beta\gamma$)	Accelerate G protein signaling, mediate fast desensitization (KCTD 12/12b), increase agonist potency (KCTD 12/16)	Turecek et al. (2014), Fritzius and Bettler (2019)
Peripheral components			
RGS proteins	G proteins ($G\alpha_{i/o}$), $GABA_BRs$ (GB1/GB2)	Negatively modulate G protein ($G\alpha_{i/o}$) signaling	Benians et al. (2005), Fowler et al. (2007)
Gα inhibitory interacting protein (GINIP)	G protein ($G\alpha_i$)	Promotes G protein signaling	Gaillard et al. (2014)
Ubiquitin-specific protease 14 (USP14)	GB1 (2nd intracellular loop)	Regulates post-endocytotic deubiquitination and degradation of $GABA_BRs$	Lahaie et al. (2016)
G protein-coupled receptor interacting scaffolding protein (GISP)	GB1 (C-terminus)	Promotes $GABA_BR$ surface expression	Kantamneni et al. (2007)
Shroom 4 (shrm4)	GB1 (C-terminus)	Facilitates $GABA_BR$ trafficking to cell surface	Zapata et al. (2017)
Ras-associated protein 1 (Rap1)	GB1 (C-terminus)	Promotes $GABA_BR$ surface expression by stimulating receptor recycling	Zhang et al. (2015)
Marlin-1	GB1 (C-terminus)	Regulates GB2 protein synthesis or vesicular trafficking?	Couve et al. (2004)
NEM-sensitive fusion (NSF) protein	GB1 (C-terminus), GB2 (C-terminus)	Facilitates PKC-mediated $GABA_BR$ desensitization	Pontier et al. (2006)

(continued)

Table 1 (continued)

	Interaction site(s)	*Impact(s) on signaling*	*References*
Syntaxin-1A	GB1 (C-terminus), N-type channels, G proteins (Gβγ)	Facilitates Gβγ-mediated inhibition of N-type channels, regulates probability of synaptic vesicle fusion	Jarvis et al. (2000), Vertkin et al. (2015)
Rat homologue of B2-1/cytohesin-1 (msec-7-1)	GB1 (C-terminus, LL motif)	Promotes $GABA_BR$ surface expression	Restituito et al. (2005)
Prenylated Rab acceptor 1 domain family, member 2 (PRAF2)	GB1 (C-terminus, LL/RSRR motifs)	Intracellular retention of GB1 in ER	Doly et al. (2016)
Coat protein I complex (COPI)	GB1 (C-terminus, RSRR motif)	Intracellular retention of GB1 (facilitate retrograde transport of GB1 from Golgi to ER?)	McMahon and Mills (2004), Brock et al. (2005), Bettler and Tiao (2006)
14-3-3 proteins	GB1 (C-terminus, RSRR motif), KCTD8/16	Disrupt $GABA_BR$ heterodimerization, decouple $GABA_BRs$ from GIRK channels	Couve et al. (2001), Brock et al. (2005), Laffray et al. (2012), Workman et al. (2015), Schwenk et al. (2016)
Tenascin/HNK-1	GB1 (N-terminus)	Inhibit of postsynaptic $GABA_BR$-dependent signaling	Saghatelyan et al. (2003)
CCAAT/enhancer-binding protein homologous protein (CHOP)	GB1a (N-terminus), GB2 (C-terminus)	Intracellular retention of $GABA_BRs$ in ER	Steiger et al. (2004), Sauter et al. (2005)
Fibulin-2	GB1a (SD1)	Receptor anchoring?	Blein et al. (2004)
β-Amyloid precursor protein (APP)	GB1a (SD1)	Facilitates GB1a/GB2 receptor axonal trafficking	Schwenk et al. (2016), Dinamarca et al. (2019)
Secreted β-amyloid precursor protein (sAPP)	GB1a (SD1)	Acts as an agonist/positive allosteric modulator on GB1a/GB2 receptors?	Dinamarca et al. (2019), Rice et al. (2019)
PILR-associating neural protein (PIANP)	GB1a (SD1)	Anchors GB1a/GB2 receptors at presynaptic terminals?	Schwenk et al. (2016), Dinamarca et al. (2019)
Adherence junction-associated protein 1 (AJAP1)	GB1a (SD1)	Anchors GB1a/GB2 receptors at presynaptic terminals?	Schwenk et al. (2016), Dinamarca et al. (2019)
G protein-coupled receptor kinases 4 and 5 (GRK4/5)	GB2	Promote agonist-induced desensitization	Perroy et al. (2003), Kanaide et al. (2007)
Multi-PDZ domain protein 1 (Mupp1)	GB2 (C-terminus)	Promotes $GABA_BR$ surface stability and signaling	Balasubramanian et al. (2006)

(continued)

Table 1 (continued)

	Interaction site(s)	*Impact(s) on signaling*	*References*
β-Filamin	GB2 (C-terminus)	Anchors $GABA_BRs$ to the cytoskeleton	Bettler et al. (2004)
Plakophilin-related *armadillo* repeat protein-interacting PDZ protein (PAPIN)	GB2 (C-terminus)	Promotes $GABA_BR$ surface stability and signaling	Balasubramanian et al. (2006)
Tamalin	GB2 (C-terminus)	[a]	Kitano et al. (2002)
Inactive dipeptidyl-peptidases 6 and 10 (DPP-6/10)	KCTD12	[a]	Schwenk et al. (2016)
Calsyntenin-3	APP	Link APP/$GABA_BR$ to kinesin-1 motor to facilitate axonal trafficking	Schwenk et al. (2016), Dinamarca et al. (2019)
c-Jun N-terminal kinase-interacting protein 3 (JIP-3)	APP	Link APP/$GABA_BR$ to kinesin-1 motor to facilitate axonal trafficking	Schwenk et al. (2016), Dinamarca et al. (2019)
Amyloid-like protein 2 (APLP2)	APP	[a]	Schwenk et al. (2016)
Integral membrane proteins 2B and 2C (ITM2B/C)	APP	[a]	Schwenk et al. (2016)
Potassium chloride cotransporter (KCC2)	[a]	$GABA_BR$ agonism triggers internalization of KCC2, altering driving force for Cl^--permeable $GABA_ARs$	Wright et al. (2017)
Neuroligin-3	[a]	[a]	Schwenk et al. (2016)
Reticulocalbin-2	[a]	[a]	Schwenk et al. (2016)
Synaptotagmin-11	[a]	[a]	Schwenk et al. (2016)
CLC-10	[a]	[a]	Schwenk et al. (2016)
Neuron-specific gene family members 1 and 2 (NSG1/2)	[a]	[a]	Schwenk et al. (2016)
Calnexin	[a]	[a]	Schwenk et al. (2016)

[a]Not determined
"?" represents presumed interaction sites or impacts on signaling

presynaptically in axon terminals, while GB1b is expressed postsynaptically in dendritic spines (Kasten et al. 2015). However, GB1a is also expressed postsynaptically in dendritic branches, although it is mostly excluded from dendritic spines (Vigot et al. 2006, Kasten et al. 2015). In line with their distinct subcellular distribution patterns, studies in hippocampal neurons from $GB1a^{-/-}$ and $GB1b^{-/-}$ mice revealed that presynaptic (GB1a/GB2) $GABA_BRs$ mediate inhibition of

neurotransmitter release, while postsynaptic (predominantly GB1b/GB2) $GABA_BRs$ generate slow inhibitory postsynaptic currents (Vigot et al. 2006).

GB1a is longer than GB1b (961 vs 841aa), as it contains two N-terminal protein interaction motifs, known as sushi domains (Lee et al. 2010). Sushi domains are highly conserved among species, are present in several GPCRs, and mediate protein interactions in a wide array of adhesion proteins (Grace et al. 2004, Lehtinen et al. 2004). Interestingly, the two GB1a sushi domains (SD1 and SD2) are structurally distinct (Blein et al. 2004), which may help to explain the different protein interactions observed between domains (Blein et al. 2004, Biermann et al. 2010, Hannan et al. 2012, Schwenk et al. 2016, Dinamarca et al. 2019, Rice et al. 2019). In addition to stabilizing GB1a/GB2 receptors at the cell surface (Hannan et al. 2012, 2016), the GB1a sushi domains are necessary and sufficient for axonal transport. Sushi domain mutations prevent GB1a from reaching axon terminals, and fusing the sushi domains to metabotropic glutamate receptor 1 (mGluR1) enables the somatodendritic protein to traffic down axons (Biermann et al. 2010). Thus, sushi domains act as axonal targeting signals, interacting with proteins to facilitate presynaptic transport. Several studies have identified a variety of proteins that directly or indirectly interact with the GB1a sushi domains (Table 1), with known or unknown functional influence. For example, experiments combining affinity purifications with mass spectrometry identified a number of proteins in the rodent brain that associate with the GB1a sushi domains. Of those identified, the β-amyloid precursor protein (APP), adherence junction-associated protein 1 (AJAP-1), and PILRα-associated neural protein (PIANP) directly bind, in a mutually exclusive manner, to GB1a SD1 (Schwenk et al. 2016, Dinamarca et al. 2019). Binding of APP to SD1 is necessary for vesicular trafficking of $GABA_BRs$ to axon terminals and presynaptic receptor function (Dinamarca et al. 2019). APP binds kinesin-1 adaptors of the c-Jun N-terminal kinase-interacting protein (JIP) and calsyntenin (CSTN) protein families to link APP/GB1a complexes to kinesin-1 motors that drive axonal transport (Valdés et al. 2012, Schwenk et al. 2016, Dinamarca et al. 2019, Fritzius and Bettler 2019). APP/GB1a complexes also stabilize APP at the cell surface to limit endosomal processing of APP to amyloid-beta (Aβ), a major component of plaques in Alzheimer's disease patients (Dinamarca et al. 2019). Taken together, APP/GB1a complex formation links $GABA_BR$ axonal transport to Aβ formation and supports the notion that dysregulated axonal trafficking (Kins et al. 2006, Thinakaran and Koo 2008) and reduced $GABA_BR$ expression (Chu et al. 1990, Iwakiri et al. 2005, Puthiyedth et al. 2016) observed in Alzheimer's disease may promote Aβ production (Dinamarca et al. 2019). The secreted form of APP (sAPP) has also been reported to bind GB1a SD1 and activate presynaptic $GABA_BRs$ in hippocampal neurons to inhibit neurotransmitter release (Rice et al. 2019). The sAPP-induced inhibition of neurotransmitter release was prevented by a $GABA_BR$ antagonist, suggesting that sAPP may function as an agonist or positive allosteric modulator of the $GABA_BR$ (Rice et al. 2019). In contrast to this report however, another study reported that neither APP nor sAPP influenced $GABA_BR$-dependent G protein signaling in a heterologous system (Dinamarca et al. 2019). Thus, further investigation is warranted to clarify the functional relevance of sAPP-$GABA_BR$ interactions.

Beyond GB1a and GB1b, many of the GB1c-n isoforms have only been detected at the mRNA level, and some are not conserved among species (Bettler et al. 2004, Jiang et al. 2012, Xu et al. 2014). While the physiological relevance of these isoforms remains unclear, there is a body of research to suggest that certain splice variants may be capable of fine-tuning endogenous $GABA_BR$ signaling in diverse ways – from forming functional receptors to inhibiting receptor association or function. For example, GB1c contains a single sushi domain (SD2) but shares a similar expression pattern to GB1a in the human brain (Pfaff et al. 1999, Jiang et al. 2012). When co-expressed with GB2, GB1c can form functional receptors in HEK-293 cells, suggesting that it may form a functional receptor in the rat brain, where it is expressed at the protein level (Pfaff et al. 1999). While both sushi domains are necessary for GB1a/GB2 stability, it is interesting to note that either GB1a sushi domain is sufficient for axonal transport (Biermann et al. 2010, Hannan et al. 2012). Thus, putative GB1c/GB2 receptors could theoretically function as presynaptic receptors.

GB1e/g/h/i/j/l/m/n may exist as secreted proteins, as they lack cytoplasmic domains and most, if not all, transmembrane domains (Jiang et al. 2012). GB1e is expressed at a relatively low level in the CNS of both humans and rodents but is a primary isoform in a variety of peripheral tissues. When expressed in heterologous systems, GB1e is both secreted and membrane-associated. While GB1e cannot form functional receptors when expressed with GB2, the strong interaction between the subunits is sufficient to disrupt the normal association between GB1a and GB2, but not sufficient to disrupt GB1a/GB2 receptor-mediated signaling through downstream effectors (Schwarz et al. 2000). GB1j is comprised of the two sushi domains plus 72 amino acids and is secreted when expressed in HEK-293 cells (Tiao et al. 2008). Purified sushi domains of GB1j (lacking the 72aa) impaired the inhibitory effect of $GABA_BRs$ on evoked and spontaneous glutamate release, but did not disrupt postsynaptic $GABA_BR$ activity in hippocampal neurons (Tiao et al. 2008). Although the entire protein was not studied, it was proposed that the sushi domains may scavenge an extracellular binding partner of GB1a that retains GB1a/GB2 receptors in presynaptic terminals. Given the recent identification of protein assemblies that interact with GB1a sushi domains to facilitate axonal transport (Table 1), it is theoretically possible that GB1 isoforms that contain sushi domains (GB1c/e/f/g/h/i/j) may function as dominant negative inhibitors of GB1a axonal transport and subsequent presynaptic $GABA_BR$ activity. When expressed in *Xenopus* oocytes, human-cloned GB1l and GB1m, but not GB1k, inhibited GB1a/GB2 receptor-mediated K^+ currents (Lee et al. 2010).

2.5 Oligomerization

At the cell surface, $GABA_BRs$ can exist in an equilibrium between heterodimers, tetramers, and higher-order oligomers in both heterologous systems and native neurons (Maurel et al. 2008, Schwenk et al. 2010, Comps-Agrar et al. 2011,

Gassmann and Bettler 2012, Calebiro et al. 2013). $GABA_BR$ heterodimers assemble by random collision into higher-order oligomers through weak and transient GB1-GB1 interactions (Comps-Agrar et al. 2011, Calebiro et al. 2013, Stewart et al. 2018, Xue et al. 2019). Destabilizing oligomers using competitors of the GB1-GB1 interaction, or a GB1 mutant, revealed different G protein coupling efficiencies depending on the oligomeric state of the $GABA_BR$ – suggesting a negative functional cooperativity among heterodimers within larger oligomers (Comps-Agrar et al. 2011). In addition to the reduced G protein coupling efficiency, recent reports also suggest that adjacent GABA-binding sites within $GABA_BR$ oligomers are not simultaneously occupied (Stewart et al. 2018).

2.6 *$GABA_BR$ Signalosome*

There is general consensus that $GABA_BRs$ function within macromolecular signaling complexes and that diverse protein interactions within these complexes can influence receptor activity, pharmacology, and localization. Proteomic approaches have identified an array of proteins that make up the $GABA_BR$ interactome and contribute to the functional diversity of native $GABA_BRs$ (Table 1) (Schwenk et al. 2010, 2016, Lujan and Ciruela 2012, Turecek et al. 2014, Fritzius and Bettler 2019). Many of the $GABA_BR$-interacting proteins show spatially and temporally restricted expression patterns throughout the brain, supporting the existence of dynamic and modular $GABA_BR$ complexes (Schwenk et al. 2016). Proteins within $GABA_BR$ complexes can be arranged in a hierarchy – from core components to peripheral components.

The receptor "core" is comprised of GB1, GB2, the heterotrimeric G protein, and K^+ channel tetramerization domain (KCTD) proteins. Obligate receptor components include GB1, GB2, and the heterotrimeric G protein, which represent the minimal components required for receptor signaling (Fritzius and Bettler 2019). Heterotrimeric G proteins critically link $GABA_BR$ heterodimers to primary downstream effectors (Gassmann and Bettler 2012, Fritzius and Bettler 2019). While $GABA_BRs$ can function without KCTD proteins, KCTD proteins are primary interactors that stably associate with $GABA_BRs$ in the brain (Schwenk et al. 2010, Turecek et al. 2014, Fritzius et al. 2017). Indeed, native neuronal KCTD proteins and $GABA_BR$ subunits robustly co-immunopurify with one another under stringent solubilization conditions (Schwenk et al. 2016).

Components of the receptor core anchor a multitude of "peripheral" components to form larger macromolecular complexes. Peripheral components include a wide variety of proteins that interact with GB1, GB2, the heterotrimeric G protein, or KCTD proteins (Tables 1 and 2). Some peripheral proteins influence receptor activity, pharmacology, or localization, while the functional relevance of others remains unknown (Schwenk et al. 2016, Fritzius and Bettler 2019).

Table 2 Potential $GABA_BR$ effectors

Effectors	*Interaction site(s)*	*Function(s)*	*References*
Adenylyl cyclase	$G\alpha_{i/o}$ (AC-I, III, V, VI, VIII, IX)	Inhibit cAMP production	Sadana and Dessauer (2009), Halls and Cooper (2017), Terunuma (2018)
	Gβγ (AC-I)	Inhibit cAMP production	
	Gβγ (AC-II, IV)	Stimulate cAMP production	
G protein-gated inwardly rectifying K^+ (GIRK) channel	Gβγ, GB1/GB2?	Generate inhibitory postsynaptic currents, shunt excitatory input, inhibit action potential backpropagation, suppress dendritic Ca^{2+} spikes	David et al. (2006), Fowler et al. (2007), Ciruela et al. (2010), Lüscher and Slesinger (2010), Gassmann and Bettler (2012)
Voltage-gated Ca^{2+} channel	Gβγ, KCTD16 (N-type channels)	Inhibit Ca^{2+} influx, suppress neurotransmitter release, suppress dendritic Ca^{2+} spikes	Gassmann and Bettler (2012), Schwenk et al. (2016)
		Facilitate Ca^{2+} influx, enhance neurotransmitter release (R-type and L-type channels)	Workman et al. (2013), Karls and Mynlieff (2015), Zhang et al. (2016)
		Inhibition of N-type channels can suppress BK channels	Garaycochea and Slaughter (2016)
Transient receptor potential vanilloid 1 (TRPV1) channel	GB1a	GB1 reverts TRPV1 sensitization	Hanack et al. (2015)
Hyperpolarization-activated cyclic nucleotide-gated 2 (HCN2) channel	KCTD16	Shorten the duration of inhibitory postsynaptic potentials	Schwenk et al. (2016)
TREK-2 channel	AKAP (tethers PKA to TREK-2 channels)	Reduced PKA-mediated tonic inhibition of TREK-2 channels triggers enhanced TREK-2 channel activity and postsynaptic hyperpolarization	Deng et al. (2009)
Metabotropic glutamate receptor 1 (mGluR1)	[a]	Extracellular Ca2+ interacts with $GABA_BRs$ to increase glutamate sensitivity of mGluR1	Hirono et al. (2001), Tabata et al. (2004)
		$GABA_BRs$ potentiate mGluR1/transient receptor potential canonical 3 (TRPC3) channel-mediated slow excitatory postsynaptic currents	Tian and Zhu (2018)

(continued)

Table 2 (continued)

Effectors	*Interaction site(s)*	*Function(s)*	*References*
M_2 muscarinic receptor (M_2R)	GB2 (C-terminus)	Facilitates M_2R signaling by preventing agonist-induced M_2R/GIRK channel co-internalization	Boyer et al. (2009)
Ca^{2+}-sensing receptor (CaR)	GB1, GB2	$GABA_BRs$ modulate CaR cell surface expression and signaling	Chang et al. (2007)
Transient receptor potential melastatin 3 (TRPM3) channel	Gβγ	$GABA_BRs$ inhibit TRPM3 channels	Badheka et al. (2017), Quallo et al. (2017)
$GABA_AR$ γ2s subunit	GB1	Increases GB1 cell surface expression, enhances $GABA_BR$ agonist-induced internalization	Balasubramanian et al. (2004)
ATF4/CREB2 and ATFx	GB1 (C-terminus)	$GABA_BR$-mediated transcriptional regulation	Nehring et al. (2000), White et al. (2000), Vernon et al. (2001)

[a]Not determined
"?" represents presumed interaction sites or impacts on signaling

3 $GABA_BR$-Dependent Signaling in Neurons

$GABA_BRs$ are expressed throughout the brain and are positioned within neurons at both postsynaptic (dendritic spines and shafts) and presynaptic (axon terminals) sites (Hammond and Mott 2015). In general, $GABA_BR$ activation inhibits neurons through G protein-dependent modulation of enzymes and ion channels. For example, activation of postsynaptic $GABA_BRs$ evokes a slow hyperpolarization of the postsynaptic membrane via activation of G protein-gated inwardly rectifying K^+ (Kir3/GIRK) channels (Lüscher and Slesinger 2010, Gassmann and Bettler 2012). Activation of presynaptic $GABA_BRs$ suppresses neurotransmitter release primarily through inhibition of voltage-gated Ca^{2+} channels (VGCCs) and reduced Ca^{2+} influx (Gassmann and Bettler 2012). Presynaptic $GABA_BRs$ function as either autoreceptors on GABAergic terminals or heteroreceptors on terminals releasing other neurotransmitters. Thus, presynaptic $GABA_BRs$ may be activated by GABA released from GABAergic terminals, or spillover of GABA from neighboring terminals, to suppress neurotransmitter release (Harrison et al. 1988, Wu and Saggau 1995, Boyes and Bolam 2003, Gassmann and Bettler 2012). By blocking the release of different types of neurotransmitters, $GABA_BRs$ can have excitatory or inhibitory influences at the circuit level.

3.1 *$GABA_BR$ Coupling to G Proteins*

Heterotrimeric G proteins mediate signaling by coupling receptors to enzymes, ion channels, and other effector proteins. The heterotrimeric G protein is comprised of three distinct subunits (α, β, γ); 35 subunits (16 Gα, 5 Gβ, 14 Gγ) have been identified in humans (Milligan and Kostenis 2006, Hillenbrand et al. 2015). Inactive heterotrimeric G proteins (Gαβγ) associate with $GABA_BRs$ through direct interactions with GB2 and KCTD proteins (Schwenk et al. 2016, Frangaj and Fan 2018, Fritzius and Bettler 2019). Selective coupling of heterotrimeric G proteins to $GABA_BRs$ is primarily determined by the Gα subunit. Studies using *N*-ethylmaleimide (NEM), antisense knockdown, and G protein toxins helped reveal that prototypical $GABA_BRs$ couple to pertussis toxin (PTX)-sensitive G proteins, including most members of the $G\alpha_i$ and $G\alpha_o$ ($G\alpha_{i/o}$) families (Morishita et al. 1990, Knott et al. 1993, Odagaki et al. 2000, Odagaki and Koyama 2001, Milligan and Kostenis 2006).

Gα subunits may guide functional coupling of $GABA_BRs$ to different effectors. In general, adenylyl cyclase is predominately regulated by $G\alpha_i$, while GIRK channels and VGCCs are largely regulated by $G\alpha_o$. In heterologous systems, constitutively active mutants of $G\alpha_{i1-3}$ proteins inhibited adenylyl cyclase, while $G\alpha_o$ mutants did not (Wong et al. 1992). In a reconstituted system using G proteins extracted from bovine cerebral cortex, $GABA_BR$ coupling to adenylyl cyclase was shown to involve $G\alpha_i$ ($G\alpha_{i1}$ or $G\alpha_{i2}$), but not $G\alpha_o$ (Nishikawa et al. 1997). In transfected HEK-293 cells, functional coupling of $GABA_BRs$ to GIRK channels preferentially involved $G\alpha_o$ and $G\alpha_{i2}$ (Leaney and Tinker 2000). Interestingly, GB1a/GB2 receptors predominantly signaled through $G\alpha_o$, while GB1b/GB2 receptors signaled equally through $G\alpha_o$ and $G\alpha_{i2}$ (Leaney and Tinker 2000). Slice electrophysiological studies using myristoylated G protein peptide inhibitors in ventrolateral periaqueductal gray neurons revealed that $GABA_BR$-GIRK currents are mediated by $G\alpha_{o1}$, but not $G\alpha_{i1-3}$ (Mcpherson et al. 2018). $G\alpha_o$, but not $G\alpha_i$, was also reported to couple $GABA_BRs$ to VGCCs in dorsal root ganglion neurons (Campbell et al. 1993, Menon-Johansson et al. 1993). While less is known regarding the contribution of specific Gβ and Gγ subunits to $GABA_BR$-effector coupling, Gβ2γ3 was identified as a mediator of $GABA_BR$-GIRK signaling (Schwindinger et al. 2012). In addition, Gβ1, Gβ2, and Gγ2 co-immunopurified with native neuronal $GABA_BRs$, suggesting their potential involvement in $GABA_BR$ signal transduction (Schwenk et al. 2016).

There is some evidence that $GABA_BRs$ may also couple to PTX-insensitive G proteins. Intracellular recordings in neurons from rat hippocampal slices showed that exposure to PTX did not fully prevent the baclofen-induced suppression of excitatory postsynaptic potentials (EPSPs), suggesting that presynaptic $GABA_BR$-dependent inhibition may be mediated through PTX-sensitive and PTX-insensitive G proteins (Potier and Dutar 1993). Interestingly, a recent proteomics study revealed that native $GABA_BRs$ co-immunopurify with the PTX-insensitive member of the $G\alpha_{i/o}$ family, $G\alpha_z$ (Simonds 1999, Schwenk et al.

2016). While no functional evidence currently exists to support $GABA_BR$-$G\alpha_z$ coupling, studies in heterologous systems show that $G\alpha_z$ can regulate adenylyl cyclase, GIRK channels, and VGCCs through several $G\alpha_{i/o}$-coupled GPCRs (Jeong and Ikeda 1998, Wettschureck and Offermanns 2005). Activation of $GABA_BRs$ during a narrow window of development has also been reported to increase intracellular Ca^{2+} in a subset of neonatal hippocampal neurons through $G\alpha_q$ signaling and protein kinase C (PKC) α (PKCα) activation (Carter and Mynlieff 2004, Bray and Mynlieff 2009, Karls and Mynlieff 2015). Since $GABA_BRs$ do not functionally couple to PTX-insensitive $G\alpha_q$ to activate phospholipase C (PLC) in heterologous systems, it remains unclear how functional coupling may occur in neurons (Franek et al. 1999).

3.2 $GABA_BR$ Regulation of Effectors

$GABA_BRs$ regulate the activity of a variety of effectors through direct or indirect interactions (Table 2). Here, we will review the functional relevance of $GABA_BR$-mediated regulation of three prototypical effectors – GIRK channels, VGCCs, and adenylyl cyclase.

3.2.1 GIRK Channels

GIRK channels are homo- or heterotetramers formed by four subunits (GIRK1–4); GIRK1–3 show broad and overlapping expression throughout the CNS, while GIRK4 is primarily found in the heart (Yang et al. 1995, Karschin et al. 1996, Luján and Aguado 2015). While multiple GIRK channel subtypes are present throughout the rodent brain, the GIRK1/2 heterotetramer is generally considered the prototypical neuronal GIRK channel (Luján et al. 2014). GIRK channels are predominantly distributed within the somatodendritic compartment, at both perisynaptic and extrasynaptic sites (Luján and Aguado 2015). Here, they mediate the postsynaptic inhibitory effect of multiple neurotransmitters through $G\alpha_{i/o}$-coupled GPCRs, including $GABA_BRs$ (Andrade et al. 1986, Misgeld et al. 1995, Lüscher et al. 1997, Lüscher and Slesinger 2010, Luján et al. 2014). Interestingly, some ultrastructural and functional data suggest that $GABA_BRs$ and GIRK channels colocalize on axon terminals and that $GABA_BR$-GIRK signaling inhibits neurotransmission (Ladera et al. 2008, Fernández-Alacid et al. 2009, Michaeli and Yaka 2010, Luján et al. 2014). However, others did not find evidence that presynaptic $GABA_BRs$ activate GIRK channels to inhibit neurotransmission (Lüscher et al. 1997, Takahashi et al. 1998).

$GABA_BR$-GIRK signaling has been detected in many neuron types throughout the brain (Lüscher and Slesinger 2010, Luján et al. 2014). $GABA_BRs$ activate GIRK channels through Gβγ dimers (Reuveny et al. 1994, Wickman et al. 1994, Lüscher and Slesinger 2010, Whorton and MacKinnon 2013), whereby direct binding of Gβγ

to GIRK channels enhances gating by stabilizing an interaction between the channel and phosphatidylinositol-4,5-bisphosphate (PIP_2), a cofactor required for channel gating (Logothetis et al. 1987, Huang et al. 1998, Luján et al. 2014). Activation of GIRK channels evokes a slow hyperpolarizing conductance via K^+ efflux that can shunt excitatory input (Nicoll 2004), inhibit backpropagation of action potentials, and block the generation of dendritic Ca^{2+} spikes (Leung and Peloquin 2006). The critical role of GIRK channels in tempering cellular excitability is evident in GIRK2$^{-/-}$ mice, which are hyperactive and susceptible to spontaneous seizures (Signorini et al. 1997, Blednov et al. 2001). These behavioral phenotypes are similarly observed in GB1$^{-/-}$ and GB2$^{-/-}$ mice, underlining the importance of both forms of inhibitory signaling throughout the brain (Gassmann et al. 2004).

$GABA_B$R-GIRK coupling efficiency can differ in individual neurons based on GIRK channel subunit composition (Luján et al. 2014). For example, dopamine neurons of the ventral tegmental area (VTA) have a lower $GABA_B$R-GIRK coupling efficiency than adjacent GABA neurons. Thus, lower concentrations of baclofen (or GABA) inhibit GABA neurons, while higher concentrations are required to directly inhibit dopamine neurons. The absence of GIRK1 and presence of GIRK2 and GIRK3 in VTA dopamine neurons underlies this effect (Cruz et al. 2004).

There is evidence that $GABA_B$Rs, G proteins, and GIRK channels form macromolecular signaling complexes that enable specific and rapid signaling upon receptor activation (Lüscher and Slesinger 2010, Luján et al. 2014). $GABA_B$Rs, $G\alpha_{i/o}$-type G proteins, and GIRK channels all associate with lipid rafts, suggesting that they may interact together (Oh and Schnitzer 2001, Koyrakh et al. 2005, Becher et al. 2008). $GABA_B$Rs and GIRK channels also co-cluster in the dendrites of rodent hippocampal neurons (Kulik et al. 2006, Booker et al. 2013, Fajardo-Serrano et al. 2013) and cerebellar neurons (Ciruela et al. 2010, Luján et al. 2017). Immunoprecipitation experiments revealed $GABA_B$R/GIRK and $G\alpha_o$/GIRK co-assemblies in heterologous systems (Clancy et al. 2005, David et al. 2006, Ciruela et al. 2010) and $GABA_B$R/GIRK co-assemblies in the mouse cerebellum (Ciruela et al. 2010, Luján et al. 2017). Evidence in support of direct protein interactions largely comes from biochemical assays in heterologous systems. BRET/FRET experiments revealed close interactions (<100 Å) between $GABA_B$Rs and GIRK2 homotetramers, GIRK1/4 or GIRK1/3 heterotetramers, and $G\alpha_o$ proteins (David et al. 2006, Fowler et al. 2007, Ciruela et al. 2010).

Some functional data also support the possibility of a pre-coupling of components in the form of a macromolecular complex. GIRK channels expressed in heterologous systems or native neurons can signal even in the absence of receptor activation, supporting the possibility that some signaling components are pre-coupled (Kahanovitch et al. 2017). Increasing the surface expression of $GABA_B$Rs in *Xenopus* oocytes reduced this basal GIRK channel activation, likely via downregulation of GIRK channel surface expression during constitutive $GABA_B$R internalization, as has been reported elsewhere (Padgett et al. 2012, Hearing et al. 2013, Lecca et al. 2016). This could indicate physical interactions among $GABA_B$Rs and GIRK channels and perhaps a pre-coupling between components of this signaling cascade.

There is also evidence against the existence of pre-coupled macromolecular complexes and in favor of a collision-coupling mode of $GABA_BR$-GIRK signaling. For example, increasing the surface expression of $GABA_BRs$ in *Xenopus* oocytes accelerated GIRK channel activation, suggesting that $GABA_BRs$ or G proteins can diffuse freely in the membrane to activate GIRK channels (Kahanovitch et al. 2017). Furthermore, unlike the direct interactions reported in heterologous systems, native neuronal $GABA_BRs$ and GIRK channels did not co-immunopurify with one another in a high-resolution proteomics study (Schwenk et al. 2016). Thus, the mode of coupling between components of this signalosome remains unclear. Taken together, these results suggest that a putative $GABA_BR$-G protein-GIRK complex may be dynamic, allowing for dissociation and reassociation of components (Kahanovitch et al. 2017). The formation of dynamic complexes, with low-affinity and/or transient interactions, could explain why $GABA_BRs$ and GIRK channels did not associate in vivo (Schwenk et al. 2016).

3.2.2 Voltage-Gated Ca^{2+} Channels

VGCCs are regulated by many $G\alpha_{i/o}$-coupled GPCRs, including $GABA_BRs$. VGCCs are typically closed at resting membrane potentials but are opened by membrane depolarization, leading to Ca^{2+} influx. Ca^{2+} influx depolarizes the cellular membrane, facilitates synaptic vesicle release, and, as a secondary messenger, regulates diverse physiological properties (Proft and Weiss 2015). VGCCs are composed of pore-forming subunits encoded by ten mammalian genes. Seven genes encode the high-voltage-activated Ca^{2+} channel subfamily including L-type ($Ca_V1.1$ to 1.4), P/Q-type ($Ca_V2.1$), N-type ($Ca_V2.2$), and R-type ($Ca_V2.3$) channels, while three genes encode low-voltage-activated T-type ($Ca_V3.1$–3.3) channels (Proft and Weiss 2015). In general, $GABA_BRs$ inhibit N- and P/Q-type channels in most neurons and L-, T-, and R-type channels in select neuron populations (Maguire et al. 1989, Chalifoux and Carter 2011, Proft and Weiss 2015).

$GABA_BR$ activation inhibits N- and P/Q-type channels in presynaptic terminals of both glutamatergic and GABAergic neurons, as well as R-type channels in some glutamatergic neurons (Wu and Saggau 1995, Proft and Weiss 2015, Burke and Bender 2019). Inhibition of presynaptic VGCCs reduces Ca^{2+} influx and decreases the probability of neurotransmitter release (Burke and Bender 2019). $GABA_BRs$ inhibit VGCCs through direct interactions between Gβγ and the channel (Herlitze et al. 1996, Waard et al. 1997, Burke and Bender 2019). Mechanistically, Gβγ binding to VGCCs slows channel activation kinetics and induces a positive shift in the voltage dependence to inhibit Ca^{2+} influx (Bean 1989, Colecraft et al. 2000). Gβγ-mediated inhibition can be relieved by strong depolarization or eventual dissociation of Gβγ from the channel (Colecraft et al. 2000, 2001, Proft and Weiss 2015). Postsynaptic $GABA_BRs$ also inhibit several VGCC subtypes in dendrites and spines (Pérez-Garci et al. 2006, Chalifoux and Carter 2011, Booker et al. 2018). Postsynaptic $GABA_BR$-VGCC signaling prevents dendritic Ca^{2+} spikes to reduce cellular

excitability and limit the actions of Ca^{2+} as a secondary messenger (Malenka 1991, Pérez-Garci et al. 2006, Chalifoux and Carter 2011, Brini et al. 2014, Booker et al. 2018).

Similar to GIRK channels, $GABA_BRs$ and VGCCs have been proposed to form signaling complexes that facilitate tight functional coupling through membrane-delimited Gβγ interactions. FRET experiments revealed that GB1a/GB2 receptors associate with Gβγ and N-type channels in hippocampal pyramidal neuron boutons, suggesting the formation of signaling complexes that facilitate $GABA_BR$/VGCC-mediated presynaptic inhibition (Laviv et al. 2011). In line with this, a high-resolution proteomics approach showed that native neuronal N-type channels assemble with GB1a/GB2 receptors (Schwenk et al. 2016). Electrophysiological, biochemical, and ultrastructural evidence also support the existence of postsynaptic signaling complexes. $GABA_BRs$ co-assemble and co-cluster with P/Q-type channels in dendritic shafts of cerebellar Purkinje neurons (Luján et al. 2017) and co-cluster with L-type channels in dendrites of hippocampal somatostatin interneurons to inhibit postsynaptic Ca^{2+} influx (Booker et al. 2018).

While $GABA_BR$-VGCC signaling has well-documented inhibitory influences on neurons, under certain conditions it can also exert excitatory influences. For example, presynaptic VGCC inhibition often suppresses the release of inhibitory neurotransmitters (e.g., GABA, glycine) to disinhibit downstream neurons. $GABA_BR$-mediated inhibition of N-type channels in rat retinal neurons also suppressed big conductance Ca^{2+}-activated K^+ (BK) channels, which led to a net increase in neuronal excitability (Garaycochea and Slaughter 2016). As mentioned earlier, $GABA_BRs$ activated L-type channels via $G\alpha_q$ signaling and PKCα activation in neonatal hippocampal neurons (Carter and Mynlieff 2004, Bray and Mynlieff 2009, Karls and Mynlieff 2015). A similar activation of L-type channels through $GABA_BRs$ has been reported in response to N-methyl-D-aspartate receptor (NMDAR) blockade (Workman et al. 2013). Lastly, $GABA_BRs$ activated R-type channels on medial habenula neurons to facilitate Ca^{2+} influx and trigger neurotransmitter release into the interpeduncular nucleus (Zhang et al. 2016, Koppensteiner et al. 2017).

3.2.3 Adenylyl Cyclase

Adenylyl cyclase catalyzes the synthesis of cyclic AMP (cAMP), a key second messenger that regulates diverse cellular processes (Smit and Iyengar 1998, Halls and Cooper 2017). Ten adenylyl cyclase isoforms are expressed throughout the mammalian brain – nine transmembrane isoforms (AC-I–IX) and one soluble isoform (AC-X) (Sadana and Dessauer 2009, Halls and Cooper 2017). While all transmembrane isoforms can be stimulated by direct interactions with $G\alpha_s$, $G\alpha_{i/o}$ proteins directly inhibit AC-I, AC-III, AC-V, AC-VI, AC-VIII, and AC-IX. The Gβγ dimers also inhibit AC-I but can stimulate AC-II and AC-IV (Sadana and Dessauer 2009, Halls and Cooper 2017, Terunuma 2018). Thus, $GABA_BRs$ can

bidirectionally regulate adenylyl cyclase activity through typical $G\alpha_{i/o}$- or Gβγ-mediated inhibition or atypical Gβγ-mediated stimulation.

Several early studies had shown that $GABA_BR$ agonists inhibit basal or forskolin-stimulated adenylyl cyclase activity in neurons via PTX-sensitive G proteins (Wojcik and Neff 1984, Knight and Bowery 1996, Simonds 1999, Bettler et al. 2004). Others found that $GABA_BRs$ can stimulate adenylyl cyclase-induced cAMP production during co-activation of $G\alpha_s$-coupled receptors by norepinephrine, isoprenaline, histamine, or vasoactive intestinal polypeptide (Bettler et al. 2004). This atypical Gβγ-mediated stimulation of adenylyl cyclase (AC-II and AC-IV) requires the presence of active $G\alpha_s$, thus demonstrating a form of G protein crosstalk between $GABA_BRs$ and $G\alpha_s$-coupled GPCRs that augments cAMP production (Simonds 1999, Bowery et al. 2002, Calver et al. 2002). The $GABA_BR$-mediated bidirectional regulation of cAMP levels was confirmed in vivo using microdialysis in freely moving rats (Hashimoto and Kuriyama 2002).

Typical $GABA_BR$-induced reduction in cAMP and subsequent protein kinase A (PKA) activity influence several downstream processes. Presynaptic reductions in cAMP levels inhibit vesicle fusion and spontaneous neurotransmitter release (Sakaba and Neher 2003, Rost et al. 2011). Postsynaptic reductions in PKA activity alleviate an A-kinase anchoring protein (AKAP)-dependent tonic inhibition of TREK2 channels (Deng et al. 2009), decrease the Ca^{2+} permeability of NMDARs (Chalifoux and Carter 2010), enhance the magnitude of tonic $GABA_AR$ currents (Connelly et al. 2013), and influence gene expression (Ghorbel et al. 2005, Fukui et al. 2008, Schwirtlich et al. 2010). Taken together, $GABA_BR$-dependent regulation of adenylyl cyclase is poised to influence diverse cellular processes across short and long timeframes – by modifying neuronal excitability and synaptic transmission, altering levels of intracellular secondary messengers (cAMP, Ca^{2+}), and regulating gene expression.

4 Regulation of $GABA_BR$-Dependent Signaling in Neurons

Tight control over the timing and strength of $GABA_BR$-dependent signaling is crucial for establishing a proper inhibitory tone that balances excitation. In this regard, signaling through $GABA_BRs$ is subject to regulation via a myriad of mechanisms.

4.1 Desensitization

Desensitization is a common regulatory mechanism of GPCRs to prevent overstimulation. For many GPCRs, desensitization involves direct phosphorylation of the receptor by GPCR kinase (GRK), followed by arrestin binding and dynamin-dependent and clathrin-mediated endocytosis (Gurevich and Gurevich 2019).

Internalized receptors accumulate in endosomal sorting compartments where they may either be dephosphorylated and recycled back to the cell surface or targeted to lysosomes for degradation (Benke et al. 2012, Iacovelli and De Blasi 2013, Lefkowitz 2013).

While prolonged activation of $GABA_BRs$ induces desensitization of the receptor response, $GABA_BR$ desensitization does not involve receptor internalization via the classical GRK phosphorylation and arrestin recruitment pathway. Rather, surface stability of $GABA_BRs$ is regulated through a variety of phosphorylation-independent and phosphorylation-dependent mechanisms (Fairfax et al. 2004, Grampp et al. 2008, Benke et al. 2012, Raveh et al. 2015). Although GRKs do not phosphorylate $GABA_BRs$, GRK4 and GRK5 still promote agonist-induced desensitization of the $GABA_BR$ response (Perroy et al. 2003, Fairfax et al. 2004, Kanaide et al. 2007). Since GRK4 and GRK5 directly associated with GB2, where they competed with the G protein for binding, they were proposed to induce desensitization by uncoupling the G protein from the $GABA_BR$ (Benke et al. 2012, Raveh et al. 2015). This interaction may be highly cell specific, as biochemical analyses reveal that GRK4 and GRK5 are minimally expressed in tissues that have high $GABA_BR$ expression, including the cerebral cortex and hippocampus (Sallese et al. 2000, Sato et al. 2015). GRK2 has also been reported to induce desensitization of several inhibitory GPCRs through a phosphorylation-independent mechanism involving the sequestration of βγ (Raveh et al. 2010, 2015). In a heterologous system, GRK2 increased desensitization of $GABA_BR$-GIRK currents by ~30% (Turecek et al. 2014).

4.2 Phosphorylation

Unlike many GPCRs, $GABA_BR$ activity is not correlated with the overall phosphorylation state of the receptor, as phosphorylation of different residues influences $GABA_BR$ activity in distinct ways (Perroy et al. 2003, Terunuma 2018). There are five known phosphorylation sites on $GABA_BRs$ that regulate endocytosis, surface stability, and desensitization. These include serine 867 (S867) and S917/923 on GB1 and S783 and S892 on GB2. Several kinases mediate phosphorylation at these sites.

4.2.1 CaMKII

Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) phosphorylates S867 on primarily GB1b, leading to the dynamin-dependent endocytosis of $GABA_BRs$ that couple to GIRK channels (Guetg et al. 2010). Glutamatergic signaling downregulates $GABA_BRs$ (Guetg et al. 2010, Maier et al. 2010), in part through the activation of NMDARs that enhance CaMKII-mediated phosphorylation of S867 to promote $GABA_BR$ internalization (Guetg et al. 2010). Indeed, blockade of either

CaMKII activity or phosphorylation of S867 was sufficient to prevent $GABA_BR$ internalization in hippocampal neurons (Guetg et al. 2010, Maier et al. 2010).

4.2.2 AMPK

AMP-activated protein kinase (AMPK) is a serine-threonine kinase that functions as an energy sensor that is activated by increased cellular levels of AMP due to high metabolic activity, or during anoxia or ischemia (Carling 2005, Carling et al. 2011). AMPK binds to the C-terminus of GB1, where it can phosphorylate two sites on GB1 (S917/923) and one site on GB2 (S783) (Kuramoto et al. 2007). The physiological relevance of AMPK-induced phosphorylation at all three sites was examined by measuring AMPK-mediated $GABA_BR$-GIRK coupling in HEK-293 cells. Phosphorylation of S783 on GB2 reduced desensitization of $GABA_BRs$ and enhanced $GABA_BR$-GIRK coupling by stabilizing receptors at the plasma membrane. Ischemic brain injury enhanced S783 phosphorylation in the hippocampus of rats, and S783 phosphorylation promoted neuronal survival of cultured hippocampal neurons after chemical anoxia (Kuramoto et al. 2007). Therefore, AMPK-mediated phosphorylation of S783 may play a neuroprotective role in limiting excitotoxicity by maintaining $GABA_BR$ inhibitory tone during times of high metabolic stress or ischemic injury (Kuramoto et al. 2007).

AMPK-mediated phosphorylation of S783 is bidirectionally regulated by glutamatergic signaling through NMDARs (Terunuma et al. 2010, Terunuma 2018). Transient activation of NMDARs enhances AMPK activity and promotes S783 phosphorylation, while prolonged NMDAR activation promotes S783 dephosphorylation (Terunuma et al. 2010). Prolonged NMDAR activity activates protein phosphatase 2A (PP2A), which dephosphorylates S783 and targets $GABA_BRs$ for lysosomal degradation, thus reducing surface expression and $GABA_BR$ function. Concurrent activation with $GABA_BRs$ prevents the NMDAR/PP2A-mediated reduction in $GABA_BR$ surface expression, likely via membrane hyperpolarization or decreased Ca^{2+} permeability of NMDARs (Terunuma et al. 2010). Thus, glutamatergic and GABAergic signaling delicately control the phosphorylation state of $GABA_BRs$ to regulate intracellular trafficking and cell surface stability. Interestingly, studies using S783A knock-in mice, in which mutation of serine to alanine decreases $GABA_BR$ degradation, revealed that the S783A mutation selectively enhanced postsynaptic, but not presynaptic, $GABA_BR$ activity (Terunuma et al. 2014). This suggests that presynaptic receptors are highly stable and have lower rates of endocytosis than postsynaptic receptors that have higher rates of phosphorylation-dependent internalization.

4.2.3 PKA

PKA phosphorylates the cytoplasmic tail of GB2 at S892, leading to increased $GABA_BR$ surface stability and reduced slow desensitization in HEK-293 and

hippocampal cells (Couve et al. 2002). Prolonged activation of $GABA_B$Rs inhibits adenylyl cyclase to reduce PKA activity and S892 phosphorylation, which coincides with increased endocytosis-independent $GABA_B$R degradation (Fairfax et al. 2004). $GABA_B$R degradation induced by chronic exposure to baclofen is attenuated by either PKA activation or co-stimulation of $G\alpha_s$-coupled β-adrenergic receptors (Couve et al. 2002, Fairfax et al. 2004, Benke 2010). Thus, PKA-induced phosphorylation of S892 and $GABA_B$R surface stability are bidirectionally regulated by G protein signaling cascades that modulate PKA activity.

4.2.4 PKC

PKC has been reported to phosphorylate GB1 at an unknown site in Chinese hamster ovary cells. Activation of $GABA_B$Rs enhances PKC recruitment to the plasma membrane, induces phosphorylation of GB1, and disrupts the direct interaction between NEM-sensitive fusion (NSF) proteins and $GABA_B$Rs to facilitate agonist-induced, internalization-independent desensitization (Pontier et al. 2006).

4.3 Ubiquitination

Ubiquitination is a posttranslational modification that involves covalent attachment of ubiquitin to a target protein, generally directing the protein to proteasomes or lysosomes for degradation (Sarker et al. 2011). Many GPCRs undergo reversible ubiquitin modifications that regulate receptor degradation, among other functions (Dores and Trejo 2012, Cottrell 2013, Kommaddi and Shenoy 2013, Kennedy and Marchese 2015). Ubiquitination is reported to regulate $GABA_B$R trafficking from the endoplasmic reticulum to the cell surface via increased proteasomal degradation. Lys(48)-linked polyubiquitination of lysines 767/711 in the GB2 C-terminus promotes constitutive proteasomal degradation of $GABA_B$Rs in cultured cortical neurons, and inactivation of these sites increases cell surface receptor levels and enhances $GABA_B$R signaling (Zemoura et al. 2013).

Ubiquitination of GB1 is also reported to control lysosomal degradation of $GABA_B$Rs. GB1 is ubiquitinated at multiple sites by Mind bomb-2 (MIB2), a ubiquitin ligase catalyzing Lys(63)-linked ubiquitination, which promotes lysosomal degradation of $GABA_B$Rs (Zemoura et al. 2016). Mutational inactivation of putative GB1 ubiquitination sites prevented lysosomal degradation. Interestingly, MIB2-induced ubiquitination is believed to contribute to the glutamate-induced downregulation of $GABA_B$Rs (Zemoura et al. 2016). A recent follow-up study supports this notion, revealing that MIB2-induced GB1 ubiquitination is largely dependent on the phosphorylation state of S867 on GB1. CaMKIIβ-induced S867 phosphorylation promotes, while S867 dephosphorylation inhibits, Lys(63)-linked ubiquitination of GB1 (Zemoura et al. 2019).

PKC has also been reported to promote ubiquitination, internalization, and degradation of $GABA_BRs$. Cell surface $GABA_BRs$ undergo PKC-mediated constitutive ubiquitination and subsequent internalization, and deubiquitination of the receptor is catalyzed post-endocytically by USP14, a deubiquitinase that directly interacts with GB1 to target $GABA_BRs$ for lysosomal degradation (Lahaie et al. 2016).

4.4 KCTD Proteins

The four KCTD proteins (KCTD8, 12, 12b, 16) assemble as homo- or heteromeric pentamers on the C-terminus of GB2 (Fritzius et al. 2017, Pinkas et al. 2017, Fritzius and Bettler 2019, Zuo et al. 2019), where they stabilize G proteins at the receptor and regulate the kinetics of G protein-dependent signaling (Turecek et al. 2014, Zheng et al. 2019). KCTD proteins accelerate the onset of $GABA_BR$-GIRK currents, and KCTD12 and KCTD16 additionally increase agonist potency, as seen by a reduced EC_{50} value of baclofen-evoked GIRK currents (Schwenk et al. 2010). KCTD12 and KCTD12b also induce fast desensitization of $GABA_BR$-GIRK currents by directly binding receptor-activated Gβγ dimers to uncouple Gβγ from GIRK channels (Schwenk et al. 2010, Turecek et al. 2014, Fritzius et al. 2017, Fritzius and Bettler 2019, Zheng et al. 2019). Interestingly, PKA-mediated phosphorylation of S892 on GB2 can regulate KCTD12-induced fast desensitization (Adelfinger et al. 2014). PKA activation in hippocampal neurons slows, while PKA inhibition accelerates, KCTD12-induced fast desensitization of $GABA_BR$-GIRK currents. PKA fails to regulate desensitization in knock-in mice with a serine 892 to alanine mutation (S892A), demonstrating that phosphorylation of S892 slows KCTD12-induced fast desensitization in vivo (Adelfinger et al. 2014). In addition to regulating G protein signaling kinetics, KCTD proteins also scaffold effector channels and other proteins at the $GABA_BR$ (Table 1). For example, N-type Ca^{2+} channels, hyperpolarization-activated cyclic nucleotide-gated 2 (HCN2) channels, and 14-3-3 proteins associate with $GABA_BRs$ through direct interactions with KCTD16 (Schwenk et al. 2016).

4.5 RGS Proteins

Regulator of G protein signaling (RGS) proteins are GTPase-accelerating proteins (GAPs) that facilitate termination of G protein signaling by promoting hydrolysis of GTP on active Gα to enable reassembly of the heterotrimeric G protein complex (Anderson et al. 2009, Gerber et al. 2016). The mammalian RGS protein superfamily is divided into eight subfamilies (RZ, R4, R7, R12, RA, GED, GRK, SNX) based on amino acid sequence or structural similarity. While structural diversity among RGS proteins does explain the existence of noncanonical cell signaling roles, the RGS

homology domain that is critical for accelerating GTPase activity is highly conserved among many members (Siderovski and Willard 2005, Gerber et al. 2016, Squires et al. 2018). RGS proteins across several subfamilies have been shown to regulate the kinetics of G protein-dependent signaling through $GABA_BRs$.

4.5.1 R7 RGS/Gβ5

The R7 RGS protein family is composed of four members (RGS6, RGS7, RGS9, RGS11) that play critical roles in fundamental neuronal processes, including vision, motor control, reward behavior, and nociception (Anderson et al. 2009). R7 RGS proteins form obligate heterodimers with G protein β5 (Gβ5) through interactions at their Gγ-like domains (Snow et al. 1999, Witherow et al. 2000, Hollinger and Hepler 2002). RGS/Gβ5 heterodimers can then form reversible complexes with adaptor proteins, including R7-binding protein (R7BP) (Drenan et al. 2005, Martemyanov et al. 2005, Grabowska et al. 2008, Patil et al. 2018). When palmitoylated, R7BP anchors the heterodimeric complex at the plasma membrane and prevents RGS protein degradation (Drenan et al. 2005, 2006, Jia et al. 2011). R7BP also facilitates the functional association of RGS/Gβ5 with GIRK channels to promote deactivation of G proteins (Xie et al. 2010, Jia et al. 2014, Ostrovskaya et al. 2014). Indeed, genetic ablation of either RGS6, RGS7, Gβ5, or R7BP prolongs deactivation kinetics of $GABA_BR$-GIRK currents (Xie et al. 2010, Maity et al. 2012, Ostrovskaya et al. 2014). Ablation of RGS7 or R7BP also enhanced the coupling efficiency of $GABA_BR$-GIRK signaling, increasing the potency of baclofen-induced GIRK currents (Ostrovskaya et al. 2014).

In line with their functional association, biochemical, electrophysiological, and ultrastructural evidence support the existence of macromolecular complexes formed of RGS7/Gβ5, $GABA_BRs$, and GIRK channels on dendritic spines of hippocampal CA1 pyramidal neurons (Xie et al. 2010, Fajardo-Serrano et al. 2013). Insights from the RGS7-Gβ5-R7BP crystal structure reveal that the orientation of the complex is compatible with macromolecular assemblies involving $GABA_BRs$ and GIRK channels (Patil et al. 2018).

In addition to forming complexes with R7BP, RGS7/Gβ5 can also assemble with G protein-coupled receptor 158 (GPR158) (Orlandi et al. 2012, Ostrovskaya et al. 2018). Formation of either complex is mutually exclusive and facilitates trafficking of RGS7 to the plasma membrane (Orlandi et al. 2012). The ability of RGS7 to negatively regulate $GABA_BR$ signaling through GIRK channels or P/Q/N-type channels is enhanced by R7BP but opposed by GPR158 (Ostrovskaya et al. 2018). Interestingly, the RGS7/Gβ5-GPR158 complex has been reported to suppress homeostatic regulation of cAMP by $GABA_BRs$ (Orlandi et al. 2018). Altogether, this suggests that RGS7/Gβ5 dimers exist in two separate complexes at the plasma membrane that may guide RGS7-meditated regulation toward particular effector systems.

4.5.2 R4 RGS Proteins

Two members of the R4 RGS subfamily (RGS2 and RGS4) have been implicated in negatively regulating $GABA_BR$-GIRK signaling in neurons. RGS2 reduces the coupling efficiency of $GABA_BRs$ with heteromeric GIRK2/3 channels in VTA dopamine neurons. Evidence from immunoelectron microscopy and slice electrophysiology in GIRK subunit-specific knockout mice suggests that the effect of RGS2 on $GABA_BR$-GIRK signaling uniquely requires the GIRK3 subunit, and FRET analysis revealed direct interactions between RGS2 and GIRK3 (Labouèbe et al. 2007).

RGS4 has been proposed to form a signaling complex with $GABA_BRs$ to terminate $GABA_BR$-GIRK signaling. Double immunohistochemistry and immunoprecipitation assays revealed that RGS4 and $GABA_BRs$ associate together in the prefrontal cortex and hypothalamus, and FRET analysis in transfected HEK-293 cells indicated direct interactions between RGS4 and either GB1 or GB2 (Fowler et al. 2007, Kim et al. 2014). RGS4 enhances GIRK channel deactivation rates within a second of agonist application in vitro, and RGS4 expression in GIRK-transfected CHO cells mimics the fast deactivation kinetics observed in hippocampal neurons and atrial myocytes (Doupnik et al. 1997). RGS4 has also been reported to limit crosstalk between two $G\alpha_{i/o}$-coupled receptors, $GABA_BRs$ and A_2 adenosine receptors (A_2Rs), in pyramidal neurons of the prefrontal cortex. Within single dendritic spines, and through inhibition of PKA, $GABA_BR$ activation inhibits NMDARs, while A_2R activation inhibits AMPARs. RGS4 appears capable of limiting interference between the two receptors' neuromodulatory functions, as blocking RGS4 activity with either a small molecule inhibitor or an intracellular anti-RGS4 antibody enables crosstalk between pathways. This raises the intriguing possibility that RGS4 dysfunction in schizophrenia could disrupt pathway segregation and promote crosstalk that drives aberrant function (Gyorgy Lur et al. 2015).

4.5.3 RGS12

RGS12 negatively regulates presynaptic $GABA_BR$ signaling through N-type channels in chick dorsal root ganglion neurons (Schiff et al. 2000). In addition to its canonical GAP activity, RGS12 binds to the synprint region of N-type channels to directly modulate Ca^{2+} signaling (Schiff et al. 2000, Richman et al. 2005).

5 Plasticity of $GABA_BR$-Dependent Signaling

Native $GABA_BRs$ are multi-protein complexes with a remarkable diversity in protein composition across space and time (Malitschek et al. 1998, Fritschy et al. 1999, Schwenk et al. 2016). This endows functional diversity to $GABA_BR$-mediated

signaling across brain regions and cell types (Cruz et al. 2004, Gassmann and Bettler 2012, Terunuma 2018) and even between sexes (Al-Dahan et al. 1994, Kelly et al. 2003, Marron Fernandez de Velasco et al. 2015). $GABA_BR$-mediated signaling is also plastic, changing throughout development (Moran et al. 2001, Luo et al. 2011, Dlouhá et al. 2013, Karls and Mynlieff 2015, Cai et al. 2017), during pathophysiological states (Fritzius and Bettler 2019), or in response to particular experiences (Terunuma 2018). Potential mechanisms underlying differences or changes in $GABA_BR$ function can be remarkably diverse, as alterations in the expression/structure/function of either core and peripheral proteins that make up $GABA_BR$ complexes, effector proteins, or regulatory proteins may have a dramatic influence at the molecular, cellular, and organismal level. In addition to examples of $GABA_BR$ plasticity described earlier, listed below are a few proposed plasticity mechanisms, among the many that have been reported.

5.1 Phosphorylation-Dependent Plasticity

Phosphorylation-dependent changes in $GABA_BR$ and GIRK channel surface availability underlie several reports of $GABA_BR$ plasticity. As discussed earlier, sustained glutamatergic or GABAergic signaling can downregulate $GABA_BRs$ through several mechanisms, including receptor phosphorylation. Exposure to drugs of abuse can also induce phosphorylation-dependent changes in $GABA_BR$ activity in vivo. For example, both acute and repeated exposure to cocaine suppressed $GABA_BR$-GIRK signaling in VTA dopamine neurons and prelimbic cortex pyramidal neurons, respectively, via phosphorylation-dependent internalization of $GABA_BRs$ and/or GIRK channels (Arora et al. 2011, Hearing et al. 2013). Exposure to inescapable footshocks similarly suppressed $GABA_BR$-GIRK signaling in the lateral habenula through increased PP2A activity and internalization of $GABA_BRs$ and GIRK channels (Lecca et al. 2016).

5.2 Plasticity of 14-3-3 Proteins

Multiple members of the 14-3-3 family of scaffolding proteins directly or indirectly interact with $GABA_BRs$ (Couve et al. 2001, Schwenk et al. 2016) and have been proposed to regulate $GABA_BR$ structure and function. For example, 14-3-3ζ is overexpressed in the dorsal horn of neuropathic rats, where it inhibits $GABA_BR$ signaling through K^+ channels and contributes to pain sensitization. Biochemical, electrophysiological, and ultrastructural evidence suggest that 14-3-3ζ disrupts $GABA_BR$-dependent signaling by dissociating $GABA_BR$ heterodimers at the plasma membrane (Laffray et al. 2012). Exposure to NMDAR antagonists, acting as rapid antidepressants, increases the surface stability of 14-3-3η, which decouples $GABA_BR$ signaling from GIRK channels in the hippocampus of socially defeated

(model of depression) rodents (Workman et al. 2015). The existence of several other $GABA_BR$-interacting 14-3-3 isoforms raises the intriguing possibility of similar interactions throughout the CNS (Schwenk et al. 2016).

5.3 Plasticity of RGS Regulation

As mentioned earlier, RGS2 negatively regulates the $GABA_BR$-GIRK coupling efficiency in VTA dopamine neurons. Chronic exposure to either gamma hydroxybutyrate (GHB) or morphine reduced RGS2 transcription and enhanced the coupling efficiency of $GABA_BR$-GIRK signaling in VTA dopamine neurons of mice (Labouèbe et al. 2007). Another interesting example involves plasticity of GPR158, an RGS7/Gβ5-binding protein that suppresses homeostatic regulation of cAMP by $GABA_BRs$ (Orlandi et al. 2018). GPR158 is upregulated in the prefrontal cortex of both humans with major depressive disorder and mice exposed to chronic stress (Sutton et al. 2018). Viral overexpression of GPR158 in the mouse prefrontal cortex induced depressive-like behaviors, while constitutive GPR158 ablation produced an antidepressant effect (Sutton et al. 2018). Thus, GPR158 plasticity and $GABA_BR$-mediated dysregulation of cAMP levels in the prefrontal cortex may play a prominent role in stress-induced depression.

6 Concluding Remarks

$GABA_BRs$ are obligate heterodimers that interact with G proteins, effectors, and a wide variety of proteins to form spatially and temporally modular complexes that impart functional diversity to $GABA_BR$-dependent signaling throughout the brain. Given the vital roles $GABA_BRs$ play in regulating synaptic transmission and behavior, as well as their links to disease, it is critical to understand the dynamic structure and function of the $GABA_BR$ signalosome. Further research aiming to identify the functional roles of components within $GABA_BR$ complexes, as well as mechanisms underlying regulation/modulation of $GABA_BR$-dependent signaling, will occupy the field for many years. A deeper understanding of the relationships between the $GABA_BR$ signalosome and pathophysiological states will yield insights that are essential for drug discovery and development efforts. While drugs targeting $GABA_BRs$ are currently used on- and off-label to treat several disorders, broadening drug design to more selectively target components within $GABA_BR$ complexes, or interactions between components, will likely increase the therapeutic potential of new medicines (Berry-Kravis et al. 2018, Fritzius and Bettler 2019).

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$GABA_B$ Receptor Chemistry and Pharmacology: Agonists, Antagonists, and Allosteric Modulators

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Contents

Abstract The $GABA_B$ receptors are metabotropic G protein-coupled receptors (GPCRs) that mediate the actions of the primary inhibitory neurotransmitter, γ-aminobutyric acid (GABA). In the CNS, GABA plays an important role in behavior, learning and memory, cognition, and stress. GABA is also located throughout the gastrointestinal (GI) tract and is involved in the autonomic control of the intestine and esophageal reflex. Consequently, dysregulated $GABA_B$ receptor signaling is associated with neurological, mental health, and gastrointestinal disorders; hence, these receptors have been identified as key therapeutic targets and are the focus of multiple drug discovery efforts for indications such as muscle spasticity

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disorders, schizophrenia, pain, addiction, and gastroesophageal reflex disease (GERD). Numerous agonists, antagonists, and allosteric modulators of the $GABA_B$ receptor have been described; however, Lioresal® (Baclofen; β-(4-chlorophenyl)-γ-aminobutyric acid) is the only FDA-approved drug that selectively targets $GABA_B$ receptors in clinical use; undesirable side effects, such as sedation, muscle weakness, fatigue, cognitive deficits, seizures, tolerance and potential for abuse, limit their therapeutic use. Here, we review $GABA_B$ receptor chemistry and pharmacology, presenting orthosteric agonists, antagonists, and positive and negative allosteric modulators, and highlight the therapeutic potential of targeting $GABA_B$ receptor modulation for the treatment of various CNS and peripheral disorders.

Keywords $GABA_B$ receptor pharmacology · Orthosteric and allosteric modulators · Therapeutic target

1 Introduction

γ-aminobutyric acid (GABA) is one of the most widely distributed amino acid neurotransmitters in the central nervous system (CNS), acting as the primary neurotransmitter responsible for neuronal inhibition. GABA activities are mediated through two distinct classes of receptors; ionotropic $GABA_A$ and $GABA_A$-ρ (formerly known as $GABA_C$, and prominently expressed in the retina (Naffaa et al. 2017)) and metabotropic $GABA_B$ receptors (Bowery et al. 2004). $GABA_A$ and $GABA_A$-ρ ionotropic receptor subunits form ion channels that are selectively permeable to anions like chloride and are responsible for the transient and rapid component of inhibitory postsynaptic potentials (Sigel and Steinmann 2012). Whereas, the metabotropic $GABA_B$ receptors belong to the superfamily of G-protein-coupled receptors (GPCRs) and mediate the slow and prolonged component of synaptic inhibition via indirect gating of neuronal K^+ and Ca^{2+} channels and lowering levels of other second messenger targets like cAMP (Bowery et al. 2002).

$GABA_B$ receptors are broadly expressed and distributed in the CNS. They are located pre- and postsynaptically; activation of presynaptic $GABA_B$ receptors by GABA located on GABAergic terminals (autoreceptors) inhibits the release of GABA, while activation of presynaptic $GABA_B$ receptors located on other nerve terminals (heteroreceptors) inhibits the release of several other neurotransmitters such as glutamate and bioactive peptides. In contrast, activation of postsynaptic receptors activate K^+ channels and induce slow inhibitory postsynaptic potentials (Benarroch 2012). $GABA_B$ receptors are also located in the periphery along the gastrointestinal (GI) tract where they regulate intestinal motility, gastric emptying, gastric acid secretion, and esophageal sphincter relaxation (Clarke et al. 2018; Lehmann et al. 2010; Ong and Kerr 1984). Dysregulated $GABA_B$ receptor function has been implicated in a variety of neurodegenerative diseases and

pathophysiological disorders, including Parkinson's disease (Nambu 2012; Tyagi et al. 2015), Alzheimer's disease (Rice et al. 2019; Sun et al. 2020), Huntington's disease (Kim and Seo 2014), epilepsy (Billinton et al. 2001a; Teichgräber et al. 2009), spasticity (Francisco et al. 2001; Basmajian 1975; Korsgaard 1976), pain (Neto et al. 2006; Enna and McCarson 2006; Murai et al. 2019), anxiety (Kalinichev et al. 2017; Li et al. 2015) and depression (Cryan and Kaupmann 2005; Felice et al. 2012; Jacobson et al. 2018), schizophrenia (Glausier and Lewis 2017; Nair et al. 2020), narcolepsy (Black et al. 2014; Szabadi 2015), and addiction (Agabio and Colombo 2014, 2015; Agabio et al. 2018; Maccioni and Colombo 2019; Ranson et al. 2020). Owing to their presence in the gastrointestinal tract these receptors are also implicated in a variety of GI disorders such as gastroesophageal reflux disease (GERD) (Clarke et al. 2018; Lehmann et al. 2010; Ong and Kerr 1984; Lehmann 2009; Symonds et al. 2003).

2 Brief History

While GABA was discovered in the mammalian brain in 1950 (Awapara 1950; Roberts and Frankel 1950), it was not recognized as an inhibitory neurotransmitter until 1967 (Krnjević and Schwartz 1966; Dreifuss et al. 1969). Early attempts to interrogate the GABA system led to the development of the synthetic agonist, β-(4-chlorophenyl)-γ-aminobutyric acid, a poorly brain penetrant derivative of GABA better known as baclofen (Keberle et al. 1969). In 1968 the identification of the first GABA receptor antagonist "bicuculline" was reported (Curtis et al. 1970), and in 1987, bicuculline and GABA receptor agonists such as isoguvacine facilitated the cloning of the ionotropic $GABA_A$ receptor, a pentameric ligand gated ion channel (Schofield et al. 1987).

The existence of the $GABA_B$ receptors (so named to distinguish it from the $GABA_A$ receptor) first emerged in 1979. Dr. Norman Bowery and colleagues discovered that GABA blocks the release of neurotransmitters such as norepinephrine from nerve terminals but this effect was not blocked by bicuculline, instead it mimicked the effects of baclofen. It was also discovered that baclofen does not interact with the $GABA_A$ site (Bowery et al. 1979, 1980, 1981). A third GABA receptor with pharmacology distinct from $GABA_A$ and $GABA_B$ was identified in 1986 by virtue of the GABA response, "Cl-current blocked by picrotoxin," being both bicuculline and baclofen insensitive (Johnston 1986). This receptor was named $GABA_C$ (now referred to as $GABA_A$-ρ) and was later cloned in 1991 (Polenzani et al. 1991). However, it was almost 20 years since being identified that the $GABA_B$ receptor was cloned using expression cloning and radioligand binding of a high affinity antagonist (1997) by the Bettler group (Kaupmann et al. 1997). Thus, reagents that modulate the GABA receptors facilitated the cloning of, and have since defined those receptors; the ionotropic receptors $GABA_A$ and $GABA_A$-ρ are defined as "bicuculline-sensitive, isoguvacine-sensitive" and

"bicuculline-insensitive, baclofen-insensitive" respectively, and the metabotropic $GABA_B$ receptor is defined as "bicuculline-insensitive, baclofen-sensitive."

3 Structure and Signaling

In common with other GPCRs, the $GABA_B$ receptor is an integral membrane protein that spans the cellular membrane with seven helices that are linked by three extracellular loops and three intracellular loops and possesses an extracellular N-terminus and an intracellular C-terminus. $GABA_B$ receptors are structurally related to metabotropic glutamate receptors (mGluRs), and together with the calcium-sensing receptor (CaSR), some pheromone and taste receptors, and orphan GPCRs (receptors with no known ligands), belong to the family C (or family III) of GPCRs (Bowery et al. 2002). Common to the members of family C GPCRs is the large extracellular N-terminus that contains a domain homologous to the periplasmic amino acid binding proteins (PBPs) found in bacteria. The X-ray structure of $GABA_B$ receptor PBP-like domains revealed an orthosteric ligand binding pocket that is made up of two globular lobes separated by a hinge region. The two lobes (LB1 and LB2) close upon ligand binding, much like a Venus flytrap does when touched by an insect, hence the globular domains in family C GPCRs are also referred to as "Venus flytrap" (VFT) domains (Galvez et al. 1999); an agonist binds and stabilizes the closed (active) conformation of the VFT, whereas an antagonist stabilizes and retains the VFT subunit in the open (inactive) configuration.

To date molecular cloning has identified two main $GABA_B$ receptor subunits, namely $GABA_{B1}$ and $GABA_{B2}$ which arise from distinct genes (Kaupmann et al. 1997, 1998). At the protein level $GABA_{B1}$ and $GABA_{B2}$ receptors share 35% identity and 54% similarity over their approximate length of 950 amino acid residues and both subunits are highly conserved across mammalian species, sharing 90–95% sequence homology between human, pig, rat, and mouse (Kaupmann et al. 1997). An active functional $GABA_B$ receptor with high affinity for agonist ligands depends upon the formation of a heterodimer between $GABA_{B1}$ and $GABA_{B2}$ receptor subunits (Kaupmann et al. 1998; Marshall et al. 1999; Jones et al. 1998). The association of the receptor subunits occurs, at least in part, through a coiled-coil motif found in the respective carboxyl termini of $GABA_{B1}$ and $GABA_{B2}$ subunits. It has been demonstrated in recombinant systems that $GABA_{B1}$ is unable to reach the cell surface in the absence of the $GABA_{B2}$ subunit because $GABA_{B1}$ contains endoplasmic retention motifs in its carboxy tail that are masked only upon heterodimerization with $GABA_{B2}$ subunit (Couve et al. 1998; Pagano et al. 2001). Interestingly, all orthosteric agonists and antagonists bind to the $GABA_{B1}$VFT and not to the $GABA_{B2}$ subunit VFT. Upon binding, an agonist induces conformational changes in the $GABA_{B1}$ subunit which by virtue of its physical interaction with the $GABA_{B2}$ subunit promotes conformational changes in the latter subunit allowing it to couple to its cognate G-protein promoting functional responses within the cell

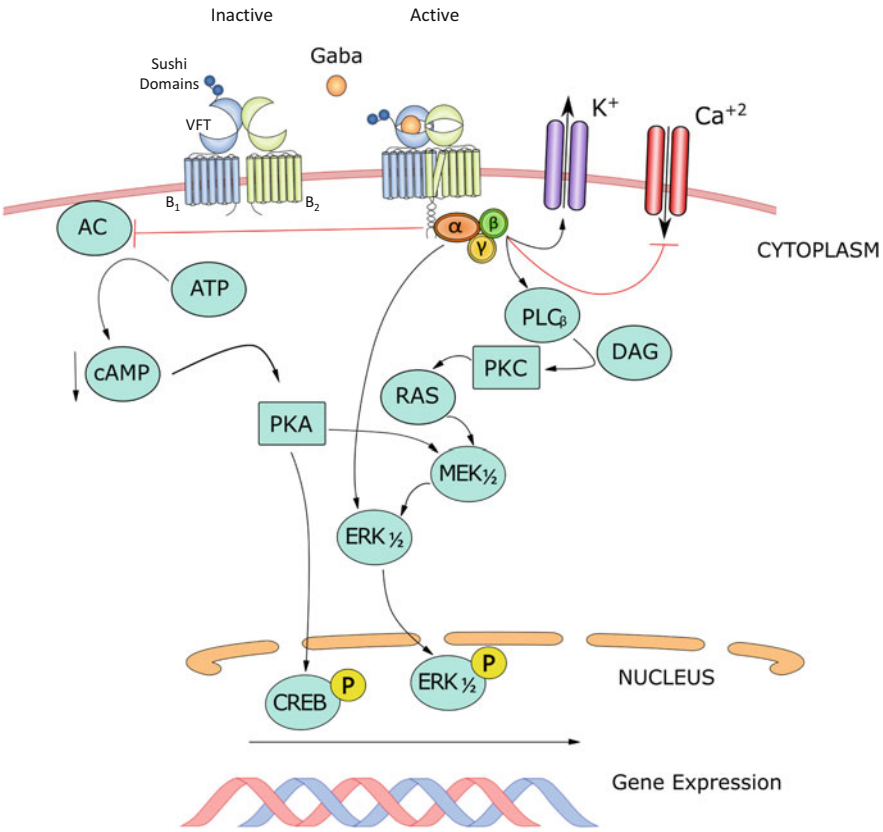


Fig. 1 Molecular diversity and signaling capacity of the GABA$_B$ receptor

(Galvez et al. 2001; Margeta-Mitrovic et al. 2001; Robbins et al. 2001; Duthey et al. 2002).

GABA$_B$ receptors provide a crucial component of inhibitory neurotransmission mainly via coupling to heterotrimeric $G_{i/o}$ type G-proteins, activation of which results in a Gα-mediated inhibition of cAMP production and a Gβγ-mediated modulation of the activity of ion channels such as high voltage-activated Ca^{2+} (Ca_v) channels and G protein-coupled inwardly rectifying Kir3-type potassium channels (GIRKs) (Morishita et al. 1990; Nishikawa et al. 1997). In rare cases and non-neuronal cells, GABA$_B$ receptor activation can promote increases in intracellular calcium either via activation of phospholipase C and store-operated channels or by inducing Ca^{2+} release from internal stores (Meier et al. 2008; New et al. 2006). Furthermore, GABA$_B$ receptor activation has been reported to induce phosphorylation of the Extracellular-signal Regulated protein Kinase 1/2 ($ERK_{1/2}$) in cerebellar neurons, as well as in the CA1 field of the mouse hippocampus (Tu et al. 2007; Vanhoose et al. 2002). Thus, GABA$_B$ receptor couples to multiple intracellular signal transduction pathways (Fig. 1) regulating ion homeostasis as well as MAPK

signaling leading to downstream effects that include blocked neurotransmitter release and hyperpolarization of neurons (Bowery et al. 2002; Bettler et al. 2004), and the modulation of autonomic control of the intestine and esophageal reflex (Clarke et al. 2018; Ong and Kerr 1984; Lehmann 2009; Symonds et al. 2003).

4 Molecular Diversity and Complexity

Molecular diversity in the $GABA_B$ receptor system arises from expression of multiple $GABA_{B1}$ subunit isoforms of which 14 mammalian isoforms ($GABA_{B(1a\text{-}1n)}$) exist between various animal species and are generated by differential transcription or splicing (Bettler et al. 2004), whereas the $GABA_{B2}$ receptor encodes a singular form of the receptor (Bettler et al. 2004; Billinton et al. 2001b). The two predominant $GABA_{B1}$ isoforms, termed $GABA_{B(1a)}$ and $GABA_{B(1b)}$, are generated by use of alternative transcription start sites, whereas other less abundant isoforms such as $GABA_{B(1c)}$, and $GABA_{B(1e)}$ are generated by alternative splicing. Only the $GABA_{B(1a)}$ and $GABA_{B(1b)}$ variants have been identified as components of the native receptor $GABA_{B1}$/$GABA_{B2}$ complex. Although the identification of these variants is suggestive of pharmacologically distinct $GABA_B$ receptors, Ng and colleagues reported that the anticonvulsant gabapentin acts as an agonist at $GABA_{B(1a)}$ but not $GABA_{B(1b)}$ (Bertrand et al. 2001; Ng et al. 2001), this has been widely disputed as heterodimers comprised of either $GABA_{B(1a)}$/$GABA_{B2}$ or $GABA_{B(1b)}$/$GABA_{B2}$ are pharmacologically indistinguishable in heterologous systems (Jensen et al. 2002; Lanneau et al. 2001) and to date, no $GABA_B$ receptor ligand differentiates between these molecular variants. However, studies facilitated by the generation of $GABA_{B1}$ isoform-specific knockout mice (Vigot et al. 2006) demonstrated that $GABA_{B1a}$- and $GABA_{B1b}$-containing receptors have distinct functions owing to their different locations within neurons, where $GABA_{B1a}$ receptors are predominantly located presynaptically on axonal terminals and $GABA_{B1b}$ postsynaptically on dendritic spines. Consequently, global $GABA_{B1}$ receptor isoform knockout mice exhibit a wide spectrum of isoform-specific behaviors. For example, using the isoform-specific knockout mice, Vigot et al. showed that $GABA_{B1a}$ and not $GABA_{B1b}$ receptor was involved in impaired synaptic plasticity in hippocampus long-term potentiation (Vigot et al. 2006). It was also shown by Perez-Garci and colleagues that $GABA_{B1b}$ was responsible for mediating postsynaptic inhibition of Ca^{2+} spikes, whereas presynaptic inhibition of GABA release was mediated by $GABA_{B1a}$ (Pérez-Garci et al. 2006). Hence, based on numerous in vivo findings, the existence of pharmacologically distinct $GABA_B$ receptors has been proposed (Pinard et al. 2010).

$GABA_{B(1a)}$ and $GABA_{B(1b)}$ differ primarily in their extracellular amino-terminal domains by a pair of sushi domains only present in the $GABA_{B(1a)}$ subunit of the $GABA_{B1(a)}$/$GABA_{B2}$ heteromer (Bettler et al. 2004; Hawrot et al. 1998). Sushi domains, or short consensus repeats, are conserved protein domains commonly involved in protein–protein interactions mostly found in proteins involved in cell–

cell adhesion. In the context of the $GABA_B$ receptor, the sushi domains have been shown to play a role in targeting the $GABA_{B(1a)}$ receptor to specific subcellular regions by means of interaction of these motifs with proteins in the extracellular matrix or on the surface of neighboring cells (Hannan et al. 2012). The diversity in $GABA_{B1}$ isoforms may therefore provide a means for targeted subcellular localization and/or coupling to distinct intracellular signaling pathways while also providing, in part, an explanation for the complex and diverse physiology effects of the GABA/$GABA_B$ receptor axis observed in neuronal tissue and in vivo (Bettler and Tiao 2006).

The molecular complexity of the $GABA_B$ receptor is further enhanced through association of the receptor with numerous trafficking, effector, and regulatory proteins, as well as other membrane-bound receptors. For example, the extracellular matrix protein, fibulin-2, has been shown to bind to the first sushi domain of the $GABA_{B(1a)}$ and target this receptor to axon terminals of excitatory synapses (Blein et al. 2004). Likewise, amyloid precursor protein (APP), amyloid precursor protein-like 2 (APLP2), and adherence junction associated protein-1 (AJAP1) interact with the sushi domains and are also anticipated to direct axonal subcellular localization of the $GABA_{B(1a)}$/$GABA_{B2}$ receptor complex (Dinamarca et al. 2019). Whereas $GABA_{B(1b)}$-containing heteromers more frequently show dendritic localization (Vigot et al. 2006).

Furthermore, a subfamily of the potassium channel tetramerization domain (KCTD) proteins (KCTD 8, 12, 12b, and 16) has been shown to exclusively and constitutively interact with the $GABA_{B2}$ carboxy-terminus acting as auxiliary subunits of the receptor to regulate the kinetics and outcome of G-protein signaling (Bartoi et al. 2010; Schwenk et al. 2010). For example, the KCTD12 and 12b subunits mediate desensitization of the receptor, whereas KCTD8 and 16 regulate non-desensitizing activities. The receptor, KCTD subunits, and G-protein combined form the core receptor signaling complex required for normal function of inhibitory brain circuits. Recently, Zuo et al., reported a high-resolution crystal structure of the KCTD16 oligomerization domain in complex with a $GABA_{B2}$ C-terminal peptide and together with mutational analysis defined the interface between KCTD16 and $GABA_{B2}$ revealing a potential regulatory site that modulates $GABA_B$ receptor activity (Zuo et al. 2019).

Other proteins have been reported to transiently associate with the $GABA_B$ receptor either directly through $GABA_{B1}$ or $GABA_{B2}$ carboxy terminal domains, which include transcription factors (i.e., ATF-4 (CREB2) and CHOP (Gadd153) (Nehring et al. 2000; Ritter et al. 2004; Sauter et al. 2005)) and scaffolding and adaptor proteins (i.e., MUPP1, 14-3-3 protein, and NSF (Balasubramanian et al. 2007; Couve et al. 2001; Pontier et al. 2006)) or indirectly through multiprotein complexes, which include neuroligin-3, synaptotagmin-11, and calnexin (Schwenk et al. 2016). Novel functions of the $GABA_B$ receptor also arise through crosstalk with other membrane receptors such as $GABA_A$, mGluR1, NMDA, IGF-1, and TrkB receptors. For a more comprehensive description of the $GABA_B$ receptor interactome, see (Benke 2013; Fritzius and Bettler 2020).

Recent biophysical and structural studies have demonstrated that $GABA_B$ receptors can form higher-order multimeric receptor complexes and this has been shown to occur in both heterologous systems and in brain membranes. These multimers comprise oligomers of $GABA_{B1}$ and $GABA_{B2}$ heteromers that self-assemble through association of their $GABA_{B1}$ subunits into tetramers (dimers of dimers) and octamers (dimers of tetramers) (Comps-Agrar et al. 2011, 2012; Maurel et al. 2008). Tetramers were found to decrease $G\alpha_i$-protein coupling efficiency suggesting that the multimers exhibit negative cooperativity between heterodimers (Calebiro et al. 2013; Stewart et al. 2018). It has emerged that the core $GABA_{B1/B2}$ receptor not only assembles with itself (oligomerization) but can also form supercomplexes with other multiprotein complexes that are likely spatiotemporally regulated in response to neuronal and developmental cues (Fritzius and Bettler 2020). The role of higher-order receptor complexes in $GABA_B$ receptor function and physiology requires further investigation to determine the functional relevance of $GABA_B$ receptor oligomerization in native tissue.

5 Agonists

As mentioned previously, the synthesis of the GABA analogue baclofen (-β-(4-chlorophenyl)-GABA; Fig. 2) in 1962 as the prototypical $GABA_B$ receptor agonist (Keberle et al. 1964) has greatly facilitated the molecular and biochemical characterization of this receptor. Indeed, baclofen has served as an invaluable tool in elucidating the electrophysiological and behavioral responses linked to the $GABA_B$ receptor system revealing its versatility as a drug target to treat a wide variety of diseases (Bowery 1993; Froestl et al. 1995a, b). Owing to its extensive therapeutic potential, numerous attempts to improve baclofen's pharmacokinetic properties and

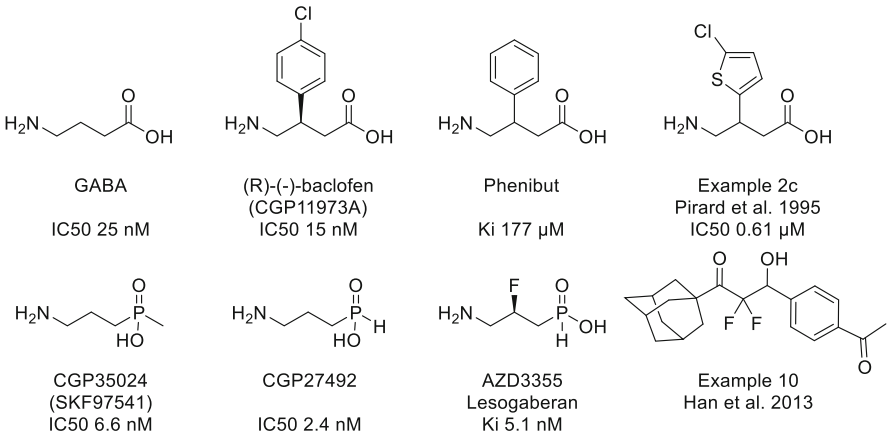


Fig. 2 Exemplar chemical structures of $GABA_B$ receptor full agonists

potency while maintaining selectivity have been pursued, but flat structure-activity relationships (SAR) around baclofen have resulted in very limited success. Of the 217 $GABA_B$ receptor-associated molecules reported in ChemBL Database (CHEMBL n.d.; Mendez et al. 2019), 55 compounds (42 agonists and 13 antagonists) are identified as being active at the $GABA_B$ receptor, most of which are chemically classified as analogues of either GABA or baclofen. However, the SAR investigations and the pharmacological properties of the resulting baclofen analogues have revealed important information regarding the chemical characteristics that endow baclofen with its activity at the $GABA_B$ receptor.

Following the resolution of baclofen in 1978 into the two enantiomers, (R)-(−)-baclofen and (S)-(+)-baclofen (Olpe et al. 1978; Weatherby et al. 1984) (CGP11973A and CGP11974A, respectively), in 1995, Froestl et al., demonstrated that the observed physiological effects of baclofen are stereoselective. They showed that the pharmacological action of baclofen is mediated by the R-(−)-enantiomer as R-(−)-baclofen (also known as Arbaclofen; Fig. 2) inhibits the binding of [^{3}H]-baclofen to $GABA_B$ receptors in cat cerebellum with an IC_{50} of 15 nM, while the S-(+)-enantiomer and racemic mixture display >100-fold and 3-fold higher IC_{50}, respectively (Froestl et al. 1995a). Many analogues of (R)-(−)-baclofen have been generated to interrogate the role of the carboxylic acid, amine, and p-chlorophenyl groups in attempts to increase potency and improve pharmacokinetic properties; as a consequence, more agonists, partial agonists, and antagonists have been discovered (Froestl 2010).

The first analogues that proved to be more potent than baclofen were generated by replacing the carboxylic acid portion of GABA with phosphinic acid residues to generate full agonists, CGP35024 (SKF97541) (Froestl et al. 1995a) and CGP27492 (Chapman et al. 1993) (Fig. 2), which have greater or equal affinity than baclofen for the $GABA_B$ receptor and IC_{50}s of 2 nM and 5 nM (Froestl et al. 1995a; Patel et al. 2001; Bon and Galvan 1996; Seabrook et al. 1990), respectively. Later SAR efforts investigated the replacement of the p-chlorophenyl group of baclofen with heterocycles. The absence of the chlorine atom from baclofen produces another potent $GABA_B$ receptor agonist, phenibut, and like baclofen, the majority of the agonist activity at the $GABA_B$ receptor is attributed to (R)-phenibut. Substitution with a 2-chlorothienyl group also provides an active albeit weaker agonist ($IC_{50} \sim 0.6$ μM) (Example 2c; Fig. 2) as determined in the [3H] baclofen displacement assay (Bolser et al. 1995). Further SAR and molecular modeling studies strongly implicated the p-chlorophenyl group (and its heteroaromatic substituents) as critical in the binding of baclofen and its analogues to the $GABA_B$ receptor (Costantino et al. 2001).

The phosphonous acid derivative, [(2R)-3-amino-2-fluoropropyl]phosphinic acid (AZD3355; Fig. 2) is a high affinity, non-brain penetrant analogue of baclofen that was developed by AstraZeneca and recently evaluated in clinical trials for the treatment of gastroesophageal reflux disease (GERD) under the generic name Lesogaberan® (Bredenoord 2009). AZD3355 has an EC_{50} of 9 nM compared to GABA's EC_{50} of 160 nM, and an increased binding affinity with a K_i of 5 nM versus GABA's 110 nM for inhibition of [^{3}H]-GABA binding in rat brain (Niazi et al. 2011).

It has been suggested that the low structural diversity of the existing orthosteric $GABA_B$ receptor ligands may be due to the conformational space having not been fully explored (Evenseth et al. 2019). In the early 2000s extensive mutagenesis studies on the extracellular domain of the $GABA_{B1}$ receptor subunit identified critical residues in LB1 and LB2 that are key for both agonist and antagonist binding (Galvez et al. 1999, 2000; Kniazeff et al. 2002). More recently, the first X-ray crystal structures of the $GABA_B$ receptor heterodimeric complex between the extracellular VFT domains of $GABA_{B1}$ and $GABA_{B2,}$ alone and in complex with bound agonists and antagonists were reported by Zuo et al., providing a more detailed understanding of how ligands act on the receptor (Geng et al. 2013). They demonstrated that in the inactive "apo" state and antagonist-bound state the VFT domains of both subunits adopt an open conformation whereas in the active "agonist-bound" state only the $GABA_{B1}$ subunit binds agonist and on doing so adopts a closed conformation.

Knowledge gained from these studies has since facilitated the development of a novel class of compounds that bind the orthosteric site of this receptor. In 2013, Colby et al. reported the discovery of $GABA_B$ receptor agonists comprised of β-hydroxy difluoromethyl ketones that represent the only structurally distinct $GABA_B$ receptor agonists as they lack the carboxylic acid or amino group of GABA (Example 10; Fig. 2) (Han et al. 2013). Additional analogues of the β-hydroxy difluoromethyl ketones have since been analyzed by the Colby laboratory, and docking models using the X-ray structures solved by Zuo et al. strongly suggest that these difluoromethyl ketones have similar binding modes to the orthosteric agonists (Sowaileh et al. 2018). Although some preliminary in vivo data suggest these compounds warrant further investigation as potential anxiolytic drugs (Han et al. 2013), their clinical utility has yet to be explored.

More recently, Mao and colleagues reported on Cryo-EM structures of the full-length inactive antagonist-bound and active agonist-bound in complex with $G\alpha_i$ protein of the $GABA_B$ receptor. This work further supports the findings that agonist binding stabilizes the closure of the $GABA_{B1}$ VFT domain (Geng et al. 2013). The Cryo-EM studies further revealed that agonist binding to $GABA_{B1}$ VFT domain induces rearrangement of the transmembrane (TM) interface between the $GABA_B$ subunits and this in turn promotes opening of the third intracellular loop in the $GABA_{B2}$ subunit allowing it to bind $G\alpha_i$ (Mao et al. 2020). Collectively, the structural studies of Zuo et al. and Mao et al. provide a deeper insight into $GABA_B$ receptor activation that will greatly assist in the design of novel modulators of the receptor.

6 Partial Agonists

Partial agonists are ligands that have varying degrees of intrinsic activities and affinity at their cognate receptors. They bind to and activate the receptor but elicit submaximal cell/tissue responses of the system relative to that produced by a full agonist. The naturally occurring GABA metabolite, γ-hydroxybutyric acid (GHB)

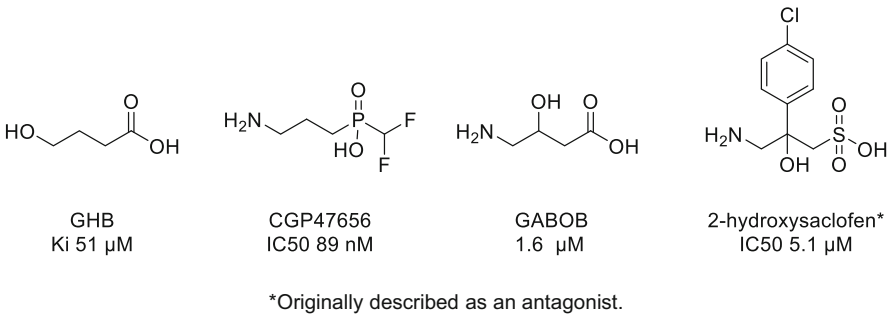


Fig. 3 Exemplar structures of $GABA_B$ receptor partial agonists

(Fig. 3), exhibits partial agonism at the $GABA_B$ receptor and is used clinically to treat symptoms of narcolepsy, alcohol dependence and withdrawal, and also used illicitly as a drug of abuse. However, experiments performed in $GABA_{B1}$ receptor null mice clearly show that not all the in vivo effects of GHB are $GABA_B$ receptor-mediated (Wellendorph et al. 2005). GHB has both low and high affinity receptor targets in the brain. The high affinity binding site is well characterized but has yet to be incontrovertibly identified. Whereas, it is well established that the $GABA_B$ receptor is the low affinity binding site where GHB acts as a partial agonist (Wong et al. 2004). Several studies demonstrated this finding including those of Mathivet et al. (1997); using binding experiments GHB was shown to have $K_i \sim 100$ μM compared to baclofen $K_i \sim 5$ μM (Mathivet et al. 1997); and Lingenhoehl et al. (1999); using recombinant systems expressing $GABA_{B1}/GABA_{B2}$ heteromer together with Kir3 channels in xenopus oocytes showed that GHB activated these receptors with an $EC_{50} \sim 5$ mM and a maximal stimulation of 69% relative to baclofen. Furthermore, three $GABA_B$ receptor competitive antagonists, CGP5426A, 2-hydroxysaclofen, and CGP35348 each completely blocked the GHB-evoked response further supporting GHB is a weak, partial agonist (Lingenhoehl et al. 1999).

Returning to the baclofen analogues, as mentioned CGP35024/SKF97541 (-γ-aminopropyl(methyl)phosphinic acid) is a potent agonist harboring a methyl substituent on the phosphinic acid moiety. Exchanging the methyl group for a difluoromethyl group produces CGP47656 (γ-aminopropyl(difluoromethyl)-phosphinic acid) (Fig. 3), rendering the molecule a partial agonist at the $GABA_B$ receptor as demonstrated by measuring binding affinities (Urwyler et al. 2005), the release of GABA from rat cortex (Froestl et al. 1995a; Gemignani et al. 1994), or the cholinergic twitch contraction in guinea pig ileum (Marcoli et al. 2000). Replacing the aromatic substituent at the 3-position of baclofen with a hydroxyl group also produces partial agonistic activity as seen in 4-amino-3-hydroxybutanoic acid (GABOB) (Fig. 3), with (R)-(−)-GABOB being tenfold less potent than racemic baclofen in binding experiments from rat brain isolates (Hinton et al. 2008).

As noted above, CGP35348 (Fig. 4) and 2-hydroxysaclofen (Fig. 3) (Kerr et al. 1988) have previously been described as $GABA_B$ receptor neutral competitive

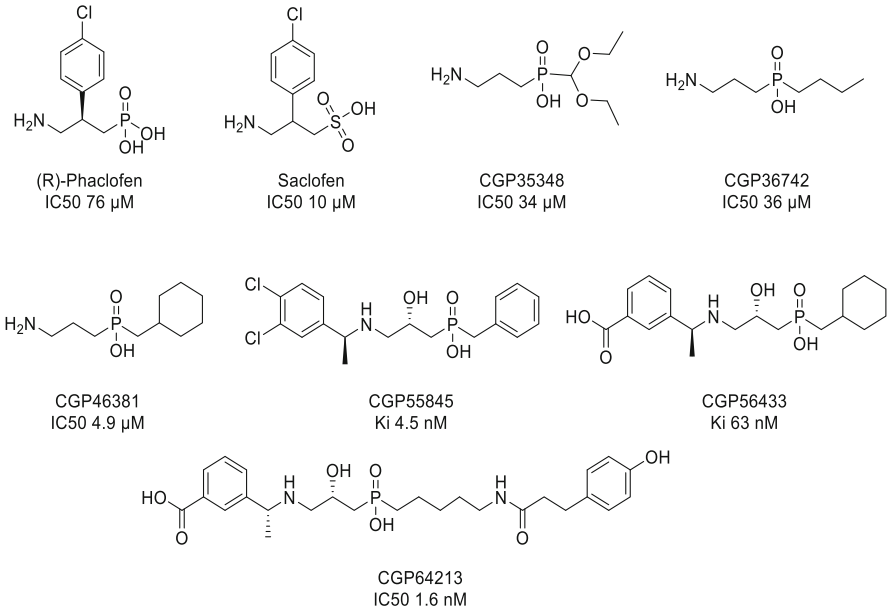


Fig. 4 Exemplar chemical structures of $GABA_B$ receptor antagonists

antagonists, having no intrinsic activity of their own and accordingly do not stimulate [^{35}S]-GTPγS-binding to membranes derived from CHO cells stably expressing the $GABA_B$ receptor. However, in the presence of CGP7930 or GS39783 (positive allosteric modulators, PAMs, of $GABA_B$ receptor) each "antagonist" stimulated [^{35}S]-GTPγS-binding to $GABA_B$ receptors with maximum efficiency of 31% and 35% of maximum GABA effect, respectively (Urwyler et al. 2005). A more sensitive assay measuring GABA/$GABA_B$ receptor-mediated inhibition of forskolin-stimulated cAMP accumulation revealed that CGP35348 and 2-hydroxysaclofen can have intrinsic partial agonistic activity in certain assay conditions that is enhanced by the PAMs. Thus, the PAMs revealed partial agonistic activity of compounds that otherwise appear to be devoid of intrinsic activity. Furthermore, the same experiments revealed that CGP7930 and GS39783 also possess intrinsic, low partial agonistic activity (Urwyler et al. 2005), an observation also reported by Binet et al. (Binet et al. 2004).

7 Antagonists

Following the 1979 discovery of a "bicuculline-insensitive, baclofen-sensitive" GABA receptor, efforts were immediately undertaken to design antagonists for this receptor. It was in the late 1980s that the first $GABA_B$ receptor antagonists were described. (R)-Phaclofen (Fig. 4), the phosphonic acid analogue of baclofen,

was one of the first discovered antagonists and was shown to block the slow inhibitory postsynaptic potential in the rat hippocampus establishing the physiological importance of this receptor (Dutar and Nicoll 1988). This discovery was closely followed by the discovery of saclofen and (S)-2-hydroxysaclofen (Fig. 3), sulphonic analogues of baclofen (Kerr et al. 1987). (R)-Phaclofen has a low affinity (~130 μM) for the receptor in radioligand binding experiments using rat brain membranes (Kerr et al. 1987), whereas (S)-2-hydroxysaclofen is tenfold more potent an antagonist at the $GABA_B$ receptor than (R)-phaclofen in this assay.

In addition to their significant contributions in interrogating $GABA_B$ receptor function and pharmacological activity, as with agonists, preclinical studies strongly support $GABA_B$ receptor antagonists having clinical importance in the treatment of various CNS disorders. $GABA_B$ receptor antagonists have been shown to suppress absence seizures in preclinical animal models of epilepsy (Bernasconi et al. 1992; Ostojić et al. 2013; Marescaux et al. 1992; Snead 3rd 1992), improve learning and memory (Bianchi and Panerai 1993; Lasarge et al. 2009; Mondadori et al. 1993) and have also been widely shown to have antidepressant-like activity in animal models (Cryan and Kaupmann 2005; Felice et al. 2012; Jacobson et al. 2018; Cryan and Slattery 2010; Frankowska et al. 2007; Mombereau et al. 2004; Nowak et al. 2006) along with a rescue of withdrawal from drugs of abuse-induced stress (Vlachou et al. 2011). Anhedonia, a common symptom of both psychostimulant withdrawal and depression, appears to be the key to the role of $GABA_B$ receptor in these disorders, as previously described by Markou and colleagues (Markou et al. 1992, 1998). Furthermore, the $GABA_B$ receptor has been shown to play a role in the regulation of glucose homeostasis in vivo (Bonaventura et al. 2012), $GABA_B$ receptor antagonism as well as receptor knockout mice shows improved glucose-stimulated insulin secretion (Bonaventura et al. 2008; Braun et al. 2004).

Following the discovery of phaclofen and 2-hydroxysaclofen, additional antagonists were discovered leading to CGP35348 (3-aminopropyl(diethoxymethyl)-phosphinic acid), a potent $GABA_B$ receptor antagonist and the first shown to penetrate the blood-brain barrier; CGP36742 (3-aminopropyl(n-butyl)phosphinic acid), the first orally bioavailable antagonist; and CGP46381, the phosphinic acid bearing a methylcyclohexyl group. However, like their predecessors, these compounds have low affinity (high μM range) for the $GABA_B$ receptor as does the chemically distinct SCH50911 (Bolser et al. 1995) (Fig. 5). As a result of SAR studies during the generation of these compounds, it was discovered that the nature of the alkyl substituent on the phosphinic acid plays a critical role in ligand activity. For example, a methyl substituent is present on the potent agonist CGP35024 (Fig. 2); when this is replaced with the difluoromethyl group of CGP47656 (3-aminopropyl(difluoromethyl)phosphinic acid), a decrease in activity at the $GABA_B$ receptor is observed with CGP47656 (Fig. 3) acting as a partial agonist (Froestl et al. 1995a; Urwyler et al. 2005; Gemignani et al. 1994; Marcoli et al. 2000). Increases in size of the substituent as with the butyl group in CGP36742 (Fig. 4) result in a derivative that displays antagonist activity at the $GABA_B$ receptor. Hence, very modest structural modifications to the baclofen core can lead to

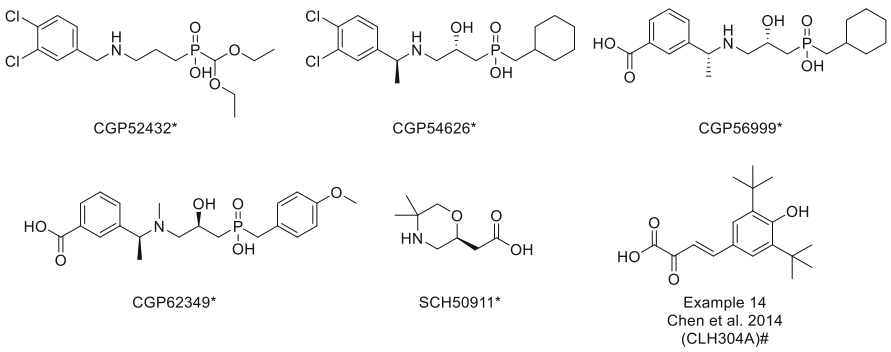


Fig. 5 Exemplar chemical structures of $GABA_B$ receptor inverse agonists

significant changes in ligand activity ranging from potent agonism to partial agonism to antagonism at the $GABA_B$ receptor (Pirard et al. 1995).

More potent antagonists have been developed since, displaying IC_{50} values in the nanomolar range. The radical shift in potency was achieved by substituting the amino group of existing $GABA_B$ modulators with benzyl substituents (3,4-dichlorobenzyl or 3-carboxybenzyl) as in CGP55845 and CGP56433, respectively. Other representatives of this generation of antagonists include CGP54626 (Fig. 4), and CGP62349, CGP52432, CGP56999, CGP54626, CGP64213 (Fig. 5); all highly potent antagonists and all demonstrating learning and memory-improving effects (Lasarge et al. 2009; Getova and Dimitrova 2007). These antagonists may also have significant clinical potential in absence epilepsy (Bernasconi et al. 1992; Marescaux et al. 1992; Snead 3rd 1992) as mice overexpressing the $GABA_{B1a}$ isoform exhibit characteristics associated with atypical absence epilepsy (Stewart et al. 2009).

8 Inverse Agonists

Given that GPCRs are believed to exist in equilibrium between inactive and active conformational states in which there is a continuum of structural conformations ranging from having no activity to being maximally active, these receptors have the potential to be active in the absence of an activating ligand, a phenomenon termed "constitutive activity." Ligands that stabilize the fully "inactive" conformation, thereby eliminating any intrinsic/constitutive activity the receptor may have, are referred to as "inverse agonists" (Berg and Clarke 2018; Kenakin 2004). Many GPCR-targeted drugs were initially characterized as "neutral" or "silent" antagonists as their discovery predated inverse agonism as a pharmacological concept. It is now estimated that at least 15% of compounds classified as antagonists have some

intrinsic activity and that these drugs confer their therapeutic efficacy by reducing constitutive receptor activity (Urwyler et al. 2005; Grunewald et al. 2002; Hirst et al. 2003; Mukherjee et al. 2006).

In the context of the $GABA_B$ receptor, constitutive receptor activity has been demonstrated to modulate neurotransmitter release and neuronal excitability in the absence of GABA. For example, in cerebellar Purkinje cells, $GABA_B$ receptor has been shown to interact with extracellular calcium ions to increase the sensitivity of the glutamate receptor 1 (mGluR1) to its endogenous ligand, glutamate, by forming a complex with the mGlu1R (Tabata et al. 2004). The use of a selective $GABA_B$ receptor inverse agonist could serve to eliminate enhanced glutamate mediated mGluR1 activity which has been identified as an avenue with therapeutic potential for the treatment of fragile X syndrome (Niswender and Conn 2010).

As noted, compounds CGP52432, CGP54626, CGP56999, CGP62349 (Fig. 5) are closely related, sharing the same core structure, and were originally identified as competitive antagonists at the $GABA_B$ receptor. As antagonists, these compounds have the ability to block GABA/$GABA_B$ receptor-mediated inhibition of forskolin-stimulated cAMP in $GABA_B$ receptor expressing recombinant systems. However, following receptor desensitization resulting from sustained exposure to GABA, the activity of this family of compounds switches from antagonism to inverse agonism as demonstrated by the CGP54626-promoted increase in cAMP production. The atypical SCH50911 antagonist that lacks large hydrophobic substituents behaved in a similar manner (Gjoni and Urwyler 2009). Likewise, the structurally distinct CLH304a previously reported as a negative allosteric modulator (NAM; Fig. 5) (Chen et al. 2014) has also since been reported to exhibit inverse agonist properties in the absence of an agonist (Sun et al. 2016).

9 Allosteric Modulators

While endogenous neurotransmitter GABA agonists (i.e., baclofen) and antagonists (i.e., phaclofen) bind to the orthosteric site (VFT domain) in the $GABA_{B1}$ subunit, it is now widely accepted that the $GABA_B$ receptor modulators identified so far act at allosteric sites (binding sites topographically distinct from the orthosteric ligand binding site) and bind the transmembrane region of the $GABA_{B2}$ subunit. Allosteric modulators (AMs) are basically classified as either positive allosteric modulators (PAMs) or negative allosteric modulators (NAMs). PAMs that possess intrinsic agonist activity are referred to as "ago-PAMs." A third class of allosteric ligand has been described that binds to the receptor but has no intrinsic activity and no apparent effect on endogenous ligand activity, hence it is referred to as a "silent allosteric modulator" (SAM) (Burford et al. 2013). Importantly, since orthosteric and allosteric ligands bind to topographically distinct sites of the receptor, both ligands can interact with the receptor simultaneously and thus, each ligand can affect the binding (binding cooperativity) and the intrinsic activity (activation cooperativity) of

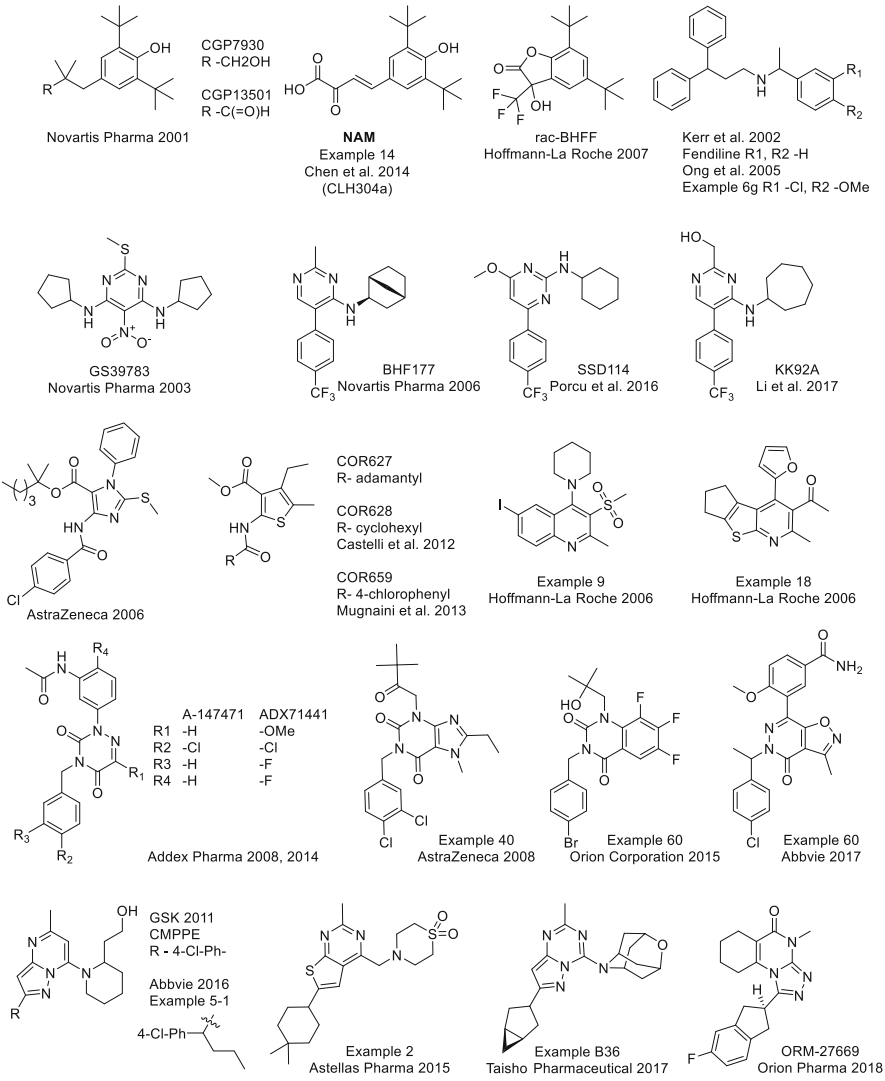
the other. Theoretical models describing these interactions have been discussed extensively elsewhere (Keov et al. 2011; May et al. 2004).

Allosteric sites are attractive therapeutic targets because molecules that bind to these sites can act in concert with an orthosteric ligand, and in doing so are believed to offer several advantages over the use of orthosteric ligands alone (Kenakin and Miller 2010). As allosteric modulators typically rely on the presence of the endogenous ligand, they have the ability to modify receptor activity in a spatial and temporal manner by acting in concert with the endogenous receptor ligand (Kenakin and Miller 2010). Therefore, allosteric modulators are believed to have the potential to induce fewer side effects, as they simply modulate endogenous ligand-mediated receptor activation. In addition, upon prolonged exposure, allosteric modulators are less likely to induce GPCR desensitization compared to an orthosteric agonist, and as such, are less likely to induce drug tolerance.

Allosteric modulators of the $GABA_B$ receptor have generated significant attention for their therapeutic potential in the treatment of alcohol and drug addiction, anxiety, depression, muscle spasticity, epilepsy, pain, and gastrointestinal disorders (Urwyler 2011). It is postulated that the use of a PAM (or ago-PAM) will achieve a more desirable pharmacological signaling profiling and physiological responses by enhancing GABA-mediated receptor signaling rather than artificially stimulating the receptor with an exogenous agonist such as baclofen. Furthermore, $GABA_B$ receptor allosteric modulators hold the promise of more favorable pharmacokinetics compared to baclofen including improved bioavalability and brain exposure as well as cytotoxicity. Hence, the potential advantages of $GABA_B$ receptor allosteric modulation have led to the development of numerous small molecule allosteric modulators, the majority of which are PAMs.

While many of the described $GABA_B$ receptor PAMs are structurally distinct, based on the core structure they can be sorted into several groups (Fig. 6; each row representing a distinct structural class). The discovery of $GABA_B$ receptor PAMs was pioneered and first reported by Novartis scientists, Urwyler and colleagues, in 2001. These researchers demonstrated that small molecule CGP7930 (2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol; discovered in a high throughput screening campaign) (Urwyler et al. 2001) potentiated GABA-stimulated [^{35}S]-GTPγS-accumulation in membrane preparations derived from CHO cells stably expressing the $GABA_B$ receptor. Using various combinations of wildtype and mutant $GABA_B$ subunits, Binet and colleagues investigated the mode of action of CGP7930, determining that the heptahelical domain (HD) of $GABA_{B2}$ was an absolute requirement for CGP7930 PAM action and that CGP7930 could also activate a truncated $GABA_{B2}$ subunit corresponding to the HD only (Binet et al. 2004).

In 2003, Novartis reported on another group of structurally distinct $GABA_B$ receptor PAMs, centered around GS39783 (*N,N′*-dicyclopentyl1–2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine). Like CGP7930, GS39783 was found to potentiate both affinity and maximal effects of GABA in biochemical and electrophysiological assay systems (Urwyler et al. 2003). Dupuis et al. studied point mutations in the TM region of $GABA_{B2}$ to identify the residues within the HD that



***NAM** CLH304a; an example of how subtle structural changes can change ligand activity

Fig. 6 Exemplar chemical structures of $GABA_B$ receptor allosteric modulators

interact with GS39783 and found that mutations G706T and A708P in TM6 were necessary and sufficient for GS39783 mediated agonist activation (Dupuis et al. 2006). Hence, both CGP7930 and GS39783 were found to bind to sites distinct from known agonist and antagonist receptor binding sites, and to require the presence of the $GABA_{B2}$ receptor subunit.

These findings prompted the pursuit of other molecules with similar PAM activities and consequently, numerous $GABA_B$ receptor allosteric modulators have been reported in the scientific and patent literature (accessible in SciFinder and Espacenet) over the past two decades. Roche scientists further developed the Novartis compounds by generating systematic modifications of CGP7930 structure and arrived at the bicyclic structure of rac-BHFF (Malherbe et al. 2008). Interestingly, it was found that both CGP7930 and rac-BHFF have intrinsic agonist activity, and distinct and differentiating ligand-induced signaling profiles compared to baclofen (Koek et al. 2013). Optimization of the genotoxic lead structure of the pyrimidine derivative of GS39783 led to the development of non-toxic $GABA_B$ receptor PAMs, such as BHF177 (N-[(1R,2R,4S)-bicyclo[2.2.1]heptan-2-yl]-2-methyl-5-[4-(trifluoromethyl)phenyl]-4-pyrimidinamine) reported by Novartis in 2006 (Floersheim et al. 2006). A decade later additional analogs from this series were reported by Porcu et al. (SSD114) (Porcu et al. 2016) and by our research group and collaborators Li et al. (KK-92A and approximately 100 additional analogs) (Li et al. 2017).

Substituted 5-membered heterocycles represent a substantial group of $GABA_B$ receptor PAMs, with the first examples of structurally novel modulators reported by AstraZeneca in patents aiming at the development of drugs for the treatment of gastrointestinal diseases (Bauer et al. 2005). Specific examples presented in the patents initially focused on imidazole derivatives that expanded in scope by scaffold hopping to cover other five-membered core heterocycles such as pyrazoles, oxazoles, and thiazoles. In 2011, a group led by Corelli identified COR627, COR628 (Castelli et al. 2012), and COR659 (Mugnaini et al. 2013) as $GABA_B$ receptor PAMs that displayed significant activity in vitro as $GABA_B$ receptor PAMs by potentiating [^{35}S]-GTPγS-binding induced by GABA while failing to exhibit intrinsic agonist activity. While the thiophene-based core of the active molecule differs from those reported by AstraZeneca, the substitution pattern resembles other representative molecules in this group.

Extensive work of Hoffman-La Roche resulted in the identification of additional classes of $GABA_B$ receptor PAMs disclosed in a series of patents published in 2006. The reported active molecules are based on a quinoline (Malherbe et al. 2006) or thieno[2,3-b]pyridine (Malherbe et al. 2007) as core heterocycles. A closely related set of $GABA_B$ receptor PAMs was reported in 2009 in an AstraZeneca patent (Cheng and Karle 2008). A separate group of $GABA_B$ receptor modulators represent a series of substituted triazinediones developed by Addex Pharma (Riguet et al. 2007). The Addex lead compound, ADX71441, is an orally available small molecule that demonstrated excellent preclinical efficacy and tolerability in several rodent models of pain, addiction, and overactive bladder (OAB) and has also proven efficacy in a genetic model of Charcot-Marie-Tooth Type 1A disease (CMT1A) (Cao and Zhang 2020).

In patents from 2008 and 2009 AstraZeneca scientists disclosed a new group of $GABA_B$ receptor PAMs based on bicyclic pyrimidinedione core, namely xanthines (Cheng et al. 2008a) and pteridine-2,4(1H,3H)-diones (Cheng et al. 2008b). Related structures were disclosed in 2015 by Orion Corporation (Prusis et al. 2015) and

Abbvie in 2017. In 2011, GlaxoSmithKline reported CMPPE, a novel moiety that positively modulated GABA-evoked in vitro [^{35}S]-GTPγS-binding signal with an EC_{50} value of 2.57 μM. The compound showed mild efficacy in a food consumption test in rats, modest in vivo potentiation of baclofen-induced muscle relaxation in mice, and poor metabolic stability in liver microsomal systems (Perdona et al. 2011). Other companies followed the CMPPE track with a range of modulators containing a modified core structure as the substitution pattern. Astellas Pharma reported a series of thieno[2,3-d]pyrimidines in 2015 (Shiraishi et al. 2014), whereas Abbvie (Faghih et al. 2016) and Richter Gedeon maintained pyrazolo[1,5-a]pyrimidinyl core in their series (WO 2018167630). In a single patent Taisho Pharmaceutical (Borza et al. 2018) covered analogs with the core heterocycle replaced by pyrazolo[1,5-a][1,3,5] triazine in addition to substituted pyrazolo[1,5-a]pyrimidines. ORM-27669, reported by Orion Pharma in 2017, with its tricyclic core structure containing [1,2,4]triazolo [4,3-a]pyrimidin-7(8H)-one represents a more original scaffold (de Miguel et al. 2019). Pretreatment with ORM-27669 reversed ethanol-induced neuroplasticity and attenuated ethanol drinking but had no effects on cocaine-induced neuroplasticity or self-administration.

Fendiline (Fig. 6) and its related arylalkylamines represent another unique structural class reported to be potential $GABA_B$ receptor PAMs. First reported as a non-selective calcium channel blocker and as a positive allosteric modulator of extracellular Ca^{2+} sensing receptors (CaSRs) (Nemeth et al. 1998), Fendiline is an FDA-approved (albeit obsolete) drug used in the treatment of coronary heart disease. Although not $GABA_B$ receptor specific, this compound is noteworthy as Ong and Kerr evaluated activity of Fendiline and its analogues as PAMs of $GABA_B$ receptors (Kerr et al. 2002, 2006) and demonstrated that the most potent analogue, (+)-N-1-(3-chloro-4-methoxyphenyl)ethyl-3,3-diphenylpropylamine) exhibited an EC_{50} of 30 nM in modulating baclofen-mediated function using grease gap recording in rat neocortical slices (Ong et al. 2005). However, direct action of Fendiline on $GABA_B$ receptor activity has been disputed (Urwyler et al. 2004) and further investigations are needed to determine the mechanism by which arylalkylamines enhance $GABA_B$ receptor-mediated responses.

$GABA_B$ receptor negative allosteric modulators (NAMs) have also been proposed as potential lead compounds for development into therapeutics for disorders such as CNS hyperexcitability-related disorders including epilepsy, anxiety, nerve damage, and low cognitive ability. Interestingly, modifications of $GABA_B$ receptor PAM CGP7930 (Fig. 6) led to discovery of the first $GABA_B$ receptor NAM, CLH304a, reported by Chen and colleagues in 2014 (Chen et al. 2014; Sun et al. 2016). CLH304a decreased agonist GABA-induced maximal effect of IP_3 production in HEK293 cells overexpressing $GABA_B$ receptor and Gαqi9 proteins without changing the EC_{50}. Moreover, it inhibited baclofen-induced $ERK_{1/2}$ phosphorylation and also blocked CGP7930-induced $ERK_{1/2}$ phosphorylation in HEK293 cells overexpressing $GABA_B$ receptor. This indicated that CLH304a (and some analogues) may be allosteric modulators, as orthosteric antagonists like CPG54626 are unable to attenuate PAM mediated signaling. Indeed, it was demonstrated that

the compounds of interest bound to an allosteric site, negatively regulating orthosteric agonist mediated signaling (Chen et al. 2014).

10 Probe Dependency

An important aspect of allosteric modulation to be taken into consideration is that the extent and direction (positive or negative) of the interaction between the orthosteric and allosteric ligands depends on which orthosteric ligand is present; a phenomenon known as "probe-dependency," this is important as many individual GPCRs respond to multiple endogenous ligands (May et al. 2004; Kenakin 2005). For the $GABA_B$ receptor, only having one known endogenous ligand, probe-dependency might be considered irrelevant. However, the potential combination of an allosteric modulator with a synthetic therapeutic such as baclofen must also consider the possibility of probe-dependent effects on receptor signaling and function.

Indeed, it has been demonstrated that baclofen shows improved efficacy and an increased therapeutic window when administered in combination with $GABA_B$ receptor PAMs (Maccioni et al. 2012). In preclinical studies, treatment with $GABA_B$ receptor PAMs GS39783 and rac-BHFF potentiated the activity of low doses of baclofen in relation to alcohol seeking behaviors (Maccioni et al. 2015). Hence, the ability of PAMs to reduce the effective dose of baclofen not only has the potential to improve efficacy in disease relevant measures, but also to expand the therapeutic window of this drug by reducing the accompanying adverse side effects. Thus, leveraging the probe-dependent effects of treatment with multiple receptor ligands has the potential to "fine-tune" receptor signaling and facilitate the development of improved strategies to target the $GABA_B$ receptor.

11 Biased Agonism/Functional Selectivity

It is well established that any given ligand for a GPCR does not simply possess a single defined efficacy; rather, a ligand possesses multiple efficacies, depending on the specific downstream signal transduction pathway being investigated. This diversity is believed to be the result of conformational changes induced in the GPCR that are ligand-specific and hence receptors can adopt various conformations that preferentially activate/modulate one signaling pathway to the exclusion of others; a phenomenon referred to as "functional selectivity" or "ligand bias" (Kenakin 2017; Smith et al. 2018; Spangler and Bruchas 2017). Conceptually, as with allosteric modulation, functional selectivity is an appealing mechanism of therapeutic intervention, as modulating only a select subset receptor signaling pathway may allow for the development of drugs that demonstrate therapeutic efficacy without recruiting pathways that lead to downstream adverse side effects (Kenakin and Miller 2010).

Functional selectivity can be achieved by modulating the receptor with a single ligand or with multiple ligands. Our own studies identified a PAM, namely KK-92A (4-(cycloheptylamino)-5-(4-(trifluoromethyl) phenyl)pyrimidin-2-yl) methanol) that exhibits pathway-selective differential modulation of $GABA_B$ receptor signaling when compared to the structurally related allosteric modulator BHF177 (Sturchler et al. 2017). Using recombinant cell-based systems overexpressing the $GABA_B$ receptor, KK-92A exhibited similar activity to BHF177 in potentiating GABA-induced $GABA_B$ receptor-mediated inhibition of forskolin-stimulated cAMP production and GABA-induced increase in intracellular Ca^{2+} levels. However, in contrast to BHF177, in the absence of GABA, KK-92A exhibited intrinsic activity with regard to $ERK_{1/2}$ phosphorylation achieving ~70% maximum efficacy relative to GABA maximum efficacy (Li et al. 2017), demonstrating ago-PAM activity and pathway-selective effects.

12 $GABA_B$ Receptor-Targeted Pharmaceuticals

The presence of functional $GABA_B$ receptors in mammalian brain and the gastrointestinal tract has been known for more than 30 years. Given the widespread distribution of GABA and the $GABA_B$ receptor in the CNS and periphery, it is not surprising that activation of the $GABA_B$ receptor provokes a host of physiological responses, and as a consequence, dysregulation of $GABA_B$ receptor activity was proposed to be associated with various CNS diseases such as mood disorders (Kalinichev et al. 2017; Li et al. 2015; Felice et al. 2012; Jacobson et al. 2018; Cryan and Slattery 2010), epilepsy (Billinton et al. 2001a; Teichgräber et al. 2009), addiction (Agabio and Colombo 2014, 2015; Agabio et al. 2018; Ranson et al. 2020; Maccioni et al. 2015), Parkinson's disease (Nambu 2012; Tyagi et al. 2015), Alzheimer's disease (Rice et al. 2019; Sun et al. 2020), Huntington's disease (Kim and Seo 2014) as well as peripheral diseases such as gastroesophageal reflux disease (GERD) (Clarke et al. 2018; Lehmann et al. 2010). More recently, GABA has emerged as a tumor signaling molecule in the periphery that controls tumor cell proliferation (Young and Bordey 2009; Zhang et al. 2014; Jiang et al. 2012), and stimulation of $GABA_B$ receptor signaling has been proposed as a novel target for the treatment and prevention of pancreatic cancer (Schuller et al. 2008; Schuller 2018; Al-Wadei et al. 2012). Numerous studies have shown potential clinical benefit of targeting the $GABA_B$ receptor in the treatment of various CNS and peripheral disorders, yet there is still only one therapeutic agent used clinically that selectively activates the $GABA_B$ receptor, namely baclofen (Lioresal®).

As discussed, baclofen was originally synthesized in 1962 by chemists at Ciba, Switzerland in an attempt to generate a more lipophilic, brain penetrant GABA mimetic (Keberle et al. 1964). It was assessed in the treatment of epilepsy but failed to show sufficient efficacy in the clinic. However, as a consequence of an incidental finding in that it had positive effects on muscle spasticity (Hudgson and Weightman 1971), baclofen (Lioresal®) has been in clinical use since 1972, gaining FDA

approval in 1977; long before its molecular target, the $GABA_B$ receptor was discovered and its mechanism of action identified. As previously mentioned, it has also shown therapeutic utility in a wide range of other off-label indications including addiction and was recently approved in Europe and Australia for the treatment of alcoholism (Agabio et al. 2018) but side effects such as sedation, nausea, muscle weakness, and rapid onset of tolerance limit its use (Kent et al. 2020).

With baclofen, an improvement over GABA regarding blood-brain barrier permeability was achieved, however, baclofen still has low brain penetration attributed to rapid efflux via the organic anion transporter (OAT3) (Ohtsuki et al. 2002). In parallel to Ciba's efforts in the 1960s to synthesize a GABA mimetic, a Russian team (Perekalin et al) synthesized a phenyl derivative of GABA, namely phenibut (-β-phenyl-γ-aminobutyric acid) that exhibits improved brain penetration over baclofen. Phenibut (Cirocard®) has been in clinical use in Russia and some Eastern European countries (not FDA-approved in USA) as a tranquilizer and cognition enhancer (nootropic) since the 1960s and is still used for these indications as well as for the treatment of mood and sleep disorders, PTSD, and a variety of neuropsychiatric diseases (Lapin 2001). However, phenibut suffers from many of the same liabilities as baclofen; sedation, muscle weakness, nausea, tolerance, and more recently has gained attention for its abuse potential (Jouney 2019).

Although baclofen has been in clinical use since 1972, it is far from an "ideal" drug; in addition to the unwanted side effects mentioned above, it also suffers from poor pharmacokinetic properties, including low brain penetration, limited absorption, short duration of action, rapid clearance from the blood, and narrow therapeutic window (Kent et al. 2020). Despite the lack of good "drug-like" qualities, the clinical success of baclofen has prompted numerous campaigns towards the identification and development of new and improved compounds that modulate the $GABA_B$ receptor and significant advances have been made. In 2009, XenoPort (now Arbor Pharmaceuticals) introduced Arbaclofen Placaril (XP19986), a transported prodrug of (R)-(−)-baclofen designed to possess a more favorable pharmacokinetic profile. Arbaclofen is absorbed throughout the intestinal tract and is rapidly converted to (R)-(−)-baclofen in tissues. It has been evaluated in Phase III clinical trials for GERD and multiple sclerosis, but these trials were discontinued in 2011 and 2013, respectively, due to lack of efficacy. It also reached Phase III trials in fragile X syndrome (FXS) but did not meet the primary outcome of improved social avoidance in FXS (Berry-Kravis et al. 2017). However, an extended release formula of Arbaclofen (Arbaclofen-ER; Ontinua®) developed by Osmotica is under FDA review as of July 2020 for the treatment of spasticity in multiple sclerosis. Also, two independent clinical trials evaluating benefit of Arbaclofen in children and adults with autism spectrum disorder (ASD) were initiated in 2019 (NCT03682978 and NCT03887676, respectively).

While XenoPort reported on Arbaclofen Placaril, AstraZeneca reported AZD3355 (Lesogaberan®; Fig. 2), a high affinity analogue of baclofen that was developed and evaluated in clinical trials for the treatment of GERD (Bredenoord 2009). AZD3355 is restricted peripherally and has a half-life of ~11 h in blood (Niazi et al. 2011). Unfortunately, Phase IIb clinical trials were terminated owing to

lack of efficacy in GERD patients. As AZD3355 is not brain penetrant and devoid of unwanted CNS effects, with no other adverse effects reported, indicating that it is safe in humans, it has been proposed that Lesogaberan® could be repurposed for the treatment of type 1 diabetes; targeting the $GABA_B$ receptor in β cells to promote β-cell survival (Tian et al. 2017).

GHB (γ-hydroxybutyric acid) is approved in some countries and used clinically for the treatment of narcolepsy-related catalepsy (Xyrem®) (Szabadi 2015) and rarely alcoholism (Alcover®) (Keating 2014). GHB also has the potential for abuse and is used illicitly as a recreational drug and intoxicant (Busardò and Jones 2015). Although GHB itself is not FDA-approved for medical use, the first generic version of Xyrem®, sodium oxybate (the sodium salt of GHB), recently (2017) received FDA approval to treat symptoms of narcolepsy including excessive daytime sleepiness and narcolepsy with cataplexy.

The first (and only to the best of our knowledge) clinical investigation of $GABA_B$ receptor antagonists was an open trial with SGS742 (CGP36742; Fig. 4) (Bullock 2005) (Froestl et al. 2004). Even though its potency is low ($IC_{50} \approx 40$ μM (Froestl et al. 1995b)), many preclinical studies showed benefit with SGS742 for spatial memory improvement (Helm et al. 2005), the treatment of depression (Nowak et al. 2006), and arrest of cortical seizures (Mares and Kubova 2008). The initial Phase II clinical trial, conducted in mild cognitive impairment patients, showed that SGS742 significantly improved attention, in particular choice reaction time and visual information processing as well as working memory (Froestl et al. 2004). However, a second Phase II trial was undertaken in mild to moderate Alzheimer's disease patients and no statistically significant improvement was detected prompting the termination of the development program. The clinical implications of modulating the $GABA_B$ receptor are outlined in Table 1.

13 Concluding Remarks

The $GABA_B$ receptor and its physiological roles are extremely complex, consequently, dysregulation of this receptor is involved in a broad range of diseases, and as such the $GABA_B$ receptor is considered a highly attractive therapeutic target for the development of new anti-epileptic, antidepressant, analgesic, and anxiolytic drugs, as well as for the treatment of cognitive disorders, drug addiction, and depression. However, at present only one compound that targets the orthosteric site of $GABA_B$ receptor is in clinical use, namely baclofen (Lioresal™); used to treat muscle spasticity in multiple sclerosis, and more recently used off-label for alcohol addiction. Unfortunately, side effects such as sedation, muscle weakness, nausea, and the lack of efficacy observed in other indications, i.e., fragile X syndrome, limit its therapeutic use. In addition to unwanted side effects baclofen also suffers from low brain penetration, limited absorption, rapid tolerance, short duration of action, and narrow therapeutic window. As described earlier, numerous small molecule agonists, antagonists, and allosteric modulators of the $GABA_B$

Table 1 Current therapeutic use and potential clinical utility of $GABA_B$ receptor modulators

Pharmacology	[a]Therapeutic use/[b]clinical potential	Approved drug/[c]clinical trial	References
Agonists/positive allosteric modulators	[a]Muscle rigidity and spasticity	Baclofen (Lioresal®) Arbaclofen-ER (Ontinua®)	(Francisco et al. 2001; Basmajian 1975; Korsgaard 1976; Coffey et al. 1993)
	[a]GERD	Baclofen (Lioresal®)	(Clarke et al. 2018; Lehmann et al. 2010; Ong and Kerr 1984; Lehmann 2009; Symonds et al. 2003)
	[a]Charcot-Marie tooth type 1A		(Cao and Zhang 2020; Dyer 2013)
	[a]PTSD/tranquilizer/nootropic	Phenibut (Cirocard®) (Eastern Europe only)	(Lapin 2001; Drake et al. 2003)
	[a]Cough suppression		(Chung 2015; Martvon et al. 2020)
	[a]Alcoholism and addiction	Sodium oxybate/GHB/(Alcover®), Baclofen (Lioresal®)	(Agabio and Colombo 2014, 2015; Agabio et al. 2018; Maccioni and Colombo 2019; Ranson et al. 2020)
	[b]Anxiety		(Kalinichev et al. 2017; Li et al. 2015)
	[b]Epilepsy		(Billinton et al. 2001a; Teichgräber et al. 2009)
	[b]Cataplexy	Sodium oxybate/GHB (Xyrem®)	(Black et al. 2014; Szabadi 2015)
	[b]Binge eating disorder		(Broft et al. 2007; Tsunekawa et al. 2019)
	[b]Parkinson's disease		(Nambu 2012; Tyagi et al. 2015)
	[b]Schizophrenia		(Glausier and Lewis 2017; Nair et al. 2020)
	[b]Huntington's disease		(Kim and Seo 2014; Kleppner and Tobin 2001)
	[b]Spatial learning and memory		(Modaberi et al. 2019; Sahraei et al. 2019)
	[b]Autism spectrum disorder (ASD)	Arbaclofen [c]NCT03682978, [c]NCT03887676	(Veenstra-VanderWeele et al. 2017; Frye 2014)
	[b]Fragile X syndrome (FXS)		(Berry-Kravis et al. 2017; Zhang et al. 2015)
	[b]Alzheimer's disease		(Rice et al. 2019; Sun et al. 2020)
	[b]Analgesic (fibromyalgia)	[c]NCT03092726	(Neto et al. 2006; Enna and McCarson 2006; Murai et al. 2019)
	[b]Pancreatic cancer		(Young and Bordey 2009; Zhang et al. 2014; Jiang et al. 2012;

(continued)

Table 1 (continued)

Pharmacology	[a]Therapeutic use/[b]clinical potential	Approved drug/[c]clinical trial	References
			Schuller et al. 2008; Schuller 2018; Al-Wadei et al. 2012)
	[b]Type 1 diabetes		(Tian et al. 2017)
Antagonists/ negative allosteric modulators	[b]Depression/mood disorders		(Cryan and Kaupmann 2005; Felice et al. 2012; Jacobson et al. 2018)
	[b]Type 2 diabetes		(Bonaventura et al. 2008, 2012; Braun et al. 2004)
	[b]Absence epilepsy/ seizures		(Bernasconi et al. 1992; Ostojić et al. 2013)
	[b]Mild cognitive impairment and memory		(Lasarge et al. 2009; Mondadori et al. 1993)
	[b]Succinic semi-aldehyde dehydrogenase (SSADH) deficiency	[c]NCT02019667	(Cortez et al. 2004; Didiášová et al. 2020)

[a]Therapeutic use
[b]Clinical potential
[c]Clinical trail

receptor have been described in the scientific and patent literature that have been developed for their therapeutic potential; positive allosteric modulators, for example, have been proposed to mitigate the unwanted side effects and reduce tolerance but have yet to be approved for clinical use. Hence, identification of novel drugs targeting the $GABA_B$ receptor that display improved efficacy and pharmacokinetic properties and with a safer side effect profile is the subject of intense research and many industrial scale drug discovery efforts.

As mentioned, the multifaceted $GABA_B$ receptor is extremely complex. However, the same complexity that has historically hindered development of $GABA_B$ receptor-targeted therapeutics now provides the potential for discovery of $GABA_B$ receptor disease-specific therapeutics. For example, $GABA_B$ receptor subtype-selective ligands are highly desirable not only to dissect the physiological role of the predominant receptor subtypes, $GABA_{B1(a)/2}$ and $GABA_{B1(b)/2}$, but also to facilitate the development of more finely-tuned mode-of-action drugs to treat various diseases. From a drug discovery perspective, it may be possible to selectively modulate $GABA_{B(1a)}$ containing heteroreceptors by targeting their sushi domains, case in point; amyloid precursor protein (APP) binds to the N-terminal sushi domain of $GABA_{B(1a)}$ and acts as an axonal trafficking factor for $GABA_B$ receptors, it has been proposed that prevention of APP binding to this domain may interfere with $GABA_B$ receptor-mediated inhibition of glutamate release and thereby enhance cognitive function in patients with Alzheimer's disease and intellectual disabilities. Likewise, the discovery of functionally selective ligands for the different $GABA_B$ receptor effectors would provide powerful tools to identify a unique signaling profile that results in the desired in vivo effects without recruiting the adverse side effects.

Alternatively, recent biophysical and structural studies have greatly improved our understanding of the structural basis of $GABA_B$ receptor activation and modulation, and proteomic studies have identified receptor-associated proteins that work in concert with the receptor to orchestrate a variety of molecularly and functionally distinct multiprotein "signalosome" complexes, while providing spatiotemporal control of receptor activity. These findings also present new opportunities for drug discovery, modulating specific protein:protein interactions mediated through sushi domains of $GABA_{B1(a)}$ (as outlined above), C-terminal domain of $GABA_B1$ and/or $GABA_{B2}$; or KCTD subunits, all present potential target sites for designing drugs that selectively interfere with receptor function for disease-specific therapeutic intervention.

Thus, the successful collaboration between medicinal chemistry and pharmacology together with significant advances in our understanding of $GABA_B$ receptor structure and activation mechanisms has drug hunters well-poised for the discovery and development of chemically and mechanistically novel therapeutics targeting the multi-tasking $GABA_B$ receptor for the treatment of a wide variety of disease states.

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$GABA_B$ Receptors and Drug Addiction: Psychostimulants and Other Drugs of Abuse

Xiaofan Li and Paul A. Slesinger

Contents

Abstract Metabotropic $GABA_B$ receptors ($GABA_BRs$) mediate slow inhibition and modulate synaptic plasticity throughout the brain. Dysfunction of $GABA_BRs$ has been associated with psychiatric illnesses and addiction. Drugs of abuse alter $GABA_B$ receptor ($GABA_BR$) signaling in multiple brain regions, which partly contributes to the development of drug addiction. Recently, $GABA_BR$ ligands and positive allosteric modulators (PAMs) have been shown to attenuate the initial

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rewarding effect of addictive substances, inhibit seeking and taking of these drugs, and in some cases, ameliorate drug withdrawal symptoms. The majority of the anti-addiction effects seen with $GABA_BR$ modulation can be localized to ventral tegmental area (VTA) dopamine neurons, which receive complex inhibitory and excitatory inputs that are modified by drugs of abuse. Preclinical research suggests that $GABA_BR$ PAMs are emerging as promising candidates for the treatment of drug addiction. Clinical studies on drug dependence have shown positive results with $GABA_BR$ ligands but more are needed, and compounds with better pharmacokinetics and fewer side effects are critically needed.

Keywords Dopamine · Positive allosteric modulators (PAMs) · Synaptic plasticity · Ventral tegmental area (VTA)

1 Introduction

γ-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the brain that activates both ionotropic $GABA_A$ receptors ($GABA_ARs$) and metabotropic $GABA_B$ receptors ($GABA_BRs$) (Chebib and Johnston 1999). While $GABA_ARs$ are chloride channels that mediate fast inhibitory postsynaptic currents (IPSCs), $GABA_BRs$ mediate slower inhibition by activating the $G_{i/o}$ G proteins and downstream second messengers (Odagaki and Koyama 2001; Chebib and Johnston 1999). $GABA_BRs$ are dimeric proteins consisting of two subunits, $GABA_BR1$ which contains the ligand-binding site, and $GABA_BR2$ which contains a binding site for allosteric modulators and is responsible for G protein coupling (Liu et al. 2004; Galvez et al. 1999, 2000; Kniazeff et al. 2002; Binet et al. 2004; Dupuis et al. 2006). The $GABA_BR2$ C-terminal domain also associates with auxiliary subunits called K^+ channel tetramerization domain (KCTD) proteins, which modulate the activation and desensitization kinetics of $GABA_BRs$ (Schwenk et al. 2010; Turecek et al. 2014; Seddik et al. 2012). Activation of postsynaptic $GABA_BRs$ leads to opening of G protein-activated inwardly rectifying K^+ (GIRK) channels (Luscher et al. 1997; Gahwiler and Brown 1985), resulting in membrane hyperpolarization and shunting excitatory currents. Activation of presynaptic $GABA_BRs$ leads to inhibition of voltage-gated Ca^{2+} (Ca_V) channels, suppressing neurotransmitter release (Thompson and Gahwiler 1992; Takahashi et al. 1998). In addition, the $G\alpha_{i/o}$ subunits inhibit adenylyl cyclase and cAMP-dependent signaling (Enna 2001). $GABA_BR$ activation contributes to synaptic plasticity, either facilitating or inhibiting long-term potentiation (LTP) depending on whether the $GABA_BRs$ act pre- or postsynaptically (Ulrich and Bettler 2007; Heaney and Kinney 2016; Morrisett et al. 1991; Olpe et al. 1993; Davies and Collingridge 1996; Davies et al. 1991). Conversely, neuronal activity also dynamically regulates $GABA_BR$ surface expression and functional signaling (Bettler and Tiao 2006). A growing body of evidence implicates $GABA_BR$ dysfunction in various psychiatric illnesses, and supports the therapeutic potential of

$GABA_BR$ ligands in treating these conditions, as well as movement and neurodegenerative disorders (Kumar et al. 2013; Bowery 2006). While this is discussed in more detail in other chapters of this book, here we focus on the involvement of $GABA_BR$ in drug addiction. Drugs of abuse have been shown to alter $GABA_BR$ signaling in many brain regions, which can last for prolonged periods of time. Some of these changes contribute to the development of behavioral and psychological manifestations of addiction, while others may be compensatory mechanisms to restore normal circuit function. The significant role the $GABA_BR$ plays in the reward circuit offers the possibility of using pharmacological $GABA_BR$ modulation to rescue circuit malfunction in addiction.

2 $GABA_BR$ Signaling in the Reward Circuit

Dopamine (DA) neurons in the VTA are crucial players in reward and motivated behaviors (Ranaldi 2014; Baik 2013). VTA DA neurons mediate the initial reinforcing effects of drugs of abuse, and long-lasting adaptations in their synaptic inputs and intrinsic activity partially underlie various behavioral manifestations of drug addiction (Luscher and Malenka 2011; Self 2004; Wanat et al. 2009; Francis et al. 2019). Glutamatergic inputs onto VTA DA neurons are known to be potentiated by drugs of abuse (see Sect. 5.3) (Overton et al. 1999; Saal et al. 2003; Ungless et al. 2001; Kalivas 1995). VTA DA neurons also receive GABAergic inputs from both local VTA GABA interneurons, as well as afferents from many subcortical regions including the nucleus accumbens (NAc), ventral pallidum (VP), rostromedial tegmental nucleus (RMTg), and lateral hypothalamus (LH) (Fig. 1) (Blacktop et al. 2016; Soden et al. 2020). Recent studies combining optogenetics and pharmacology have attempted to resolve the specific receptors mediating these inhibitory inputs (Nieh et al. 2015, 2016; Polter et al. 2018; Matsui et al. 2014; Edwards et al. 2017), which will be discussed in more detail below. Some other brain regions involved in reward function such as the amygdala, prefrontal cortex (PFC), lateral habenula, dorsal and median raphe nuclei also express $GABA_BRs$ (Margeta-Mitrovic et al. 1999), but the functional role of $GABA_BR$ signaling in these regions in reward processes is less well studied.

2.1 The VTA Microcircuit

Both $GABA_ARs$ and $GABA_BRs$ are expressed on VTA DA and GABA neurons (Ciccarelli et al. 2012). There are some indications that the two receptor types are activated by distinct inputs to the VTA, since the $GABA_A$ and $GABA_B$ IPSPs occur independently of each other and are differentially modulated by pharmacological agents (Sugita et al. 1992). VTA DA and GABA neurons have different sensitivities to $GABA_A$ and $GABA_B$ agonists as a result of differences in receptor subunit

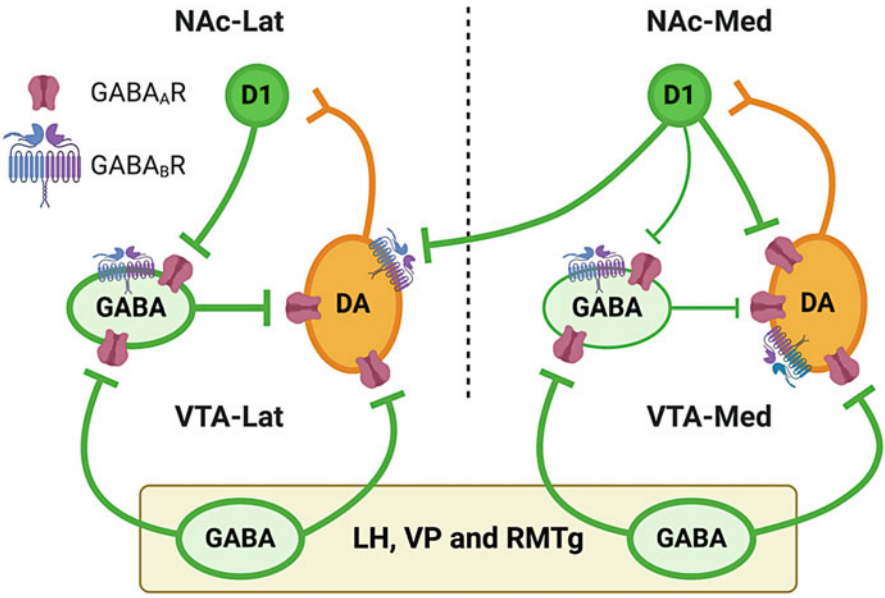


Fig. 1 Inhibitory inputs to VTA neurons. Main inhibitory inputs onto VTA dopamine neurons and the receptors that mediate inhibition are illustrated. D1: medium spiny neurons (MSN) expressing dopamine D1 receptor; GABA: VTA GABA interneurons; DA: VTA dopamine neurons; LH: lateral hypothalamus; VP: ventral pallidum; RMTg: rostromedial tegmental nucleus; Lat: lateral; Med: medial. Note that $GABA_A$R-mediated inhibitions from D1 MSNs to VTA GABA neurons and from VTA GABA neurons to VTA DA neurons are weaker in VTA-Med compared to VTA-Lat (Yang et al. 2018)

composition and coupling efficiency to GIRK channels, respectively (Tan et al. 2010; Cruz et al. 2004). Furthermore, VTA GABA interneurons inhibit DA neurons preferentially through $GABA_A$ receptors (Fig. 1) (Edwards et al. 2017; Polter et al. 2018). These facts should be taken into consideration when interpreting the effects of intra-VTA application of GABAergic drugs, as $GABA_A$R and $GABA_B$R agonists/antagonists can have bidirectional effects on DA neuron activity depending on whether the DA or GABA neurons are primarily affected (Laviolette and van der Kooy 2001; Laviolette et al. 2004; Cruz et al. 2004; Xi and Stein 1998). Nevertheless, in most in vivo studies $GABA_B$R ligands appear to bypass the VTA microcircuit and act directly on DA neurons. For example, infusion of baclofen (a $GABA_B$R agonist) locally into the VTA revealed decreases in DA release both within the VTA (Klitenick et al. 1992) and in the NAc (Westerink et al. 1996). DA release and locomotor activation in response to morphine, cocaine, and amphetamine were also blocked by intra-VTA baclofen (Kalivas et al. 1990; Klitenick et al. 1992; Leite-Morris et al. 2004). On the other hand, intra-VTA infusion of $GABA_B$R antagonist elicits an increase in DA level (Giorgetti et al. 2002). This $GABA_B$R-mediated inhibition of DA signaling serves as the basis for targeting $GABA_B$Rs in the treatment of drug addiction.

Recently, $GABA_BR$ signaling in VTA DA neurons has been shown to be more nuanced than previously thought. Lateral VTA DA neurons that project to lateral NAc shell have significantly larger baclofen-induced currents than medial VTA DA neurons which project to medial NAc shell (Yang et al. 2018). Interestingly, $GABA_BR1$ expression detected by in situ hybridization is relatively uniform across different regions of the midbrain (Edwards et al. 2017), suggesting that other factors may determine the functional variability of $GABA_BR$ signaling in subpopulations of VTA DA neurons.

2.2 GABAergic Inputs to the VTA

A wide range of subcortical regions send GABAergic projections to the VTA, and in most cases they preferentially synapse on local GABA neurons in the VTA (Soden et al. 2020). This pattern of innervation provides a potential mechanism of feedforward disinhibition of dopamine neurons which can switch them from tonic to burst firing to signal reward (Soden et al. 2020; Paladini and Tepper 1999). The major sources of GABAergic input to VTA with relevance to addiction are discussed below.

The RMTg is a GABAergic nucleus located just caudal to the VTA, and is therefore sometimes referred to as the tail of the VTA (tVTA) (Kaufling et al. 2009; Jhou et al. 2009b). GABAergic axons from RMTg neurons project to VTA/SNc DA neurons and provide tonic $GABA_AR$-mediated inhibition of DA neuron activity (Matsui and Williams 2011; Lecca et al. 2012). RMTg neurons are activated by noxious stimuli through innervation by glutamatergic lateral habenular neurons (Jhou et al. 2009a). Opioids and cannabinoids inhibit RMTg neurons and result in disinhibition of DA neurons (Lecca et al. 2012). The opioid sensitivity is specifically mediated by μ-opioid receptors, which are densely expressed on RMTg neurons (Jhou et al. 2009b; Matsui and Williams 2011). Interestingly, persistent induction of the transcription factor ΔFosB was observed in RMTg following chronic exposure to cocaine and amphetamine, but not morphine (Perrotti et al. 2005). Additional studies are needed to determine whether GABA release from RMTg afferents also activates $GABA_BRs$.

The LH sends both glutamatergic and GABAergic axons to the VTA (Kallo et al. 2015; Nieh et al. 2015). While both DA and GABA neurons of the VTA receive excitatory input from LH, the GABAergic LH-VTA projection appears to preferentially target GABA neurons (Nieh et al. 2016). Activation of this GABAergic pathway results in disinhibition of VTA DA neurons and promotes approach behavior (Nieh et al. 2016). LH-VTA GABAergic projection also encodes cue-reward predictions to regulate learning (Sharpe et al. 2017). Picrotoxin-sensitive $GABA_AR$ IPSCs have been observed in VTA neurons following LH activation, whereas the presence of $GABA_BR$ IPSCs has not yet been tested (Nieh et al. 2016).

The VP has reciprocal connections with VTA (Faget et al. 2016; Taylor et al. 2014). GABAergic inputs from the VP provide a tonic inhibition on VTA DA

neuron firing (Floresco et al. 2003), although this pathway can also be acutely activated by reward-associated cues (Smith et al. 2009). Silencing the GABAergic VP to VTA projection disrupts reinstatement of cocaine seeking, which is not recapitulated by $GABA_A$ antagonist (gabazine)-mediated direct disinhibition of VTA DA neurons, suggesting that the GABAergic VP afferents likely also inhibit non-DA neurons in the VTA (Mahler et al. 2014). Whether GABAergic VP afferents signal through $GABA_A$Rs or $GABA_B$Rs on VTA neurons has not been established.

In the NAc, dopamine D1 receptor-expressing medium spiny neurons (D1R-MSNs) send direct projections to the VTA, and comprise the "direct" pathway. Initial studies found that D1R-MSNs preferentially target non-DA neurons, in particular GABA neurons, even though a weak connection to DA neurons also exists (Bocklisch et al. 2013; Xia et al. 2011; Matsui et al. 2014). This pathway signals through $GABA_A$Rs and is inhibited by μ-OR activation (Xia et al. 2011; Matsui et al. 2014). Optogenetic stimulation of the D1R-MSN terminals failed to evoke $GABA_B$R IPSCs in VTA neurons, which can nevertheless be produced by electrical stimulation (Xia et al. 2011). A more recent study, however, identified a NAc to VTA DA connection that signals through $GABA_B$Rs (Edwards et al. 2017). The authors confirmed that NAc to VTA projection also inhibits GABA neurons via $GABA_A$Rs. Importantly, by combining immuno-electron microscopy and electrophysiological measurements on the spatio-temporal dynamics of $GABA_B$R activation, Edwards et al. (2017) showed that NAc axon terminals form symmetric synapses on DA cell bodies and dendrites, and that $GABA_B$Rs on DA neurons are activated by synaptic release of GABA. It will be important to determine whether the same population of NAc MSNs contact both DA and GABA neurons in the VTA. In contrast to the NAc input, VTA GABA neuron to DA neuron connection is mediated largely by $GABA_A$Rs, with only a small $GABA_B$R component (Edwards et al. 2017). Interestingly, VTA GABA neurons can inhibit presynaptic glutamate release onto DA neurons through $GABA_B$Rs, but not $GABA_A$Rs (Chen et al. 2015). $GABA_B$R-mediated inhibition of DA neuron terminal activity and DA efflux has also been reported in the NAc (Saigusa et al. 2012; Pitman et al. 2014; Xi et al. 2003).

A recent study by Yang et al. (2018) provided more precise anatomical resolution to the NAc-VTA connectivity on the medial-lateral axis (Fig. 1). Medial VTA (VTA-Med) DA neurons form reciprocal connections with NAc medial shell (NAc-Med), while lateral VTA (VTA-Lat) DA neurons are reciprocally connected with NAc lateral shell (NAc-Lat) (Yang et al. 2018). Interestingly, the two pathways show little overlap. Importantly, D1R-MSNs from NAc-Lat inhibit non-DA neurons (likely GABA neurons) more strongly than DA neurons in VTA-Lat, resulting in an overall excitation of VTA-Lat DA neurons (Yang et al. 2018). In contrast, NAc-Med D1R-MSNs equally target DA and non-DA neurons and when stimulated result in an overall inhibition of VTA-Med DA neurons. The inhibitory control by D1R-MSNs described above is mediated by $GABA_A$Rs, while $GABA_B$R-mediated inhibition is induced by NAc-Med, but not NAc-Lat stimulation in about half of NAc-Lat projecting DA neurons, but not in NAc-Med projecting DA neurons (Yang et al. 2018). The amplitudes of $GABA_B$R IPSCs (Yang et al. 2018) were smaller than in

Edwards et al. (2017), a difference that could partly be explained by a more widespread expression of ChR2 virus. What is the functional role of this NAc-Med to VTA-Lat $GABA_BR$ pathway? Intra-VTA antagonism of $GABA_BR$ with CGP 35348 removed the anxiety-like phenotype induced by NAc-Med D1R-MSN stimulation, and at the same time revealed a rewarding effect of the stimulation (Yang et al. 2018). This is consistent with $GABA_BRs$ having a reward-suppressing effect in the VTA (Willick and Kokkinidis 1995).

In summary, while a wide range of GABAergic inputs to the VTA has been identified, their full characterization remains incomplete. Currently, it is well established that these inputs can activate $GABA_ARs$ on VTA DA and GABA neurons to drive direct inhibition and disinhibition of DA neurons, respectively. However, with the exception of NAc, the ability of these inputs to activate $GABA_BRs$ in the VTA and contribute to drug addiction has not been explored, despite ample evidence that VTA neuron activity is modulated by $GABA_BR$ ligands. Future studies should address this gap in order to understand more completely GABAergic control of DA neuron activity.

3 Impact of Addictive Drugs on $GABA_BR$ Signaling in the Reward Circuit

$GABA_BR$ signaling undergoes activity-dependent plasticity and drug-evoked changes. A recent review by Lalive and Lüscher (2016) provides a good description of these processes and some underlying mechanisms. Here, we focus on the impact of these drug-induced alterations in $GABA_BR$ signaling on neurotransmission in the brain reward circuit.

3.1 Psychostimulants

Cocaine acutely modulates $GABA_BR$ signaling in the VTA in a bidirectional manner. In acutely prepared VTA slices, relatively low concentrations of cocaine (~0.1 μM) inhibit $GABA_BR$ inhibitory postsynaptic potentials (IPSPs) on VTA DA neurons via presynaptic 5-HT receptors, leading to disinhibition of DA neurons (Cameron and Williams 1994). On the other hand, cocaine at 1 μM or higher concentrations blocks dopamine reuptake and increases the level of extracellular dopamine, which activates D1 receptors on afferent GABA terminals to facilitate $GABA_BR$ IPSP (Cameron and Williams 1993, 1994; Lacey et al. 1990; Brodie and Dunwiddie 1990). Apart from immediate action, psychostimulants can also lead to long-lasting changes in $GABA_BR$ signaling, depending on the length of drug exposure (acute vs. chronic) and time of measurement (early vs. late withdrawal). A single injection of cocaine (15 or 30 mg/kg) decreases $GABA_BR$-GIRK signaling

in VTA DA neurons for 3–4 days, possibly through a downregulation of surface GIRK channels (Arora et al. 2011). Furthermore, a single injection of cocaine (15 mg/kg) or amphetamine (10 mg/kg) increased AMPAR/NMDAR ratio in DA neurons, but not GABA neurons in the VTA (Saal et al. 2003; Ungless et al. 2001). This results in an altered excitation/inhibition balance on VTA DA neurons, enhancing DA signaling in the short term. On the other hand, a single cocaine or methamphetamine injection also diminished $GABA_BR$ signaling in VTA GABA neurons (Padgett et al. 2012), perhaps leading to more local GABA release as a compensatory mechanism to dampen VTA DA activity.

Chronic psychostimulant exposure results in altered $GABA_BR$ function within the VTA and other brain regions. $GABA_BR$-GIRK signaling in VTA DA neurons was diminished by either methamphetamine self-administration (Sharpe et al. 2014) or repeated non-contingent injections (Munoz et al. 2016). Suppression of $GABA_BR$-GIRK signaling was also observed in layer 5/6 pyramidal neurons of dorsal medial prefrontal cortex (mPFC) following repeated cocaine treatment (Hearing et al. 2013). This is consistent with the impaired ability of intra-mPFC baclofen to inhibit glutamate transmission (Jayaram and Steketee 2004) and cocaine-induced locomotor activity (Steketee and Beyer 2005). In the VTA, however, microdialysis analysis of somatodendritic DA and glutamate release in freely moving rats revealed a different story. Intra-VTA infusion of $GABA_BR$ antagonist CGP 55845A showed that at baseline, DA release is under tonic $GABA_BR$ inhibition while glutamate release is not (Giorgetti et al. 2002). After repeated amphetamine injections, both DA and glutamate release in the VTA was affected by increased $GABA_BR$ inhibitory tone (Giorgetti et al. 2002). Interestingly, this was seen during the early withdrawal period (3 days after last drug injection) but not at late withdrawal times (10–14 days after last drug injection), consistent with the finding that synaptic plasticity in the VTA that accompany psychostimulant sensitization is transient (Zhang et al. 1997). In the dorsolateral septal nucleus (DLSN), a brain region also implicated in reward function (Olds and Milner 1954), presynaptic $GABA_BR$ function was impaired after prolonged cocaine exposure, leading to enhancement of GABA and glutamate release in this area (Shoji et al. 1997). On the other hand, postsynaptic $GABA_BR$ function was not altered by chronic cocaine in DLSN (Shoji et al. 1997). In short, alterations in $GABA_BR$ function by psychostimulants can vary by brain region and timing.

Chronic cocaine exposure also decreased $G_{i\alpha}$ and $G_{o\alpha}$ G protein levels in VTA, NAc, and locus coeruleus (LC) (Nestler et al. 1990), and decreased functional coupling of $GABA_BRs$ to G proteins in VTA and NAc (Kushner and Unterwald 2001; Xi et al. 2003). Similarly, chronic amphetamine also attenuated $GABA_BR$ G protein coupling in NAc in late withdrawal (Zhang et al. 2000). Altogether, the diminished $GABA_BR$ signaling promotes behavioral sensitization to the drug, even though $GABA_BR$ expression may remain unaltered (Li et al. 2002a). Cocaine withdrawal can also indirectly reduce $GABA_BR$ transmission through impairing presynaptic GABA release, as seen at entopeduncular nucleus to lateral habenula (LHb) synapses (Tan et al. 2018). Reduced $GABA_BR$ transmission leads to higher excitability of LHb neurons, and in combination with enhanced excitatory input as a

result of diminished $GABA_AR$ signaling, they contribute to the negative symptoms of cocaine withdrawal (Tan et al. 2018).

Animals that self-administer drugs of abuse can develop neuroadaptations associated with motivated responding and craving for the drug that are absent in animals passively receiving administered drug. Autoradiographic analysis using a $GABA_BR$ antagonist, [^{3}H]CGP 54626 revealed a large decrease in binding in a wide range of brain regions following a 10-day withdrawal in animals self-administering cocaine, but not in yoked animals that received non-contingent injections of cocaine (Frankowska et al. 2008). Decreases in GABABR binding were observed in the PFC, dorsal striatum, NAc, amygdala, hippocampus, VTA and substantia nigra, all regions important in the reward circuit. Whether the decreased $GABA_BR$ levels in these areas contribute to craving and relapse will require further study.

3.2 Nicotine

To date, there are few reports describing the impact of nicotine on $GABA_BR$ signaling. One group found that subcutaneous injection of 0.4 mg/kg nicotine for 14 days in rats abolished the baclofen-mediated inhibition of electrically evoked DA release from VTA slices (Amantea and Bowery 2004), and reduced $GABA_BR$ coupling to G proteins in the mPFC and NAc (Amantea et al. 2004). However, another study that chronically infused a much higher dose of nicotine (3.16 mg/kg/day for 7 days prior to and during testing) found that the ability of intra-VTA $GABA_BR$ agonist to elevate the threshold for intracranial self-stimulation (ICSS) is similar in vehicle and nicotine-treated rats (Paterson et al. 2005a, b). While the first two studies examined $GABA_BR$ function during nicotine withdrawal, the last study was done during nicotine administration, which may contribute at least partly to the discrepancies in these findings.

$GABA_BR1$ and $GABA_BR2$ RNA expression levels have been examined in rats chronically exposed to oral nicotine or cigarette smoke. A decrease in $GABA_BR1$ expression was observed in the hippocampus following both chronic nicotine and cigarette smoke (Li et al. 2002b). Interestingly the length of smoke exposure was negatively correlated with the degree of decrease in $GABA_BR1$ RNA. In the PFC, chronic nicotine led to a small decline in both $GABA_BR1$ and $GABA_BR2$ RNA expression, while cigarette smoke increased the level of both receptor subunits (Li et al. 2004). Whether the discrepancies are due to different route of exposure or ingredients other than nicotine in the cigarette smoke remains to be investigated.

3.3 Opioids

Acute morphine promotes glutamate release onto VTA DA neurons by removing $GABA_BR$ inhibition of glutamatergic inputs (Chen et al. 2015). Chronic morphine treatment, however, increases $GABA_BR$ signaling in the VTA during withdrawal, which in turn reduces glutamate release and leads to a decrease in DA neuron activity (Manzoni and Williams 1999). This may result from 1) enhanced GABA release during withdrawal (Bonci and Williams 1997) and 2) increased coupling efficiency of $GABA_BR$ to GIRK channels through downregulation of RGS2, a regulator of G protein signaling (Labouebe et al. 2007). Chronic infusion of morphine via osmotic pumps has also been reported to increase both $GABA_BR1$ and $GABA_BR2$ immunoreactivity in the globus pallidus (GP) and substantia nigra pars reticulata (SNr) although the functional implication of these changes is still unclear (Negrete-Diaz et al. 2019). On the other hand, chronic morphine treatment has also been shown to decrease the activity of inhibitory $G_{i/o}$ G proteins in the locus coeruleus (Selley et al. 1997). Since $GABA_BR$ and μ-opioid receptor both couple to $G_{i/o}$ G proteins, $GABA_BR$ signaling is potentially affected in this region and should be directly tested.

3.4 Cannabis

Tetrahydrocannabinol (THC) is the principal psychoactive cannabinoid in cannabis and is a partial agonist for the cannabinoid receptor CB1, which signals via $G_{i/o}$ as does $GABA_BR$ (Howlett et al. 1986; Bidaut-Russell et al. 1990). Chronic THC treatment resulted in CB1 downregulation and desensitization in most brain regions, whereas $GABA_BR$-stimulated G protein activation was not affected (Sim et al. 1996; Selley et al. 2004). However, a heterologous attenuation of adenylyl cyclase inhibition was observed in mouse cerebellum for $GABA_BR$ and adenosine A1 receptor following long-term THC treatment (Selley et al. 2004). This may contribute to the cross-tolerance to motor coordination deficits with cannabinoid, $GABA_BR$, and A1R agonists. Garcia-Gil et al. (1999) demonstrated that perinatal exposure to THC in male and female rats potentiates the motor inhibitory effect of baclofen in adult animals, despite the fact that baclofen-stimulated G protein activation was not changed, especially in the substantia nigra (Garcia-Gil et al. 1999). Whether $GABA_BR$ signaling in the reward circuit is altered by THC remains to be investigated.

3.5 Summary

In summary, drugs of abuse induce plasticity in $GABA_BR$ signaling in various parts of the brain, and these changes can take place at the level of RNA expression, receptor trafficking, G protein coupling, as well as the effector proteins. It appears that some of the changes contribute to the progression to addiction, while others may be compensatory responses to other changes in neurotransmission. Studies on psychostimulant-induced alterations in $GABA_BR$ signaling have been most abundant, whereas for other drugs we are still at the beginning of such investigations. It is important to figure out which of the plastic changes are universal mechanisms to all drugs of abuse, and which ones are drug-specific. Such knowledge will be beneficial for devising therapeutic interventions targeted at $GABA_BRs$.

4 Effects of Genetic Manipulations of $GABA_BR$ on Animal Models of Drug Addiction

Mice lacking either the $GABA_BR1$ or $GABA_BR2$ subunit have been generated and studied on the Balb/c genetic background (Gassmann et al. 2004; Schuler et al. 2001). Unfortunately, premature death in early adulthood was observed in knockout (KO) mice on other backgrounds such as C57BL6/J, preventing behavioral characterization (Prosser et al. 2001). Both $GABA_BR1$ and $GABA_BR2$ KO mice display hyperlocomotion, hyperalgesia, and spontaneous epileptic seizures. $GABA_BR1$ KO mice also have an anxious and antidepressant-like phenotype (Mombereau et al. 2004). Relatively few studies have examined the effects of $GABA_BR$ KO mice on drug addiction.

A series of studies conducted by Varani et al. on the $GABA_BR1$ KO in combination with pharmacological manipulations of $GABA_BRs$ have demonstrated a role for $GABA_BRs$ in mediating acute effects of nicotine as well as nicotine withdrawal symptoms with respect to reward and anxiety (Varani et al. 2012, 2014, 2015, 2018). $GABA_BRs$ are required for the acute locomotor, antinociceptive, and anxiolytic effects of nicotine, as well as the neurochemical changes that give rise to anxiety in nicotine withdrawal. On the other hand, $GABA_BR$ activity counteracts the rewarding effect of nicotine (Le Foll et al. 2008; Varani et al. 2018). While the specific brain regions where $GABA_BR$ activity is important for the nicotine effects are still elusive, nicotine-dependent changes in c-fos and BDNF expression in several brain areas involved in anxiety are absent in $GABA_BR1$ KO (Varani et al. 2012, 2014, 2015), and nicotine-induced increase in c-fos expression in NAc and VTA is potentiated in $GABA_BR1$ KO (Varani et al. 2018).

The two alternatively spliced isoforms of $GABA_BR1$ subunit, $GABA_BR1a$ and $GABA_BR1b$, localize to the presynaptic and postsynaptic membrane, respectively (Biermann et al. 2010; Vigot et al. 2006). $GABA_BR1b$ lacks the N-terminal sushi repeats that mediate axonal targeting in $GABA_BR1a$ (Kaupmann et al. 1997;

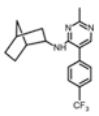
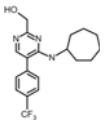
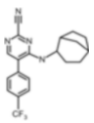
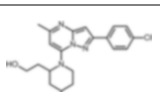
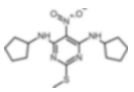
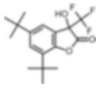
Biermann et al. 2010). In the hippocampus, $GABA_BR1a$-containing heteroreceptors mediate inhibition of glutamate release while $GABA_BR1b$-containing heteroreceptors mediate postsynaptic inhibition (Vigot et al. 2006). Isoform-specific knockouts showed differential effects on locomotor response to cocaine: While $GABA_BR1b$ KO mice had higher basal locomotion, they failed to develop sensitization; $GABA_BR1a$ KO had higher acute locomotor response as well as sensitization to cocaine (Jacobson et al. 2016).

Selective deletion of $GABA_BRs$ in VTA DA neurons has been carried out by injecting an adeno-associated virus expressing Cre recombinase under the tyrosine hydroxylase (TH) promoter (AAV-TH-iCre) into the VTA of mice with floxed $GABA_BR1$. This manipulation decreased baclofen currents in DA neurons and enhanced locomotor response to cocaine, while basal locomotion and morphine-induced locomotion were unaltered (Edwards et al. 2017). Rifkin et al. (2018) used a different approach to investigate the effect of reduced $GABA_BR$-activated GIRK currents on drug sensitivity. A conditional knockout of sorting nexin 27, which regulates surface expression of GIRK channels, led to reduced baclofen-induced currents in VTA DA neurons and increased sensitivity to cocaine-dependent locomotor sensitization (Rifkin et al. 2018).

5 Effects of Pharmacological Manipulations on Animal Models of Drug Addiction: Agonists, Antagonists, and Positive Allosteric Modulators (PAMs)

Research in the past few decades has yielded substantial evidence for the therapeutic potential of $GABA_BR$ ligands in treating a variety of neurological disorders (Bowery 2006; Heaney and Kinney 2016; Jacobson et al. 2018; Kumar et al. 2013). At the moment, baclofen is the only $GABA_BR$ agonist approved for human use as a muscle relaxant. In 2018, it was also approved in France for treating alcohol use disorder, despite controversy regarding its efficacy and safety (Braillon et al. 2020; de Beaurepaire et al. 2018). Recently several groups have attempted to validate its effect for treating abuse of addictive substances by using animal models. The common hypothesis is that activating $GABA_BRs$ on VTA DA neurons dampen their activity, thus reducing the rewarding effect of drugs of abuse and suppressing the motivation to work for them. Indeed, baclofen has been shown to dose-dependently reduce DA release in the NAc shell evoked by cocaine, nicotine, and morphine (Fadda et al. 2003). $GABA_BR$ agonists other than baclofen as well as antagonists have also been studied in the context of drug addiction. A thorough review of the chemistry and pharmacology of these ligands can be found in a recent book chapter (Froestl 2010). A potential issue with $GABA_BR$ orthosteric agonists (e.g., baclofen) is the induction of side effects, including sedation, weakness, vertigo, and headache (Agabio and Colombo 2015; Tyacke et al. 2010). Positive allosteric modulators (PAMs) may provide a remedy for this problem. In general,

Table 1 List of $GABA_BR$ PAMs and chemical names

Name	Chemical name	Structure	Citation
BHF177	*N*-[(1*R*,2*R*,4*S*)-bicyclo[2.2.1]hept-2-yl]-2-methyl-5-[4-(trifluoromethyl)phenyl]-4-pyrimidinamine		Guery et al. (2007)
KK-92A	(4-(cycloheptylamino)-5-(4-(trifluoromethyl)-phenyl)pyrimidin-2-yl) methanol (analog of BHF177)		Li et al. (2017)
NVP998	(4-(bicyclo(2.2.1)heptylamino()-5-(4-(trifluoromethyl)phenyl)pyrimidin-2-yl) nitrile (analog of BHF177)		Sturchler et al. (2017)
CGP7930	3-(3′,5′-Di-*tert*-butyl-4′-hydroxy)phenyl-2,2-dimethylpropanol		Urwyler et al. (2001)
CMPPE	2-{1-[2-(4-chlorophenyl)-5-methylpyrazolo[1,5-a] pyrimidin-7-yl]-2-piperidinyl}ethanol		Perdona et al. (2011)
GS39783	*N,N′*-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine		Urwyler et al. (2003)
rac-BHFF	(*R,S*)-5,7-di-*tert*-butyl-3-hydroxy-3-trifluoromethyl-3*H*-benzofuran-2-one		Malherbe et al. (2008)

allosteric modulators bind to a region of the $GABA_BR$ outside of the ligand-binding site, where they induce conformational changes that either increase (i.e., positive, PAMs) or decrease (i.e., negative, NAMs) the effects of GABA. Therefore, they do not possess intrinsic agonistic activity, but can modulate the effect of endogenous GABA release on $GABA_BRs$. As a result, they are expected to produce fewer side effects compared to orthosteric $GABA_BR$ agonists (Filip et al. 2015). PAMs also appear to activate the receptor without inducing desensitization, which is partially responsible for the development of tolerance (Sturchler et al. 2017). CGP7930 (Urwyler et al. 2001) and GS39783 (Urwyler et al. 2003) were the first $GABA_BR$ PAMs discovered through compound screening in a $GTP\gamma^{35}S$ binding assay (Table 1). They increased both the potency and efficacy of GABA at $GABA_BR$, suggesting that they enhance not only agonist binding, but also receptor-G protein coupling (Urwyler 2011). The $GABA_BR$ PAM binding site was mapped to the transmembrane domain of the $GABA_BR2$ subunit (Binet et al. 2004; Dupuis et al. 2006), Since then, new $GABA_BR$ PAMs have been identified by screening

compound libraries or structural analogs of existing PAMs in GTPγ^{35}S binding or functional $GABA_BR$ signaling assays (Table 1). In this section we will review studies that tested the effects of $GABA_BR$ ligands and PAMs in animal models of addiction for the common drugs of abuse. There are also a few other recent reviews on this topic (Filip et al. 2015; Filip and Frankowska 2008; Vlachou and Markou 2010; Phillips and Reed 2014).

5.1 Psychostimulants

5.1.1 Amphetamine and Methamphetamine

Baclofen given systemically (2–4 mg/kg) dose-dependently blocks the development and expression of locomotor sensitization to amphetamine (Bartoletti et al. 2004, 2005; Cedillo and Miranda 2013). A $GABA_BR$ PAM, CGP 7930, potentiated the effect of low dose (2 mg/kg) baclofen in blocking both the development and expression of amphetamine sensitization (Cedillo and Miranda 2013). GS39783, another $GABA_BR$ PAM, blocked expression of amphetamine conditioned place preference (CPP) without affecting locomotion (Halbout et al. 2011). In rats self-administering amphetamine, 1.8–5.6 mg/kg baclofen reduced responding under both fixed ratio (FR) and progressive ratio (PR) schedule of reinforcement, which was accompanied by an attenuation of amphetamine-induced DA level increase in the NAc (Brebner et al. 2005).

Baclofen (1.25–5 mg/kg) dose-dependently attenuated both the acquisition and expression of methamphetamine CPP (Li et al. 2001). Methamphetamine-induced CPP is resistant to extinction, but 2 mg/kg baclofen given after daily extinction sessions facilitated the extinction (Voigt et al. 2011a). Interestingly, two home cage injections of $GABA_BR$ PAMs (CGP 7930 or GS39783) in rats with established methamphetamine CPP abolished the expression of CPP in a subsequent test (Voigt et al. 2011a, b). This implicates $GABA_BRs$ in the short-term maintenance of memories associated with methamphetamine conditioning. Regarding methamphetamine self-administration, 2.5 and 5 mg/kg baclofen injection reduced break points for all doses of methamphetamine on a PR schedule, indicating diminished motivation to work for the drug without apparent motor impairment (Ranaldi and Poeggel 2002). There is also evidence that baclofen rescues cognitive deficits induced by methamphetamine. For example, methamphetamine-induced impairments of prepulse inhibition and novel object recognition were ameliorated by systemic baclofen at 2 mg/kg (Arai et al. 2009).

5.1.2 Cocaine

Baclofen (2 mg/kg) prevented cocaine-conditioned locomotion as well as stimulus-induced glutamate release in the NAc (Hotsenpiller and Wolf 2003). Both the

development and expression of locomotor sensitization to cocaine were also reduced by baclofen as well as another $GABA_BR$ agonist, SKF 97541 (Frankowska et al. 2009). Intra-mPFC baclofen blocked acute locomotor activation by cocaine and the development of sensitization, but not the expression of sensitization (Steketee and Beyer 2005). The $GABA_BR$ PAM GS39783 modestly attenuated the development of cocaine sensitization, and also blocked cocaine-induced activation of DARPP-32 and CREB in the NAc (Lhuillier et al. 2007).

The impact of $GABA_BR$ ligands on cocaine self-administration has been extensively studied for more than two decades. Roberts et al. (1996) showed in rats trained to self-administer IV cocaine and tested on a progressive ratio task, that 2.5 mg/kg baclofen significantly reduced the break points for all cocaine doses (0.18 to 1.5 mg/kg/inj). In comparison, baclofen only slightly decreased responding for food reward. This suggests that at low dosages, baclofen can specifically dampen the reinforcing effect of cocaine without causing sedation or general disruption of performance, which typically emerges at 5 mg/kg (Roberts et al. 1996). In a subsequent study, the authors used a discrete trials procedure to probe baclofen's effect on the initiation of cocaine self-administration (Roberts and Andrews 1997). When each discrete trial was separated by 30 min, rats showed a clear diurnal pattern of cocaine taking where most of the infusions (1.5 mg/kg/inj) happen during the dark phase. The likelihood of cocaine taking is low but increasing at the beginning of the dark phase, and much higher in the middle of the dark phase. Baclofen (2.5 mg/kg) injection given at either time point strongly suppressed drug taking. A range of baclofen doses (1.25–5.0 mg/kg) were able to suppress cocaine taking while having no effect on food taking on a second lever.

A study from Campbell et al. (1999) showed that baclofen treatment prior to the 7-h cocaine self-administration session (FR1) suppressed cocaine intake in a dose-dependent manner. 2.5 and 5 mg/kg baclofen were effective in reducing cocaine infusions especially in the first 4 h of the session. The suppression effect was also stronger for the lower dose of cocaine (0.2 mg/kg/inj) compared to the higher dose (0.4 mg/kg/inj). In comparison, cocaine-primed reinstatement was more effectively blocked by baclofen at 1.25 & 2.5 mg/kg. Similarly, the Roberts group also found that baclofen had a greater effect in suppressing lower unit dose (0.75 mg/kg/inj) of cocaine intake on a FR1 schedule. Interestingly, the suppression can be attributed to a period of non-responding at the beginning of the session, instead of a reduced rate of responding (Brebner et al. 2000a). Regarding cue-induced reinstatement of cocaine seeking, 2.5 & 5 mg/kg baclofen was able to suppress responding without affecting food seeking. However, it is less effective on cocaine+cue primed reinstatement (Froger-Colleaux and Castagne 2016).

To gain more insights on the mechanism of baclofen-mediated reduction in drug intake/seeking, local infusions of baclofen into different brain regions have been carried out in several studies. One study found that intra-VTA baclofen injection was three times more potent than intra-NAc or intra-striatum baclofen in suppressing cocaine self-administration (1.5 mg/kg/inj) on a progressive ratio task (Brebner et al. 2000b). Another study showed that intra-NAc or intra-VTA injection of baclofen, but not intra-dorsal striatum injection, decreased responding for cocaine (0.66 mg/kg/inj) on an FR5 schedule (Shoaib et al. 1998). Interestingly, in a more recent report

by Backes and Hemby (2008), intra-VTA injection of the $GABA_AR$ antagonist picrotoxin was found to inhibit cocaine self-administration to an extent comparable to that seen with baclofen, and that the effect of picrotoxin could be blocked by the $GABA_BR$ antagonist 2-hydroxysaclofen. This suggests that picrotoxin likely acts by disinhibiting VTA GABA neurons in the VTA, which in turn release GABA that act on $GABA_BRs$ on DA neurons.

CGP 44532, a highly selective and high-affinity $GABA_BR$ agonist, dose-dependently decreased cocaine self-administration on a progressive ratio schedule as well as on a discrete trials procedure with little effect on food self-administration (Brebner et al. 1999), similar to what has been reported for baclofen (Roberts and Andrews 1997; Brebner et al. 2000a). This is likely not due to an anhedonia state induced by CGP 44532, since it was found to be hedonically neutral in a brain stimulation reward (BSR) rate-frequency paradigm (Dobrovitsky et al. 2002). Cocaine-induced potentiation of BSR, however, was reduced by CGP 44532 in a dose-dependent manner (Dobrovitsky et al. 2002). When tested in baboons, CGP 44532 decreased cocaine-primed reinstatement of cocaine seeking to a degree comparable to that seen for baclofen (Weerts et al. 2007). However, both agonists reduced responding for food as much as for cocaine under an FR10 schedule (Weerts et al. 2005).

Several $GABA_BR$ PAMs have been tested on cocaine self-administration, usually in direct comparison with $GABA_BR$ agonists. $GABA_BR$ PAM CGP 7930 has been shown to reduce cocaine self-administration without affecting food responding across a range of doses (10–100 mg/kg), while high doses of the $GABA_BR$ agonists baclofen (5 mg/kg) and SKF 97541 (0.3 mg/kg) decreased food responding (Filip et al. 2007). A similar pattern was found for cocaine or cue-induced reinstatement, where CGP 7930 dose-dependently reduced cocaine seeking but not food seeking, while both agonists reduced cocaine but also food seeking at high doses (Filip and Frankowska 2007). Intriguingly, the same group found that while SCH 50911 ($GABA_BR$ antagonist) blocked the effects of $GABA_BR$ agonists and PAM on cocaine self-administration, it also decreased cue-induce reinstatement for cocaine but not for food (Filip and Frankowska 2007; Filip et al. 2007). Whether the latter phenomenon may be due to a possible rewarding property of SCH 50911 or its partial agonistic properties at the $GABA_BRs$ is yet uncertain. Another $GABA_BR$ PAM GS39783 reduced responding for cues previously paired with cocaine when administered i.p. at 30 and 100 mg/kg, and did not suppress locomotor activity at the lower dose (30 mg/kg) (Halbout et al. 2011). In contrast, baclofen significantly decreased locomotion at the minimal effective dose (2.5 mg/kg) for reducing cocaine seeking (Halbout et al. 2011). GS39783 also attenuated cocaine's rewarding effect in that it reduced the threshold lowering effect of cocaine on ICSS (Slattery et al. 2005). Unlike baclofen which elevated ICSS threshold, GS39783 has no effect on the threshold, suggesting that it is hedonically neutral. When compared side-by-side, CGP 7930 is somewhat more effective than GS39783 in reducing responding for cocaine across different self-administration schedules, including PR, FR1 as well as discrete trials (DT), a difference that may stem from the difference in drug bioavailability (Smith et al. 2004). CMPPE, a relatively new $GABA_BR$ PAM (Perdona et al.

2011) abolished cue-induced cocaine reinstatement with no sign of sedation or body weight loss, which would be observed with a high dose (3 mg/kg) of baclofen (Vengeliene et al. 2018). Non-sedative doses of the $GABA_BR$ PAM rac-BHFF (Malherbe et al. 2008) attenuated cocaine self-administration and prevented the cocaine-induced increase in AMPAR/NMDAR ratio in VTA DA neurons (de Miguel et al. 2018).

An important caveat of these self-administration studies is that they were performed on male animals only, even though in humans, women are more likely to progress from initial drug use to addiction (Van Etten and Anthony 1999). So far only a few papers have touched on the sex differences in the effect of $GABA_BR$ ligands on cocaine self-administration. It was noted that baclofen has a greater effect in decreasing the acquisition rate and percentage of cocaine self-administration in female rats than in males (Campbell et al. 2002). The relationship between cocaine use in adolescent and adulthood is also sexually dimorphic: males that display escalating self-administration in adolescence develop habit-based inflexible behaviors in adulthood, while in females it was not the escalating animals, but ones with low response rates that later develop behavioral inflexibility (DePoy et al. 2016). Baclofen similarly reduced drug seeking in these animals when they were re-exposed to cocaine-associated context in adulthood (DePoy et al. 2016). When female rats were selectively bred for high (HiS) versus low (LoS) saccharin intake, a pattern emerged in that HiS rats exhibited more cocaine self-administration and higher reinstatement than LoS rats. While baclofen was effective in reducing cocaine-primed reinstatement in both groups, it potentiated cocaine intake escalation in HiS rats but attenuated it in LoS rats during long-access sessions (Holtz and Carroll 2011). This highlights the potential complication of individual variability which is especially relevant in human clinical trials in interpreting the effects of $GABA_BR$ modulation on drug addiction. More studies are needed in the future on sex differences for drug addiction and treatment.

5.2 Nicotine

A recent review by Jacobson et al. (2018) provides an excellent summary of preclinical studies on the effects of $GABA_BR$ compounds on nicotine addiction. Nicotine can directly activate VTA DA neurons through nicotinic acetylcholine receptors, or enhance glutamate transmission onto DA neurons (Mansvelder et al. 2002; Zhao-Shea et al. 2011). Therefore, it is conceivable that $GABA_BR$ activation on VTA DA neurons should counteract the reinforcing effect of nicotine. Indeed, intra-VTA infusion of baclofen as well as the $GABA_AR$ agonist muscimol reduced nicotine self-administration in rats (Corrigall et al. 2000). Systemic baclofen and CGP 44532 have been reported to decrease nicotine self-administration on both FR and PR schedules, block nicotine CPP, and suppress cue-induced reinstatement although high doses of these drugs frequently affect responding for food as well (Paterson et al. 2004; Paterson et al. 2005b; Le Foll et al. 2008). Interestingly, the

anxiolytic and anxiogenic effects produced by low and high doses of nicotine require $GABA_BR$ activity, since they are blocked by saclofen (Varani and Balerio 2012), consistent with the absence of nicotine's anxiolytic and anxiogenic responses in the $GABA_BR1$ KO (Varani et al. 2012, 2014).

$GABA_BR$ PAMs generally show more specificity than $GABA_BR$ agonists in attenuating nicotine's stimulant and rewarding effects or nicotine self-administration without nonspecific effects on locomotion or food seeking. GS39783 was able to block the acquisition of nicotine CPP and nicotine-induced ΔFosB in the NAc (Mombereau et al. 2007); it abolished nicotine-induced hyperlocomotion without affecting basal locomotion (Lobina et al. 2011), and it potentiated the effect of a sub-effective dose of the $GABA_BR$ agonist CGP 44532 on nicotine reinforcement without affecting food responding (Paterson et al. 2008). CGP 7930 also specifically blocked nicotine's locomotor and rewarding effects (Lobina et al. 2011; Paterson et al. 2008). BHF177, a $GABA_BR$ PAM characterized by Guery et al. (2007) (Table 1), decreased responding for nicotine under FR and PR schedules without affecting food responding, blocked cue-induced nicotine but not food seeking, and abolished the reward-enhancing effect of nicotine on ICSS (Paterson et al. 2008; Vlachou et al. 2011). A novel analog of BHF177 named KK-92A exhibited even better selectivity for inhibiting nicotine taking and seeking over food responding (Li et al. 2017). A recent study by Sturchler et al. compared the efficacies of GS39783, BHF177 and a structural analog NVP998 both in vitro and in vivo (Sturchler et al. 2017). Interestingly these PAMs exhibit different functional influence on intracellular signaling pathways and their effects show species selectivity. NVP998 displays highest PAM activity at the human receptor while it has no effect on nicotine self-administration in rats. This underscores the importance of testing potential drugs in nonhuman primate models before moving on to clinical research.

5.3 Opioids

The main mechanism of opioid reward is through inhibition of midbrain GABA neurons via μ-opioid receptors, and resulting disinhibition of VTA DA neuron activity (Johnson and North 1992; Corre et al. 2018; Margolis et al. 2014; Matthews and German 1984; Gysling and Wang 1983). Not surprisingly, $GABA_BR$ agonists have been reported to block the initial rewarding effect of opioids, and the effect can be localized to the VTA. For example, morphine-induced CPP was dose-dependently inhibited by intra-VTA infusion of baclofen prior to morphine injection during acquisition, an effect that was suppressed by the $GABA_BR$ antagonist saclofen (Tsuji et al. 1996). Morphine-induced locomotor activation and sensitization, as well as NAc c-fos activation were also dose-dependently blocked by intra-VTA baclofen (Leite-Morris et al. 2004). Baclofen given systemically (2.5 & 5 mg/kg) likewise inhibited the development of morphine sensitization and morphine-induced DA release in the NAc (Fu et al. 2012). In animals that have developed morphine CPP, systemic baclofen treatment after, but not before extinction sessions,

dose-dependently facilitated the extinction process, presumably by affecting the reconsolidation of morphine-related memories (Heinrichs et al. 2010). Injection of baclofen into the cerebral ventricles decreased morphine self-administration while the $GABA_BR$ antagonist phaclofen increased self-infusions (Ramshini et al. 2013). Another group also showed that systemic baclofen (1.8 mg/kg) decreased morphine self-administration under FR1 schedule, and that $GABA_BR$ antagonist SCH 50911 blocked this effect (Yoon et al. 2007). Similarly for heroin, systemic baclofen dose-dependently decreased heroin self-administration, and the higher dose (1 mg/kg) of baclofen completely prevented acquisition of self-administration, even when baclofen treatment was discontinued after a week (Xi and Stein 1999). Baclofen also abolished heroin-induced DA release in the NAc, an effect partially blocked by intra-VTA saclofen. Intra-VTA infusion of baclofen (2 μg), however, increased heroin self-administration in well-trained animals, which likely indicates a compensatory response to reduced heroin reward at low baclofen dosage (Xi and Stein 1999). Intra-NAc baclofen on the other hand did not affect heroin self-administration. The effects of $GABA_BR$ PAMs on opioid addiction have so far not been reported.

Opioid withdrawal results in a wide range of physiological and psychological symptoms, which may precipitate relapse. Rodents also display withdrawal signs following naloxone-precipitated withdrawal from chronic morphine treatment. Systemic baclofen (2 or 20 mg/kg) treatment before naloxone injection attenuated behavioral signs of morphine withdrawal in multiple studies (Bexis et al. 2001; Pedron et al. 2016; Diaz et al. 2006). Infusion of baclofen into the locus coeruleus also dose-dependently decreased withdrawal signs (Riahi et al. 2009).

5.4 Other Drugs/Reinforcers

Propofol is a PAM of the $GABA_AR$ and is widely used as an intravenous anesthetic. It has abuse potential in humans and is self-administered in rats (LeSage et al. 2000). The rewarding effect of propofol is postulated to be the result of its activation of $GABA_ARs$ on VTA GABA neurons, leading to disinhibition of VTA DA neurons. Baclofen, either systemic (3 mg/kg) or intra-VTA (50 or 100 ng/side) suppressed propofol self-administration under FR1 schedule without affecting motor activities or food-maintained responses (Yang et al. 2011).

In rats trained to self-stimulate the medial forebrain bundle (MFB) upon discriminative cue presentation, $GABA_BR$ agonist baclofen, but not a $GABA_AR$ agonist or NMDAR agonist, infused into the VTA resulted in a rightward shift of the current intensity-operant response curve, indicating a dampening of the rewarding effect of MFB stimulation (Willick and Kokkinidis 1995). In another study, $GABA_BR$ agonist CGP 44532, $GABA_BR$ antagonists CGP 56433A and CGP 51176 all increased thresholds for brain stimulation reward (Macey et al. 2001). In addition, the antagonists had additive effects when given with the agonist, instead of blocking its effect. This paradoxical result could be attributed to $GABA_BR$ action at pre- versus post-synaptic sites (Macey et al. 2001).

5.5 Stress and Addiction

Stress is a common trigger of relapse in drug addiction (Sinha 2008). The role of $GABA_BRs$ in stress-induced reinstatement of drug seeking has been investigated in animal models. Intra-VTA infusion of the $GABA_BR$ antagonist 2-hydroxysaclofen prevented reinstatement of cocaine seeking induced either by stress or intra-VTA injection of corticotropin-releasing factor (CRF) (Blacktop et al. 2016). In comparison, intra-VTA bicuculline ($GABA_AR$ antagonist) had no effect on cocaine reinstatement. Stress-induced reinstatement depends on CRF actions in the VTA. CRF can exert opposing actions through the two receptor types, CRF-R1 and CRF-R2 (Williams et al. 2014). Within the VTA, CRF is known to enhance glutamate release (Wang et al. 2005), increase AMPAR/NMDAR ratio in DA neurons (Ungless et al. 2003), as well as promoting GABA release (Williams et al. 2014) and potentiating GIRK-mediated inhibitory currents in DA neurons upon D2R and $GABA_BR$ activation (Beckstead et al. 2009). The balance of these effects can be altered by cocaine self-administration, in that CRF-R2-mediated facilitation of GABA release becomes suppressed and the overall effect of CRF shifts to excitation (Williams et al. 2014). Due to the complexity of GABAergic signaling in the VTA, it is difficult to conclude whether the $GABA_BR$ signaling responsible for stress-induced cocaine reinstatement acts on DA neurons, local interneurons, or presynaptic terminals. Measurements of VTA neuron activity and DA release may help clarify the mechanism.

Chronic restraint stress facilitated the development of morphine CPP, in that stressed mice required lower doses and less conditioning sessions to develop a preference for morphine (Meng et al. 2014). Baclofen injection (1.25 or 2.5 mg/kg) 30 min before morphine conditioning abolished the emergence of stress-precipitated morphine CPP (Meng et al. 2014). However, it was not determined whether baclofen would also block the morphine CPP that develops over more pairings regardless of stress pre-exposure. In separate experiments, 2.5 mg/kg baclofen facilitated extinction of morphine CPP in stressed mice, and it prevented forced swim-induced reinstatement of morphine CPP in both stressed and non-stressed mice (Meng et al. 2014).

There are several instances where baclofen was used in conjunction with the $GABA_AR$ agonist muscimol to inhibit neural activity of a specific brain region in a drug addiction paradigm. Baclofen/muscimol injection into the NAc core or shell suppressed context-induced reinstatement of cocaine seeking (Fuchs et al. 2008). Baclofen/muscimol injection into the bed nucleus of the stria terminalis (BNST), a key convergence point of cues and stress, reduced cue and/or yohimbine stress-induced reinstatement of cocaine seeking (Buffalari and See 2011). The LHb encodes aversive and anxiogenic states, and when inactivated by baclofen/muscimol resulted in reduced cue-induced cocaine reinstatement in the presence of yohimbine stress (Gill et al. 2013). Finally, baclofen/muscimol inactivation of BLA or ventral hippocampus prevented stress-potentiated reacquisition of nicotine self-administration (Yu and Sharp 2015). Whether $GABA_BR$ modulation alone in

these brain regions is sufficient to induce these behavioral effects remains to be investigated.

5.6 Summary

So far, preclinical research has revealed a general pattern that $GABA_BR$ agonists and PAMs are capable of (1) attenuating the initial reinforcing/stimulating effect of addictive drugs and blocking certain aspects of drug-induced plasticity; (2) reducing self-administration of drugs on various reinforcement schedules; (3) reducing drug seeking induced by cue, stress, or drug re-exposure; (4) attenuating withdrawal symptoms for opioids. The first three effects have been localized to the VTA, since intra-VTA application of $GABA_BR$ ligands usually recapitulates the effects seen with systemic application. Furthermore, $GABA_BR$-mediated inhibition of DA neurons and resulting decrease in DA release is the principle mechanism of these anti-reward effects. On the other hand, $GABA_BR$ signaling can also mediate some neural processes that contribute to drug addiction, especially in relation to stress and anxiety during drug abstinence. Compared with $GABA_BR$ agonists, $GABA_BR$ PAMs in most cases demonstrated comparable or better performance with less undesirable nonspecific effects. Novel $GABA_BR$ PAMs are continuously being discovered and can be tested in the context of drug addiction. Future studies should also investigate the pharmacokinetics and off-target activity of $GABA_BR$ PAMs, which are critical determinants of their clinical applicability. So far it is not known whether other allosteric binding sites exist on $GABA_BR$ or whether PAM binding affects modulation by auxiliary subunits (KCTDs) (Filip et al. 2015), which also interact with the $GABA_BR2$ subunit (Zuo et al. 2019; Zheng et al. 2019). Mapping of the $GABA_BR$ PAM binding site(s) will allow for in silico screening of new compounds based on homology models, which may help accelerate the drug discovery process.

6 Clinical Studies of $GABA_BR$ Modulators in Drug Addiction

For recent discussions on clinical trials using baclofen or other drugs that act indirectly through the $GABA_BR$ for the treatment of substance abuse, see (Tyacke et al. 2010; Brebner et al. 2002; Phillips and Reed 2014; Agabio and Colombo 2015). To date, baclofen is the only $GABA_BR$ ligand for which clinical data exist on drug addiction treatment, although human studies using the $GABA_BR$ PAM ADX 71441 developed by Addex Therapeutics (Kalinichev et al. 2017) for treating cocaine use disorder have recently been funded by the NIH. In 2018 baclofen was authorized for marketing as a treatment for alcohol use disorder in France although

its effectiveness and safety have been questioned by a number of clinical studies (Braillon et al. 2020; Palpacuer et al. 2018; Chaignot et al. 2018). With the encouraging preclinical results discussed earlier, in the coming years we should expect to see more $GABA_BR$ PAMs in clinical studies.

Baclofen has been most extensively tested for cocaine use disorders. An open label study in Los Angeles on 10 patients found that 20 mg baclofen given three times per day (20 mg t.i.d.) decreased cocaine craving and use (Ling et al. 1998). A subsequent randomized placebo-controlled trial by the same group however found no significant effect of baclofen (20 mg t.i.d.) on cocaine craving although cocaine use was reduced as confirmed by urine testing (Shoptaw et al. 2003). On the other hand, in a few other studies cocaine craving in cocaine-dependent individuals was reported to be suppressed by baclofen (60 mg per day) (Kaplan et al. 2004; Haney et al. 2006). In addition, an fMRI study on cocaine-dependent individuals demonstrated a baclofen-induced suppression of neural activation by subliminal cocaine cues, but not sexual or aversive cues (Young et al. 2014). This provides a potential mechanism for baclofen's inhibition of cocaine craving. Interestingly, the subjective "high" of cocaine was consistently found to be unaffected by baclofen (Rotheram-Fuller et al. 2007; Haney et al. 2006; Lile et al. 2004; Ling et al. 1998). Discrepant results were reported concerning baclofen modulation of the cardiovascular effects of cocaine. While one study found a high dose of baclofen (60 mg) attenuated cocaine (50 mg)-induced increase in heart rate (Haney et al. 2006), another study showed that baclofen plus amantadine (30 mg and 100 mg t.i.d.) did not impact heart rate or blood pressure changes with 20 and 40 mg cocaine (Rotheram-Fuller et al. 2007). Yet another study reported only an increase in systolic blood pressure with 45 mg cocaine, which was not affected by up to 30 mg of baclofen (Lile et al. 2004).

A multi-center double-blind study involving 160 individuals with severe cocaine dependence tested the efficacy of an 8-week treatment with baclofen (60 mg/day) on cocaine use (Kahn et al. 2009). Unfortunately, there was no difference between baclofen and placebo groups in cocaine use (self-reported and urine test confirmed) or craving. While the severity of cocaine dependence may have contributed to the negative result, it has also been suggested that baclofen is more likely to help prevent relapse than to initiate abstinence (Phillips and Reed 2014; Tyacke et al. 2010; Brebner et al. 2002). Indeed, as discussed above, baclofen does not alter the subjective experience in taking cocaine, but rather reduces craving during abstinence. In addition, the 60 mg/day dose that was recommended for treating spasticity was well tolerated across aforementioned studies and may need to be gradually augmented to achieve better effects on addiction treatment. In line with this, a randomized double-blind trial of baclofen (20 mg t.i.d.) on methamphetamine dependent individuals revealed a significant treatment effect only among those who reported taking a higher percentage of baclofen (Heinzerling et al. 2006).

The acute effect of baclofen (20 mg) on cigarette smoking has been studied on smokers using a double-blind within-subject design (Cousins et al. 2001). In the 3-h ad libitum smoking period following drug administration, the number of cigarettes smoked and nicotine craving were not changed by baclofen. Baclofen did however decrease liking of cigarette smoking and produced a sedative/relaxing feeling. It

would be of interest to see whether this acute change in subjective experience would have long-term impact if baclofen is repeatedly paired with smoking. A more recent double-blind placebo-controlled trial examined the effects of prolonged baclofen treatment (9 weeks) on cigarette smoking (Franklin et al. 2009). Here a higher dose of baclofen was used, i.e. 80 mg per day in 20 mg doses, which was titrated up over 12 days. Baclofen was significantly more effective in reducing the number of cigarettes smoked per day although a decline in smoking over the 9 weeks was seen in the placebo group as well. Importantly, baclofen did not produce more side effects than placebo, except mild sedation. The authors attributed this lack of side effects to the gradual increase of baclofen dose at the beginning of the study. These findings are very encouraging and suggest that baclofen could potentially be used for smoking relapse prevention. Future studies should also monitor cigarette consumption after cessation of baclofen treatment, as it has been reported that abrupt discontinuation of baclofen can produce withdrawal symptoms and relapse into cocaine use (Kaplan et al. 2004).

There are very few clinical studies so far evaluating baclofen's effect on opioid dependence and withdrawal, possibly due to the availability of methadone and buprenorphine as treatment options. One randomized double-blind clinical trial compared the effects of baclofen (40 mg/day) and alpha-2A adrenergic receptor agonist clonidine (0.8 mg/day) on opiate addicts undergoing detoxification, and showed that baclofen was more effective in treating both the physical and mental symptoms of withdrawal (Akhondzadeh et al. 2000). The side effects and retention rate for the two drugs were similar while clonidine generated more problems relating to hypotension (Ahmadi-Abhari et al. 2001). These studies suggest that baclofen may serve as a better detoxification medication than clonidine. However, the lack of placebo control in this trial prevented a direct evaluation of baclofen's intrinsic effects. Subsequently, the same group conducted a 12-week long double-blind placebo-controlled trial to evaluate the effect of 60 mg/day baclofen on opioid withdrawal (Assadi et al. 2003). It was found that retention rate was significantly higher for the baclofen group, and that baclofen treatment was significantly better than placebo in managing withdrawal and depressive symptoms. However, the rates of opioid-positive urine did not differ between the groups. One drawback of the present studies is the high dropout rate during the trial. In the future, larger clinical trials should be conducted to help validate these findings.

Baclofen generally does not produce subjective effects indicative of abuse potential in humans. However, in cannabis users trained to discriminate THC from placebo, baclofen alone (50 mg) was able to substitute for the THC discriminative stimulus (Lile et al. 2012). This suggests that baclofen may help relieve some symptoms associated with cannabis abstinence, such as craving and anxiety. Future studies should evaluate the potential of baclofen and other $GABA_BR$ ligands in treating cannabis use disorders. Thus far, one study focusing on daily marijuana smokers found that baclofen (60, 90 mg/day) dose-dependently reduced craving for marijuana and tobacco during active smoking, yet it did not impact mood during abstinence and did not prevent relapse (Haney et al. 2010). Actually, baclofen led to

slight worsening of cognitive performance and early wake-ups during marijuana abstinence.

In summary, clinical studies have demonstrated some preliminary success as well as limitations for baclofen in addiction treatments. Baclofen appears to help ameliorate craving and withdrawal symptoms, and can help prevent relapse especially at high doses. It may best serve as a compliment to existing forms of therapy and medication, instead of a stand-alone treatment. Whether baclofen is also effective in treating multidrug addiction will require more investigation. One limitation of baclofen is its relatively short pharmacokinetic profile, which necessitates multiple (3–4) doses per day. Typical dosage used is 60 mg per day given in three doses although this was based on the recommendation for spasticity and should be adjusted to achieve the best outcome for treating addiction. Many studies start with a lower dose and titrate it up over the course of a few days, and this seems to help reduce side effects. Another GABAergic drug vigabatrin induces a more prolonged increase in GABA levels due to its molecular mechanism as an irreversible inhibitor of GABA transaminase (Tyacke et al. 2010). However, the lack of $GABA_BR$ specificity gives rise to a wide range of side effects. With the development of newer $GABA_BR$ agonists and PAMs and the accumulating evidence that they are comparably or more effective than baclofen in suppressing addiction-like behavior while having less side effects in animal models, more efforts should be put in to push these drugs towards clinical research. In addition, the development of drugs that target signaling molecules downstream of $GABA_BRs$ may also provide options for treating addiction.

7 Conclusions

$GABA_BR$ signaling plays important roles in the reward circuit, and advancing techniques are enabling more in-depth dissection of the nuanced interactions between drugs of abuse and $GABA_BR$ signaling. On the other hand, pharmacological research, both preclinical and clinical, has revealed great potential for $GABA_BR$ agonists and PAMs as a supplemental treatment for drug addiction, mainly in suppressing craving, relapse, and withdrawal symptoms. Further research is needed to find the most efficacious, specific, and practical $GABA_BR$ agent for human use, as well as to understand the potential long-term side effects due to $GABA_BR$ modulation in various parts of the brain.

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$GABA_B$ Receptors and Alcohol Use Disorders: Preclinical Studies

August F. Holtyn and Elise M. Weerts

Contents

Abstract Preclinical research over the past several decades has demonstrated a role for the γ-aminobutyric $acid_B$ ($GABA_B$) receptor in alcohol use disorder (AUD). This chapter offers an examination of preclinical evidence on the role of the $GABA_B$ receptor on alcohol-related behaviors with a particular focus on the $GABA_B$ receptor agonist baclofen, for which effects have been most extensively studied, and positive allosteric modulators (PAMs) of the $GABA_B$ receptor. Studies employing rodent and non-human primate models have shown that activation of the $GABA_B$ receptor can reduce (1) stimulating and rewarding effects of alcohol; (2) signs of alcohol withdrawal in rats made physically dependent on alcohol; (3) acquisition and maintenance of alcohol drinking under a two-bottle alcohol versus water choice procedure; (4) alcohol intake under oral operant self-administration procedures; (5) motivational properties of alcohol measured using extinction and progressive ratio procedures;

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(6) the increase in alcohol intake after a period of alcohol abstinence (the alcohol deprivation effect or ADE); and (7) the ability of alcohol cues and stress to reinstate alcohol seeking when alcohol is no longer available. Baclofen and $GABA_B$ PAMs reduce the abovementioned behaviors across different preclinical models, which provides strong evidence for a significant role of the $GABA_B$ receptor in alcohol-related behaviors and supports development of medications targeting $GABA_B$ receptors for the treatment of AUD. This chapter highlights the value of examining mechanisms of alcohol-related behaviors across multiple animal models to increase the confidence in identification of new therapeutic targets.

Keywords Alcohol · Animal models · Baclofen · GABA · Positive allosteric modulators

1 $GABA_B$ Receptors and Alcohol Use Disorders: Preclinical Studies

Alcohol use disorder (AUD) can be a chronic, relapsing condition with many individuals returning to heavy drinking after detoxification and abstinence. Advances in our understanding of the behavioral, neurobiological, genetic, and environmental mechanisms that perpetuate heavy alcohol use will shed light on the most promising methods to promote sustained reductions in alcohol use. Central to the advancement of our understanding of these mechanisms, and their interactions, is the use of preclinical animal models that capture key aspects of AUD. While it is not possible to model all aspects of the human disorder in animals, models have been developed to study different features related to AUD. Examining these different features separately affords more precise experimental control of alcohol exposure and allows for control over the multiple, confounding influences involved in the development and maintenance of AUD.

In considering animal models, it is important to remember that models are constantly evolving as we learn more about AUD and its diagnosis. For example, the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorder*s (DSM-IV) had two distinct disorders, alcohol abuse and alcohol dependence, with specific diagnostic criteria for each. Diagnosis of alcohol dependence differed from the historical use of the term "dependence," which referred to a physiological adaption to chronic alcohol consumption in which alcohol withdrawal syndrome occurred when alcohol use was discontinued. This differentiation is important as physical dependence is a function of dose and duration of drug exposure, and occurs outside of substance use disorders (e.g., medications for pain, anxiety, or depression). The fifth edition of the DSM (DSM-5) now has a single disorder called AUD with mild, moderate, and severe subclassifications based on the number of diagnostic criteria met. AUD includes a range of drinking patterns and behavioral

components, and spans a continuum of severity. Thus, the use of multiple animal models that capture different features of AUD and encompass this continuum is important.

The present chapter offers an examination of preclinical evidence on the role of the $GABA_B$ receptor in alcohol-related behaviors with a particular focus on $GABA_B$ receptor agonists and positive modulators, for which effects have been most extensively studied. This includes baclofen, a $GABA_B$ receptor agonist that has been studied in rodents and non-human primates, and positive allosteric modulators of $GABA_B$ receptors ($GABA_B$ PAMs) – CGP7930, GS39783, BHF177, rac-BHFF, ADX71441, CMPPE, COR659, and ORM-27669 – studied primarily in rodent models. $GABA_B$ PAMs bind to sites of the $GABA_B$ receptor that are distinct from the binding site of endogenous GABA or direct agonists, such as baclofen. $GABA_B$ PAMs have no or modest intrinsic agonist activity; they activate the $GABA_B$ receptor system only when and where endogenous GABA has been released and can potentiate the action of endogenous GABA (or agonists; Urwyler 2016). The following sections review preclinical evidence featuring baclofen (see Table 1 for study details) and $GABA_B$ PAMs (see Table 2 for study details) and are organized by the procedures utilized.

2 Locomotor Activity

Acutely, alcohol produces a biphasic effect on spontaneous locomotor behavior in rodents, where lower doses stimulate activity and very high doses suppress activity and produce a loss of the righting reflex (i.e., the rodent no longer flips onto its feet when placed on its back) (Pohorecky 1977). Alcohol-induced stimulation of locomotor activity has been used to model the stimulating and euphorigenic effects of alcohol in humans. It has been proposed that a drug that can reduce alcohol-induced stimulation of locomotor activity in rodents may also reduce the euphorigenic effects of alcohol in humans (Wise and Bozarth 1987). In a seminal study, acute intraperitoneal (i.p.) administration of 5 mg/kg baclofen prior to alcohol administration prevented alcohol-induced stimulation of locomotor activity in mice (Cott et al. 1976). This finding was replicated in subsequent studies showing that acute administration of baclofen prior to alcohol prevented increases in locomotor activity induced by low-to-moderate doses of alcohol in several different rat and mouse strains (Arias et al. 2009; Boehm II et al. 2002; Broadbent and Harless 1999; Chester and Cunningham 1999; Holstein et al. 2009a; Humeniuk et al. 1993; Quintanilla et al. 2008; Shen et al. 1998). Acute administration of the $GABA_B$ PAM GS39783 (30 mg/kg, i.p.) also has been shown to suppress locomotor activity induced by acute administration of 2 g/kg alcohol in mice (Kruse et al. 2012).

Table 1 Description of studies examining effects of the $GABA_B$ receptor agonist, baclofen, on alcohol-related behaviors

Reference	Dose and route	Treatment duration	Alcohol-related behavior	Experimental procedure	Alcohol dose	Animal	Effect	Outcome
Cott et al. (1976)	5 mg/kg, i.p.	Acute	Locomotor activity	Locomotor activity induced by acute alcohol	2.4 g/kg, i.p.	Female NMRI mice	Decrease	Locomotor activity (activity counts)
Quintanilla et al. (2008)	R(+)-baclofen: 1, 2, and 3 mg/kg, i.p.	Acute	Locomotor activity	Locomotor activity induced by acute alcohol	0.5 g/kg, i.p.	Male and female UChB rats	Dose-dependent decrease	Locomotor activity (number of square crossings)
Arias et al. (2009)	1, 1.5, and 2.5 mg/kg, s.c.	Acute	Locomotor activity	Locomotor activity induced by acute alcohol	2.5 g/kg, i.g.	Male and female preweanling Sprague-Dawley rats	Decrease at 1 and 2.5 mg/kg	Locomotor activity (number of beam breaks)
Humeniuk et al. (1993)	Baclofen: 0.25, 0.5, and 1 mg/kg, i.p.; (+)-baclofen: 0.5, 1, and 5 mg/kg, i.p.; (−)-baclofen: 0.25, 0.5, 0.75, and 1 mg/kg, i.p.	Acute	Locomotor activity	Locomotor activity induced by acute alcohol	1.75 g/kg, i.p.	Female BALB/c mice	Baclofen: dose-dependent decrease; (+)-baclofen: no change; (−)-baclofen: decrease at 0.75 and 1 mg/kg	Locomotor activity (activity counts)
Shen et al. (1998)	0.625, 1.25, 2.5, and 5 mg/kg, i.p.	Acute	Locomotor activity	Locomotor activity induced by acute alcohol	2 g/kg, i.p. (FAST mice); 1.5 g/kg, i.p. (C57BL/6J, and DBA/2J mice)	Male FAST, C57BL/6J, and DBA/2J mice	Dose-dependent decrease (FAST and DBA/2J mice); no alcohol-induced locomotor activity (C57BL/6J mice)	Locomotor activity (distance traveled)

Broadbent and Harless (1999)	5, 6.25, and 7.5 mg/kg, i.p.	Repeated (injections given before each activity trial)	Locomotor activity	Locomotor activity induced by repeated alcohol (4 activity trials at 48-h intervals)	2 g/kg, i.p.	Male DBA/2J mice	Dose-dependent decrease	Locomotor activity (activity counts)
Boehm II et al. (2002)	0.69, 1.39, and 2.77 μg, i.c.v.	Acute	Locomotor activity	Locomotor activity induced by acute alcohol	2 g/kg, i.p.	Male and female FAST mice	Decrease at 1.39 and 2.77 μg	Locomotor activity (distance traveled)
Boehm II et al. (2002)	0.01 and 0.02 μg, intra-VTA	Acute	Locomotor activity	Locomotor activity induced by acute alcohol	2 g/kg, i.p.	Male FAST and SLOW mice	Dose-dependent decrease (anterior VTA) in FAST mice	Locomotor activity (distance traveled)
Holstein et al. (2009b)	0.625, 1.25, 2.5, and 5 mg/kg, i.p.	Acute	Locomotor activity	Locomotor activity induced by acute alcohol	1 g/kg, i.p.	Male FAST mice	Decrease at 5 mg/kg (FAST-1 mice); no change (FAST-2 mice)	Locomotor activity (distance traveled)
Chester and Cunningham (1999)	2.5, 5, and 7.5 mg/kg, i.p.	Repeated (before each alcohol conditioning session)	Conditioned place preference (CPP) and locomotor activity	Eight conditioning sessions, four with alcohol and four with saline	2 g/kg, i.p.	Male DBA/2J mice	No change in CPP; dose-dependent decrease in locomotor activity	Time on grid floor paired with alcohol and locomotor activity (activity counts)
Bechtholt and Cunningham (2005)	25 and 50 ng, intra-VTA	Acute	Conditioned place preference (CPP)	Eight conditioning sessions, four with alcohol and four with saline	2 g/kg, i.p.	Male DBA/2J mice	Decrease	Time on grid floor paired with alcohol
Colombo et al. (2000)	10, 20, and 40 mg/kg, i.p.	Acute	Alcohol withdrawal	Withdrawal from intragastric alcohol gavage	20% w/v	Male Wistar rats	Dose-dependent decrease	Withdrawal severity score

(continued)

Table 1 (continued)

Reference	Dose and route	Treatment duration	Alcohol-related behavior	Experimental procedure	Alcohol dose	Animal	Effect	Outcome
Humeniuk et al. (1994)	5, 10, and 20 mg/kg, i.p.	Acute	Alcohol withdrawal	Withdrawal from alcohol-containing diet	6% v/v	Female C57BL/6 mice	No change in hypothermia, tremor, and tail arch; increase in convulsive behavior at 10 and 20 mg/kg	Hypothermia, tremor, tail arch, and convulsions
File et al. (1991)	1.25 and 2.5 mg/kg, i.p.	Acute	Alcohol withdrawal	Withdrawal from alcohol-containing diet	10% v/v	Male Lister rats	Decreased anxiogenic withdrawal responses and aggression; decreased tremor at 2.5 mg/kg	Anxiogenic withdrawal responses (time spent in social interaction and in open arms of plus maze), aggression (number of aggressive acts), and tremor
Knapp et al. (2007)	1.25 and 2.5 mg/kg, i.p.	Acute	Alcohol withdrawal	Withdrawal from alcohol-containing diet	7% w/v	Sprague-Dawley rats	Attenuation of decreased social interaction	Time spent in social interaction
Knapp et al. (2007)	1.25 mg/kg, i.p.	Acute (during the third withdrawal)	Alcohol withdrawal	Withdrawal from cycled alcohol-containing diet	4.5% w/v	Sprague-Dawley rats	Attenuation of decreased social interaction	Time spent in social interaction

Knapp et al. (2007)	1.25, 2.5, and 5 mg/kg, i.p.	Repeated (during the first and second withdrawal)	Alcohol withdrawal	Withdrawal from cycled alcohol-containing diet	4.5% w/v	Sprague-Dawley rats	Attenuation of decreased social interaction at 2.5 and 5 mg/kg dose	Time spent in social interaction
Knapp et al. (2007)	1.25, 2.5, and 5 mg/kg, i.p.	Repeated (once daily for 2 days prior to stress sessions)	Alcohol withdrawal	Restraint stress and withdrawal from alcohol-containing diet	4.5% w/v	Sprague-Dawley rats	Attenuation of decreased social interaction at 2.5 and 5 mg/kg doses	Time spent in social interaction
Knapp et al. (2007)	5 mg/kg, i.p.	Repeated (2 injections 5 days apart plus one injection prior to a stress session)	Alcohol withdrawal	Restraint stress and withdrawal from cycled alcohol-containing diet	4.5% w/v	Sprague-Dawley rats	Attenuation of decreased social interaction	Time spent in social interaction
Colombo et al. (2002)	1 and 3 mg/kg, i.p.	Repeated (once daily for 10 consecutive days)	Acquisition of alcohol drinking	Two-bottle alcohol versus water choice (unlimited access)	10% v/v	Male sP rats	Decrease at 3 mg/kg	Alcohol intake (g/kg)
Colombo et al. (2005)	1 mg/kg baclofen, i.p. with and without 0.5 mg/kg naltrexone, i.p.	Repeated (once daily for 10 consecutive days)	Acquisition of alcohol drinking	Two-bottle alcohol versus water choice (unlimited access)	10% v/v	Male sP rats	Decrease at 1 mg/kg baclofen + 0.5 mg/kg naltrexone	Alcohol intake (g/kg)
Smith et al. (1992)	10 mg/kg, i.p.	Repeated (once daily for 10 days; every other day)	Acquisition of alcohol drinking	Two-bottle alcohol versus water choice (limited access; every other day)	2, 4, 6, 8, and 10% v/v (increasing across days)	Male Long-Evans rats	Increase	Alcohol intake (g/kg)

(continued)

Table 1 (continued)

Reference	Dose and route	Treatment duration	Alcohol-related behavior	Experimental procedure	Alcohol dose	Animal	Effect	Outcome
Quintanilla et al. (2008)	R(+)-baclofen: 1, 2, and 3 mg/kg, i.p.	Acute	Alcohol drinking	Two-bottle alcohol versus water choice (limited access)	10% v/v	Female UChB rats	Dose-dependent decrease	Alcohol intake (g/kg)
Quintanilla et al. (2008)	R(+)-baclofen: 1, 2, and 3 mg/kg, i.p.	Repeated (once daily for 4 consecutive days)	Alcohol drinking	Two-bottle alcohol versus water choice (unlimited access)	10% v/v	Male and female UChB rats	Dose-dependent decrease	Alcohol intake (g/kg)
Kemppainen et al. (2012)	3, 10, and 30 ng, microinjections into the VP	Acute	Alcohol drinking	Two-bottle alcohol versus water choice (limited access; every other day)	10% v/v	Male AA rats	Dose-dependent decrease	Alcohol intake (g/kg)
Daoust et al. (1987)	3 mg/kg, i.p.	Repeated (once daily for 14 consecutive days)	Alcohol drinking	Three-bottle alcohol versus water choice (unlimited access)	12% v/v	Male Long-Evans rats	Decrease	Alcohol intake (g/kg)
Boas et al. (2012)	1.25, 2.5, and 5 mg/kg, i.p.	Repeated (once daily for 2 consecutive days)	Alcohol drinking	Three-bottle alcohol versus water choice (unlimited access)	5% and 10% v/v	Male Swiss mice	Decrease at 1.25 mg/kg in "light drinkers"	Alcohol intake (g/kg)

Stromberg (2004)	2.5, 5, and 7.5 mg/kg, i.p. with and without 1.0 mg/kg naltrexone, i.p.	Repeated (once daily for 2 consecutive days alone followed by once daily for 2 consecutive days with naltrexone)	Alcohol drinking	Two-bottle alcohol versus water choice (limited access)	8% v/v	Male Wistar rats	Decrease at 5 mg/kg and at all doses in combination with naltrexone	Alcohol intake (g/kg)
Stromberg (2004)	2.5, 5, and 7.5 mg/kg, i.p.	Repeated (once daily for 4 consecutive days)	Alcohol drinking	Two-bottle alcohol versus water choice (limited access)	8% v/v	Male Wistar rats	Dose-dependent decrease	Alcohol intake (g/kg)
Kasten et al. (2015)	R(+)-baclofen: 10 mg/kg, i.p.; S(−)-baclofen: 10 mg/kg, i.p.	Acute	Alcohol drinking	Two-bottle alcohol versus water choice (unlimited access)	10% v/v	Male and female HAP1 mice	R(+)-baclofen: decrease; S(−)-baclofen: increase	Alcohol intake (g/kg)
Peters et al. (2012)	2.5 mg/kg, i.p.	Acute	Alcohol drinking	Two-bottle alcohol versus water choice (unlimited access)	8% v/v	Male C57BL/6N mice	Decrease in socially stressed and non-socially stressed mice	Alcohol consumed (% of baseline)
Smith et al. (1999)	10 mg/kg, i.p.	Repeated (once daily for 5 days, every other day)	Alcohol drinking	Two-bottle alcohol versus water choice (limited access; every other day)	10% v/v	Male Long-Evans rats	Increase	Alcohol intake (g/kg and volume consumed), preference for alcohol (preference ratio)

(continued)

Table 1 (continued)

Reference	Dose and route	Treatment duration	Alcohol-related behavior	Experimental procedure	Alcohol dose	Animal	Effect	Outcome
Colombo et al. (2000)	2.5, 5, and 10 mg/kg, i.p.	Repeated (once daily for 14 consecutive days)	Alcohol drinking	Two-bottle alcohol versus water choice (unlimited access)	10% v/v	Male sP rats	Dose-dependent decrease	Alcohol intake (g/kg)
Colombo et al. (2004)	0.5 and 1 mg/kg, i.p.	Acute	Alcohol drinking	Morphine and WIN 55,212-2 induced alcohol drinking: two-bottle alcohol versus water choice (unlimited access)	10% v/v	Male sP rats	Dose-dependent decrease	Alcohol intake (g/kg)
Kasten et al. (2015)	R(+)-baclofen: 1, 3, and 10 mg/kg, i.p.; S(−)-baclofen: 1, 3, 10, and 30 mg/kg, i.p.	Acute	Binge-like drinking	Drinking in the dark	20% v/v	Male C57BL/6J mice	R(+)-baclofen: decrease at 10 mg/kg; S(−)-baclofen: increase at 10 mg/kg	Alcohol intake (g/kg)
Moore et al. (2009)	0.01 and 0.02 μg, intra-VTA	Acute	Binge-like drinking	Drinking in the dark	20% v/v	Male C57BL/6J mice	Decrease (anterior VTA)	Alcohol intake (g/kg)
Crabbe et al. (2017)	R(+)-baclofen: 5 and 10 mg/kg, i.p.	Acute	Binge-like drinking	Drinking in the dark	20% v/v	Male and female HDID mice	Dose-dependent decrease	Alcohol intake (g/kg)
Tanchuck et al. (2011)	2.5 or 5 mg/kg, i.p.	Acute	Binge-like drinking	Scheduled high alcohol consumption (SHAC)	5% v/v	Male WSC mice	Dose-dependent decrease	Alcohol intake (g/kg)

Colombo et al. (2003a)	1, 1.7, and 3 mg/kg, i.p.	Acute	Relapse-like drinking	Alcohol deprivation effect (7 days of alcohol deprivation after two-bottle alcohol versus water choice; unlimited access)	10% v/v	Male sP rats	Dose-dependent decrease of ADE	Alcohol intake (g/kg)
Colombo et al. (2006)	1 mg/kg, i.p.	Acute	Relapse-like drinking	Alcohol deprivation effect (14 days of alcohol deprivation after four-bottle alcohol versus water choice; (unlimited access)	10%, 20%, and 30% v/v	Male sP rats	Decrease of ADE	Alcohol intake (g/kg)
Vengeliene et al. (2018)	1 and 3 mg/kg, i.p.	Repeated (five injections across alcohol deprivation and return to alcohol access)	Relapse-like drinking	Alcohol deprivation effect (repeated deprivation periods after four-bottle alcohol versus water choice; (unlimited access)	5%, 10%, and 20% v/v	Male Wistar rats	Decrease at 3 mg/kg	Alcohol intake (g/kg)
Dean et al. (2012)	1 mg/kg baclofen, s.c. with and without 3 mg/kg loperamide, i.g.	Acute	Operant self-administration	FR 2	10% v/v (in 0.04% saccharine)	Male Wistar rats	Decrease with and without loperamide (potentiation by loperamide)	Alcohol self-administration (percent change from baseline)

(continued)

Table 1 (continued)

Reference	Dose and route	Treatment duration	Alcohol-related behavior	Experimental procedure	Alcohol dose	Animal	Effect	Outcome
Anstrom et al. (2003)	1.8, 3.2, and 5.6 mg/kg, i.p.	Acute	Operant self-administration	FR 1	10% v/v	Male Long-Evans rats	Dose-dependent decrease	Alcohol intake (g/kg)
Janak and Michael Gill (2003)	0.3, 1, and 3 mg/kg, i.p.	Acute	Operant self-administration	FR 1	10% v/v	Male Long-Evans rats	Decrease by 1 and 3 mg/kg	Lever presses
Walker and Koob (2007)	0.5, 1, 2, and 4 mg/kg, i.p.	Acute	Operant self-administration	FR 1	10% w/v	Dependent and non-dependent male Wistar rats	Dose-dependent decrease	Number of reinforcers and alcohol intake (g/kg)
Lorrai et al. (2016)	Baclofen: 3 mg/kg, i.p.; R(+)-baclofen: 0.75, 1.5, and 3 mg/kg, i.p.; S(−)-baclofen: 6, 12, and 24 mg/kg; i.p.	Acute	Operant self-administration	Concurrent schedule of alcohol and water reinforcement (FR 4 alcohol lever, FR 1 water lever)	15% v/v	Male sP rats	Baclofen: decrease at 3 mg/kg; R(+)-baclofen: decrease at 1.5 and 3 mg/kg; S(−)-baclofen: no change	Lever presses and alcohol intake (g/kg)
Besheer et al. (2004)	1, 3, 10, and 17 mg/kg, i.p.	Acute	Operant self-administration	Concurrent schedule of alcohol and water reinforcement (FR 1 alcohol lever, FR 1 water lever)	10% v/v	Male C57BL/6J mice	Decrease at 10 and 17 mg/kg	Lever presses on the alcohol lever
Liang et al. (2006)	2 and 3 mg/kg, i.p.	Acute	Operant self-administration	FR 3	10% v/v	Male P rats	Decrease at 3 mg/kg	Lever presses

Maccioni et al. (2005)	1.7 and 3 mg/kg, i.p.	Acute	Operant self-administration	FR 4	15% v/v	Male sP rats	Decrease at 3 mg/kg	Lever presses, alcohol intake (g/kg), and latency to first response
Maccioni et al. (2018)	0.03, 0.1, and 0.3 μg; intra-VTA	Acute	Operant self-administration	FR 4	15% v/v	Male sP rats	Decrease at 0.1 and 0.3 μg (posterior VTA)	Lever presses and alcohol intake (g/kg)
Orrù et al. (2012)	1.25, 2.5, and 5 mg/kg, i.p.	Acute	Operant self-administration	FR 3	9% v/v (alcoholic beer)	Male C57BL/6J mice	Decrease at 5 mg/kg	Lever presses and alcohol intake (g/kg)
Williams et al. (2016)	0.3, 1, and 3 mg/kg; i.p.	Acute	Operant self-administration	Stress (yohimbine)-induced self-administration	10% w/v	Male Wistar rats	Decrease at 1 and 3 mg/kg	Number of reinforcers and alcohol intake (g/kg)
Williams et al. (2016)	0.3, 1, and 3 mg/kg, i.p.	Acute	Operant self-administration	FR 1	10% w/v	Male Wistar rats	Decrease at 3 mg/kg	Number of reinforcers and alcohol intake (g/kg)
Maccioni et al. (2012)	1, 1.7, and 3 mg/kg, i.p.	Acute	Operant self-administration	FR 4	15% v/v	Male P, sP, and AA rats	Decrease at 1.7 and 3 mg/kg (P rats) and decrease at 3 mg/kg (sP and AA rats)	Lever presses and alcohol intake (g/kg)
Maccioni et al. (2012)	1, 1.7, and 3 mg/kg, i.p.	Acute	Operant self-administration (motivation to consume alcohol)	PR	15% v/v	Male P, sP, and AA rats	Decrease at 1.7 and 3 mg/kg (P and sP rats) and decrease at 3 mg/kg (AA rats)	PR breakpoint and cumulative responses

(continued)

Table 1 (continued)

Reference	Dose and route	Treatment duration	Alcohol-related behavior	Experimental procedure	Alcohol dose	Animal	Effect	Outcome
Maccioni et al. (2008b)	1 and 3 mg/kg, i.p.	Acute	Operant self-administration (motivation to consume alcohol)	PR	15% v/v	Male sP rats	Dose-dependent decrease	PR breakpoint and cumulative responses
Walker and Koob (2007)	2 mg/kg, i.p.	Acute	Operant self-administration (motivation to consume alcohol)	PR	10% w/v	Dependent and non-dependent male Wistar rats	Decrease	PR breakpoint and cumulative responses
Colombo et al. (2003b)	1, 2, and 3 mg/kg, i.p.	Acute	Motivation to consume alcohol	Within-session extinction	10% v/v	Male sP rats	Dose-dependent decrease	Extinction responses
Duke et al. (2014)	0.3, 0.56, 1, 1.8, and 2.4 mg/kg; i.m.	Acute	Motivation to consume alcohol	Within-session extinction	4% w/v	Male baboons	Decrease at 1.8 and 2.4 mg/kg	Extinction responses
Maccioni et al. (2008a)	3 mg/kg, i.p.	Acute	Reinstatement of alcohol seeking	Cue-induced alcohol seeking	15% v/v	Male sP rats	Decrease	Lever presses, latency to first response, and response rate
Vengeliene et al. (2018)	1 and 3 mg/kg, i.p.	Acute	Reinstatement of alcohol seeking	Cue-induced alcohol seeking	10% v/v	Male Wistar rats	Dose-dependent decrease	Nose pokes

Tanchuck et al. (2011)	2.5 or 5 mg/kg, i.p.	Acute	Operant seeking and self-administration	Sipper	10% v/v	Male C57BL/6J mice	Seeking: decrease at 5 mg/kg; drinking: no change	Seeking: lever presses; drinking: alcohol intake (g/kg)
Czachowski et al. (2006)	0.3, 1, and 3 mg/kg, i.p.	Repeated (once daily for 3 consecutive days)	Operant seeking and self-administration	Sipper	10% v/v	Male Long-Evans rats	Seeking: decrease at 3 mg/kg; drinking: increase at 1 mg/kg	Seeking: lever presses and latency to first lever press; drinking: alcohol intake (g/kg)
Duke et al. (2014)	0.1, 0.3, 0.56, 1.0, 1.8, and 2.4 mg/kg; i.m.	Acute	Operant seeking and self-administration	Three-component chained schedule	4% w/v	Male baboons	Seeking: no change; drinking: decrease at 1.8 and 2.4 mg/kg	Seeking: lever presses and latency to first lever press; drinking: alcohol intake (g/kg)
Holtyn et al. (2017)	0.1, 0.32, 0.56, 1.0, and 1.8 mg/kg; i.m.	Repeated (once daily for 10 consecutive days across alcohol deprivation and return to alcohol access)	Operant seeking and self-administration	Three-component chained schedule	4% w/v	Male baboons	Seeking and drinking: no change	Seeking: lever presses and latency to first lever press; drinking: alcohol intake (g/kg) and number of drinks in drinking bout

(continued)

Table 1 (continued)

Reference	Dose and route	Treatment duration	Alcohol-related behavior	Experimental procedure	Alcohol dose	Animal	Effect	Outcome
Holtyn et al. (2017)	1.8 mg/kg, i.m.	Repeated (once daily for 5 consecutive days during ongoing alcohol access)	Operant seeking and self-administration	Three-component chained schedule	4% w/v	Male baboons	Seeking: no change; drinking: decrease	Seeking: lever presses and latency to first lever press; drinking: alcohol intake (g/kg) and number of drinks in drinking bout

Drug administration routes: *i.p.* intraperitoneal, *s.c.* subcutaneous, *i.c.v.* intracerebroventricular, *i.g.* intragastric, *i.m.* intramuscular, *VTA* ventral tegmental area, *VP* ventral pallidum; rat and mouse strains: *UChB* University of Chile bibulous, *sP* Sardinian alcohol-preferring, *AA* Alko Alcohol, *HAP1* high-alcohol-preferring 1, *WSC* withdrawal seizure control, *HDID* high drinking in the Dark, *P* Indiana alcohol-preferring; experimental procedures: *ADE* alcohol deprivation effect, *FR* fixed ratio, *PR* progressive ratio

Table 2 Description of studies examining effects of positive allosteric modulators (PAMs) of the GABA$_B$ receptor on alcohol-related behaviors

Reference	Drug	Dose and route	Treatment duration	Alcohol-related behavior	Experimental procedure	Alcohol dose	Animal	Effect	Outcome
Kruse et al. (2012)	GS39783	1, 3, 10, and 30 mg/kg, i.p.	Acute	Locomotor activity	Locomotor activity induced by acute alcohol	2.0 g/kg, i.p.	Male DBA/2J mice	Decrease at 30 mg/kg	Locomotor activity (total distance traveled)
Kruse et al. (2012)	GS39783	30 mg/kg, i.p.	Repeated (once daily for 11 consecutive days prior to alcohol injection)	Locomotor activity	Locomotor activity induced by repeated alcohol	2.5 g/kg, i.p.	Male DBA/2J mice	Increase at 30 mg/kg	Locomotor activity (total distance traveled)
Kruse et al. (2012)	GS39783	30 mg/kg, i.p.	Acute (after sensitization had developed)	Locomotor activity	Locomotor activity induced by repeated alcohol	2.5 g/kg, i.p.	Male DBA/2J mice	No change	Locomotor activity (total distance traveled)
de Miguel et al. (2018)	Rac-BHFF	30 mg/kg, i.p.	Repeated (before each alcohol conditioning session)	Conditioned place preference (CPP)	Eight conditioning sessions, four with alcohol and four with saline	0.5 g/kg, i.p.	Male C57BL/6J mice	No change	Time shifts between post- and pre-conditioning times spent in the alcohol-paired compartment
de Miguel et al. (2018)	ORM-27669	100 mg/kg, i.p.	Repeated (before each alcohol conditioning session)	Conditioned place preference (CPP)	Eight conditioning sessions, four with alcohol and four with saline	0.5 g/kg, i.p.	Male C57BL/6J mice	No change	Time shifts between post- and pre-conditioning times spent in the alcohol-paired compartment

(continued)

Table 2 (continued)

Reference	Drug	Dose and route	Treatment duration	Alcohol-related behavior	Experimental procedure	Alcohol dose	Animal	Effect	Outcome
Orru et al. (2005)	GS39783	6.25, 12.5, and 25 mg/kg, i.g.	Repeated (once daily for 5 consecutive days)	Acquisition of alcohol drinking	Two-bottle alcohol versus water choice (unlimited access)	10% v/v	Male sP rats	Dose-dependent decrease	Alcohol intake (g/kg)
Orru et al. (2005)	CGP7930	25, 50, and 100 mg/kg, i.g.	Repeated (once daily for 5 consecutive days)	Acquisition of alcohol drinking	Two-bottle alcohol versus water choice (unlimited access)	10% v/v	Male sP rats	Dose-dependent decrease	Alcohol intake (g/kg)
Orru et al. (2005)	GS39783	50 and 100 mg/kg, i.g.	Repeated (once daily for 5 consecutive days)	Alcohol drinking	Two-bottle alcohol versus water choice (unlimited access)	10% v/v	Male sP rats	Decrease at 100 mg/kg	Alcohol intake (g/kg)
Loi et al. (2013)	Rac-BHFF	50, 100, and 200 mg/kg, i.g.	Repeated (once daily for 7 consecutive days)	Alcohol drinking	Two-bottle alcohol versus water choice (unlimited access)	10% v/v	Male sP rats	Dose-dependent decrease	Alcohol intake (g/kg)
Orru et al. (2005)	CGP7930	50 and 100 mg/kg, i.g.	Repeated (once daily for 5 consecutive days)	Alcohol drinking	Two-bottle alcohol versus water choice (unlimited access)	10% v/v	Male sP rats	Decrease at 100 mg/kg	Alcohol intake (g/kg)

Hwa et al. (2014)	ADX71441	3, 10, 17 mg/kg, i.g.	Acute	Alcohol drinking	Two-bottle alcohol versus water choice (repeated cycles of limited access)	20% w/v	Male C57BL/6J mice	Decrease at 10 and 17 mg/kg	Alcohol intake (g/kg)
Hwa et al. (2014)	ADX71441	3, 10, 30 mg/kg, i.g.	Acute	Binge-like drinking	Drinking in the dark	20% w/v	Male C57BL/6J mice	Dose-dependent decrease	Alcohol intake (g/kg)
Colombo et al. (2015)	GS39783	25, 50, and 100 mg/kg, i.g.	Acute	Binge-like drinking	Four-bottle alcohol versus water choice (limited and unpredictable access)	10%, 20%, and 30% v/v	Male sP rats	Decrease	Alcohol intake (g/kg)
de Miguel et al. (2018)	Rac-BHFF	30 mg/kg, i.p.	Acute	Binge-like drinking	Drinking in the dark	20% v/v	Male C57BL/6J mice	Decrease	Alcohol intake (g/kg)
de Miguel et al. (2018)	ORM-27669	100 mg/kg, i.p.	Acute	Binge-like drinking	Drinking in the dark	20% v/v	Male C57BL/6J mice	Decrease	Alcohol intake (g/kg)
Linsenbardt and Boehm (2014)	GS39783	30 mg/kg, i.p.	Acute	Binge-like drinking	Drinking in the dark	20% v/v	Male C57BL/6J mice	Decrease	Alcohol intake (g/kg)
Vengeliene et al. (2018)	CMPPE	10 and 30 mg/kg, i.p.	Repeated (five injections across alcohol deprivation and return to alcohol access)	Relapse-like drinking	Alcohol deprivation effect (repeated deprivation periods after four-bottle alcohol versus water choice; unlimited access)	5%, 10%, and 20% v/v	Male Wistar rats	Decrease of ADE	Alcohol intake (g/kg)

(continued)

Table 2 (continued)

Reference	Drug	Dose and route	Treatment duration	Alcohol-related behavior	Experimental procedure	Alcohol dose	Animal	Effect	Outcome
Augier et al. (2017)	ADX71441	1, 3, 10, and 30 mg/kg, i.p.	Acute	Operant self-administration	FR 2–3	20% v/v	Dependent and non-dependent male Wistar rats	Dose-dependent decrease (higher potency in alcohol-dependent rats)	Lever presses and reinforcers earned
Lorrai et al. (2019)	GS39783	25, 50, and 100 mg/kg, i.g.	Acute	Operant self-administration	FR 4	15% v/v	Male and female sP rats	Dose-dependent decrease	Lever presses and alcohol intake (g/kg)
Maccioni et al. (2017)	GS39783	2.5, 5, and 10 mg/kg, i.p.	Acute	Operant self-administration	FR 4	15% v/v	Male sP rats	Decrease at 5 and 10 mg/kg	Lever presses and alcohol intake (g/kg)
Maccioni et al. (2007)	GS39783	25, 50, and 100 mg/kg, i.g.	Acute	Operant self-administration	FR 4	15% v/v	Male sP rats	Dose-dependent decrease	Lever presses and alcohol intake (g/kg)
Maccioni et al. (2015)	GS39783	50 mg/kg, i.g.	Repeated (once daily for 10 consecutive days)	Operant self-administration	FR 4	15% v/v	Male sP rats	Decrease	Lever presses and alcohol intake (g/kg)
Maccioni et al. (2015)	GS39783 and baclofen	5 mg/kg, i.g. (GS39783) and 1 mg/kg, i.p. (baclofen)	Acute	Operant self-administration	FR 4	15% v/v	Male sP rats	Decrease	Lever presses and alcohol intake (g/kg)

Maccioni et al. (2012)	GS39783	25, 50, and 100 mg/kg, i.g.	Acute	Operant self-administration	FR 4	15% v/v	Male P, sP, and AA rats	Decrease at all three doses (P and sP rats) and decrease at 50 and 100 mg/kg (AA rats)	Lever presses and alcohol intake (g/kg)
Maccioni et al. (2010b)	Rac-BHFF	50, 100, and 200 mg/kg, i.g.	Acute	Operant self-administration	FR 4	15% v/v	Male sP rats	Dose-dependent decrease	Lever presses and alcohol intake (g/kg)
Maccioni et al. (2015)	Rac-BHFF	50 mg/kg, i.g.	Repeated (once daily for 5 consecutive days)	Operant self-administration	FR 4	15% v/v	Male sP rats	Decrease	Lever presses and alcohol intake (g/kg)
Maccioni et al. (2015)	Rac-BHFF and baclofen	5 mg/kg, i.g. (rac-BHFF) and 1 mg/kg, i.p. (baclofen)	Acute	Operant self-administration	FR 4	15% v/v	Male sP rats	Decrease	Lever presses and alcohol intake (g/kg)
Liang et al. (2006)	CGP7930	10 and 20 mg/kg, i.p.	Acute	Operant self-administration	FR 3	10% v/v	Male P rats	Decrease at 20 mg/kg	Lever presses
Liang et al. (2006)	CGP7930 and baclofen	10 mg/kg (CGP7930) and 2 mg/kg (baclofen), i.p.	Acute	Operant self-administration	FR 3	10% v/v	Male P rats	Decrease	Lever presses
Maccioni et al. (2018)	CGP7930	5, 10, and 20 μg; intra-VTA	Acute	Operant self-administration	FR 4	15% v/v	Male sP rats	Decrease at 10 and 20 μg	Lever presses and alcohol intake (g/kg)
Maccioni et al. (2009)	BHF177	12.5, 25, and 50 mg/kg, i.g.	Acute	Operant self-administration	FR 4	15% v/v	Male sP rats	Decrease at 25 and 50 mg/kg	Lever presses and alcohol intake (g/kg)

(continued)

Table 2 (continued)

Reference	Drug	Dose and route	Treatment duration	Alcohol-related behavior	Experimental procedure	Alcohol dose	Animal	Effect	Outcome
Orrù et al. (2012)	BHF177	3.75, 7.5, 15, and 30 mg/kg, i.p.	Acute	Operant self-administration	FR 3	9% v/v (alcoholic beer)	Male C57BL/6J mice	Decrease at 15 and 30 mg/kg	Lever presses and alcohol intake (g/kg)
Maccioni et al. (2019a)	COR659	2.5, 5, and 10 mg/kg, i.p.	Repeated (once daily for 10 consecutive days)	Operant self-administration	FR 4	15% v/v	Male sP rats	Decrease at 5 mg/kg and 10 mg/kg	Lever presses and alcohol intake (g/kg)
Maccioni et al. (2017)	COR659	2.5, 5, and 10 mg/kg, i.p.	Acute	Operant self-administration	FR 4	15% v/v	Male sP rats	Decrease at 5 and 10 mg/kg	Lever presses and alcohol intake (g/kg)
Maccioni et al. (2019b)	CMPPE	2.5, 5, and 10 mg/kg, i.p.	Acute	Operant self-administration	FR 5	15% v/v	Female sP rats	Decrease at 5 and 10 mg/kg	Lever presses and alcohol intake (g/kg)
Maccioni et al. (2017)	GS39783	2.5, 5, and 10 mg/kg, i.p.	Acute	Operant self-administration (motivation to consume alcohol)	PR	15% v/v	Male sP rats	No significant change	PR breakpoint and cumulative responses
Maccioni et al. (2008b)	GS39783	25, 50, and 100 mg/kg, i.g.	Acute	Operant self-administration (motivation to consume alcohol)	PR	15% v/v	Male sP rats	Dose-dependent decrease	PR breakpoint and cumulative responses
Augier et al. (2017)	ADX71441	3 and 10 mg/kg, i.p.	Acute	Operant self-administration (motivation to consume alcohol)	PR	20% v/v	Male Wistar rats	Dose-dependent decrease	PR breakpoint

Maccioni et al. (2012)	GS39783	25, 50, and 100 mg/kg, i.g.	Acute	Operant self-administration	PR	15% v/v	Male P, sP, and AA rats	Decrease at all three doses (P and sP rats) and no significant change (AA rats)	PR breakpoint and cumulative responses
Maccioni et al. (2009)	BHF177	12.5, 25, and 50 mg/kg, i.g.	Acute	Operant self-administration (motivation to consume alcohol)	PR	15% v/v	Male sP rats	Decrease at 50 mg/kg	PR breakpoint and cumulative responses
Maccioni et al. (2017)	COR659	2.5, 5, and 10 mg/kg, i.p.	Acute	Operant self-administration (motivation to consume alcohol)	PR	15% v/v	Male sP rats	Decrease at 5 and 10 mg/kg	PR breakpoint and cumulative responses
Maccioni et al. (2019b)	CMPPE	2.5, 5, and 10 mg/kg, i.p.	Acute	Operant self-administration (motivation to consume alcohol)	PR	15% v/v	Female sP rats	Decrease at 5 and 10 mg/kg	PR breakpoint and cumulative responses
Maccioni et al. (2010a)	GS39783	25, 50, and 100 mg/kg, i.g.	Acute	Operant self-administration	Sipper	15% v/v	Male sP rats	Seeking: decrease at 100 mg/kg in response requirement achieved and no significant change in latency; drinking: decrease at all three doses	Seeking: response requirement achieved and latency to first response; drinking: number of licks and alcohol intake (g/kg)

(continued)

Table 2 (continued)

Reference	Drug	Dose and route	Treatment duration	Alcohol-related behavior	Experimental procedure	Alcohol dose	Animal	Effect	Outcome
Maccioni et al. (2019a)	COR659	2.5, 5, and 10 mg/kg, i.p.	Repeated (once daily for 10 consecutive days)	Reinstatement of alcohol seeking	Cue-induced alcohol seeking	15% v/v	Male sP rats	Dose-dependent decrease	Lever presses
Maccioni et al. (2019b)	CMPPE	2.5, 5, and 10 mg/kg, i.p.	Acute	Reinstatement of alcohol seeking	Cue-induced alcohol seeking	15% v/v	Female sP rats	Decrease at 5 and 10 mg/kg	Lever presses
Augier et al. (2017)	ADX71441	3 and 10 mg/kg, i.p.	Acute	Reinstatement of alcohol seeking	Cue- and stress (footshock)-induced alcohol seeking	20% v/v	Male Wistar rats	Dose-dependent decrease	Lever presses
Vengeliene et al. (2018)	CMPPE	10 and 30 mg/kg, i.p.	Acute	Reinstatement of alcohol seeking	Cue-induced alcohol seeking	10% v/v	Male Wistar rats	Dose-dependent decrease	Nose pokes

Drug administration routes: *i.p.* intraperitoneal, *i.g.* intragastric, *VTA* ventral tegmental area; rat strains, *sP* Sardinian alcohol-preferring, *AA* Alko alcohol, *P* Indiana alcohol-preferring; experimental procedures: *ADE* alcohol deprivation effect, *FR* fixed ratio, *PR* progressive ratio

3 Place Conditioning

Place conditioning can be used to study the rewarding and aversive effects of drugs. The place conditioning apparatus used for rodents is typically a 2- or 3-sided chambered compartment with dividers between sides; each side of the box has distinct environmental cues (e.g., floor texture, color). Animals are treated with a drug/dose and placed in one side of the compartment (CS+); vehicle administrations are paired with the other side of the compartment (CS−). After repeated drug-CS+ or vehicle-CS− pairings, the divider is removed and drug-free animals are tested for side preferences. A conditioned place preference (CPP) assesses the rewarding effects of drug administration by measuring increased approach and contact behaviors to the location containing the distinct environmental cues previously paired with the drug and is measured as increased time spent in the drug-paired compartment during the drug-free test.

The effects of activation of the $GABA_B$ receptor on CPP have been mixed. An early study reported that the repeated administration of baclofen (before each conditioning session) did not affect the acquisition of CPP associated with 2 g/kg alcohol in mice (Chester and Cunningham 1999). In a subsequent study, acute microinjection of baclofen into the ventral tegmental area (VTA) prior to preference testing decreased CPP associated with 2 g/kg alcohol in mice (Bechtholt and Cunningham 2005). One study has investigated effects of $GABA_B$ PAMs on CPP using the positive modulators ORM-27669 and rac-BHFF. In that study, the repeated administration of ORM-27669 or rac-BHFF (before each conditioning session) did not significantly change the acquisition of CPP associated with 0.5 g/kg alcohol in mice. The injection-free test session was conducted 48 h after the last conditioning session, which may have contributed to the lack of effects of ORM-27669 and rac-BHFF (de Miguel et al. 2018). Effects of baclofen and $GABA_B$ PAMs on CPP have not been examined in rats.

4 Alcohol Withdrawal Syndrome

Heavy drinkers who abstain from alcohol use may experience alcohol withdrawal, which can include mild to moderate symptoms such as tremors, irritability, or anxiety to more severe symptoms such as delirium tremens, hallucinations, and seizures (Saitz 1998). When animals are exposed to a period of chronic alcohol consumption or administration, followed by a period of abstinence in which access to alcohol is withheld, animals often show signs of withdrawal (e.g., tremors, anxiety-like behavior, seizures, irritability/aggression) that can resemble those observed in humans experiencing alcohol withdrawal (Becker 2000). In rats made physically dependent on alcohol via repeated intragastric alcohol administration, baclofen has been shown to reduce tremors and seizures (Colombo et al. 2000). Baclofen was also effective in the reduction of anxiety-like behaviors and tremors in

rats made physically dependent on alcohol by prolonged exposure to an alcohol-containing diet (File et al. 1991; Knapp et al. 2007). However, in mice made physically dependent by exposure to an alcohol-containing diet, baclofen did not affect tremors or tail arch, and induced convulsive behavior (Humeniuk et al. 1994).

5 Alcohol Drinking

5.1 Acquisition and Maintenance of Alcohol Drinking

A common method to assess alcohol drinking is to give rodents access to alcohol and water concurrently in two separate bottles in their home cages and measure consumption of both fluids. This two-bottle "alcohol versus water" choice regimen allows for measurement of the amount of alcohol that animals will consume voluntarily when alcohol and water are both available. Access to alcohol can be unlimited (i.e., 24 h/day) or limited (i.e., <24 h/day or access on alternating days). Outbred rodents (e.g., Swiss mice, Long-Evans rats, Wister rats) rarely consume sufficient amounts of alcohol to achieve a blood alcohol level (BAL) of 0.08% or more (Leeman et al. 2010). High alcohol intake and BALs exceeding 0.08% can be obtained in inbred mouse (e.g., C57BL/6N; high-alcohol-preferring, HAP mice) and rat (e.g., Sardinian alcohol-preferring, sP; University of Chile bibulous, UChB; Alko Alcohol, AA rats) lines selectively bred for high alcohol drinking, preference, or blood alcohol levels (Crabbe 2008), and such lines have been used extensively to investigate novel pharmacotherapies, often in comparison to non-preferring or low drinking strains.

Several studies have used the two (or more)-bottle "alcohol versus water" choice regimen to investigate effects of baclofen on the acquisition and maintenance of alcohol drinking. Water availability allows for evaluation of whether a change in alcohol intake after administration of a test drug produced a specific effect on alcohol. Some studies (Colombo et al. 2002, 2005) have shown that the repeated administration of baclofen during initial access to alcohol decreased acquisition of alcohol drinking in Sardinian alcohol-preferring (sP) rats given unlimited access to alcohol. During initial access to alcohol, sP rats will typically escalate alcohol intake over several sessions eventually reaching asymptote (Colombo et al. 2002, 2005). However, baclofen increased acquisition of alcohol drinking in another study using outbred Long-Evans rats given limited alcohol access (Smith et al. 1992). In most studies, acute or repeated administration of baclofen reduced ongoing alcohol drinking in several different rat (UChB, AA, sP, Long-Evans, and Wistar rats) and mouse (Swiss, C57BL/6N, and HAP mice) strains (Boas et al. 2012; Colombo et al. 2000, 2004; Daoust et al. 1987; Kasten et al. 2015; Kemppainen et al. 2012; Peters et al. 2012; Quintanilla et al. 2008; Stromberg 2004). In the studies in which baclofen reduced the acquisition or maintenance of alcohol drinking, baclofen either did not change water intake or increased water intake so that total fluid intake remained unchanged, indicating a specific reductive effect on alcohol intake (Boas

et al. 2012; Colombo et al. 2000, 2002, 2004, 2005; Kasten et al. 2015; Kemppainen et al. 2012; Peters et al. 2012; Quintanilla et al. 2008; Stromberg 2004).

A few studies have used the two-bottle "alcohol versus water" choice regimen to investigate effects of $GABA_B$ PAMs on the acquisition and maintenance of alcohol drinking. In those studies, repeated treatment with GS39783 and CGP7930 during initial access to alcohol decreased acquisition of alcohol drinking in alcohol-preferring sP rats (Orru et al. 2005). Repeated administration of GS39783, CGP7930, and rac-BHFF also reduced ongoing alcohol drinking in sP rats; these effects were accompanied by increases in daily water intake, leaving daily total fluid intake unchanged (Loi et al. 2013; Orru et al. 2005). Exposure to repeated cycles of alcohol access and deprivation (i.e., chronic intermittent alcohol access) can generate high levels of voluntary alcohol drinking in rodents (Crabbe et al. 2011; Hwa et al. 2011). Acute administration of the $GABA_B$ PAM ADX71441 reduced alcohol intake, without altering water intake, in alcohol-preferring C57BL/6J mice repeatedly given intermittent alcohol access (Hwa et al. 2014).

5.2 Binge-Like Drinking

Binge drinking (drinking "too much, too fast") is defined by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) as alcohol consumption sufficient to achieve a blood alcohol level of 80 mg/dL (0.08%) or more within a 2–3 h drinking period; this corresponds to consumption of about 0.8–1 g/kg. There are two mouse models of binge-like drinking in which alcohol is voluntarily consumed – "drinking in the dark" (DID) (Rhodes et al. 2005) and "scheduled high alcohol consumption" (SHAC) (Finn et al. 2005) – that have been used to investigate effects of baclofen on alcohol consumption. Under the DID procedure, brief periods of alcohol access (2–4 h/day) are provided at fixed times during the early period of the dark phase of the light/dark cycle. Under the SHAC procedure, water-restricted mice are given daily access to water for periods of fluid availability ranging from 4 to 10 h; every third day, access to alcohol is provided during the initial 30 min of fluid availability followed by access to water during the remainder of the fluid-availability period. Acute administration of baclofen reduced binge-like drinking in both models in several different mouse strains, including C57BL/6J, high DID (HDID), and withdrawal seizure control (WSC) mice (Crabbe et al. 2017; Kasten et al. 2015; Moore et al. 2009; Tanchuck et al. 2011).

A few studies have used the DID procedure to investigate effects of $GABA_B$ PAMs on binge-like drinking. In those studies, acute administration of GS39783, ADX71441, rac-BHFF, and ORM-27669 reduced binge-like drinking in C57BL/6J mice (de Miguel et al. 2018; Hwa et al. 2014; Linsenbardt and Boehm 2014). Acutely administered GS39783 also has been shown to reduce binge-like drinking in sP rats exposed to limited and unpredictable alcohol access during the dark phase of the light/dark cycle. Water and food intake were unchanged (Colombo et al. 2015).

Primate models have been developed that generate high levels of voluntary alcohol intake and can produce binge-like drinking patterns (Baker et al. 2014, 2017; Kaminski et al. 2008; Katner et al. 2004, 2007; Weed et al. 2008; Weerts et al. 2006). However, these models have not been well utilized to investigate effects of baclofen or $GABA_B$ PAMs. Only two studies have examined effects of baclofen using these primate models (Duke et al. 2014; Holtyn et al. 2017; see the "appetitive-consummatory procedures" section for a description of these studies).

5.3 Relapse-Like Drinking

When animals are exposed to a period of alcohol drinking, followed by a period of abstinence in which access to alcohol is withheld (i.e., deprivation), animals often show a transient but marked increase in alcohol-drinking behavior upon return to alcohol access. This phenomenon, termed the "alcohol deprivation effect" or ADE, was first described in rats by Sinclair and Senter under the two-bottle "alcohol versus water" choice regimen (Sinclair and Senter 1968). Since this initial finding, the ADE has been observed in mice (Khisti et al. 2006; Melendez et al. 2006) and non-human primates (Kornet et al. 1990; Weerts et al. 2006) under both two (or more)-bottle "alcohol versus water" choice procedures (Spanagel et al. 1996; Wolffgramm and Heyne 1995) and operant self-administration procedures (Heyser et al. 1997; Hölter et al. 1997). The magnitude of the ADE is a function of the duration of alcohol abstinence in mice, rats, and non-human primates (Middaugh et al. 2000; Rodd-Henricks et al. 2000; Weerts et al. 2006), and in rodents, is augmented with repeated alcohol deprivations (Oster et al. 2006; Rodd et al. 2003). It does not appear that physical dependence is a primary factor in expression of the ADE; animals that show the ADE do not show signs of a withdrawal syndrome upon alcohol abstinence (Heyser et al. 1997), and when withdrawal symptoms are present, the ADE still occurs after symptoms have dissipated (Cicero et al. 1971; Waller et al. 1982).

Four rodent studies have shown treatment with baclofen or the $GABA_B$ PAM CMPPE to reduce ADE under the two-bottle "alcohol versus water" choice regimen. In two studies, acute administration of baclofen upon return to alcohol access after 7 (Colombo et al. 2003a) or 14 (Colombo et al. 2006) days of alcohol abstinence reduced ADE. Baclofen effects were specific to alcohol intake, as food and water intake and spontaneous locomotor activity were unchanged. In the third study, rats were exposed to repeated alcohol deprivations interspersed with long periods of alcohol access. Repeated administration of baclofen (3 mg/kg) or CMPPE (10 and 30 mg/kg) during the final alcohol deprivation and return to alcohol access decreased ADE. The reduction in ADE was accompanied by a decrease in spontaneous locomotor activity for baclofen (suggestive of nonspecific effects), but not CMPPE (Vengeliene et al. 2018).

6 Operant Alcohol Self-Administration

Operant self-administration paradigms are commonly used to study voluntary alcohol intake in laboratory animals. Under this procedure, an animal performs a response, such as pressing a lever, to obtain access to alcohol, and reinforcement is demonstrated when self-administration of alcohol is greater when compared to self-administration of the vehicle (what alcohol is mixed in). Baclofen and $GABA_B$ PAM effects on alcohol self-administration have been investigated using a number of different self-administration procedures (described below), including the fixed and progressive ratio procedures, appetitive-consummatory procedures ("sipper" and chained schedule of alcohol reinforcement procedures), and extinction and reinstatement of alcohol seeking procedures.

6.1 Fixed Ratio (FR) Procedure

Under the prototypical fixed ratio (FR) procedure, a specific number of responses are required to obtain access to alcohol. The response requirement to gain access to alcohol remains unchanged within and across sessions. Several studies using the FR procedure have shown that acute and repeated treatment with baclofen reduced both the number of lever responses for alcohol and amount of self-administered alcohol in several different rat and mouse strains (Anstrom et al. 2003; Besheer et al. 2004; Dean et al. 2012; Janak and Michael Gill 2003; Liang et al. 2006; Lorrai et al. 2016; Maccioni et al. 2005, 2012, 2018; Orrù et al. 2012; Walker and Koob 2007; Williams et al. 2016). Similar findings have been reported with $GABA_B$ PAMs: acute treatment with CGP7930, GS39783, rac-BHFF, BHF177, ADX71441, COR659, and CMPPE (Augier et al. 2017; Liang et al. 2006; Lorrai et al. 2019; Maccioni et al. 2007, 2009, 2010b, 2012, 2017, 2018, 2019b; Orrù et al. 2012) and repeated treatment with GS39783, rac-BHFF, and COR659 (Maccioni et al. 2015, 2019a) reduced both the number of lever responses for alcohol and amount of self-administered alcohol in rodents. Some of the $GABA_B$ PAMs (BHF177, rac-BHFF, and CMPPE) had effects that were selective for alcohol as self-administration of alternative reinforcers (e.g., sucrose or saccharin solutions) were unchanged, while others (COR659 and ADX71441) had non-selective effects (Augier et al. 2017; Maccioni et al. 2017).

Some studies also have investigated effects of combining low doses of $GABA_B$ PAMs and baclofen. In the initial study of CGP7930 (Liang et al. 2006), the combination of low doses of CGP7930 (10 mg/kg, i.p.) and baclofen (2 mg/kg, i.p.) decreased alcohol self-administration in alcohol-preferring Indiana P rats. In the same study, 10 mg/kg CGP7930 administered alone and 2 mg/kg baclofen administered alone were ineffective at reducing self-administration. This finding was extended in subsequent studies showing that "ineffective" doses of GS39783 (5 mg/kg, i.g.) or rac-BHFF (5 mg/kg, i.g.) and baclofen (1 mg/kg, i.p.) reduced

both the number of lever responses for alcohol and amount of self-administered alcohol in rats (Maccioni et al. 2015). Both drug combinations (GS39783 + baclofen and rac-BHFF + baclofen) were selective for alcohol (i.e., sucrose self-administration in a control group did not change after administration of the drug combinations).

6.2 Progressive Ratio (PR) Procedure

Under the within-session progressive ratio (PR) procedure, the number of responses required to obtain access to alcohol is progressively increased after each delivery of alcohol. The "breakpoint," or the lowest ratio not completed, is taken as a measure of the motivational properties of alcohol. Acute administration of baclofen reduced the breakpoint for alcohol and the cumulative (overall) number of responses in several rat strains (Maccioni et al. 2008b, 2012; Walker and Koob 2007). However, treatment with baclofen also tended to reduce self-administration of sucrose or saccharin solutions comparable to the self-administration of alcohol (Anstrom et al. 2003; Colombo et al. 2003b; Janak and Michael Gill 2003; Maccioni et al. 2005, 2008b, 2012). Acute administration of the $GABA_B$ PAMs GS39783, ADX71441, BHF177, COR659, and CMPPE reduced the breakpoint for alcohol in several rat strains, including Wistar, sP, and P rats (Augier et al. 2017; Maccioni et al. 2008b, 2009, 2012, 2017, 2019b). Treatment with GS39783 and BHF177 did not alter breakpoints for a nondrug, sucrose solution, whereas COR659 reduced breakpoints for sucrose.

6.3 Extinction and Reinstatement of Alcohol Seeking Procedures

In the extinction and reinstatement procedures, alcohol seeking is observed under conditions in which access to alcohol has been discontinued. Under the extinction procedure, the cues presented are those that previously had been directly paired with alcohol self-administration. Animals trained to self-administer alcohol will eventually stop responding if access to alcohol is discontinued. The persistence of responding (e.g., the highest number of responses) provides a quantifiable measure of the extent to which stimuli previously associated with alcohol maintain responding in its absence. Acute administration of baclofen decreased lever pressing during extinction of alcohol seeking in rats (Colombo et al. 2003b) and non-human primates (Duke et al. 2014). The ability of a pharmacotherapy to facilitate extinction may be important to the development of medications targeting alcohol cravings and urges to drink.

The reinstatement paradigm measures the ability of an alcohol-associated stimulus (cue-induced reinstatement), a priming dose of alcohol or another drug (drug-

induced reinstatement), or a stressor such as a footshock (stress-induced reinstatement) to reinstate responding (i.e., alcohol seeking) following extinction (i.e., in the absence of alcohol). These procedures are designed to model conditions that can trigger craving and relapse. Two studies in rats showed that acute administration of baclofen prior to presentation of alcohol-associated stimuli reduced reinstatement of alcohol seeking (Maccioni et al. 2008b; Vengeliene et al. 2018). Various $GABA_B$ PAMs have shown efficacy in reducing alcohol- and stress-induced reinstatement of alcohol seeking in sP and Wistar rats. Acute pretreatment with ADX71441 and CMPEE prior to presentation of alcohol-associated stimuli or a stressor (footshock) reduced reinstatement of alcohol seeking (Augier et al. 2017; Maccioni et al. 2019b; Vengeliene et al. 2018), and repeated treatment with COR659 similarly reduced cue-induced reinstatement of alcohol seeking (Maccioni et al. 2019a).

6.4 Appetitive-Consummatory Procedures

The "sipper" procedure and the chained schedule of alcohol reinforcement (CSR) procedure have been developed to separately examine seeking and consumption under conditions of ongoing alcohol availability. In these procedures, seeking is defined as responding that produces the opportunity to consume alcohol. Under the typical sipper procedure, developed by Samson et al. (1998), a specific number of responses (e.g., 30 lever presses) were required to gain access to alcohol. Lever responses in this phase were defined as appetitive alcohol seeking. Once the response requirement was completed, alcohol was freely available for a fixed duration (e.g., 20 min), which encompassed the consummatory phase. In two studies that used the sipper procedure, baclofen reduced alcohol seeking but did not reduce alcohol intake in male C57BL/6J mice and male Long-Evans rats (Czachowski et al. 2006; Tanchuck et al. 2011). One additional study used the "sipper" procedure to examine effects of the $GABA_B$ PAM GS39783 on alcohol seeking and consumption (Maccioni et al. 2010a). In that study, acute treatment with GS39783 decreased both alcohol seeking and consumption in male sP rats. Selectively of effects of GS39783 on alcohol seeking and consumption were not investigated (Maccioni et al. 2010a).

The CSR, developed by Holtyn et al. (2014), Kaminski et al. (2008), and Weerts et al. (2006), consisted of three sequential components – each contained different schedule requirements – that modeled different phases of alcohol anticipation, seeking, and consumption. Fulfilling the schedule requirement in each successive component was necessary to progress to the next component with alcohol available for self-administration only in the final component. The CSR can include fixed ratio and within-session or across-session progressive ratio manipulations. Under the CSR, baboons consume significant amounts of alcohol (~1.0 g/kg per day) to reach BALs exceeding 0.08% (i.e., binge drinking) and maintain this level of consumption 7 days per week for prolonged periods (Holtyn et al. 2014; Kaminski et al. 2008). In the two studies that used the CSR procedure, administration of baclofen during active daily drinking decreased alcohol self-administration

behaviors and total consumption, but did not alter seeking responses (Duke et al. 2014; Holtyn et al. 2017). In contrast, when baclofen treatment was initiated during abstinence, baclofen did not significantly alter alcohol self-administration upon return to alcohol access conditions (Holtyn et al. 2017). Baclofen did, however, facilitate extinction of responses previously reinforced by alcohol or by a non-alcohol reinforcer (Tang, a calorically equivalent, orange-flavored beverage) under within-session extinction conditions, particularly during early extinction (Duke et al. 2014). Non-selective effects of baclofen on the non-alcohol reinforcer (i.e., Tang) and transient side effects (i.e., vomiting, decreased food intake, and lethargy) also were reported at the highest doses tested.

7 Discussion

The present chapter describes and highlights preclinical research on the role of the $GABA_B$ receptor on alcohol-related behaviors. The therapeutic effects of the $GABA_B$ receptor agonist, baclofen, in preclinical animal models of alcohol reinforcement, motivation, and self-administration are well established. Despite the promising results in preclinical models, the use of baclofen in clinical practice may be limited by side effects, such as sedation and motor incoordination (Garbutt 2018). Such effects were also suggested by some preclinical research. For example, doses of baclofen that reduced alcohol self-administration potentiated the sedative effects of alcohol, even at non-sedative doses of alcohol, and also reduced locomotor activity (Besheer et al. 2004), or reduced self-administration of a non-alcoholic reinforcer such as an orange-flavored sweet beverage (Duke et al. 2014; Holtyn et al. 2017). Positive allosteric modulation of the $GABA_B$ receptor produced an effect on alcohol self-administration and consumption similar to that produced by baclofen. A potential advantage of $GABA_B$ PAMs is that they may possess a wider therapeutic window compared to full agonists, such as baclofen. In general, the $GABA_B$ PAMs have been shown to reduce alcohol self-administration at doses lower than those inducing sedation and motor-incoordination.

To examine the selectivity of effects, preclinical studies have compared self-administration and consumption of alcohol versus alternative, non-alcohol reinforcers (e.g., sucrose or saccharin solutions) following treatment with baclofen and $GABA_B$ PAMs. The selectivity of the reducing effect of baclofen on alcohol self-administration and consumption has been shown to be mixed, as treatment with baclofen reduced consumption and self-administration of alternative, non-alcohol reinforcers in some studies (Anstrom et al. 2003; Czachowski et al. 2006; Janak and Michael Gill 2003; Maccioni et al. 2005, 2008b; Tanchuck et al. 2011). The majority of studies examining $GABA_B$ PAMs have shown they selectively reduced alcohol consumption and did not reduce consumption of other non-alcohol reinforcers (Maccioni and Colombo 2019). Both baclofen and $GABA_B$ PAMs may be useful treatments to promote alcohol abstinence and reduce drinking if it occurs, although nonspecific effects of baclofen and some $GABA_B$ PAMs must be considered.

The use of preclinical animal models is critical to understanding the development of AUD and the consequences of chronic alcohol exposure. Furthermore, these models provide valuable tools for the concurrent testing of potential pharmacotherapies for AUD in a variety of paradigms. It is our contention that this concurrent testing will increase the probability of developing a wider spectrum of efficacious pharmacotherapies that can benefit a greater majority of individuals with AUD and help identify the best candidates for advancement for in-human testing. The preclinical studies reviewed in the present chapter have greatly increased our understanding of the role of the $GABA_B$ receptor in the control of alcohol-related behaviors. The demonstration that baclofen and $GABA_B$ PAMs modify alcohol-related behaviors across multiple animal models allows greater confidence in the generality of the findings.

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$GABA_B$ Receptors and Alcohol Use Disorders: Clinical Studies

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Contents

Abstract Harmful alcohol use and alcohol use disorders (AUD) result in major health and community burden worldwide, yet treatment options are limited. Novel pharmacotherapies are urgently required, and treatments involving $GABA_B$ receptors have been used in treating alcohol-related disorders. This chapter will review the clinical evidence of $GABA_B$ pharmacotherapies, such as baclofen and γ-hydroxybutyric acid. This includes the use of these treatments in individuals experiencing alcohol withdrawal symptoms and outlining the outcomes of studies

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of alcohol relapse prevention relapse including case studies, comparative studies and randomised controlled trials. Laboratory research investigating biobehavioural effects of baclofen will also be summarised and polymorphisms associated with baclofen treatment, and safety concerns of $GABA_B$ treatments will be addressed. In summary, pharmacological treatments targeting $GABA_B$ receptors such as baclofen may be modestly effective in the management of alcohol use disorder, but safety concerns limit the widespread applicability of the currently available agents.

Keywords Alcohol use disorder · Alcohol withdrawal · Baclofen · Neuroimaging · Pharmacotherapy · Psychophysiology · Randomised controlled trial · Treatment

1 Introduction

Harmful alcohol use is a pervasive worldwide issue linked to significant health and community burden, accounting for 5.3% of all deaths and a causal factor in over 200 disease and injury conditions (World Health Organization 2018a). Globally, an estimated 6.2% of men and 1.2% of women have alcohol use disorders (AUDs) (World Health Organization 2018a). Diagnostic criteria for AUD have been developed and progressively validated and are included in the International Classification of Diseases (World Health Organization 2018b) and the Diagnostic and Statistical Manual of the American Psychiatric Association (American Psychiatric Association 2013). The cardinal features of AUD are loss of control over alcohol use and continuing use despite evident harms. Other important diagnostic criteria result from neuroadaptation leading to increased tolerance of the effects of alcohol and a characteristic withdrawal syndrome upon cessation of alcohol consumption (American Psychiatric Association 2013). Alcohol-related liver disease is a particularly important consequence of alcohol use (Thursz et al. 2018). Liver disease is the leading cause of alcohol-related mortality (Asrani et al. 2019), and, conversely, alcohol is the leading cause of advanced liver disease worldwide (Rehm et al. 2013). Liver disease complicates the pharmacological management of alcohol use disorder (AUD) because drug metabolism may be impaired in the presence of liver failure.

While alcohol use disorders are leading causes of preventable death, treatment options are still limited. Currently, there are few pharmacological treatments specifically indicated for alcohol dependence in Europe, the USA and Australia, including acamprosate, naltrexone, nalmefene and disulfiram. These agents have been extensively evaluated in a large number of double-blind, randomised controlled trials (RCTs), a study design that randomly assigns participants to receive either a placebo (or active placebo) or treatment, and considered the "gold-standard" research design for evaluating treatment efficacy. RCTs of these agents have generally demonstrated modest effect sizes for use in treatment of AUD (Johnson 2008), and treatment

uptake in the community is low (Morley et al. 2016). There is thus an urgent need for the development of novel agents for the treatment of alcohol use disorder that are both more effective and more appealing to the target population.

2 Overview of Pharmacotherapies Modulating the GABAB Receptor

A number of current therapeutic drugs influence $GABA_A$ transmission including pregabalin, gabapentin and the benzodiazepines, but few act principally on $GABA_B$ receptors, the focus of this review. Accordingly, we restrict this review of $GABA_B$ receptor treatments for alcohol use disorders to agents that directly act upon the $GABA_B$ receptor such as baclofen and γ-hydroxybutyric acid.

2.1 Baclofen

Baclofen is a selective $GABA_B$ receptor agonist that is emerging as a potential treatment for alcohol dependence. Baclofen has minimal liver metabolism (~10–15%), and few reported hepatic side effects (Addolorato et al. 2007), making this drug of particular interest amongst those with alcohol-related liver disease. Chronic alcohol use results in a downregulation of GABA receptor activity and disinhibition of the dopaminergic pathway. $GABA_B$ receptors are expressed on dopamine, and GABA neurons and preclinical studies have demonstrated that baclofen can diminish self-administration of alcohol, maintenance and reinstatement of alcohol-drinking behaviour (Agabio and Colombo 2014), possibly due to inhibition of the mesolimbic reward system.

There has been expanded utilisation of baclofen in the treatment of alcohol dependence, particularly in Europe. Prescriptions for baclofen significantly increased between 2007 and 2013 with a large proportion being initiated in primary care (Chaignot et al. 2015). The provision of a temporary recommendation for baclofen to be prescribed to alcohol-dependent patients was granted in France in 2014, and further approval was granted in October 2018 for use of baclofen in alcohol-dependent patients up to a dose of 80 mg/day (Rolland et al. 2019). Nonetheless, there is ongoing controversy in the field, and, among the few high-quality trials in the literature, the results are mixed. The superiority of baclofen over placebo is yet to be established (Agabio et al. 2018), and baclofen has been recommended as a second-line treatment for alcohol use disorders for individuals who do not respond to other treatments, though it can be an effective first-line treatment for those with contraindications to currently approved medications.

2.2 γ-Hydroxybutyric Acid

Sodium oxybate is the sodium salt of γ-hydroxybutyric acid (GHB). It has been applied as a treatment for AUD since the early 1990s, particularly in European countries such as Italy and Austria (Keating 2014). GHB is a short-chain fatty acid structurally similar to GABA and is a weak agonist at the $GABA_B$ receptor (Sewell and Petrakis 2010). It may be converted to GABA leading to direct activation of $GABA_A$ and $GABA_B$ receptors, leading to sedative and anxiolytic manifestations that also resemble alcohol-like effects (Bay et al. 2014). GHB is also a potent agonist at the excitatory GHB receptor (Cash et al. 1999). It is likely that GABAergic activity mediates the therapeutic effect of GHB on withdrawal and relapse in AUD (Sewell and Petrakis 2010). As alcohol withdrawal results from reduced GABAergic activity in the central nervous system, exogenous GHB may ameliorate alcohol withdrawal through both its conversion to GABA and indirect activation of $GABA_A$ receptors. GHB's efficacy in relapse prevention may be as an effective agonist treatment, as it affects the $GABA_A$ receptor in a similar fashion to alcohol. This effect is analogous to the use of methadone for treatment of opioid dependence.

GHB can have a biphasic response upon dopamine release according to dose concentration, which can increase the risk of overdose when GHB is used as a treatment for AUD. At low concentrations of GHB, dopamine is released following GHB receptor stimulation. However, dopamine release is inhibited by higher doses of GHB that stimulate $GABA_B$ receptors (Caputo et al. 2009). This therefore produces a biphasic response, whereby lower doses of GHB cause euphoria, but higher doses may lead to deep sedation and potentially fatal overdose (Keating 2014). Consequently, abuse and toxicity limit the potential for therapeutic use of GHB.

One systematic review evaluating the use of GHB in the management of alcohol withdrawal syndrome and also for relapse prevention (Leone et al. 2010) was inconclusive regarding GHB's efficacy over placebo or other pharmacological treatments specifically for relapse prevention in AUD, due to an insufficient number of RCTs. Recently, an expert group of European alcohol researchers and clinicians evaluated the data for GHB in the treatment of AUD, and secondary analyses indicated GHB is effective in alcohol-dependent patients with very high-risk drinking (van den Brink et al. 2018). Nonetheless, these analyses were post hoc, and only a small number of patients were included with a wide study duration range (3–12 months).

3 Treatment for Alcohol Withdrawal Syndrome

Heavy drinkers that suddenly reduce their alcohol consumption or abstain altogether may consequently experience symptoms of alcohol withdrawal. These symptoms may range from mild to moderate effects, including tremors, anxiety, irritability and agitation, to more severe effects including hallucinations, delirium tremens and

seizures (Fiellin et al. 2002). Alcohol withdrawal syndrome (AWS), which comprises a cluster of these symptoms occurring in individuals with alcohol use disorder, can occur after decreased chronic or heavy alcohol consumption or from cessation. Resultant experienced physiological manifestations can be mild to moderate (e.g. tremor, paroxysmal sweats, fever), but more severe presentations of symptoms such as seizures and delirium can have serious outcomes, including death (Connor et al. 2016). As several of these symptoms involve disruption of several neurotransmitter systems, including inhibitory systems such as GABA, treatments for AWS aim to target these systems to reduce these symptoms.

While benzodiazepines remain the preferred treatment for AWS, their use is limited by abuse liability, tolerance, lack of efficacy in severe cases and complications related to sedation (Haber et al. 2009). Other pharmacotherapies for treating alcohol withdrawal symptoms affecting $GABA_B$ receptors have been investigated, such as baclofen, and the evidence of baclofen for treating AWS will be covered briefly. Baclofen was initially used for treatment of AWS in alcohol-dependent patients in two case report studies (a detailed examination of one or a small set of patients administered a treatment) by the same research group (Addolorato et al. 2002b, 2003). In the first study (Addolorato et al. 2002b), a relatively low dose of baclofen (10 mg day t.i.d.) was administered to five patients, who demonstrated a rapid reduction of severity of AWS symptoms, as assessed using the Clinical Institute Withdrawal Assessment for Alcohol-revised scale (CIWA-Ar) (Sullivan et al. 1989). These patients maintained abstinence with continued baclofen treatment for a subsequent 30 days, with no reported major side effects. Another single case study of a patient (Addolorato et al. 2003) presenting with severe AWS who was administered the comparatively high initial dose of baclofen (75 mg/day) demonstrated significantly decreased AWS severity within 1 h of initial administration, and after stabilisation the patient was abstinent for 30 days with a resultant lower baclofen dose (30 mg/day) with no reported side effects.

Comparative studies that compare the target treatment versus an active "gold-standard" treatment have evaluated baclofen versus benzodiazepines – considered the gold-standard comparator for AWS treatment. Addolorato et al. (2006) found that patients receiving either oral baclofen (30 mg/day for 10 days) or diazepam (0.55–0.75 mg/kg/day for 6 consecutive days, with 25% tapered dose daily for days 7–10), experienced similar reductions in AWS severity, reflected by decreased CIWA-Ar scores. Reddy et al. (2014) compared progressive reduction in baclofen doses (30 mg/day t.i.d. reducing to 10 mg/day) versus chlordiazepoxide (75 mg/day reducing to 25 mg/day) over 9 days and found both treatments decreased CIWA-Ar scores, but baclofen was the less effective treatment. Recently, Gulati et al. (2019) found comparable efficacy for baclofen (10 mg/day t.i.d.) and lorazepam (8–12 mg/day t.i.d.) in an open-label study. However, in open-label study designs, the treatment received is known to patients and researchers; this may lead to biased outcomes as compared to blinded designs such as RCTs.

There have been two RCTs assessing the efficacy of baclofen for treating AWS compared to placebo, including usual care for symptom-triggered benzodiazepine treatment. In the first study (Lyon et al. 2011), patients with moderate AWS

symptoms were randomised for a complete 3-day course of oral baclofen ($n = 18$, 30 mg/day t.i.d.) or placebo ($n = 13$), and baclofen treatment was associated with significantly reduced lorazepam use for management of AWS. In a larger study by Heppe et al. (2019) of 101 patients admitted for AWS, the same baclofen dose regimen ($n = 50$, 30 mg/day t.i.d.) compared to placebo ($n = 51$) was administered for 72 h. Baclofen did not significantly reduce progression of moderate of severe AWS in these patients, although there was a trend of baclofen reducing benzodiazepine use.

Taken together, while there is some evidence that baclofen may be effective in reducing alcohol withdrawal symptoms, it does not have clear superiority over benzodiazepines as a preferred pharmacotherapy for AWS. Two recent literature reviews similarly concluded that the evidence was insufficient to justify routine use of baclofen for AWS (Cooney et al. 2019; Liu and Wang 2019).

4 Relapse Prevention in Alcohol Use Disorders

Prevention of relapse is a major goal of pharmacotherapy of severe alcohol use disorders (Freyer et al. 2016). Relapse involves resumption of heavy alcohol consumption after a prolonged period of abstinence, and vulnerability to relapse is clinically commonly associated with intense cravings desires to drink that are often provoked by drinking-related cues (Becker 2008). It is a major obstacle to treatment efforts, and patterns of fluctuating remission and relapse are common in individuals in AUD treatment. Evidence from clinical studies of baclofen has been reported here. Baclofen has been extensively evaluated as a potential pharmacotherapy for relapse prevention in severe alcohol use disorder, with several case studies, open-label studies and RCTs. Additionally, there have been multiple meta-analyses evaluating the efficacy of baclofen (Bschor et al. 2018; Lesouef et al. 2014; Pierce et al. 2018; Rose and Jones 2018). Despite this body of work, the evidence is still equivocal regarding baclofen's superiority over placebo for relapse prevention, as presented in a recent consensus statement by leading researchers investigating baclofen (Agabio et al. 2018). Low-to-moderate baclofen doses are defined here as $\leq$60 mg/day, with high doses >60 mg/day (Pierce et al. 2018) – the latter including very high doses of more than 300 mg/day (de Beaurepaire 2014).

4.1 Baclofen: Low-to-Moderate Doses

With regard to low-to-moderate dosing regimens, the initial RCT investigating baclofen as a treatment of alcohol dependence was conducted by Addolorato et al. (2002a), which demonstrated significantly higher rates of abstinence and reduced alcohol intake after 4 weeks of baclofen (30 mg/day t.i.d.) treatment relative to placebo in 39 Italian outpatients. In a subsequent double-blinded RCT (where both

participants and researchers are blinded to treatment conditions) by the same research group (Addolorato et al. 2011), a beneficial dose-response effect of baclofen (30 mg/day versus 60 mg/day) was found, through secondary analyses, in reducing the number of drinks per drinking day after 12 weeks compared to placebo, with no reported significant differences for several alcohol dependence measures. An Australian multisite double-blinded RCT by Morley et al. (2018a) of 104 outpatients, with or without liver disease, demonstrated a greater time to lapse and relapse for baclofen-treated patients (30 mg/day or 75 mg/day) and a significant treatment effect for days abstinent compared to placebo.

Conversely, findings from a double-blinded RCT in the USA (Garbutt et al. 2010) found no beneficial effect of baclofen (30 mg/day) versus placebo in 80 alcohol-dependent patients randomised to 12 weeks of treatment. No significant effects were seen for any reported drinking outcomes, although baclofen significantly reduced subjective craving across the trial and reduced symptoms of anxiety. Similarly, Ponizovsky et al. (2015) also did not observe any beneficial treatment effect of baclofen during a 12-week RCT with a moderate dose (50 mg/day) in 64 alcohol-dependent patients. Factors that may account for the disparity in these studies' findings (Leggio et al. 2010) have included the relatively lower severity of AUD in the USA study, coupled with a high placebo response for both studies (Garbutt et al. 2010; Ponizovsky et al. 2015), as compared to studies demonstrating significant baclofen treatment effects. Indeed, a recent meta-analysis showed that baclofen was more effective for high drinking levels (Pierce et al. 2018), and similar effect was found in the Australian baclofen trial (Rombouts et al. 2019), suggesting that baclofen may have a significant beneficial effect in severe AUD cases only.

4.2 Baclofen: High-Doses

The use of high-dose baclofen was advocated by an influential anecdotal report published by French cardiologist Olivier Ameisen (Ameisen 2004). This led to widespread use of this medication, particularly in France. The first double-blinded RCT of high-dose baclofen (Müller et al. 2015) was conducted in a sample of German alcohol-dependent patients ($N = 56$) administered individually titrated baclofen doses (mean dose of 180 mg/day) versus placebo. There was a strong positive treatment effect on abstinence rates, with a number needed to treat of 2.3. Beraha et al. (2016) randomised 151 patients to high-dose baclofen (up to 150 mg/day), low dose (30 mg/day) or placebo and found no beneficial treatment effect of baclofen for time to first relapse or rates of abstinence. However, in post hoc analyses, this group did report that higher doses were associated with a longer first relapse. A long-term maintenance RCT of 180 mg/day in 320 patients by Reynaud et al. (2017) found only a trend for a positive baclofen effect versus placebo at 6-month follow-up in reducing alcohol consumption, although they reported a significant effect of baclofen to reduce craving.

4.3 Specific Populations

The effectiveness of baclofen in treating AUD may be reliant on specific patient characteristics increasing the probability of a favourable applicability of baclofen – that is, a personalised approach to pharmacotherapy in these patients. One potential reason of baclofen's differential effects on different populations may be related to greater depletion of brain GABA+ levels in specific populations, such as those with ALD, which may predicate a more effective baclofen treatment response (Morley et al. 2018b). Here, we briefly cover treatment effects of baclofen investigated in individuals with AUD and comorbid issues, including liver disease and mood disorders such as anxiety.

4.3.1 Alcohol-Related Liver Disease

Liver disease associated with alcohol is a major consequence of chronic alcohol consumption with a dose-response relationship, which can be exacerbated by conditions such as hepatitis C, with chronic infections of the hepatitis C virus leading to both acute and long-term liver damage (Goldberg et al. 2017). Current approved treatments for alcohol use disorders (e.g. disulfiram, naltrexone, nalmefene) are not suitable for patients with liver disease as they are contraindicated due to metabolism by the liver, whereas baclofen is processed primarily through the kidneys with low liver metabolism (about 15%) (Davidoff 1985). Baclofen is thus an ideal candidate for treating alcohol use disorder in patients with liver disease, and its effectiveness has been investigated in this subgroup. An RCT by Addolorato et al. (2007) demonstrated clear treatment effects of baclofen in reducing drinking outcomes specifically in Italian patients with cirrhosis ($N = 84$) in a 12-week trial, whereby patients receiving baclofen (30 mg/day t.i.d) had higher rates of abstinence and significantly reduced craving compared to placebo. This is contrasted by findings of RCT of USA military veterans (Hauser et al. 2017) with comorbid chronic hepatitis C and AUD, which found that a low dose of baclofen (30 mg/day) had no effect on days abstinent or reducing alcohol use during a 12-week trial compared to placebo. However, this sample was largely male (96.3% of sample) and had relatively low levels of baseline consumption. Most recently, Morley et al. (2018a) have confirmed the effectiveness of baclofen for treatment of AUD with high drinking levels and comorbid liver disease, and this study is the first replication of the influential findings of Addolorato et al. (2007). Furthermore, using magnetic resonance spectroscopy imaging techniques, lower cortical levels of GABA were revealed in alcohol-dependent patients with liver disease, and this suggests that a $GABA_B$ agonist may restore GABA transmission in this setting (Morley et al. 2018b).

4.3.2 Comorbid Anxiety

Morley et al. (2014) have previously reported no beneficial effect of baclofen (30 mg/day, 60 mg/day) in an RCT in 42 alcohol-dependent patients in Australia. No treatment effect was found for time to lapse or time to relapse, although a beneficial treatment effect of baclofen to reduce alcohol consumption was found in post hoc analyses in patients with a comorbid anxiety disorder. Further work by the same group (Rombouts et al. 2019) analysing a subsequent larger trial (Morley et al. 2018a) failed to observe that anxiety was a significant predictor of baclofen to reduce alcohol consumption. Garbutt et al. (2010) found that baclofen reduced anxiety levels during treatment of AUD, although this sample was not diagnosed for comorbid anxiety and did not find evidence of reduced drinking.

5 Meta-Analyses Assessing Baclofen's Efficacy

The efficacy of baclofen in treating relapse in AUD is therefore unclear. This has been further indicated through numerous meta-analyses conducted evaluating the efficacy of baclofen, with multiple meta-analyses published in 2018 alone. The inconsistency of findings between the individual trials can be partially explained by differing criteria used for study inclusion, such as the subpopulations assessed, and the reported outcomes. An earlier meta-analysis focused on RCTs using low baclofen doses of 30 mg/day (Lesouef et al. 2014) and reported that baclofen treatment had a significant effect on rates of abstinence but was limited by only including studies administering a relatively low baclofen dose. More recently, one meta-analysis determined that baclofen was associated with higher abstinence rates in AUD samples versus placebo, but not with other drinking outcomes of increased abstinence days or reduced heavy drinking days (Rose and Jones 2018). Alternatively, the largest meta-analysis identified a modest positive effect superior to placebo when considering noncomplicated AUD samples. Yet, baclofen's overall superior clinical utility was not clearly established, partly due to the significant heterogeneity among the RCTs (Bschor et al. 2018). Moreover, another meta-analysis examining alcohol-dependent samples indicates a dose-specific effect with efficacy in low doses (<60 mg/day) for achieving abstinence, but no benefit in higher doses (Pierce et al. 2018). Finally, benefit for those with high drinking levels was identified by Pierce et al. (2018), an important finding that helps explain numerous inconsistencies in the literature. In summary, it is apparent that there are mixed findings from double-blinded RCTs evaluating baclofen as a pharmacotherapy for AUD, and a consensus regarding superiority compared to placebo for treatment of AUD has not been reached.

6 Laboratory Studies Evaluating Craving and Biobehavioural Effects of Baclofen

While several studies have evaluated baclofen in treating AUD, relatively few studies have investigated the biobehavioural effects of baclofen by employing psychophysiological techniques such as neuroimaging. Assessing these effects using psychophysiological techniques may provide indications to how baclofen may reduce drinking outcomes through a deeper understanding of how baclofen acts on the central nervous system in humans. This can be identified more directly with neuroimaging techniques, comparatively indirectly through measuring peripheral measures of response as well as the interaction baclofen may have with alcohol. Moreover, as baclofen is assumed to reduce the subjective craving for alcohol, demonstrating this using controlled laboratory techniques can elucidate the biobehavioural mechanisms through which baclofen may attenuate these cravings.

An early study assessed acute dose effects of baclofen and in combination with alcohol in a non-treatment seeking heavy drinking sample using doses containing 40 mg and 80 mg and no baclofen (Evans and Bisaga 2009). Overall, baclofen did not affect subjective craving, and only modest increases in cardiovascular indices of heart rate and blood pressure were observed. Both baclofen and alcohol did increase sedation and impair cognitive performance, but there was no marked increase observed in combination.

Another laboratory study observed that baclofen-treated participants, relative to placebo, demonstrated reduced overall alcohol consumption for 2 days prior to, and during, an alcohol administration task after a 7-day trial (Leggio et al. 2013). Additionally, increased arterial blood pressure and salivation were seen during an alcohol cue reactivity task, but no medication effect for subjective and physiological responses to alcohol cues (Leggio et al. 2013). This same group has reported a lack of anti-craving or anti-reinforcing effect following baclofen administration given increased feelings of "high" and intoxication during an alcohol administration task (Farokhnia et al. 2017). Baclofen-treated participants also displayed lower heart rate during alcohol priming and administration, but few physiological differences were reported during alcohol cue reactivity (Farokhnia et al. 2017), and the doses for these two studies were relatively low (30 mg/day).

Follow-up analyses of the parent study (Farokhnia et al. 2017) found that the interindividual variability in the pharmacokinetics of baclofen greatly influenced biobehavioural outcomes, with a maximum baclofen concentration negatively correlating with cue-elicited reported alcohol craving and alcohol-induced alcohol-liking ratings (Farokhnia et al. 2018a). Moreover, when assessing baclofen effects upon feeding and stress-related neuroendocrine responses, participants in the baclofen group had higher levels of leptin compared to placebo, although no associated alcohol consumption differences (Farokhnia et al. 2018b). As leptin has a key role in mediating addictive and motivational behaviours, this may demonstrate a role of GABAergic system in the shared neurobiology of gut-brain axis behaviours and the

subsequent role of baclofen in dampening alcohol-, feeding- and stress-related responses.

Lastly, dose-specific cue responses during an alcohol cue reactivity task were examined in alcohol-dependent patients treated with low-dose (30 mg/day) or high-dose (75 mg/day) baclofen or placebo after 2 weeks of treatment (Logge et al. 2019a). A range of psychophysiological indices were employed including skin conductance, heart rate and heart rate variability. Only the high-dose baclofen-treated patients demonstrated both more dynamic cue responses to appetitive water and alcohol cues. Additionally, only those receiving high-dose showed increased recovery after cues were removed as indicated by a return of high-frequency heart rate variability levels to baseline levels before cue exposures. These two patterns of dynamic cue responses and subsequent recovery reflect adaptive parasympathetic autonomic nervous system activity which is involved in regulation of cue-elicited responses, which is advantageous to controlling cue-elicited responses in AUD (Logge et al. 2019a).

6.1 Functional Brain Activation Correlates with Baclofen

fMRI studies have assessed baclofen's effects on cue-elicited craving and brain activity in various patient samples and differing doses. Participants are shown images of associated drinking cues while in an MRI scanner, and brain activities during these images are compared to activity during control images to evaluate regions of increased activity relevant to cues (Logge et al. 2019b). These studies generally focused upon motivational pathways, including subcortical brain regions of reward implicated in drug cue reactivity and cue salience, as well as brain networks involved in processing and regulation of these cues (e.g. corticostriatal-thalamic loop circuits) (Courtney et al. 2016; Jasinska et al. 2014).

Holla et al. (2018) found that participants administered a moderate dose (60 mg/day) of baclofen for 2 weeks demonstrated increased brain activation from pretreatment to posttreatment scan to alcohol cues compared to healthy controls. This increased activation was seen in in the bilateral dorsal prefrontal cortex (PFC) and rostral anterior cingulate cortex (ACC). Increased activation of ACC and reduced insular cortex activity was also associated with longer time to lapse to first alcohol use in the baclofen-treated participants. However, this study was limited by the lack of a placebo group tested for comparison and was not completed within the context of a RCT.

An fMRI cue reactivity study of individually titrated high-dose baclofen in alcohol-dependent patients (Beck et al. 2018) showed a greater decrease in alcohol cue-elicited brain activation from pretreatment baseline to treatment scan compared to placebo-treated patients. These areas were primarily mesocorticolimbic areas (i.e. left orbitofrontal cortex, bilateral amygdala, left ventral tegmental area (VTA)). Furthermore, high-dose baclofen decreased alcohol cue-modulated

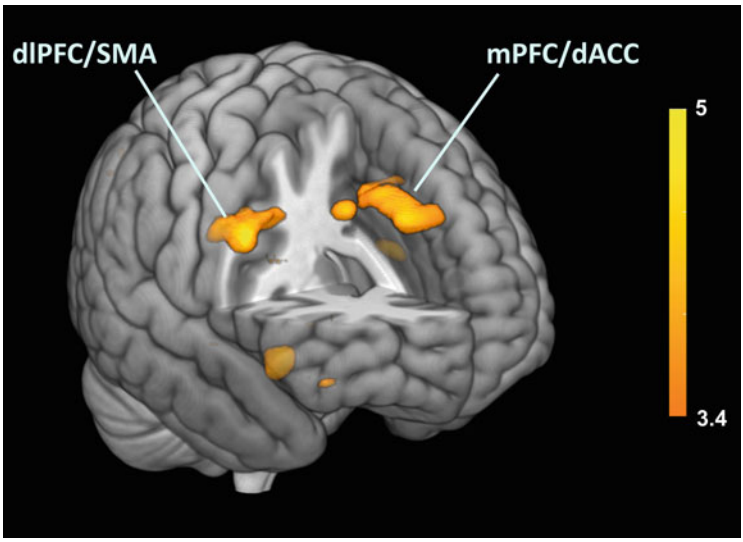

Fig. 1 Greater brain activation to alcohol cues observed in alcohol-dependent patients receiving placebo versus baclofen-treated patients (75 mg/day), showing regions of activation in key prefrontal regions implicated in drug cue reactivity including the medial prefrontal cortex (mPFC), dorsal anterior cingulate cortex (dACC), dorsolateral prefrontal cortex (dlPFC) and supplementary motor area (SMA). Displayed with $p < 0.001$ uncorrected. Colour bar indicates increasing activation with *T*-scores of 3.4–5. Adapted from Logge et al. (2019b)

functional connectivity between subcortical (i.e. VTA) and cortical regions (ACC, medial PFC), with higher rates of abstinence for baclofen-treated patients.

Logge et al. (2019b) investigated whether there were dose-specific effects of baclofen relating to cue reactivity and whether these effects were associated with clinical outcomes. Participants receiving comparatively low (30 mg/day) or high (75 mg/day) baclofen or placebo daily for 3 weeks underwent an fMRI cue reactivity session. Interestingly, there were dose-specific effects in high-dose baclofen-treated participants compared to placebo. Reduced brain activation in those receiving high-dose baclofen, as compared to placebo, was seen in the dorsolateral and medial PFC and ACC, which are key mesocorticolimbic brain regions implicated in reward in addiction (see Fig. 1). Additionally, increased alcohol cue-elicited activation in key prefrontal cortical and mesolimbic regions implicated in drug cue reactivity was associated with more heavy drinking days after the scan session in the placebo-treated patients compared to baclofen-treated patients.

In summary, assessment of psychophysiological indices of treatment effects, including brain activity, has revealed how $GABA_B$ receptor pharmacotherapies such as baclofen can modulate processes in the central and peripheral nervous systems that may lead to observed drinking outcomes such as reduced consumption. It should be noted that while these laboratory studies demonstrate dose-specific effects of baclofen, higher doses are associated with more severe side effects and are not more effective than lower doses in regard to clinical outcomes. Instead, these

studies of baclofen's biobehavioural impacts may help to inform which subpopulations may be best suited and amenable to treatment.

7 Polymorphisms Associated with Baclofen Treatment Response

Although treatment with baclofen appears to be popular in the community, there is significant heterogeneity, and not all individuals with alcohol dependence respond favourably to baclofen. Thus, improvement in the ability to predict baclofen response would have important clinical appeal. Advances in the understanding of this heterogeneity will transform the management of alcohol use disorders, generating opportunity for a personalised approach (Enoch et al. 2012; Lee et al. 2014; Wang and Wang 2016). It has been hypothesised by Enoch et al. (2016) that downregulation of $GABA_B$ receptors leading to increased synaptic GABA may increase the rewarding effects of alcohol and vulnerability for dependence. To this degree, these authors demonstrated a significant and congruent association between *GABBR1* rs29220 and alcoholism in three populations with different ethnicity (Finnish male, Plains Indian and African American sample), whereby the heterozygotes were significantly more common in alcohol-dependent participants relative to the controls. These authors noted that the *GABBR1* rs29220, a non-coding intronic single nucleotide polymorphism (SNP), could represent a tag for a common functional SNP and postulated that this polymorphism could therefore be a predictor for response to baclofen or its adverse effects in the treatment of alcoholism (Enoch et al. 2016). This association has now been identified in the recently completed Australian baclofen study (Morley et al. 2018c) such that the rs29220 polymorphism moderated the therapeutic effect of baclofen. Confirmation of this finding is anticipated in prospective and/or independent cohorts.

8 Safety Concerns with Baclofen

Baclofen is associated with generally mild side effects, but this includes dose-dependent sedation, which has a range of impacts including impairing driving skills (Hetland and Carr 2014) or cognitive performance (Evans and Bisaga 2009). Baclofen's effects can also manifest as more severe sedation, particularly at higher doses and combined with greater levels of alcohol consumption (Rolland et al. 2015). The majority of the RCTs reported no serious adverse events (SAEs) (Addolorato et al. 2002a, 2007; Beraha et al. 2016; Garbutt et al. 2010; Hauser et al. 2017; Morley et al. 2014; Müller et al. 2015; Ponizovsky et al. 2015), Those reporting SAEs (Morley et al. 2018a; Reynaud et al. 2017) were largely determined to be unrelated to study medication (for review, see Pierce et al. 2018). Poisoning

with baclofen has been increasingly reported in regions where it is more commonly used, particularly in France, where several poisoning cases have been reported after use of baclofen for treatment of AUD (Boels et al. 2017). Of these cases, several were considered serious, including four deaths. Similarly, in Australia, an increasing number of calls to the Australian Poisons information service have been documented and associated with increasing use of baclofen, presumably largely related to AUDs (Jamshidi et al. 2018). Infrequently, dose escalation is found, and baclofen has some degree of abuse liability (Dore et al. 2011). Moreover, abrupt cessation of baclofen should be avoided as there has been some association with withdrawal delirium and seizures (Nasti and Brakoulias 2011).

9 Conclusions

The $GABA_B$ receptor agonist baclofen has found a role in the treatment management of AUDs, but its application is limited by modest effect size, toxicity and some degree of abuse liability. The use of baclofen for alcohol withdrawal has limited direct supportive evidence, but it may play an adjunctive role in some cases. Understanding of the mechanisms underlying baclofen's biobehavioural effects using psychophysiological techniques may elucidate baclofen's dose-specific effects and identify suitable subpopulations. GHB is used in Italy, but abuse liability and toxicity have precluded its adoption elsewhere notwithstanding evidence of some efficacy. There have been recent developments of the therapeutic potential of positive allosteric modulators (PAMS) in treatment of AUDs. PAMs have a dual mode of action, enhancing the affinity of the $GABA_B$ receptors for GABA and agonists while simultaneously potentiating their effects (Adams and Lawrence 2007). As this is suggested to result in fewer side effects and lower tolerance compared to agonists alone, novel positive allosteric modulators appears promising, but none are currently available at the clinical level (Maccioni and Colombo 2019). Pharmacotherapies that target $GABA_B$ receptors therefore have a modest beneficial effect in in the treatment of AUDs, but coupled with concerns about related side effects, they currently have a limited applicability as a widespread treatment.

Conflicts of Interest The authors report no relevant conflicts of interest.

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$GABA_B$ Receptors and Pain

Dietmar Benke

Contents

Abstract A substantial fraction of the human population suffers from chronic pain states, which often cannot be sufficiently treated with existing drugs. This calls for alternative targets and strategies for the development of novel analgesics. There is substantial evidence that the G protein-coupled $GABA_B$ receptor is involved in the processing of pain signals and thus has long been considered a valuable target for the generation of analgesics to treat chronic pain. In this review, the contribution of $GABA_B$ receptors to the generation and modulation of pain signals, their involvement in chronic pain states as well as their target suitability for the development of novel analgesics is discussed.

Keywords Chronic pain · $GABA_B$ receptor · Hyperalgesia · Inflammatory pain · Neuropathic pain · Nociception

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1 Introduction

Pain is a highly subjective and generally unpleasant sensation that has an essential protective function to organisms. It provokes avoidance of harmful situations or stimuli and indicates potential tissue damage and illness. The importance of pain for survival is impressively demonstrated by humans insensitive to physical pain due to mutations in the voltage-gated sodium channel $Na_v1.7$. They frequently experience severe injuries in their childhood before they learn alternative strategies to avoid harmful situations (Bennett and Woods 2014). Pain has a major impact on wellbeing and can be detrimental to the quality of life when it becomes chronic, i.e. if it persists after healing of the pain-inducing injury.

There is a large body of literature indicating the involvement of $GABA_B$ receptors in the modulation of pain signals and chronic pain states. They are thus considered as promising drug targets for the development of novel analgesics. $GABA_B$ receptors are ubiquitously expressed in the nervous system and are involved in all major neurological functions and various neurological diseases (Gassmann and Bettler 2012).

$GABA_B$ receptors are heterodimeric G protein-coupled receptors comprising $GABA_{B1}$ and $GABA_{B2}$ subunits (Jones et al. 1998; Kaupmann et al. 1998; Ng et al. 1999; White et al. 1998). They are expressed in virtually all neurons at pre- as well as postsynaptic locations (Kulik et al. 2002, 2003, 2006). Binding of the neurotransmitter GABA to the receptor activates $G_{i/o}$ proteins, which in turn modulate a number of effector systems. The most prominent effect of $GABA_B$ receptors located in the soma and dendrites is the activation of G protein-coupled inwardly rectifying potassium channels (GIRK channels), resulting in the hyperpolarization of the neuronal membrane, thereby elevating the threshold for action potential generation (Luscher et al. 1997; Andrade et al. 1986; Greif et al. 2000; Otis et al. 1993). At presynaptic sites, the most obvious effect of $GABA_B$ receptors is the inhibition of voltage-gated Ca^{2+} channels, thereby reducing neurotransmitter release (Guyon et al. 2013; Mintz and Bean 1993; Chen and van den Pol 1998; Bussieres and El Manira 1999; Bean 1989; Lambert and Wilson 1996). Predominantly via these mechanisms, $GABA_B$ receptors control the excitability and activity of neurons (Gassmann and Bettler 2012).

$GABA_B$ receptors are expressed as two subtypes in the brain, $GABA_{B1a,2}$ and $GABA_{B1b,2}$. They are assembled from two isoforms of the $GABA_{B1}$ subunit ($GABA_{B1a}$ and $GABA_{B1b}$) (Kaupmann et al. 1997) in combination with the $GABA_{B2}$ subunit. $GABA_{B1a}$ and $GABA_{B1b}$ subunits are generated by alternative promoter usage (Steiger et al. 2004) and differ only in their very N-terminal domain by the addition of two sushi repeats in $GABA_{B1a}$ (Kaupmann et al. 1997; Hawrot et al. 1998). Both subtypes are differentially regulated during development (Benke et al. 1999; Fritschy et al. 1999, 2004) and serve distinct functional roles, which are most likely caused by their distinct subcellular localization (Pérez-Garci et al. 2006; Shaban et al. 2006; Vigot et al. 2006). $GABA_{B1a}$ is predominantly expressed at

presynaptic terminals, whereas $GABA_{B1b}$ is mostly present at postsynaptic sites (Vigot et al. 2006).

GABA and $GABA_B$ receptors are highly expressed in structures within pain pathways, indicating their involvement in processing pain signals at different levels (Hammond 1997; Enna and McCarson 2006). There is compelling evidence that activation of $GABA_B$ receptors mediates analgesia, whereas $GABA_B$ receptor antagonist increases the sensitivity to noxious stimuli (Hammond 1997). This not only shows that activation of $GABA_B$ receptors by exogenously applied agonists can relieve pain but also indicates the involvement of $GABA_B$ receptors in endogenous processing of pain signals. This notion is further substantiated by the observation that global deletion of $GABA_B$ receptors produces hyperalgesia (Schuler et al. 2001; Gassmann et al. 2004). Here, the role of $GABA_B$ receptors in the processing of pain signals and their potential as target for the development of analgesics is discussed.

1.1 Pain-Related Terminology

Allodynia: sensation of pain in response to a normally painless stimulus, such as light touch
Analgesic: painkiller, drug to relieve pain by achieving analgesia
Analgesia: relief from pain, loss of pain sensation
Hyperalgesia: abnormally increased sensitivity to a painful stimulus
Hypoalgesia: decreased sensitivity to a painful stimulus
Nociception: detection of painful stimuli
Nociceptor: a sensory neuron that responds to harmful or potentially damaging stimuli
Nocifensive behaviour: response to noxious or painful stimuli

2 Expression and Function of $GABA_B$ Receptors in Nociceptive Pathways

Pain is an unpleasant sensation consisting of an affective-motivational (pain unpleasantness) and a sensory component (pain intensity) (Bell 2018). While the affective-motivational component is processed in the brain, the sensory part (nociception) involves peripheral and central structures. Pain signals are generated in the periphery (e.g. skin, muscles, joints, viscera) and detected by nociceptors, which are primary afferent neurons conveying the pain signals to the spinal cord (Fig. 1). The pain signals are integrated by interneurons of the spinal cord and transmitted by projection neurons to the brainstem and diverse higher brain centres (ascending pathways) for further processing leading to pain perception. In addition, pain signals are modulated (inhibition or facilitation) by descending pathways originating in the

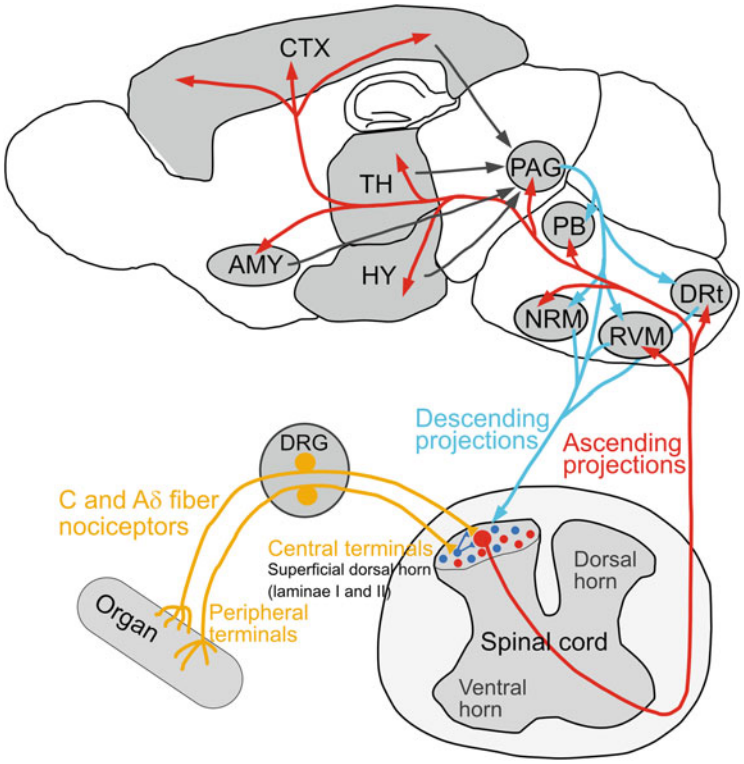

Fig. 1 Simplified schematic view of structures involved in transmitting and modulating nociceptive information relevant to $GABA_B$ receptors. Pain signals generated in the periphery (organ) are detected by nociceptors (C and Aδ fibre nociceptors, orange), which convey the signals to the dorsal horn of the spinal cord. Pain signals are extensively integrated by a complex network of excitatory (red) and inhibitory (blue) interneurons abundantly (but not exclusively) located in the superficial layers of the dorsal horn (laminae I and II). Pain signals are transmitted by projection neurons (large red dot) to the brainstem and diverse higher brain centres (ascending pathways, red lines) for further processing leading to pain perception. In addition, pain signals are modulated (inhibition or facilitation) in the dorsal horn by descending pathways (blue lines) originating in the brain. *AMY* amygdala, *CTX* cerebral cortex, *DRt* dorsal reticular nucleus, *HY* hypothalamus, *NRM* nucleus raphe magnus, *PAG* periaqueductal grey, *PB* parabrachial nucleus, *RVM* rostral ventromedial medulla

brain (White et al. 2018) (Fig. 1). Descending pathways can inhibit ascending pain signals and provide endogenous pain relief (e.g. stress-induced analgesia).

2.1 $GABA_B$ Receptors in Nociceptors

Noxious signals in peripheral tissues are sensed by so-called nociceptors. Nociceptors are glutamatergic primary afferent neurons with branched nerve endings

in the innervated tissue, responding primarily to noxious signals (heat, mechanical and chemical stimuli). Their cell bodies are located in the dorsal root ganglia (DRG), and their central axon endings synapse in the dorsal horn of the spinal cord. Nociceptors are categorized as fast-conducting (5–35 m/s) medium-diameter (1–5 μm) myelinated neurons (Aδ-fibres) and slow-conducting (<2 m/s) small-diameter (0.2–1.5 μm) unmyelinated neurons (C-fibres) (Albrecht and Rice 2010). Aδ afferents convey fast sharp and well-localized pain, while C-fibre neurons give rise to slow and diffuse pain sensation. $GABA_{B1}$ and $GABA_{B2}$ mRNAs and proteins are well expressed in nociceptors (Charles et al. 2001; Towers et al. 2000; Engle et al. 2006, 2012; Hanack et al. 2015; Yang et al. 2001; Desarmenien et al. 1984). In the DRGs, $GABA_B$ receptors are mainly localized in the somata of Aδ and C-fibres. In addition, $GABA_B$ receptors are highly expressed at central axon terminals of Aδ and C-fibres, which target the superficial dorsal horn of the spinal cord (predominantly laminae I and II).

2.1.1 $GABA_B$ Receptors in Peripheral Axon Endings of Nociceptors

$GABA_{B1}$ as well as $GABA_{B2}$ subunit proteins were detected in free nerve endings of nociceptors in dermal tissue of the hindpaw of mice (Whitehead et al. 2012). This suggests the modulation of nociceptive signals via $GABA_B$ receptors already occurs at the sites of signal generation.

Interestingly, $GABA_{B1}$, but not $GABA_{B2}$, was found at peripheral axon terminals in a subset of nociceptors expressing transient receptor potential cation channel subfamily V member 1 (TRPV1) (Hanack et al. 2015). TRPV1 is a non-selective cation channel activated by a large variety of stimuli including heat, acidic conditions and capsaicin. TRPV1 plays an important role in perception and integration of noxious stimuli in peripheral tissues as well as in the development of pathological pain (Moore et al. 2018). Different inflammatory mediators/pathways can dramatically lower the activation threshold of TRPV1 in a phosphorylation-dependent manner, leading to hyperalgesia (increased sensitivity to noxious stimuli). $GABA_{B1}$ appears to prevent the sensitization of TRPV1 by a noncanonical signalling pathway that involves neither $GABA_{B2}$ nor $G_{i/o}$ proteins (Hanack et al. 2015). Instead, $GABA_{B1}$ appears to interact with TRPV1 upon agonist activation, inducing most likely a conformational change that shields TRPV1 from sensitization by preventing its PKC-mediated phosphorylation. Application of $GABA_B$ receptor antagonists led to TRPV1 sensitization (Hanack et al. 2015), suggesting that tonic activation of $GABA_{B1}$ keeps the threshold for TRPV1 activation high, thereby preventing sensitization of the system. Thus, the interaction of $GABA_{B1}$ with TRPV1 upon activation with GABA appears to be an endogenous mechanism to inhibit sensitization of TRPV1. However, this mechanism fails under pathological inflammatory conditions and leads to peripheral hyperalgesia. As application of baclofen reduces peripheral inflammatory hyperalgesia (Hanack et al. 2015), activation of $GABA_{B1}$ has the potential to counteract hyperalgesia. It is very likely that under pathological inflammatory pain, $GABA_{B1}$ and/or the release of GABA at

peripheral nociceptor axon terminals is downregulated, cancelling the protective function of $GABA_{B1}$.

2.1.2 $GABA_B$ Receptors in Central Axon Endings of Nociceptors

The central axon endings of Aδ and C-fibres predominantly target interneurons in laminae I–II of the dorsal spinal cord, where their signals are integrated and further transmitted to supraspinal structures (Todd 2010; Zeilhofer et al. 2012b). $GABA_B$ receptors are highly expressed at the central axon terminals of nociceptors. This is indicated by the predominant expression of $GABA_{B1a}$ and $GABA_{B2}$ mRNA (Towers et al. 2000) and the finding that $GABA_{B1a/2}$ receptors are largely targeted to presynaptic axon terminals to inhibit transmitter release (Vigot et al. 2006). This notion is further substantiated by radioligand binding studies after capsaicin-induced degeneration of the afferent fibres (Price et al. 1984, 1987), transection of the dorsal afferent fibres (Price et al. 1987) or virus-mediated knock-down of $GABA_{B1a}$ in afferent fibres (Jones et al. 2005), which significantly reduced the expression of $GABA_B$ receptors in their target area, the superficial laminae of the spinal cord. From these studies, it has been estimated that about 40–50% of $GABA_B$ receptors in the superficial dorsal horn are present in primary afferent terminals. Finally, it is well established that $GABA_B$ receptor activation mediates presynaptic inhibition of Aδ and C-fibre activity, with C-fibres being more strongly affected than Aδ fibres (Ataka et al. 2000; Yang et al. 2001; Iyadomi et al. 2000; Yang and Ma 2011; Gangadharan et al. 2009; Wang et al. 2007).

Although it is quite clear that presynaptic $GABA_B$ receptors regulate glutamatergic transmission of nociceptors onto spinal cord interneurons, the contribution of these receptors to the analgesic activity of baclofen has been questioned by a recent study using a mouse line lacking $GABA_{B1}$ in nociceptors (Gangadharan et al. 2009). Conditional deletion of $GABA_{B1}$ specifically in Aδ and C-fibres (SNS-$GABA_{B1}^{-/-}$ mice) resulted in higher excitability of Aδ-fibres, but surprisingly had no effect on basal nociceptive sensitivity or on the development of chronic inflammatory and neuropathic pain (Gangadharan et al. 2009). Systemic administration of baclofen exerted a similar analgesic activity in wildtype and SNS-$GABA_{B1}^{-/-}$ mice, suggesting that $GABA_B$ receptors expressed in nociceptors have only a minor contribution to the regulation of nociception and the analgesic activity of baclofen on a global scale. It should be noted that supraspinal effects of baclofen in these experiments might have masked regulatory functions of peripheral presynaptic $GABA_B$ receptors. In addition, compensatory plastic adaptations should be taken into consideration with the long-term deletion of $GABA_{B1}$ in the SNS-$GABA_{B1}^{-/-}$ mice.

Despite this negative finding, there is solid evidence for the regulation of nociception via peripheral $GABA_B$ receptors. For instance, strong tonic activation of sciatic Aδ-fibres in the dorsolateral hindpaw of mice induces a reduced nociceptive sensitivity in dorsomedial saphenous C-fibres by a central $GABA_B$ receptor-mediated mechanism (Jones et al. 2005). This intersegmental modulation is most

likely due to the activation of GABAergic interneurons in the superficial dorsal horn by the persistently activated Aδ-fibres, which in turn activate presynaptic $GABA_B$ receptors on saphenous C-fibres, reducing excitatory transmitter release. In line with this view, virus-mediated knock-down of $GABA_{B1a}$ in primary afferents diminished sciatic Aδ-fibre generated hypoalgesia, indicating that this mechanism depends on presynaptic $GABA_B$ receptors present in central primary afferent terminals (Jones et al. 2005).

Another recent example for the involvement of peripheral presynaptic $GABA_B$ receptors in the regulation of nociception and pathological pain is the modulation of $GABA_B$ receptor activity by the Gα inhibitory interacting protein (GINIP) (Gaillard et al. 2014). GINIP was discovered by screening for markers to discriminate subpopulations of nociceptors. Its expression is restricted to two nonpeptidergic C-fibre neuron populations that express MRGPRD or TAFA4 (low-threshold mechanoreceptors), which selectively target lamina II of the dorsal horn. GINIP interacts with the activated Gαi protein and most likely stabilizes $GABA_B$ receptor signalling. Deletion of GINIP strongly impaired baclofen-induced inhibition of high-voltage-activated Ca^{2+} channels and diminished baclofen-mediated presynaptic inhibition in lamina II interneurons. Most importantly, $GINIP^{-/-}$ mice displayed prolonged mechanical hypersensitivity in a model of neuropathic pain that was, in contrast to wild-type mice, not reversed by intrathecal administration of baclofen (Gaillard et al. 2014). This illustrates the importance of peripheral $GABA_B$ receptors located at the central endings of nociceptors for the modulation of chronic pain states.

2.2 *$GABA_B$ Receptors in Dorsal Horn Interneurons and Projection Neurons*

The central axon endings from nociceptors predominantly terminate in laminae I and II of the dorsal horn where their signals are integrated by a complex network of inhibitory and excitatory interneurons. The integrated signals are then transmitted to supraspinal centres via glutamatergic projection neurons predominantly located in lamina I (Todd 2010; Zeilhofer et al. 2012b). According to the 'gate control theory of pain' postulated in 1965 by Melzack and Wall (1965), GABA/glycine-mediated inhibition by interneurons of the dorsal horn provides a 'gate' that controls the output of projection neurons to prevent their activation by innoxious stimuli. This theory is well supported by experiments ablating or inactivating populations of dorsal horn inhibitory interneuron. These experiments led to spontaneous pain behaviour and hyperalgesia (Foster et al. 2015; Duan et al. 2014).

The superficial laminae of the dorsal horn exhibit highest expression of $GABA_B$ receptors within the spinal cord. They are expressed on the axon terminals of primary afferent neurons and on pre- and postsynaptic sites of interneurons (Wang et al. 2007; Iyadomi et al. 2000). Activation of presynaptic $GABA_B$ receptors inhibits transmitter release of both glutamatergic and GABAergic neurons resulting

in a complex integration of signals via inhibition and disinhibition (Melin et al. 2013; Zheng et al. 2010; Yang and Ma 2011; Yang et al. 2015).

$GABA_B$ receptors are also expressed in a subset of lamina I projection neurons targeting the caudal ventrolateral medulla (Castro et al. 2006) and the parabrachial nucleus (Brewer and Baccei 2018). Interestingly, neonatal tissue damage in the hindpaw of mice specifically increased postsynaptic $GABA_B$ receptor function in projection neurons targeting the parabrachial nucleus in the adult animals (Brewer and Baccei 2018). Hindpaw injury in early neonatal rats induces acute hyperalgesia followed by generalized hypoalgesia (Ren et al. 2004). It is likely that the increased inhibitory postsynaptic $GABA_B$ receptor function in projection neurons targeting the parabrachial nucleus contributes to the observed reduction in pain sensitivity.

2.3 *$GABA_B$ Receptors in Supraspinal Areas*

After integration in the dorsal spinal cord, noxious signals are relayed via ascending fibre tracts (e.g. spinothalamic, spinoreticular, spinoparabrachial tracts) to diverse subcortical and cortical brain structures including the periaqueductal grey, mesencephalic and thalamic nuclei, amygdala, hypothalamus, insular cortex, anterior cingulate cortex and somatosensory cortex (Bell 2018). Accordingly, upon noxious stimuli, neuroimaging techniques revealed the activation of a complex pattern of structures across the entire brain (commonly called the 'pain matrix'), depending on the kind of stimulus as well as on the emotional state (May 2007; Legrain et al. 2011). There is evidence that this system is not specifically dedicated to 'pain' but has a more general function for processing sensory information irrespective of their origin (Legrain et al. 2011).

As $GABA_B$ receptors are ubiquitously expressed in all these structures, they most likely contribute to the processing of all kinds of noxious information in all relevant brain areas to generate the sensation of pain. In general, due to its inhibitory nature, activation of $GABA_B$ receptors predominantly has alleviating effects on pain sensation. For instance, injection of the $GABA_B$ receptor antagonist CGP 35348 into the anterior cingulate cortex (ACC), which is involved in the regulation of the affective component of pain (Fuchs et al. 2014), was associated with the development of mechanical hypersensitivity in naïve rats, whereas the $GABA_B$ receptor agonist baclofen reduced hyperalgesia in neuropathic rats (partial sciatic nerve ligation) (Migita et al. 2018). In line with this finding, application of the $GABA_B$ receptor antagonist CGP 55845 increased the excitability of ACC neurons in healthy rats (Nashawi et al. 2016). However, inhibition of $GABA_B$ receptors in the ACC had no effect on the excitability of ACC neurons in rats with mechanical allodynia induced with the anticancer drug paclitaxel, suggesting a deficiency in $GABA_B$ receptor-mediated inhibition in this chronic pain state (Nashawi et al. 2016).

Another example of $GABA_B$ receptor-mediated modulation of pain signals is the central amygdala. The central amygdala is involved in processing the emotional components of pain and directly receives signals from activated lamina I projection

neurons as well as relayed signals via neurons of the parabrachial nucleus (Neugebauer et al. 2004). Activation of $GABA_B$ receptors at axon terminals of parabrachial neurons reduced excitatory neurotransmission onto neurons in the central amygdala via inhibition of N-type Ca^{2+} channels (Delaney and Crane 2016). Furthermore, activation of $GABA_B$ receptors in the ventrobasal complex of the thalamus, which receives noxious information from neurons of the spinothalamic tract and relays them primarily to the somatosensory cortex, reduced nociception in models of acute and chronic inflammatory pain (Soares Potes et al. 2006; Potes et al. 2006a).

2.4 *$GABA_B$ Receptors in Descending Pain Control Pathways*

Supraspinal centres not only process ascending noxious signals to generate the sensation of pain but also have a top-down modulatory effect on signal processing at the level of the spinal cord dorsal horn. Descending control pathways arise from the midbrain and brainstem. They can be inhibitory (reducing pain) or facilitatory (enhancing pain) (Fig. 1). In this way, inputs from various brain areas reflecting diverse cognitive or emotional states can influence processing of noxious signals in the dorsal horn and thereby modulate the threshold for noxious signalling via ascending projection neurons.

The ventrolateral part of the periaqueductal grey (PAG), located in the midbrain, is an important integrative structure and a main site of the descending pain modulatory system. It receives input from various cortical areas, such as the thalamus, hypothalamus and amygdala as well as noxious information from ascending spinal cord projection neurons. It sends information to the spinal cord indirectly via the nucleus raphe magnus (NRM) and the area of the rostral ventromedial medulla (RVM) to the spinal cord (White et al. 2018; Lau and Vaughan 2014). $GABA_B$ receptors are located at pre- and postsynaptic sites in PAG neurons to inhibit transmitter release by inhibiting Ca^{2+} channels and mediate hyperpolarization by activating K^+ channels (Yang et al. 2003), respectively, with presynaptic $GABA_B$ receptors being more sensitive to baclofen than postsynaptic receptors (Chen et al. 2017). There seems to be a tonic inhibition of presynaptic terminals by GABA acting on presynaptic $GABA_B$ receptors (Li et al. 2017). Counterintuitively, activation of $GABA_B$ receptors in the ventrobasal PGA by injection of baclofen reduced acute pain (Levy and Proudfit 1979). Considering that stimulation of the PAG mediates analgesia (Wang et al. 2016), activation of $GABA_B$ receptors very likely mediates an overall disinhibition of PAG neurons, resulting in an excitatory output.

In contrast to the PAG, the dorsal reticular nucleus (DRt) in the medulla oblongata, which is reciprocally connected with various brain areas as well as the spinal dorsal horn, is involved in top-down facilitation of nociceptive signals (Martins and Tavares 2017). Global stimulation of the DRt enhanced, whereas inhibition reduced nociception (Almeida et al. 1996, 1999). $GABA_B$ receptors are well expressed in DRt neurons (Pinto et al. 2008) and are activated by locally

released GABA in response to peripheral inflammatory pain (Martins et al. 2015). Knock-down of $GABA_{B1a}$ as well as local injection of the $GABA_B$ receptor antagonist CGP 35348 into the DRt reduced inflammatory pain in the second phase of the formalin test, whereas injection of baclofen enhanced nociceptive behaviour (Martins et al. 2015). As $GABA_B$ receptors are predominantly expressed in enkephalinergic DRt neurons (Martins et al. 2015), local release of GABA upon noxious stimulation might activate presynaptic $GABA_B$ receptors and inhibit the release of endogenous opioids, abrogating tonic inhibition of projection neurons descending to the spinal cord.

3 $GABA_B$ Receptors and Pathological Pain

In unfortunate circumstances, nociceptive pain can become pathological, i.e. pain persists although the pain-causing insult has been removed. Chronic pain is a major health problem as it is widespread and pharmacologically difficult to address and massively affects life quality. Chronic pain is associated with profound plastic changes in peripheral and central nociceptive pathways, resulting in enhanced pain sensation to painful stimuli (hyperalgesia) or even in pain upon innocuous stimuli (allodynia) such as light touch (Yam et al. 2018). Reduced inhibitory control profoundly contributes to pain sensitization and the development of chronic pain (Zeilhofer et al. 2012a; Gwak and Hulsebosch 2011).

3.1 $GABA_B$ Receptors and Inflammatory Pain

Inflammation is an endogenous response of the body to clean up damaged tissue and promote its repair. Tissue damage triggers the production of a variety of inflammatory mediators that activate nociceptors. Prolonged inflammation results in peripheral as well as central sensitization. Peripheral sensitization is caused by excessive activation of nociceptors. In contrast, central sensitization results from plastic changes leading to enhanced excitation of neurons in the dorsal horn and the brain (Gangadharan and Kuner 2013; Basbaum et al. 2009).

Systemic application of baclofen diminished nocifensive behaviours in animal models of acute and chronic inflammatory pain (Smith et al. 1994; Patel et al. 2001; Shafizadeh et al. 1997). Thus, in principle, $GABA_B$ receptors at all anatomical levels of the pain pathway could contribute to the analgesic effects of baclofen. Indeed, there is evidence that baclofen acts at peripheral nociceptors as well as at spinal and supraspinal levels to relieve inflammatory pain. For instance, direct injection of baclofen into an inflamed rat paw produced an antinociceptive effect upon prostaglandin E2-induced hyperalgesia (Reis and Duarte 2006; Whitehead et al. 2012). The antinociceptive effect of baclofen was prevented by pre-injecting K^+ channel blockers, indicating the involvement of $GABA_B$ receptor-activated K^+ channels in

the peripheral action of baclofen (Reis and Duarte 2006). Intrathecal injection of baclofen also reduced inflammatory pain responses in rodents, demonstrating its activity at spinal cord neurons (Patel et al. 2001; Dirig and Yaksh 1995; Naderi et al. 2005). Spinal application of baclofen reduced the activity of Aβ-, Aδ- and C-fibres in rats with carrageenan-inflamed hindpaws (Sokal and Chapman 2003) and considerably diminished c-Fos expression in dorsal horn neurons (Buritova et al. 1996) consistent with increased $GABA_B$ receptor-mediated neuronal inhibition. Finally, injection of baclofen in, for example, the ventrobasal complex of the thalamus (Potes et al. 2006a, b) shows the effectiveness of baclofen at the supraspinal level.

As chronic peripheral inflammation leads to enhanced excitability of dorsal horn neurons (Menetrey and Besson 1982; Takazawa et al. 2017), it is conceivable that the altered excitation/inhibition balance may lead to plastic changes in the expression of $GABA_B$ receptors. However, currently there is no clear picture on the regulation of $GABA_B$ receptor expression and its potential functional significance in inflammatory pain states. For example, $GABA_B$ receptor mRNA (McCarson and Enna 1999) and protein levels (Sands et al. 2003) appear to be upregulated in DRGs and the dorsal horn 24 h after formalin-induced hindpaw inflammation in rats. However, the increased $GABA_{B1}$ and $GABA_{B2}$ protein levels did not translate into increased $GABA_B$ receptor function in the dorsal horn as measured by [^{35}S] GTPγS binding (Sands et al. 2003). On the other hand, a decrease of $GABA_B$ receptors in the dorsal horn was observed 3–4 weeks after inducing inflammation (Castro-Lopes et al. 1995), and, finally, in a rat model of orofacial inflammation, $GABA_B$ receptors were downregulated in the trigeminal ganglion (Liu et al. 2019). Thus, there seems to be no general rule for the regulation of $GABA_B$ receptor expression in inflammatory pain states. Instead, a potential regulation of $GABA_B$ receptor expression might underlie specific temporal and spatial conditions.

Besides regulating neuronal excitability, $GABA_B$ receptors appear to be involved in other processes in inflammatory pain states. For instance, spinal $GABA_B$ receptors also appear to modulate the local inflammatory response as shown in a mouse model of arthritis (Bassi et al. 2016). Intrathecal injection of baclofen increased neutrophil recruitment to the inflamed knee joint in a p38 MAPK-dependent manner. Thus, in addition to the well-established analgesic activity, activation of spinal $GABA_B$ receptors also appears to exert a pro-inflammatory effect. This should be taken into account in the case of development analgesic drugs for the treatment of chronic inflammation targeting $GABA_B$ receptors.

Another example is the control of interleukin 1β release from satellite glial cells in trigeminal ganglia. $GABA_B$ receptors were recently found to be expressed in satellite glial cells in trigeminal ganglia where they control the activity of inwardly rectifying K^+ channels (Takeda et al. 2015). Satellite glial cells ensheath trigeminal neurons, are activated by neurotransmitters released from trigeminal neurons and appear to play an important role in the development of hyperalgesia in response to inflammatory stimuli via release of interleukin 1β (Takeda et al. 2009). Injection of baclofen into the trigeminal ganglion after inducing orofacial inflammation reduced mechanical allodynia, prevented downregulation of $GABA_B$ receptors in satellite glial cells

and inhibited satellite glial cell activation as well as interleukin 1β release (Liu et al. 2019). This study provides intriguing indications that glial $GABA_B$ receptors adjacent to nociceptors may play a role in the regulation of orofacial inflammation. However, this mechanism might be restricted to the trigeminal ganglia since satellite glia cells in DRG neurons appear not to express $GABA_B$ receptors (Engle et al. 2012).

3.2 *$GABA_B$ Receptors and Neuropathic Pain*

Neuropathic pain originates from nerve injury and diseases that lead to peripheral or central neuronal damage such as diabetes (Schreiber et al. 2015), cancer (Davis 2018), multiple sclerosis (Khan and Smith 2014) and HIV (Aziz-Donnelly and Harrison 2017). Neuropathic pain is associated with a multitude of plastic alterations in the CNS leading to enhanced excitation and central sensitization (Gangadharan and Kuner 2013; Sandkuhler 2009). These include a prominent reduction of GABAergic inhibitory control (Zeilhofer et al. 2012a). Peripheral nerve damage significantly reduced GABA levels as well as GABA release in the dorsal horn (Eaton et al. 1998; Vaysse et al. 2011; Lever et al. 2003; Ibuki et al. 1997; Somers and Clemente 2002). Restoration of GABA levels by viral overexpression of GAD65 or injection of GABA-expressing cells into the dorsal horn significantly reduced neuropathic pain in rats with peripheral nerve injury (Vaysse et al. 2011; Jergova et al. 2012; Lee et al. 2007). Intrathecal injection of $GABA_A$ receptor and $GABA_B$ receptor antagonists showed that both receptors mediated the antinociceptive activity of GABA-expressing grafts (Jergova et al. 2012). Therefore, enhancing $GABA_B$ receptor activity is a promising strategy to relieve neuropathic pain.

In fact, systemic (Smith et al. 1994; Patel et al. 2001; Magnaghi et al. 2014) and intrathecal administration (Bai et al. 2014; Liu et al. 2018; Zemoura et al. 2016; Dias and Prado 2016; Hwang and Yaksh 1997; Malan et al. 2002; Lee et al. 2010; Gwak et al. 2006) of baclofen as well as injecting it into supraspinal structures (Migita et al. 2018) alleviated nociceptive responses in animal models of neuropathic pain. This implies, as in the case of chronic inflammatory pain, that $GABA_B$ receptors at all levels of the pain pathway contribute to the analgesic effect of baclofen. Interestingly, chronic intrathecal application of baclofen in a rat model of diabetic neuropathic pain normalized elevated expression levels of NR2B-containing NMDA receptors, most likely in a CREB-dependent manner (Liu et al. 2014; Bai et al. 2014). This illustrates that $GABA_B$ receptor activity not only counterbalances overexcitation but can trigger diverse pathways to affect neuronal excitability at different levels.

As neuropathic pain leads to diminished neuronal inhibition, a plastic downregulation of $GABA_B$ receptors might be a contributing factor. However, since baclofen still exhibits analgesic activity in animal models of neuropathic pain, an excessive downregulation of $GABA_B$ receptors is very unlikely.

In the rat model of streptozotocin-induced diabetes, hyperalgesia and allodynia develop 3 weeks after a single injection of streptozotocin. This is accompanied by increased glutamatergic excitatory activity in spinal cord neurons without changes in GABAergic and glycinergic inhibitory postsynaptic currents, indicating a preserved GABAergic tone under these conditions (Wang et al. 2007). However, $GABA_B$ receptor-mediated inhibition of primary afferent terminals was significantly reduced, which might be explained by downregulation of $GABA_B$ receptors at central nociceptor terminals. Indeed, a robust loss of $GABA_B$ receptors in the spinal cord was observed by Western blotting 5–7 weeks after induction of diabetes with streptozotocin (Wang et al. 2011). Because the onset of nocifensive behaviour (after 3 weeks) was not exactly paralleled by this robust $GABA_B$ receptor downregulation (starting after 5 weeks), it is unlikely that the loss of $GABA_B$ receptors is essential for the development of pain symptoms in this model of neuropathic pain.

Another example for a considerable downregulation of $GABA_B$ receptors is cancer-induced bone pain in rats (Zhou et al. 2017). In this model downregulation of $GABA_B$ receptors in the spinal cord exactly followed the time course of mechanical allodynia development. Chronic intrathecal application of baclofen partially restored $GABA_B$ receptor expression, which was associated with a reduction of elevated PKA and pCREB levels (Zhou et al. 2017). As activation of the PKA/CREB pathway contributes to the development of cancer-induced bone pain (Hang et al. 2013), there might be a link between downregulation of $GABA_B$ receptors and the expression of chronic pain in cancer-induced bone pain.

Whereas there is clear evidence for downregulation of $GABA_B$ receptors in the spinal cord in diabetes-induced and cancer-induced bone pain, inconsistent results were reported from models of neuropathic pain induced by chronic constriction of the sciatic nerve (CCI) and spinal nerve ligation (SNL). There is convincing evidence that $GABA_B$ receptors are downregulated in DRG neurons after peripheral nerve ligation (Engle et al. 2012), but no change was found in the dorsal horn (Engle et al. 2006; Smith et al. 1994; Zemoura et al. 2016). However, Wu et al. (2011) observed a strong reduction specifically in $GABA_{B1a}$ expression (expression $GABA_{B1b}$ was not affected) 7 and 14 days after spinal nerve ligation. This suggests that presynaptic $GABA_B$ receptors are mainly affected, most probably at central nociceptor terminals. Downregulation of $GABA_{B1a}$ as well as nocifensive behaviour was prevented or reduced, respectively, by intrathecal application of a p38-MAPK inhibitor (Wu et al. 2011). As p38-MAPK is specifically activated in dorsal horn microglia after spinal nerve ligation (Tsuda et al. 2004), this observation may link microglia activation to the downregulation of $GABA_{B1a}$ so far by an unknown mechanism.

The mechanism(s) involved in downregulating $GABA_B$ receptors under chronic pain conditions are currently unknown. However, it is well established that neuronal over-excitation caused by sustained activity of AMPA and NMDA receptors rapidly downregulates $GABA_B$ receptors and thereby strongly reduces $GABA_B$ receptor-mediated neuronal inhibition by enhancing lysosomal degradation of the receptors (Maier et al. 2010; Guetg et al. 2010; Kantamneni et al. 2014; Terunuma et al. 2010;

Zemoura et al. 2019). As chronic pain states are associated with considerably enhanced glutamate receptor activity, this mechanism might be one factor contributing to $GABA_B$ receptor downregulation.

Another very intriguing mechanism impairing $GABA_B$ receptor activity under conditions of neuropathic pain was proposed by Laffray et al. (2012). In the spinal nerve ligation model of neuropathic pain, the $GABA_B$ receptor interacting protein 14-3-3ζ was found to be selectively upregulated in the ipsilateral dorsal horn. Binding of 14-3-3ζ to $GABA_B$ receptors occurred at the plasma membrane and interfered with heterodimerization of the receptors. This rendered the receptors non-functional and diminished $GABA_B$ receptor signalling (Laffray et al. 2012). Preventing the interaction of $GABA_B$ receptors with 14-3-3ζ using an interfering synthetic peptide enhanced baclofen-mediated analgesia and, interestingly, partially reversed nocifensive behaviours in neuropathic rats in the absence of baclofen. This observation supports a role for diminished $GABA_B$ receptor signalling in the development of neuropathic pain.

These examples suggest a rather complex regulation of $GABA_B$ receptors during chronic pain states.

4 $GABA_B$ Receptors as Target for Treating Chronic Pain

The $GABA_B$ receptor agonist baclofen has a long history in the clinic for treating severe spasticity caused by, e.g. multiple sclerosis, spinal cord injury and stroke. It efficiently resolves spasm and relieves associated musculoskeletal pain (Slonimski et al. 2004). In addition, a variety of small clinical studies and case reports demonstrated the analgesic activity of baclofen in chronic pain states not associated with spasticity, including trigeminal neuralgia, intractable post herpetic neuralgia, complex regional pain syndrome, chronic pain after cerebral stroke, painful spinal cord lesions and neuropathic pain after peripheral nerve injury (Fromm and Terrence 1987; Fromm et al. 1984; Zuniga et al. 2000; Hosny et al. 2004; van der Plas et al. 2013; Kopsky et al. 2015; Lind et al. 2008; Herman et al. 1992; Taira and Hori 2007; Taira et al. 1995; Goto et al. 2013; Harmer and Larson 2002). Although baclofen is often successful in patients not responding to common analgesics (e.g. opioids), there are several drawbacks associated with baclofen that prevents its widespread application. Baclofen has a relatively short half-life (2–4 h) and it poorly crosses the blood-brain barrier. These characteristics require high doses for systemic application, which can cause tolerance and severe side effects (Brennan and Whittle 2008). For these reasons, baclofen is given in severe cases intrathecally with pumps. But even then side effects such as drowsiness, confusion, disturbance of speech, gastrointestinal problems, nausea, hypotension, sexual dysfunction and many others were observed (Slonimski et al. 2004). Therefore, baclofen will be reserved for severe chronic pain states resistant to common analgesics or as an adjuvant in combination with analgesics, e.g. opioids (Gatscher et al. 2002; Zuniga et al. 2000).

Because $GABA_B$ receptors are ubiquitously expressed and are involved in numerous physiological functions, their global activation will invariably be associated with unwanted effects. It is therefore rather unlikely that orthosteric $GABA_B$ receptor agonists will be successful as first-line analgesics. Novel strategies like the recent development of $GABA_B$ receptor positive allosteric modulators (Froestl 2010; Urwyler 2011) may have some potential for the generation of analgesics. Positive allosteric modulators increase the affinity and efficacy of the receptor for GABA, resulting in "use-dependent" potentiation of $GABA_B$ receptor activity. This kind of activity enhancement appears to be associated with fewer side effects (in particular sedation) in various animal models.

So far, only a very limited number of positive allosteric modulators were evaluated in animal models of acute and chronic pain (Table 1). Regarding neuropathic pain, only rac-BHFF was tested in the CCI model of neuropathic pain (Zemoura et al. 2016). Although rac-BHFF increased the paw withdrawal threshold after mechanical stimulation at a non-sedating oral dose in naïve mice, it was inactive in neuropathic mice. However, after activation of $GABA_B$ receptors with a subsaturating intrathecal dose of baclofen, rac-BHFF potentiated the analgesic effect of baclofen (Zemoura et al. 2016). This observation indicates that the reduced GABAergic tone (see above) in neuropathic mice was too low to permit efficient allosteric modulation of $GABA_B$ receptors and pain relief. Thus, in pain conditions associated with a considerably reduced GABAergic tone, allosteric modulators are not expected to display sufficient analgesic activity. They may, however, be useful to enhance the activity of low-doses of baclofen, which might result in fewer side effects.

Regarding chronic inflammatory pain, the positive allosteric modulators ADX71441 and ADX71943 reduced hyperalgesia in the formalin test and the monosodium iodoacetate model of chronic osteoarthritis (Kalinichev et al. 2014, 2017). ADX71943 very poorly crosses the blood-brain barrier and therefore exhibits, in contrast to ADX71441, no central effects (Kalinichev et al. 2014). In the model of chronic osteoarthritis ADX71943 exhibited strongest analgesic activity in the early, more inflammatory related, phase of the model and vanished in later stages presumably related to a more centrally driven neuropathic pain state (Kalinichev et al. 2017). Thus, peripherally acting allosteric modulators might be effective in acute and early inflammatory pain without a prominent central component. Such drugs are expected to lack the severe central side effects associated with baclofen.

$GABA_B$ receptors are also involved in the regulation of visceral pain (Page et al. 2006; Loeza-Alcocer et al. 2019). In acute visceral pain, the positive allosteric modulators CGP7930 (colon pain) (Brusberg et al. 2009) and ADX17441 (colon and bladder pain) (Kannampalli et al. 2017) decreased nociceptive responses. The analgesic effect of ADX17441 appeared to be predominantly mediated via peripheral and supraspinal sites since systemic and intra-cerebroventricular but not intrathecal administration was effective (Kannampalli et al. 2017). The observation that

Table 1 Drugs and strategies with $GABA_B$ receptor selective analgesic activity

Drug/ treatment	Pain model	Application	Effect	Reference
Positive allosteric modulators				
Rac-BHFF	*Neuropathic pain* (CCI)	Systemic (p.o.)	No effect	Zemoura et al. (2016)
ADX71943	*Inflammatory pain*			
	Formalin test	Systemic (p.o.)	Analgesic (peripheral)	Kalinichev et al. (2014)
	Chronic osteoarthritis	Systemic (p.o.)	Analgesic only in early phase (peripheral)	Kalinichev et al. (2017)
ADX71441	*Inflammatory pain* Chronic osteoarthritis	Systemic (p.o.)	Analgesic	Kalinichev et al. (2017)
	Visceral pain Colon distension	Systemic (i.p.)	Analgesic	Kannampalli et al. (2017)
		Spinal (i.t.)	No effect	
		Supraspinal (i.c.v.)	Analgesic	
	Bladder distension	Systemic (i.p.)	Analgesic	
		Spinal (i.t.)	No effect	
		Supraspinal (i.c.v.)	Analgesic	
CGP7930	*Visceral pain* (Colon distension)	Systemic (i.v.)	Analgesic	Brusberg et al. (2009)
Drugs with unidentified binding site on $GABA_B$ receptors				
α-Conotoxin Vc1.1	*Visceral pain* (Colon distension)	Peripheral (intra-colonic)	Analgesic	Castro et al. (2017)
Fucoidan	*Neuropathic pain* (Chemotherapeutic drug-induced)	Repeatedly systemic (i.p.)	Analgesic (most likely via upregulation of $GABA_B$ receptors)	Hu et al. (2017)
Isovaline	*Inflammatory pain* (Intraplantar PGE_2)	Peripheral (intraplantar injection)	Analgesic	Whitehead et al. (2012)
Targeting protein-protein interactions with interfering peptides				
$GABA_B$ receptor/14-3-3ζ	*Neuropathic pain* (Spinal nerve ligation)	Spinal (i.t.)	Analgesic	Laffray et al. (2012)

positive allosteric modulators of $GABA_B$ receptors show efficacy in models of both colon and bladder pain is of some importance as bladder pain syndromes exhibit co-morbidity with other functional pain syndromes such as irritable bowel syndrome (Kim and Chang 2012; Malykhina et al. 2012).

5 Conclusions

As discussed above, it is now well established that $GABA_B$ receptors play an important role in the regulation of nociception and chronic pain at the peripheral, spinal and supraspinal level. Despite the large body of literature recommending baclofen as a potent analgesic for various pain states, it is rather questionable if $GABA_B$ receptor agonists will develop into first- or second-line analgesics for the treatment of chronic pain. This is mainly because the ubiquitous expression and the various physiological functions of $GABA_B$ receptors will inevitably induce side effects upon global activation. Because of their "use-dependent" action, positive allosteric modulators promise fewer side effects than orthosteric agonists. However, clinical data on their efficacy as analgesics and on their side effect profile is lacking so far. Exclusively peripheral acting positive allosteric modulators might have a great potential for the treatment of visceral pain because they are unlikely to be associated with central side effects. This, however, presumes that visceral pain signalling can effectively be inhibited at peripheral sites as the supraspinal component will remain unaffected.

Because of the limitations of $GABA_B$ receptor agonists and allosteric modulators, a promising strategy for future drug development targeting $GABA_B$ receptors might focus on biased agonists affecting $GABA_B$ receptors but only activating selected downstream pathways of the receptor, preferably involved in the pain state of interest. An example of this strategy, for the treatment of chronic visceral pain, might be α-conotoxin Vc1.1 (or ACV1), a synthetic peptide derived from the venom of the marine cone snail *Conus victoriae*. Vc1.1 activates $GABA_B$ receptors via an unidentified site (not via the orthosteric binding site) (McIntosh et al. 2009) and selectively inhibits $Ca_V2.2$ as well as $Ca_V2.3$ (Berecki et al. 2014; Cuny et al. 2012) in a c-src tyrosine kinase-dependent manner (Callaghan et al. 2008). Peripheral (intra-colonic) administration of Vc1.1 reduced mechanical hypersensitivity by inhibition of nociceptive signalling from the colon to the spinal cord (Castro et al. 2017). Vc1.1 most likely induces a very limited set of side effects, because it displays antinociceptive activity upon peripheral administration (no central effects expected) and activates only a subset of possible downstream effectors of $GABA_B$ receptors ($Ca_V2.2.$, $Ca_V2.3$).

Another example might be the upregulation of $GABA_B$ receptors by fucoidan, a polysaccharide isolated from marine brown seaweeds. Fucoidan exerts a variety of clinically relevant activities, including anticancer and anti-inflammatory effects (Wang et al. 2019). Repeated systemic application of fucoidan was recently shown to reduce hyperalgesia in the spinal nerve ligation model of neuropathic pain via an unknown mechanism (Hu et al. 2014). In a model of chemotherapeutic drug-induced neuropathic pain, fucoidan-mediated analgesia was accompanied with the upregulation of $GABA_B$ receptor expression (Hu et al. 2017). As repeated administration of fucoidan was required for its analgesic effect, it might well be that increased $GABA_B$ receptor activity caused by its upregulation is a contributing factor.

Finally, a potential for developing novel highly specific therapeutic interventions might be the targeting of pain-specific protein-protein interactions. As discussed above (see Sect. 3.2), the group of Marc Laundry provided an excellent example of such an approach regarding $GABA_B$ receptors and neuropathic pain (Laffray et al. 2012). Ideally, the development of interfering peptides to target pain-related protein-protein interactions involving $GABA_B$ receptors requires the discovery of interactions specifically associated with the pain state of interest. Future research needs to show whether such an approach can overcome the limitations of $GABA_B$ receptor agonists.

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$GABA_B$ Receptors: Anxiety and Mood Disorders

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Contents

Abstract Gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the brain, acts at the ionotropic $GABA_A$ and $GABA_C$ receptors, and the metabotropic $GABA_B$ receptor. This chapter summarizes the studies that have investigated the role of the $GABA_B$ receptor in stress-related psychiatric disorders including anxiety and mood disorders. Overall, clinical and preclinical evidences strongly suggest that the $GABA_B$ receptor is a therapeutic candidate for depression and anxiety disorders. However, the clinical development of $GABA_B$ receptor-based drugs to treat these disorders has been hampered by their potential side-effects, particularly those of agonists. Nevertheless, the discovery of novel $GABA_B$ receptor allosteric modulators, and increasing understanding of the influence of specific intracellular $GABA_B$ receptor-associated proteins on $GABA_B$ receptor activity, may now pave the way towards $GABA_B$ receptor therapeutics in the treatment of mood and anxiety disorders.

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1 Introduction

The inhibitory action of GABA is mediated by the ionotropic $GABA_A$ and $GABA_C$ receptors, and the metabotropic $GABA_B$ receptor. The $GABA_A$ receptor is bicuculline-sensitive and the subsequent opening of its transmembrane channel which is permeable to chloride mediates rapid neuronal inhibition in the adult brain. In 1979, Norman Bowery and colleagues published the discovery of a novel type of GABA receptor that was described as being "atypical" and insensitive to the $GABA_A$ receptor antagonist bicuculline (Bowery et al. 1979). Baclofen was identified to be a potent and selective agonist of this novel receptor, and in 1980 it was demonstrated that baclofen acting on this novel receptor decreased neurotransmitter release in the central nervous system (Bowery et al. 1980). This atypical receptor described by Bowery and colleagues would later be referred to as the $GABA_B$ receptor (Hill and Bowery 1981). The $GABA_B$ receptor is a G-protein-coupled receptor that inhibits adenylate cyclase activity and mediates the slow and prolonged component of synaptic inhibition (Bowery et al. 2004). $GABA_B$ receptors are localized in most brain regions, and $GABA_{B(1)}$ receptor mRNA is detectable in almost all neuronal cell populations and is highly expressed in the limbic system (Bettler et al. 2004; McDonald et al. 2004). The receptor consists of two subunits, $GABA_{B(1)}$ and $GABA_{B(2)}$, which heterodimerise to form the functional $GABA_B$ receptor (Bettler et al. 2004). The $GABA_{B(1)}$ subunit contains the orthosteric ligand binding site, while the $GABA_{B(2)}$ subunit is responsible for G-protein activation and contains binding sites for positive allosteric modulators (Galvez et al. 2001; Bettler et al. 2004; Binet et al. 2004; Gassmann and Bettler 2012). Isoforms of the $GABA_{B(1)}$ receptor subunit have been identified (Lee et al. 2010) and the two main isoforms expressed in the brain are $GABA_{B(1a)}$ and $GABA_{B(1b)}$ which form $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ receptors, respectively (Lee et al. 2010). Structurally, $GABA_{B(1)}$ isoforms differ only by the presence of a sushi domain in the N-terminal ectodomain of the $GABA_{B(1a)}$ receptor subunit isoform (see Fig. 1).

Since its discovery, there has been a long-standing interest in the therapeutic potential of the $GABA_B$ receptor. In this review, we will summarize the studies assessing the role of the $GABA_B$ receptor in mood disorders, specifically in depression, and in anxiety disorders. Clinical and preclinical evidences supporting the role for $GABA_B$ receptors in the pathophysiology of depression and anxiety disorders will be summarized in addition to the preclinical evidence of the antidepressant and anxiolytic effects of pharmacological and genetic modulation of $GABA_B$ receptor activity. Unless otherwise stated, most of the preclinical studies discussed in this chapter have been conducted in male rodents. Since most of this evidence is from

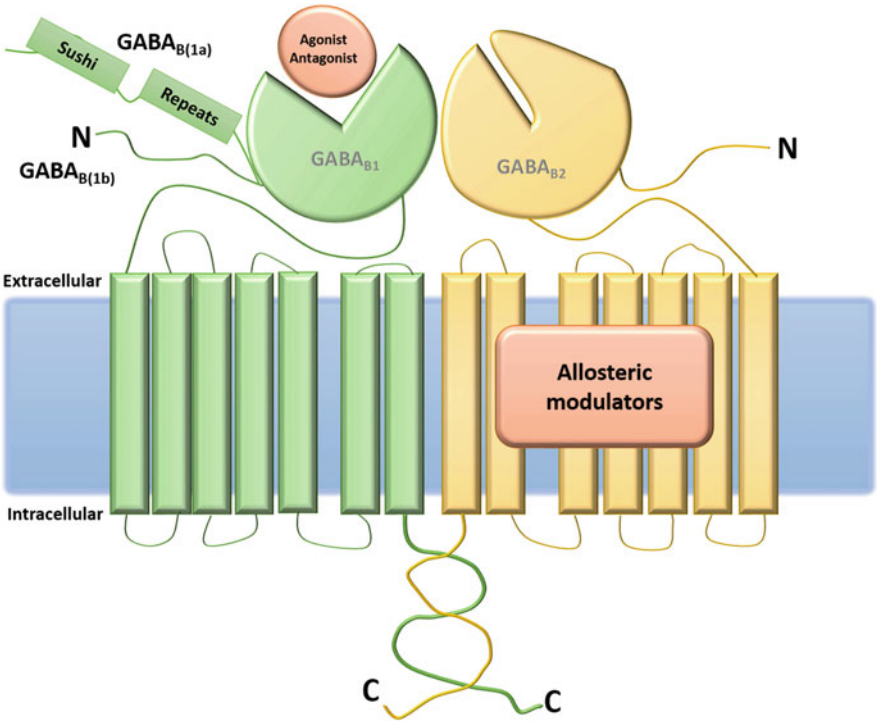

Fig. 1 Schematic representation of the $GABA_B$ receptor. $GABA_B$ receptors are composed of $GABA_{B(1)}$ and $GABA_{B(2)}$ receptor subunits that form an active heterodimer. The $GABA_{B(1)}$ receptor subunit is essential for the binding of GABA and $GABA_B$ receptor agonists and antagonists. $GABA_{B(1)}$ receptor subunit presents as two main isoforms, namely $GABA_{B(1a)}$ and $GABA_{B(1b)}$ that differ by the presence of a sushi domain in the N-terminal of the $GABA_{B(1a)}$ isoform. Adapted from Cryan and Kaupmann (2005)

preclinical studies, readers outside this research field are advised to first read Table 1, which summarizes the behavioural tests used to assess depression-, antidepressant- and anxiety-like behaviour in rodents (Cryan and Slattery 2007), prior to reading the review.

2 Role of the $GABA_B$ Receptor in the Modulation of Anxiety

2.1 Effects of $GABA_B$ Receptor Agonists and Positive Allosteric Modulators on Anxiety-Like Behaviour

Baclofen is the first described $GABA_B$ receptor agonist (Bowery et al. 1980), which was synthetized in 1962 by Heinrich Keberle in CIBA (Basel, Switzerland).

Table 1 Behavioural tests used in anxiety and depression research

Behavioural tests	Description
Anxiety	
Elevated zero maze (EZM)	Rodents are placed on the elevated zero maze consisting of two open (stressful) and two enclosed (protecting) elevated areas that form a zero or circle. Naturally, rodents display aversion to open areas. Anxiolytic drug treatment increases the number of entries and time spent in the open areas of the maze
Elevated plus maze (EPM)	Rodents are placed on the elevated plus maze consisting of two open arms and two closed arms. Naturally, rodents display aversion versus open areas. Anxiolytic drug treatment increases number of entries and time spent in the open arms
Light/dark box (LDB)	Rodents are placed in an apparatus consisting of a light and dark compartment. Naturally, rodents display aversion to illuminated areas. Anxiolytic drug treatment increases the time spent and the number of entries into the light compartment
Marble burying (MB)	Rodents are placed in a cage containing bedding and a number of novel marbles. Anxiolytic drug treatment reduces the number of marbles buried
Stress-induced hyperthermia (SIH)	Temperature is measured twice at an interval of 15 min. The difference between the first and second measurements is a physiological measure of anxiety. Anxiolytic drug treatment reduces the magnitude of the increase in temperature which is observed in the second measurement
Ultrasonic vocalizations	Rodent pups when separated from their dams produce alarm calls that are an index of anxiety. Anxiolytic drug treatment reduces the number of alarm calls
Vogel conflict test	Rodents are punished by electric shocks when trying to get either food or water, and thus the number of times the animal goes to get food or water decreases. Anxiolytic drugs increase the number of punished responses in the presence of shock as compared to unpunished responses
Staircase test	Rodents are placed in an enclosed staircase with five steps. The number of steps climbed and rearings made in a 3-min period are observed. Anxiolytic drugs reduce rearing at doses that do not reduce the number of steps climbed
Light-enhanced startle (LES)	Startle reactivity is increased by presentation of a bright light. Because LES is based on the innate aversion of rodents for bright light, it does not require training sessions. Anxiolytic drug treatment reduces startle potentiation
Fear-potentiated startle (FPS)	The test consists of two training sessions in which an aversive foot shock is paired with a neutral cue light. In the test session, presentation of this cue light is used to elicit startle potentiation. Anxiolytic drugs reduce startle potentiation
Fear conditioning (FC)	Rodents are placed in a FC box in which an aversive stimulus (electric shock) is paired with a neutral context (such as a location) or stimulus (such as a tone). This results in the expression of a fear response (freezing behaviour) in the presence of the neutral context or neutral stimulus alone. Anxiolytics reduce freezing behaviour

(continued)

Table 1 (continued)

Behavioural tests	Description
Four-plate test	Animals are exposed to a novel environment and exploratory behaviour is suppressed by the delivery of a mild electric foot shock contingent on quadrant crossing. Animals can only escape from this aversive situation by remaining motionless (passive avoidance). Anxiolytic drugs increase exploratory behaviour
Depression	
Forced swim test (FST)	Rodents placed in an inescapable container of water engage in escape oriented behaviours. After few minutes, the animal adopts an immobile posture just making sufficient movements to keep its head above water. Antidepressant drug treatment reduces time spent immobile
Tail suspension test (TST)	Mice, when suspended from the tail, will adopt an immobile posture. Antidepressant treatment decreases time spent immobile
Learned helplessness (LH)	Animals exposed to inescapable shocks subsequently fail to escape when an escape option is presented. Antidepressants increase the number of escapes
Olfactory bulbectomy (OB)	Removal of the olfactory bulbs causes several behavioural and neurochemical alterations, which are only reversed by chronic antidepressant treatment
Maternal deprivation	When rodents are separated from their dams during early postnatal life they can develop a number of depression-like behaviours. Chronic antidepressant treatment reverses those abnormal behaviours
Chronic mild stress (CMS)	Rodents are subjected to a variety of unpredictable stressors which leads to several behavioural alterations. Chronic treatment with antidepressants reverses those alterations
Social defeat stress (SDS)	Rodents are exposed to physical interaction with an unfamiliar aggressive animal, combined with sensory contact throughout the stress procedure. This protocol, repeated daily, induces depression-like behaviours that are reversed by antidepressant treatment

Adapted from Cryan and Slattery (2007)

Baclofen was formulated as an antiepileptic drug and marketed in 1972 as Lioresal. Currently, baclofen is indicated primarily to treat spasticity but it also has beneficial effects in treating pain, is used off-label in the treatment of alcohol use disorder and has been shown to inhibit the re-enforcing effects of many other addictive drugs (Bowery et al. 2002). However, there is also much preclinical evidence suggesting that $GABA_B$ receptor agonists such as baclofen may be potential therapeutic approaches to treat anxiety disorders (Cryan et al. 2005; Cryan and Slattery 2010; Felice et al. 2016) (summarized in Table 2).

Acute baclofen administration has been shown to reduce anxiety-like behaviour in several rat and mouse models (Ketelaars et al. 1988; File et al. 1991, 1992; Nastiti et al. 1991; Shephard et al. 1992; Andrews and File 1993; Amikishieva and Semendyaeva 2007; Lu et al. 2016), although some conflicting findings have also been reported. For instance, while one study reported that baclofen was effective in the Vogel conflict test (Ketelaars et al. 1988), another study reported no such effect

Table 2 Effects of $GABA_B$ receptor agonists and positive allosteric modulators (PAMs) on anxiety-like behaviours in rodents

Drug	Paradigm	Finding	References
Agonists			
Baclofen	Vogel conflict	Acute treatment ↓ anxiety in rats	File et al. (1991)
		Acute treatment ↓ anxiety in rats	Ketelaars et al. (1988)
		Acute treatment ↓ anxiety in rats	Shephard et al. (1992)
		Acute treatment ↔ in rats	Agmo et al. (1991)
		Acute treatment ↔ in rats	Li et al. (2013)
	EPM	Acute treatment ↓ anxiety in ethanol withdrawal model in rats	File et al. (1992)
		Acute treatment ↓ anxiety in mice	Amikishieva and Semendyaeva (2007)
		Injection in the AcbSh ↔ in 24 h food-deprived rats	Lopes et al. (2012)
		Acute treatment ↔ in rats	Li et al. (2013)
		Acute treatment ↔ mice	Dalvi and Rodgers (1996)
		Acute treatment ↔ in nicotine-induced anxiety in mice	Varani and Balerio (2012)
		PND 14–28 treatment ↑ anxiety in adult mice	Sweeney et al. (2014)
	EZM	Acute treatment ↔ in rats	Frankowska et al. (2007)
	LDB	Acute treatment ↔ in mice	Li et al. (2013)
	SI	Microinjection in the BLA ↔ in rats	Sanders and Shekhar (1995)
	SIH	Acute treatment ↔ in rats	Li et al. (2015)
	LES	Acute treatment ↔ in rats	Li et al. (2015)
CGP44532	Four plate	Acute treatment ↑ anxiety in mice	Partyka et al. (2007)
SKF97541	EZM	Acute treatment ↔ in rats	Frankowska et al. (2007)
PAMS			
GS39783	EZM	Acute treatment ↓ anxiety in rats, mice	Cryan et al. (2004)
		Acute, chronic treatment ↓ anxiety in mice	Mombereau et al. (2004)
	EPM	Acute, chronic treatment ↓ anxiety in rats	Cryan et al. (2004)
	SIH	Acute, chronic treatment ↓ anxiety in mice	Cryan et al. (2004)
	LDB	Acute, chronic treatment ↓ anxiety in mice	Mombereau et al. (2004)
CGP7930	EZM	Acute treatment ↓ anxiety in rats	Frankowska et al. (2007)

(continued)

Table 2 (continued)

Drug	Paradigm	Finding	References
		Acute treatment ↓ anxiety in mice	Jacobson and Cryan (2008)
	EPM	Acute treatment ↔ in rats	Jacobson and Cryan (2008)
	SIH	Acute treatment ↓ anxiety in mice	Jacobson and Cryan (2008)
	Staircase	Acute treatment ↓ anxiety in mice	Jacobson and Cryan (2008)
rac-BHFF	SIH	Acute treatment ↓ anxiety in mice	Malherbe et al. (2008)
BHF177	EPM	Acute treatment ↔ in mice	Li et al. (2013)
	LDB	Acute treatment ↔ in mice	Li et al. (2013)
	Vogel conflict	Acute treatment ↔ in mice	Li et al. (2013)
	LES	Acute treatment ↔ in rats (anxiolytic-like effects in high but not low LES-responding rats)	Li et al. (2015)
ADX71441	MB	Acute treatment ↓ anxiety in mice	Kalinichev et al. (2017)
	EPM	Acute treatment ↓ anxiety in mice, rats	Kalinichev et al. (2017)

AcbSh nucleus accumbens shell, *BLA* basolateral amygdala, *EPM* elevated plus maze, *EZM* elevated zero maze, *LDB* light/dark box, *LES* light-enhanced startle, *MB* marble burying, *PND* postnatal day, *SI* social interaction, *SIH* stress-induced hyperthermia
↓ = decreased; ↔ = no effects; ↑ = increased

(Agmo et al. 1991). However, the latter study also reported that higher doses of baclofen induced motor deficits in rats which may have reduced the number of licks, thus resulting in a potentially false negative finding in this test (Agmo et al. 1991). Similarly, Li and colleagues have reported that baclofen had sedative but not anxiolytic effects in rats in several behavioural tests (Li et al. 2015). Conflicting findings have also been reported in mice whereby baclofen was anxiolytic in some studies (Nastiti et al. 1991; Amikishieva and Semendyaeva 2007) but not in others (Dalvi and Rodgers 1996; Varani and Balerio 2012; Li et al. 2013). In one such study, baclofen increased punished drinking in the Vogel conflict test which would be indicative of an anxiolytic effect, but the authors suggest that this finding may also be due to analgesic effects of baclofen (Li et al. 2013). Motor impairing and hypothermic effects are characteristic side-effects of $GABA_B$ receptor agonists, and this likely confounds the interpretation of anxiety-related behavioural tests that are dependent on motor activity (e.g. elevated plus maze, Vogel conflict test, etc.) or body temperature (e.g. stress-induced hyperthermia) (Cryan et al. 2004). In addition, the effects of baclofen on anxiety may depend upon the developmental stage of the brain. For example, we have found that chronic treatment with R-baclofen during

early postnatal life (Postnatal day (PND) 14- PND 28) in mice induced anxiety-like behaviour in adulthood in the elevated plus maze (EPM) but not in the stress-induced hyperthermia (SIH) and marble burying (MB) tests (Sweeney et al. 2014). This suggests that during early life $GABA_B$ receptor signalling might play a functional role in programming anxiety behaviour in adulthood (Sweeney et al. 2014), although this effect might also be test-specific.

Importantly, baclofen has several side-effects including sedation or somnolence, hypothermia, vertigo and muscle relaxation (Agabio et al. 2013). Moreover, repeated administration of $GABA_B$ agonists such as baclofen can induce receptor tolerance/desensitization resulting in a reduced therapeutic response following chronic administration (Lehmann et al. 2003). Thus, there has been great interest in developing drugs that target the $GABA_B$ receptor but with a reduced side-effect profile and that would not result in tolerance. As such, positive allosteric modulators (PAMs) offer several advantages over receptor agonists such as baclofen (Christopoulos 2002): (1) PAMs target more diverse sites that are distinct from the highly evolutionary conserved orthosteric site thus potentially contributing to greater selectivity; (2) PAM binding leads to potentiation of GABA-mediated effects on the receptor rather than direct activation of the receptor; (3) saturation of allosteric binding sites does not induce downregulation or overstimulation of the target receptor; (4) PAMs are active only in tissues where the endogenous agonist is present giving a more specific drug activity. Essentially, PAMs of $GABA_B$ receptors offer the advantage of reduced risk for receptor desensitization/tolerance when compared with classical $GABA_B$ receptor agonists such as baclofen (Gjoni and Urwyler 2008, 2009).

The first $GABA_B$ receptor PAMs that were identified and characterized were CGP7930 (Urwyler et al. 2001; Adams and Lawrence 2007) and GS39783 (Urwyler et al. 2003), shortly followed by rac-BHFF (Malherbe et al. 2008), BHF177 (compound # 27 (Guery et al. 2007)), CMPPE (Perdona et al. 2011), COR627 and COR628 (Castelli et al. 2012).

Several preclinical studies have interrogated the effects of some of these $GABA_B$ receptor PAMs on anxiety-like behaviour (summarized in Table 2). Chronic and acute administration of GS39783 has been shown to induce anxiolytic-like effects with no effects on locomotion, cognition, temperature or narcosis (Cryan et al. 2004; Mombereau et al. 2004). A recent study identified the brain structures that are modulated by GS39783 under either basal or mild stress (anxiogenic) conditions which were induced by exposing mice to the open arm of an EPM (Pizzo et al. 2018). Under basal conditions, GS39783 increased c-Fos expression in the amygdala nuclei, cortical areas and periaqueductal gray (PAG) subregions, while it inhibited c-Fos expression in the dorsal raphe nucleus (DRN) (Pizzo et al. 2018). Under stress conditions (open arm exposure), GS39783 reversed stress-induced c-Fos expression in the granular cell layer of the dentate gyrus of the hippocampus, no longer increased c-Fos expression in the amygdala nor did it reduce c-Fos expression in the DRN (Pizzo et al. 2018). Together, this suggests that GS39783 modulation of anxiety may involve neural circuits involving the dentate gyrus of the hippocampus, the amygdala and the DRN.

CGP7930 has only modest anxiolytic-like effects in mice but a superior side-effect profile than $GABA_B$ receptor agonists (Frankowska et al. 2007; Jacobson and Cryan 2008). Specifically, CGP7930 was effective in the elevated zero maze (EZM) in rats (Frankowska et al. 2007) and exhibited modest anxiolytic effects in the SIH, staircase test and EZM in mice (Jacobson and Cryan 2008). However, CGP7930 had no anxiolytic effects in the EPM in mice (Jacobson and Cryan 2008).

Both Rac-BHFF and BHF177 induce anxiolytic effects in some tests but not others. Specifically, Rac-BHFF and BHF177 induced anxiolytic-like effects in the SIH test in mice and rats, a test of the physiological anxiety response (Malherbe et al. 2008; Vinkers et al. 2010; Li et al. 2015). BHF177 induced anxiolytic-like effects on light-enhanced startle (LES; a test based on the innate aversion of rodents for bright light) in high-, but not low-LES responding rats in the staircase test (Li et al. 2015) but was inactive in the EPM and light dark box test in mice (Li et al. 2013). Importantly, BHF177, at doses over 40 mg/kg, caused hypothermia in contrast to other $GABA_B$ receptor PAMs including CGP7930 and Rac-BHFF (Vinkers et al. 2010) which may have confounded findings in SIH test. On the other hand, Rac-BHFF at the same dose that induced anxiolytic-like effects in the SIH (100 mg/kg) did not enhance baclofen- and γ-hydroxybutyric acid (GHB)-induced hypothermia (Koek et al. 2010), suggesting that its effects in the SIH test are not confounded by effects of $GABA_B$ receptor modulation of body temperature. A novel $GABA_B$ receptor PAM ADX71441 has also been shown to be effective in the MB test in mice and in the EPM in mice and rats (Kalinichev et al. 2017). Recently, Rondard and colleagues (Lecat-Guillet et al. 2017) developed time-resolved fluorescence resonance energy transfer (trFRET) sensors which represent an innovative tool to screen and identify new $GABA_B$ receptors PAMs with lower side-effect profiles. Interestingly, trFRET revealed that GS39783 exhibits low intrinsic agonist activity (as expected by a PAM), whereas CGP7930 and rac-BHFF display agonist-PAMs characteristics (Lecat-Guillet et al. 2017). This finding is in agreement with behavioural studies outlined above demonstrating that GS39783 induced anxiolytic-like behavioural effects without affecting locomotion, cognition, temperature or narcosis, and suggests that this drug may be a good target for clinical development. Effects of PAMs on conditioned anxiety have also been examined. BHF177 did not affect conditioned fear responses in the fear-potentiated startle (FPS) test in rats (Li et al. 2015) and was ineffective in the Vogel conflict test (Li et al. 2013). Similarly, treatment with GS39783 did not affect conditioned fear responses in mice (Sweeney et al. 2013).

Taken together, preclinical evidence suggests that activation of the $GABA_B$ receptor may induce anxiolytic-like effects particularly in tests of innate anxiety whereby PAMs decrease innate anxiety in some tests but not others, and thus perhaps do so in a test-specific manner. Importantly, these findings may also be confounded by motor impairing and hypothermic effects.

2.2 *Effects of $GABA_B$ Receptor Loss of Function and $GABA_B$ Receptor Antagonists on Anxiety-Like Behaviour*

Given the evidence that agonists and PAMs of the $GABA_B$ receptor can exert anxiolytic effects, several studies have also interrogated the impact of genetically induced $GABA_B$ receptor loss of function and $GABA_B$ receptor antagonists on anxiety-like behaviour (summarized in Table 3).

Mice lacking either the $GABA_{B(1)}$ or $GABA_{B(2)}$ receptor subunits exhibit an anxious phenotype. Specifically, $GABA_{B(1)}{}^{-/-}$ mice were more anxious in the light dark box (LDB) test and the staircase test (Mombereau et al. 2004). In addition, these mice exhibited anxiety/panic-like behaviour in the EZM actively jumping off the maze (Mombereau et al. 2004). Similarly, mice lacking the $GABA_{B(2)}$ receptor subunit also exhibit anxiety-like behaviour in the LDB (Mombereau et al. 2005). Anxiety behaviour has also been assessed in mice lacking specific isoforms of the $GABA_{B(1)}$ receptor subunit. $GABA_{B(1a)}{}^{-/-}$ and $GABA_{B(1b)}{}^{-/-}$ mice did not exhibit altered behaviour in innate tests of anxiety including in the EPM, SIH and MB tests (Jacobson et al. 2007; O'Leary et al. 2014). Similarly, $GABA_{B(1a)}{}^{-/-}$ and $GABA_{B(1b)}{}^{-/-}$ mice that underwent early life stress (via maternal separation) or chronic stress in adulthood (via social defeat stress) did not exhibit differences in innate anxiety behaviour when compared to wild type mice (O'Leary et al. 2014). On the other hand, $GABA_{B(1a)}{}^{-/-}$ mice were unable to acquire conditioned taste aversion (CTA), whereas $GABA_{B(1b)}{}^{-/-}$ mice were unable to extinguish aversive taste memories in this test (Jacobson et al. 2006). Taken together, this suggests that loss of function of either the $GABA_{B(1)}$ or $GABA_{B(2)}$ receptor subunit increases innate anxiety, while loss of function of just one $GABA_{B(1)}$ receptor subunit isoform is not sufficient to affect innate anxiety-like behaviour. However, changes in locomotor activity can be a confounding factor of the behavioural tests, for instance $GABA_{B(1)}{}^{-/-}$ and $GABA_{B(1b)}{}^{-/-}$ (but not $GABA_{B(1a)}{}^{-/-}$) mice display hyperlocomotor activity in a new environment (Mombereau et al. 2004; O'Leary et al. 2014).

In contrast to the findings in genetically altered mice, the effects of $GABA_B$ receptor antagonists on anxiety behaviour are less clear (Table 3). Overall, however, the findings suggest that $GABA_B$ receptor antagonists can induce anxiolytic-like effects in rats (Zarrindast et al. 2001; Frankowska et al. 2007; Partyka et al. 2007) but less so in mice (Dalvi and Rodgers 1996; Mombereau et al. 2004; Sweeney et al. 2014). When given systemically to rats, $GABA_B$ receptor antagonists were effective in the EPM, EZM, Vogel conflict test and four-plate test (Zarrindast et al. 2001, Frankowska et al. 2007, Partyka et al. 2007) but were ineffective when locally administered into the basolateral amygdala or the shell of the nucleus accumbens (Sanders and Shekhar 1995; Lopes et al. 2012). In mice, chronic treatment with the $GABA_B$ receptor antagonist CGP56433A had no effect in the LDB test (Mombereau et al. 2004). Similarly, acute treatment with the $GABA_B$ receptor antagonist CGP 52432 did not have anxiolytic effects in the EPM, MB and SIH tests (Dalvi and Rodgers 1996; Sweeney et al. 2014) or in cued auditory fear conditioning (Sweeney et al. 2013). However, the $GABA_B$ receptor antagonist CGP36742 induced

Table 3 Effects of $GABA_B$ receptor inhibition or loss of function on anxiety-like behaviours in rodents

Mice	Paradigm	Finding	References
Genetic			
$GABA_{B(1)}^{-/-}$	LDB	↑ anxiety	Mombereau et al. (2004)
	Staircase test	↑ anxiety	Mombereau et al. (2004)
$GABA_{B(2)}^{-/-}$	LDB	↑ anxiety	Mombereau et al. (2005)
$GABA_{B(1a)}^{-/-}$	EPM	↔ anxiety	Jacobson et al. (2007)
		↔ anxiety	O'Leary et al. (2014)
	SIH	↔ anxiety	Jacobson et al. (2007)
		↔ anxiety	O'Leary et al. (2014)
	MB	↔ anxiety	Jacobson et al. (2007)
	FC	Generalized fear to a neutral context 24 h after training	Lynch et al. (2017)
$GABA_{B(1b)}^{-/-}$	EPM	↔ anxiety	Jacobson et al. (2007)
		↔ anxiety	O'Leary et al. (2014)
	SIH	↔ anxiety	Jacobson et al. (2007)
		↔ anxiety	O'Leary et al. (2014)
	MB	↔ anxiety	Jacobson et al. (2007)
Antagonists			
CGP 35348	EPM	Acute treatment ↔ anxiety in mice	Dalvi and Rodgers (1996)
		Acute treatment ↓ anxiety in rats	Zarrindast et al. (2001)
CGP56433A	LDB	Chronic treatment ↔ anxiety in mice	Mombereau et al. (2004)
SCH 50911	EZM	Acute treatment ↓ anxiety in rats	Frankowska et al. (2007)
CGP 36742	EPM	Acute treatment ↓ anxiety in rats	Partyka et al. (2007)
	Vogel conflict	Acute treatment ↓ anxiety in rats	Partyka et al. (2007)
	Four plate	Acute treatment ↓ anxiety in rats	Partyka et al. (2007)
CGP51176	Four plate	Acute treatment anxiety ↔ in mice	Partyka et al. (2007)
Saclofen	EPM	Injection in the AcbSh ↔ anxiety in 24 h food-deprived rats	Lopes et al. (2012)
		Acute treatment prevented nicotine-induced anxiety in mice	Varani and Balerio (2012)

(continued)

Table 3 (continued)

Mice	Paradigm	Finding	References
CGP52432	FC	Acute treatment ↔ anxiety in mice	Sweeney et al. (2013)
	EPM	PND 14–28 treatment ↔ anxiety in adult mice	Sweeney et al. (2014)

EPM elevated plus maze, *EZM* elevated zero maze, *FC* fear conditioning, *LDB* light/dark box, *LES* light-enhanced startle, *MB* marble burying, *PND* postnatal day, *SI* social interaction, *SIH* stress-induced hyperthermia, *AcbSh* nucleus accumbens shell
↓ = decreased; ↔ = no effects; ↑ = increased

anxiolytic-like effects in the four-plate test in mice (Partyka et al. 2007), and the $GABA_B$ receptor antagonist 2OH-Saclofen reversed the effects of nicotine treatment on anxiety-like behaviours in mice (Varani and Balerio 2012). In addition, the $GABA_B$ receptor antagonist CGP 36216 when administered intracerebroventricularly (ICV) or in the dorsal hippocampus or ventral hippocampus induced fear generalization in mice treated after fear memory consolidation (Lynch et al. 2017). Importantly, the clinical use of $GABA_B$ receptor antagonists has been limited mainly by their potential side-effects including pain, gastroesophageal reflux disease, drug addiction and proconvulsive action (Vergnes et al. 1997; Ghose et al. 2011).

In summary, $GABA_B$ receptor agonists and PAMs exert anxiolytic-like effects, while loss of function of the $GABA_B$ receptor ($GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice) induced anxiogenic-like effects. However, loss of function of either the $GABA_{B(1a)}$ or $GABA_{B(1b)}$ receptor subunit isoform alone did not affect anxiety-like behaviour, likely because these mice still express functional $GABA_B$ receptors ($GABA_{B(1b,2)}$ or $GABA_{B(1a,2)}$, respectively). The impact of $GABA_B$ receptor antagonists on anxiety is at present somewhat less clear but sometimes similar to agonists/PAMs appears to be anxiolytic. The precise mechanisms underlying the anxiolytic effects of both $GABA_B$ receptor antagonists, and agonists/PAMS which would be expected to have opposing pharmacological effects are not yet fully understood but may be a function of the fact that $GABA_B$ receptors are found both pre-synaptically and post-synaptically and that drugs might differ in their efficacy at these different receptor sites and at different subunits of the receptor (Cryan and Kaupmann 2005; Sun et al. 2016; Freyd et al. 2017). Nevertheless, the evidence overwhelmingly supports the $GABA_B$ receptor as a valid drug development target for the treatment of anxiety disorders.

3 Role of the $GABA_B$ Receptor in Depression and Antidepressant Action

One of the first indications that the $GABA_B$ receptor may play a role in depression came from preclinical studies reporting that chronic treatment with antidepressant drugs or repeated electroconvulsive shock upregulated $GABA_B$ receptor binding and function in the mouse and rat frontal cortex (Pilc and Lloyd 1984; Lloyd et al. 1985; Suzdak and Gianutsos 1986; Gray and Green 1987; Szekely et al. 1987; Pratt and Bowery 1993). More recently, it has been reported that chronic treatment with antidepressants (fluoxetine, phenelzine, desipramine and tranylcypromine) increased the expression of the $GABA_{B(1a)}$ receptor subunit isoform in the rat hippocampus (Sands et al. 2004). As outlined below, it has since been shown that pharmacological or genetic blockade of $GABA_B$ receptor activity exerts antidepressant-like effects. While these effects of $GABA_B$-receptor antagonist induction of antidepressant-like behaviour seem to be opposing to antidepressant-induced upregulation of the $GABA_B$ receptor, they might be due to drug selective effects on either or both presynaptic and postsynaptic $GABA_B$ receptors (Cryan and Kaupmann 2005, Sun et al. 2016, Freyd et al. 2017).

3.1 Effects of $GABA_B$ Receptor Agonists on Depression-Like Behaviour

The effects of $GABA_B$ receptor agonists on depression-related behaviours in rodents are summarized in Table 4. Several studies have reported that baclofen induced antidepressant-like behaviour in the forced swimming test (FST) in both mice and rats (Aley and Kulkarni 1989, 1990; Car and Wisniewska 2006; Frankowska et al. 2007; Khan et al. 2016). In agreement, it has also been reported that acute treatment with the $GABA_B$ receptor agonist SKF 97541, or the $GABA_B$ receptor PAM, CGP 7930, induced antidepressant-like effects in the rat FST (Frankowska et al. 2007). However, negative findings have also been reported. Indeed, the $GABA_B$ receptor agonists Phaclofen and CGP 44532, and the PAM, GS39783, did not exhibit antidepressant-like activity in the FST in mice or rats (Mombereau et al. 2004; Slattery et al. 2005; Nowak et al. 2006; Araki et al. 2016; Pesarico et al. 2016). Moreover, it was reported that chronic administration of baclofen exacerbated learned helplessness in rats (Nakagawa et al. 1996b) and that baclofen attenuated the effects of several antidepressants in the rat FST and in the learned helplessness model (Nakagawa et al. 1996a, 1996b). More recently, a study showed the baclofen inhibited the antidepressant-like effects of ketamine (which has rapid antidepressant effects) in the mouse tail suspension test (TST) (Rosa et al. 2016). Taken together, it is not yet entirely clear whether pharmacological activation of the $GABA_B$ receptor has antidepressant-like effects.

Table 4 Effects of $GABA_B$ receptor agonists and positive allosteric modulators (PAMs) on depression-like behaviours in rodents

Drug	Paradigm	Finding	References
Agonists			
Baclofen	FST	Acute treatment ↓ immobility in mice	Aley and Kulkarni (1989)
		Acute treatment ↓ immobility in mice	Aley and Kulkarni (1990)
		Acute treatment ↓ immobility in mice	Khan et al. (2016)
		Acute treatment ↓ immobility in rats	Car and Wisniewska (2006)
		Acute treatment ↓ immobility in rats	Frankowska et al. (2007)
		Acute treatment ↔ immobility in isolation reared mice	Araki et al. (2016)
	LH	Chronic treatment ↑ escape failures in rats	Nakagawa et al. (1996a)
		Chronic treatment ↑ escape failures in desipramine-treated rats	Nakagawa et al. (1996b)
	TST	Acute treatment ↑ immobility in mice treated with ketamine/ascorbic acid	Rosa et al. (2016)
PAMs			
SKF 97541	FST	Acute treatment ↓ immobility in rats	Frankowska et al. (2007)
CGP7930		Acute treatment ↓ immobility in rats	Frankowska et al. (2007)
GS39783		Acute treatment ↔ immobility in mice	Mombereau et al. (2004)
		Acute treatment ↔ immobility in mice	Slattery et al. (2005)
CGP 44532		Acute treatment ↔ immobility in mice	Nowak et al. (2006)

FST forced swim test, *LH* learned helplessness
↓ = decreased; ↔ = no effects; ↑ = increased

3.2 *Effects of $GABA_B$ Receptor Blockade or Loss of Function on Depression-Like Behaviour*

In contrast to the data on $GABA_B$ receptor agonists and PAMs, we have much stronger evidence that $GABA_B$ receptor blockade (either pharmacologically or genetically) induces antidepressant-like behaviour (see Table 5). Most studies report that chronic or acute treatment with $GABA_B$ receptor antagonists has antidepressant-like effects in both mice and rats. For instance, the $GABA_B$ receptor antagonist, CGP36742, exhibits antidepressant-like activity in mice in several behavioural tests including the FST, chronic mild stress paradigm, olfactory bulbectomy model and

Table 5 Effects of $GABA_B$ receptor inhibition or loss of function on depression-like behaviours in rodents

Mice	Paradigm	Finding	References
Genetic			
$GABA_{B(1)}^{-/-}$	FST	↓ immobility	Mombereau et al. (2004)
$GABA_{B(2)}^{-/-}$		↓ immobility	Mombereau et al. (2005)
$GABA_{B(1a)}^{-/-}$	FST	↓ immobility	O'Leary et al. (2014)
		↓immobility	Jacobson et al. (2017)
	MS	↑ susceptibility	O'Leary et al. (2014)
	SDS	↑ susceptibility	Jacobson et al. (2017)
$GABA_{B(1b)}^{-/-}$	FST	↓ immobility	O'Leary et al. (2014)
	MS	↑ resilience	O'Leary et al. (2014)
	SDS	↑ resilience	O'Leary et al. (2014)
Antagonists			
CGP36742	LH	Acute treatment ↓ depression-like behaviour in rats	Nakagawa et al. (1999)
	FST	Acute treatment ↓ immobility in mice	Nowak et al. (2006)
CGP56433A	FST	Acute treatment ↓ immobility in mice	Mombereau et al. (2004)
		Acute treatment ↓ immobility in mice	Slattery et al. (2005)
CGP51176	FST	Acute treatment ↓ immobility in mice	Nowak et al. (2006)
	Sucrose preference	Chronic treatment ↓ anhedonia in the CMS rat model	Nowak et al. (2006)
SCH 50911	FST	Acute treatment ↓ immobility in mice	Frankowska et al. (2007)
CGP52432	FST	Acute, subchronic and chronic treatment ↓ immobility in mice	Felice et al. (2012)

CMS chronic mild stress, *FST* forced swim test, *LH* learned helplessness, *MS* maternal separation, *SDS* social defeat stress
↓ = decreased; ↔ = no effects; ↑ = increased

the learned helplessness paradigm (Nakagawa et al. 1999; Nowak et al. 2006). Similarly, the $GABA_B$ receptor antagonists CGP51176, CGP51176A, CGP56433A, SCH50911 and CGP52432 also induced antidepressant-like effects in both the mouse and rat FST (Mombereau et al. 2004; Slattery et al. 2005; Frankowska et al. 2007; Felice et al. 2012). In addition, CGP51176A has also been shown to reduce stress-induced anhedonia as measured by increased sucrose consumption in the chronic mild stress rat model (Nowak et al. 2006).

Studies in genetically modified $GABA_B$ receptor mice have revealed findings similar to that observed with receptor antagonists. $GABA_{B(1)}^{-/-}$ and $GABA_{B(2)}^{-/-}$ mice exhibit an antidepressant-like phenotype in the FST (Mombereau et al. 2004,

2005). In the TST, male but not female $GABA_{B(1b)}^{-/-}$ mice displayed decreased immobility suggesting antidepressant-like phenotype whereas male and female $GABA_{B(1a)}^{-/-}$ mice exhibited increased immobility, suggesting a depression-like phenotype. In the FST, both $GABA_{B(1a)}^{-/-}$ and $GABA_{B(1b)}^{-/-}$ mice exhibited an antidepressant-like phenotype (O'Leary et al. 2014). However, male but not female $GABA_{B(1b)}^{-/-}$ mice are hyperactive in the open field test which may have contributed to the reduced immobility of males in the FST and TST (O'Leary et al. 2014). Interestingly, $GABA_{B(1a)}^{-/-}$ mice are more susceptible whereas $GABA_{B(1b)}^{-/-}$ mice are more resilient to early life stress (via maternal separation) and social defeat stress in adulthood (O'Leary et al. 2014). Specifically, $GABA_{B(1a)}^{-/-}$ mice are more susceptible to stress (maternal separation or social defeat stress) -induced anhedonia as measured in the saccharin preference and female urine sniffing tests, and were also more susceptible to social defeat stress-induced social avoidance (O'Leary et al. 2014). On the other hand, $GABA_{B(1b)}^{-/-}$ mice were resilient to stress-induced anhedonia and psychosocial stress-induced social withdrawal (O'Leary et al. 2014). In addition, $GABA_{B(1a)}^{-/-}$ but not $GABA_{B(1b)}^{-/-}$ mice exhibited a blunted 8-OH-DPAT-induced corticosterone and adrenocorticotropic hormone (ACTH) release, thus suggesting disrupted regulation of the hypothalamic-pituitary-adrenal (HPA) axis which is the neuroendocrine stress response system (Jacobson et al. 2017).

Taken together, preclinical pharmacological studies and studies using genetically altered $GABA_B$ receptor mice strongly suggest that inhibition of $GABA_B$ receptors has therapeutic potential in the treatment of depression (Alexander 2017; Jacobson et al. 2018). As described earlier, sometimes, the $GABA_B$ receptor agonist baclofen has also been shown to have antidepressant-like effects in the forced swim test (FST). The precise mechanisms underlying how opposing pharmacological manipulations (agonist vs. antagonist) could exert similar antidepressant-like effects is unknown. However, it may be a function of the fact that $GABA_B$ receptors are found both pre-synaptically and post-synaptically, and that drugs might differ in their selectivity for these differentially located $GABA_B$ receptors. The subunit composition of affected receptors might also influence behavioural responses to pharmacological agents. For example, it has been shown that mice lacking $GABA_{B(1b)}$ receptor subunit isoform exhibit a stress-resilient phenotype, while mice lacking the $GABA_{B(1a)}$ subunit are more stress-susceptible (O'Leary et al. 2014).

4 Clinical Evidence of the Role of $GABA_B$ Receptor in Mood Disorders

The preclinical evidence of the therapeutic potential of $GABA_B$ receptor modulation in the treatment of depression is also supported by clinical evidence. One of the first clinical indications of a role for the $GABA_B$ receptor in depression comes from a small study reporting that baclofen may worsen depressive like-symptoms (Post

et al. 1991). In that study, patients with primary affective disorder were chronically treated with baclofen (10–55 mg/day). Out of five patients, three exhibited increased depression during baclofen treatment and these depressive symptoms improved during baclofen withdrawal (Post et al. 1991). This baclofen-induced worsening of depressive symptoms seems counterintuitive to its antidepressant-like effects in preclinical studies. The reasons underlying this discrepancy are unclear but may relate to the fact that preclinical assessments of baclofen were not done in animal models of depression per se, e.g. stress-induced anhedonia, but were conducted using "normal" animals in the FST which is a behavioural test of antidepressant-drug-like activity and not a model of depression. Nevertheless, several studies also reported that depressed patients displayed blunted baclofen-induced growth hormone release (Marchesi et al. 1991; O'Flynn and Dinan 1993), further suggesting a role for the $GABA_B$ receptor in depression. The effects of baclofen on depression and anxiety-related clinical measures are contradictory, however, as summarized in a recent review on its off-label use to treat alcohol use disorder (Agabio and Leggio 2018).

Postmortem studies have reported regional alterations in $GABA_B$ receptor subunit expression in brains from depressed suicide victims (Ghose et al. 2011) and depressed individuals (Klempan et al. 2009). Specifically, it was reported that depressed suicide victims exhibited upregulation of the $GABA_{B(2)}$ receptor subunit in cortical and subcortical brain regions compared with non-depressed suicide victims (Klempan et al. 2009). More recently, it was reported that $GABA_{B(1)}$ and $GABA_{B(2)}$ receptor subunit expression was reduced in the superior frontal cortex of subjects with bipolar disorder (Fatemi et al. 2017). In the hippocampus of depressed patients, $GABA_{B(2)}$ gene expression was reported to be increased by 50% (Ghose et al. 2011). In addition, in the dentate gyrus of the hippocampus of these depressed patients, there was a 30% decrease in the expression of the $GABA_{B(1a)}$ receptor subunit isoform when compared with controls (Ghose et al. 2011). Interestingly, the dentate gyrus is one of just a few brain areas where neurogenesis, the birth of new neurons, occurs throughout life (Altman 1962a, b; Spalding et al. 2013; Boldrini et al. 2018; Moreno-Jimenez et al. 2019). Hippocampal neurogenesis has been implicated in the mechanism of antidepressant action (Santarelli et al. 2003; David et al. 2009; O'Leary and Cryan 2014; Miller and Hen 2015) and recently we and others reported that $GABA_B$ receptor antagonists that have antidepressant-like behavioural effects increase hippocampal neurogenesis (Felice et al. 2012; Giachino et al. 2014). We have also found that the stress-resilient behavioural phenotype of $GABAB_{(1b)}^{-/-}$ mice is accompanied by resilience to stress-induced decreases in adult hippocampal neurogenesis (O'Leary et al. 2014).

There is also evidence from human transcranial magnetic stimulation (TMS) studies that there are alterations in $GABA_B$ receptor activity in depression. The first such study suggested that $GABA_B$ neurophysiological deficits are closely related to the pathophysiology of major depressive disorder (Levinson et al. 2010). In that study, patients with major depressive disorder (MDD) exhibited decreased cortical silence, a measure of intracortical inhibition thought to be a marker of $GABA_B$ receptor neurotransmission. Other more recent studies have confirmed

that depressed patients exhibit a decreased cortical silent period (a TMS measure of $GABA_B$ receptor activity) (Veronezi et al. 2016). Accordingly, adolescents with depression and a lifetime history of suicidal behaviours exhibited impaired long-interval intracortical inhibition (LICI; which is a TMS measure of $GABA_B$ receptor-mediated inhibition) when compared to healthy adolescents and to depressed adolescents without a history of suicidal behaviour (Lewis et al. 2018). A follow-up small study by the same group reported an association between increases in $GABA_B$-mediated cortical inhibition and a reduction in suicidal ideation over time in adolescents treated for depression (Lewis et al. 2019). A paired-pulse TMS (ppTMS) study revealed that patients with treatment resistant depression (TRD) exhibit more reduced $GABA_A$ and $GABA_B$ receptor-mediated cortical inhibition compared to non-TRD patients and healthy subjects (Jeng et al. 2019), thus suggesting a potential role for $GABA_B$ receptor function in TRD. In addition, selective serotonin reuptake inhibitor (SSRI) antidepressants were shown to modulate $GABA_B$ receptor-mediated long-interval intracortical inhibition in non-TRD patients (Jeng et al. 2019), thus providing clinical evidence for a role of $GABA_B$ receptors in antidepressant action.

In contrast to depression, clinical studies interrogating a role for the $GABA_B$ receptor in anxiety disorders are sparse and the evidence is largely indirect. Nevertheless, there is strong evidence that GABAergic neurotransmission plays a role in the treatment and pathophysiology of anxiety disorders as benzodiazepines (which act on the $GABA_A$ receptor) are used to treat anxiety disorders (Nemeroff 2003). In terms of a potential role for $GABA_B$ receptors, baclofen has been shown to attenuate the anxiety that is associated with alcohol withdrawal, post-traumatic stress, panic disorder and traumatic spinal-cord lesions (Cryan et al. 2005).

In summary, both clinical and preclinical evidence strongly support a role for the $GABA_B$ receptor in depression and anxiety disorders. However, the involvement of the $GABA_B$ receptor in the pathophysiology of anxiety disorders is less explored in clinical studies when compared with depression. Indeed, the majority of clinical studies on the role of the GABAergic system in anxiety disorders are focused on the $GABA_A$ receptor. However, it is worth noting that the $GABA_B$ receptor can contribute to inhibition by also modulating $GABA_A$ receptor activity at presynaptic and postsynaptic sites (Cryan et al. 2005; Tao et al. 2013), thus suggesting a potential upstream modulating role for the $GABA_B$ receptor in anxiety disorders. Moreover, preclinical studies suggest that agonists and PAMs of the $GABA_B$ receptor have anxiolytic effects.

5 Conclusions and Perspectives

Although both preclinical and clinical studies suggest the $GABA_B$ receptor as a potential target for the development of new therapeutic approaches for mood and anxiety disorders, only one $GABA_B$ receptor-based compound, SGS272 (CGP36742, a $GABA_B$ receptor antagonist), progressed to Phase II clinical trials

and was investigated as a potential treatment for cognitive deficits (Ghose et al. 2011). To date, however, no clinical trials assessing the effects of $GABA_B$ receptor antagonists in depressed patients have ever been conducted. The development of such antagonists of the $GABA_B$ receptor for the treatment of mood disorders is mainly hampered by its potential side-effects, particularly the potential risk of proconvulsive action. However, the abundance of preclinical evidence of the antidepressant-like effects of $GABA_B$ receptor antagonists cannot be ignored, and thus novel and more selective $GABA_B$ receptor antagonists with a better side-effect profile could lead to new therapeutic approaches in the clinic. In 2014, the first negative allosteric modulator (NAM) of the $GABA_B$ receptor was generated. This was a CGP7930 analogue, called CLH304a (also named Compound 14) (Chen et al. 2014). In 2016, two additional novel NAMs, CLH391 and CLH393, were synthetized based on the structure of CLH304a (Sun et al. 2016). It would be expected that NAMs would have a better side-effect profile than antagonists and as such, the discovery of these NAMs is very promising for the development of innovative drugs that negatively modulate $GABA_B$ receptor action, and thus might have antidepressant potential with a reduced side-effect profile.

The $GABA_B$ receptor plays a key role in anxiety disorders as demonstrated by a plethora of preclinical evidence. PAMs represent promising drugs to treat anxiety-like disorders with safer side-effect profiles than $GABA_B$ receptor agonists. ADX 71441 is the first $GABA_B$ receptor PAM approved for phase I clinical trial (Kalinichev et al. 2017) indicated for alcohol use disorder, Charcot-Marie-Tooth disease and nicotine dependence. However, future clinical trials are required to evaluate the effects of PAMs in anxiety disorders.

Overall, the $GABA_B$ receptor represents a promising target to develop new therapeutic treatments for depression and anxiety disorders. Since Bowery and colleagues' discovery of the $GABA_B$ receptor in 1979, thousands of studies investigating its role in mammals and non-mammals such as the drosophila model (Manev and Dzitoyeva 2010) have been published. The introduction of genetic tools has allowed the further study of the role of $GABA_B$ receptor subunits and their isoforms in mice. Despite the drive of scientists to study the $GABA_B$ receptor, there is still a lot unknown. In particular, side-effects associated with $GABA_B$ receptor modulation hamper its path to become a relevant drug target. However, the introduction of novel tools to study the $GABA_B$ receptor (e.g. FRET-Based Sensors) and the discovery of novel $GABA_B$ receptor PAMs and NAMs will pave the way towards $GABA_B$ receptor therapeutics in human disorders such as depression and anxiety disorders. However, NAMs have yet to be tested in vivo. Intracellular $GABA_B$ receptor-associated proteins may also be important targets to modulate $GABA_B$ receptor activity because protein–protein interaction may allow more precise and temporal $GABA_B$ receptor activity modulation. Particularly, the K^+ channel tetramerization domain (KCTD) that is associated with the $GABA_{B2}$ receptor C-terminus is envisaged to be a promising target (Sereikaite et al. 2019).

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$GABA_B$ Receptors in Neurodegeneration

Alessandra P. Princivalle

Contents

Abstract GABA is the main inhibitory neurotransmitter in the mammalian central nervous system (CNS) and acts via metabotropic $GABA_B$ receptors. Neurodegenerative diseases are a major burden and affect an ever increasing number of humans. The actual therapeutic drugs available are partially effective to slow down the progression of the diseases, but there is a clear need to improve pharmacological treatment thus find alternative drug targets and develop newer pharmaco-treatments. This chapter is dedicated to reviewing the latest evidence about $GABA_B$ receptors and their inhibitory mechanisms and pathways involved in the neurodegenerative pathologies.

Keywords Alzheimer's disease · $GABA_B$ receptors · Hippocampal sclerosis · Neurodegenerative diseases · Parkinson's disease · Temporal lobe epilepsy

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1 Introduction

The $GABA_B$ receptor is the main inhibitory receptor in the mammalian brain (Curtis 1974; Krnjevic 1974). It was first described by Bowery et al. (1980) who used pharmacology techniques to identify it, but for almost 20 years afterwards no other study succeeded in confirming the presence of this receptor. In 1997 Kaupmann et al. characterised the sequence of the receptor gene, transcript, protein, and the molecular structure of the receptor. They also demonstrated the presence of alternative splice variants of the $GABA_B$ receptor; these were different in the N-terminus domain and named $GABA_{B1a}$ and $GABA_{B1b}$. One year later the same group (Kaupmann et al. 1998) and two others (Jones et al. 1998; White et al. 1998) demonstrated that this receptor was not fully functional in cells enriched with it, and they described a second $GABA_B$ receptor gene, transcript, protein, and the molecular structure of the mature fully functional receptor. These two proteins have therefore been defined as subunits of the fully functional receptor and given the names of $GABA_{B1}$ and $GABA_{B2}$. The same studies also demonstrated for the first time that, in order for a G-protein coupled receptor to be fully functional, it has to work as a dimer composed of these two subunits. In a short period of time other subunits were described (Isomoto et al. 1998) but with very minor roles and very low expression.

After the molecular characterisation of the two main subunits, DNA or RNA probes and antibodies became available or could be produced in order to study the distribution and the level of expression of both the proteins and the transcripts. Thus, many groups began to investigate distribution and expression levels of the $GABA_B$ receptors. Many of these studies were focused on specific areas of the brain and spinal cord in rodents, primates, and in humans. Animal models of various neurodegenerative diseases were used in order to shed light on the structure, expression, and physiological and pathological roles of $GABA_B$ receptors in these conditions.

Electrophysiological and pharmacological evidence demonstrated abnormalities of the $GABA_B$ receptor in many pathological conditions such as spasticity, epilepsy, anxiety, depression, and cognitive deficits (Marescaux et al. 1992; Mott and Lewis 1994; Olpe et al. 1993; Meeren et al. 2004; Stewart et al. 2009; Gassmann and Bettler 2012; Castelli and Gessa 2016). Further, more recently, involvement of the $GABA_B$ receptor has been demonstrated in neurodegenerative diseases such as Alzheimer's (Dal Prà et al. 2019; Tang 2019), amyotrophic lateral sclerosis (Schumacher et al. 2019), Huntington's (Rosas-Arellano et al. 2018; Rekik et al. 2011), Parkinson's (Hillman et al. 2012), essential tremors (Paris-Robidas et al. 2012), and autoimmune encephalitis (Moser et al. 2018; Maureille et al. 2019). In this chapter the attention is focused on the role that $GABA_B$ receptors play in epilepsy, and, more specifically, temporal lobe epilepsy associated with hippocampal sclerosis TLE-HS. Attention is also given to two major neurodegenerative diseases, Alzheimer's (AD) and Parkinson's disease (PD).

2 $GABA_B$ Receptor and Its Effects

It is well known that $GABA_B$ receptors belong to the G-protein coupled (guanine nucleotide binding protein) receptor family (Wojcik and Neff 1984; Hill et al. 1984; Hill 1985; Karbon and Enna 1985; Andrade et al. 1986) and thus are associated with slow synaptic neurotransmission. $GABA_B$ receptors were initially identified by their insensitivity to the $GABA_A$ antagonist bicuculline and their selective activation by (−)baclofen (Hill and Bowery 1981). Later, a number of compounds specific for $GABA_B$ receptors were identified, e.g., the antagonist 2-hydroxy-saclofen and the class of antagonists named CGP. Activation of $GABA_B$ receptors produces three major effects: (a) increases in postsynaptic neuronal K^+ conductance to generate long-lasting inhibitory postsynaptic potentials (Dutar and Nicoll 1988); (b) inhibition of adenylate cyclase activity, leading to a reduction in cAMP levels (Wojcik and Neff 1984; Hill et al. 1984; Hill 1985; Karbon and Enna 1985; Andrade et al. 1986; Rascol et al. 1989); (c) decrease of membrane Ca^{2+} flux $GABA_B$ receptor activation mediated by G-proteins that are members of the pertussis toxin-sensitive family $G_{i\alpha}/G_{o\alpha}$ (Odagaki et al. 2000; Odagaki and Koyama 2001). These actions are discussed separately below. More details about the history and structure of the $GABA_B$ receptors can be found in chapters "Historical Perspective on the GABAB Receptor" and "GABAB Receptor Structure", respectively.

2.1 K^+ Channels

When activated by an agonist, $GABA_B$ receptors increase K^+ conductance, producing hyperpolarisation of the cell membrane, which has been reported in various brain regions including the cortex (pyramidal cells; Connors et al. 1982; Karlsson and Olpe 1989; Luhmann and Prince 1991), hippocampus (granule cells and interneurons; Fujita 1979; Misgeld et al. 1984; Dutar and Nicoll 1988; Williams and Lacaille 1992), cerebellum (Schreurs et al. 1992; Vigot and Batini 1997), amygdala (Rainnie et al. 1991), and thalamus (Hirsch and Burnod 1987; Crunelli and Leresche 1991; Curró Dossi et al. 1992).

It has been reported that in K^+ subunit deletion, G protein-activated inwardly rectifying potassium (GIRK) channel 2 (GIRK2) mutant mice, in hippocampal neurons, postsynaptic K^+ currents induced by the $GABA_B$ receptor agonist baclofen are reduced or absent, and it was demonstrated that deletion of GIRK2 did not involve presynaptic inhibition. Therefore, GIRK-containing channels were shown not to be responsible for presynaptic effects (Lüscher et al. 1997). In contrast, a K^+ current is shown to be coupled to $GABA_B$ receptors on presynaptic terminals in hippocampal cultures (Thompson and Gähwiler 1992), so changes in membrane K^+ flux appear to be due to postsynaptic $GABA_B$ receptor activation (Saint et al. 1990).

2.2 Ca^{2+} Channels

Baclofen and GABA depress somatic Ca^{2+} currents not only in peripheral neurons (Dolphin and Scott 1986, 1987, 1990) but also in cultured mammalian hippocampal and cerebellar neurons (Huston et al. 1990; Wojcik et al. 1990; Pfrieger et al. 1994). $GABA_B$ receptor-mediated blockage of Ca^{2+} channels and coupling mechanisms are involved (Scott et al. 1991). A reduction of Ca^{2+} currents can be considered responsible for the depression of synaptic transmission by presynaptic $GABA_B$ receptors (Huston et al. 1990).

More recent evidence showed that presynaptic neurotransmitter release is indeed modulated by $GABA_B$ receptors through Ca^{2+} channels (White et al. 1998). Patch clamp measurements from a presynaptic terminal indicate that baclofen reduced Ca^{2+} currents, but had no effect on presynaptic K^+ currents and this was G-protein dependent (Takahashi et al. 1998).

2.3 Inhibition of Adenylate Cyclase

$GABA_B$ receptor agonists inhibit basal and forskolin-stimulated neuronal adenylate cyclase in brain slices (Knight and Bowery 1996), through a G-protein dependent mechanism that results in a reduced level of intracellular cAMP. When $GABA_B$ receptors are activated, one α subunit is released from the G-protein and interacts with AC to inhibit cAMP formation. The G-protein involved has been demonstrated to be $G_{i\alpha}/G_{o\alpha}$, because ADP-ribosylation of the G-protein by pertussis toxin blocked any receptor interaction (Asano and Ogasawara 1986; Xu and Wojcik 1986). The βγ subunit of the G-protein interacts with K^+ and Ca^{2+} channels and can potentiate β-adrenoreceptor-mediated cAMP production (Knight and Bowery 1996), via cross talk mechanisms (Lefkowitz 1992). More details about the physiology of $GABA_B$ receptors can be found in chapter "GABAB Receptor Signal Transduction".

3 $GABA_A$ Receptors

GABA acts also through $GABA_A$ receptors. $GABA_A$ receptors are ligand-gated Cl^- ion channels generating fast synaptic inhibition (Schofield et al. 1987; Smith and Olsen 1995). $GABA_A$ receptors can be pharmacologically distinguished by the competitive antagonist bicuculline, they are modulated by many therapeutic agents, such as benzodiazepines (BZD), and are a potential drug target for a number of neurological disorders. $GABA_A$ receptors are widely distributed in the CNS (Fritschy and Mohler 1995). The existence of multiple $GABA_A$ receptor subunits has been demonstrated by regional differences in affinity and distribution of binding

sites for BZD receptor ligands (Niddam et al. 1987; Sieghart et al. 1987; Bureau and Olsen 1990, 1993; Ruano et al. 1992).

4 $GABA_B$ in Neurodegenerative Diseases

Neurodegeneration is defined as the progressive atrophy and loss of function of neurons, which is present in neurodegenerative diseases. Neurodegenerative diseases are also characterised by deposition of proteins, due to a fault in post-translational processing, specifically defective proteolysis, leading to overproduction of misfolded proteins. There are several such proteins that undergo incorrect post-translational processing; however, for the purpose of this chapter only the major proteins involved are considered: tau, amyloid-β (Aβ), and α-synuclein.

4.1 $GABA_B$ in Alzheimer's Disease

Alzheimer's disease (AD), as mentioned above, is a neurodegenerative disease described for the first time in 1907 by the Bavarian physician and pathologist Alois Alzheimer (1864–1915). Unfortunately, since then, and over a century later, the prevalence of AD has tremendously increased. It is now the fifth most common cause of death globally. About 44 million people worldwide are living with dementia, 70% due to AD (Dumurgier and Sabia 2020). The main symptoms of AD are: loss of recent memory, disorientation to time and place, sometimes antisocial behaviour –"loss of inhibitions", lack of outward physical signs.

The symptoms observed are due to the specific regions of the brain affected by neurodegeneration, which are the hippocampus and the cortex, where the loss of neurons becomes increasingly evident with the progression of the disease (Fig. 1). The hippocampus is the centre for processing and storing memories, and cortex is the centre for high cognitive function built on memories.

The neuropathological features of AD are extracellular senile plaques made up by amyloid-β protein, and intracellular neurofibrillary tangles (NFT) made up of paired filaments and hyperphosphorylated tau protein (Fig. 2).

The majority of studies on AD are focused on the pathological processing of the amyloid precursor peptide (APP) leading to the formation of amyloid-β, the resulting build-up of amyloid plaques (Fig. 3), and also on the development of the tangles due to hyperphosphorylation of the tau protein (Fig. 4). In addition to this, recent evidence has shown that $GABA_B$ receptors also play a role in the pathology of AD.

The earliest indications for the role of $GABA_B$ receptors in AD emerged from a quantitative autoradiography binding study. In this study, a significant decrease of Bmax for $GABA_B$ receptors was reported in the cortex and hippocampus, especially in the *stratum moleculare* of the dentate gyrus (DG), the *stratum lacunosum-moleculare,* and the *stratum pyramidale* of CA1 (Chu et al. 1987a, b). Following

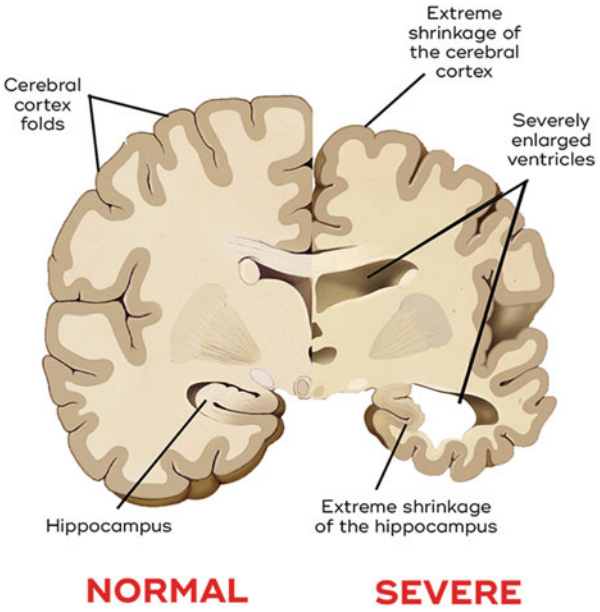

Fig. 1 Brain imaging showing a control brain on the left and a brain affected by Alzheimer's disease on the right. (Credit: Queensland Brain Institute, The University of Queensland. qbi.uq.edu.au/dementia)

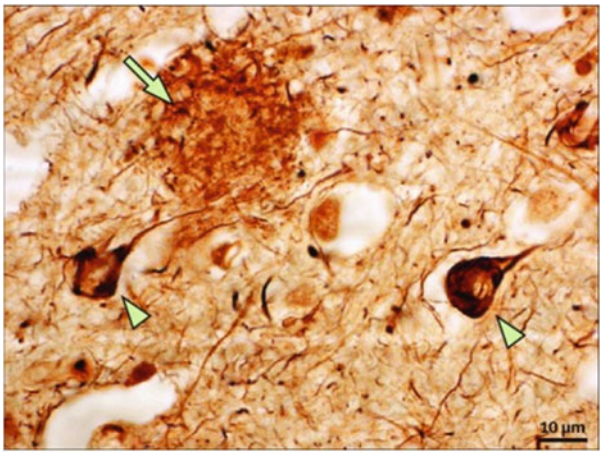

Fig. 2 Neuropathological hallmarks of Alzheimer's disease. Postmortem Bielschowsky silver staining of frontal cortex from a patient with Alzheimer's disease, showing the presence of a neuritic amyloid plaque (arrow), consisting of aggregated extracellular amyloid β fibrils, and intraneuronal neurofibrillary tangles (arrowheads), consisting of hyperphosphorylated tau protein. (Taken from Winblad et al. 2016 with permission from Elsevier journals License Number 4947750098122)

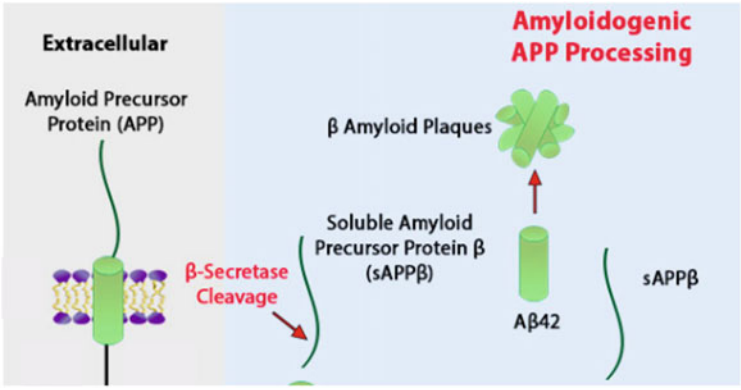

Fig. 3 Schematic representation of the amyloid-plaque formation. Adapted from https://www.biolegend.com/amyloid_precursor_protein

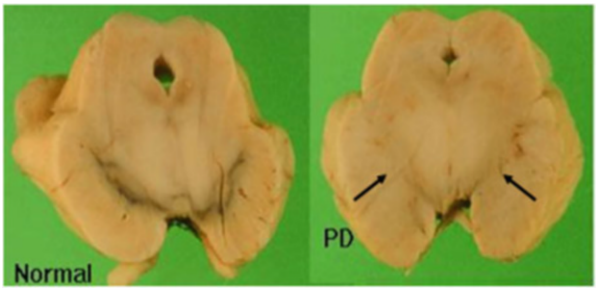

Fig. 4 Brain images showing a normal postmortem sample on the left and the loss of pigmented neurons in the pars compacta of the substantia nigra (SNpc) of a postmortem PD patient on the right (black arrows)

up from these early findings, it was reported that, in a mouse model of colchicine-infused hippocampus, the sensory memory of the mice was impaired and also the amount of GABA in the cortex was decreased. Conversely, in mice simultaneously treated with colchicine and the $GABA_B$ antagonist CGP36742, memory loss was not recorded. The researchers of that study therefore concluded that the $GABA_B$ receptor antagonist CGP36742 could be a treatment for AD (Yu et al. 1997). More experimental evidence obtained by immunohistochemistry emerged, corroborating the differential expression of $GABA_B$ receptors in varying stages of AD according to the Braak Staging. These data suggested that the expression of $GABA_{B1}$ is stable in CA1 through all the stages of the disease. In contrast, in the initial stages (Braak III/IV) of the pathology, the expression of $GABA_{B1}$ expression is higher in CA2-4, which could be interpreted as a compensatory (or self-defending) mechanism where the expression decreases with the progression of the disease (Braak V/VI), leading to neuronal death and impairment between excitation and inhibition. From these data it

can be concluded that the formation of the NFTs in the hippocampus initially induces an increased expression and, later, increasing NFT accumulation stops the expression of this $GABA_B$ receptor specifically (Iwakiri et al. 2005).

In recent data, rat ex vivo brain sections containing the hippocampus were treated with excess Aβ. These data demonstrated that, during the early stage of the disease, amyloid-β causes a dysregulation between excitatory and inhibitory neurotransmission, leading to disruption of the neuronal network. These changes are significant in the septo-hippocampal region, which processes learning and memory, according to oscillatory activity at the synapses between fimbria and CA3 (Nava-Mesa et al. 2013). This group noted that the mechanism of action of amyloid-β was localised at the postsynaptic region and presumably linked to $GABA_B$ and its K^+ and Ca^{2+} channels via GIRK channels. These data suggest that amyloid-β modifies GIRK channels in CA3 pyramidal neurons in a way that is linked to the functioning of $GABA_B$ in the modulation of the hippocampal circuit. Another study on the effect of amyloid-beta (Aβ) on gene expression demonstrated that the level of expression of GIRK2, 3, and 4 subunits was decreased, but $GABA_B$ receptor expression was unaffected. These data corroborate the previous observations showing a relationship between the effect of Aβ and K^+ channels linked to $GABA_B$ receptors (Mayordomo-Cava et al. 2015). Another study showed that in a rat streptozotocin-induced diabetic (STZ) model of sporadic AD, baclofen enhanced memory, again showing a role for $GABA_B$ receptors in AD (Pilipenko et al. 2018). One of the latest pieces of evidence that $GABA_B$ receptors play a role in AD is the link between $GABA_B$/APP and the formation of Aβ, emerging from a study on sequence-related epitopes in APP with nanomolar affinity for the sushi-domain on the N-terminal site of presynaptic $GABA_{B1a}$ receptors. This study demonstrates, by using a proteomics approach, a multiprotein complex containing APP, c-Jun N-terminal kinase-interacting protein (JIP) and calsyntenin, together with $GABA_{B1a}$. This multiprotein complex facilitates Aβ formation and blocks the axonal trafficking of presynaptic of the $GABA_B$ receptor, decreasing its expression (Dinamarca et al. 2019). In a genetic mouse model of AD expressing a chimeric mouse/human (Mo/Hu) APP-695 with mutations linked to familial AD (Oh et al. 2009), the use of various immunohistochemical techniques demonstrated a decreased expression of $GABA_{B1}$ in the cell membrane surface of the *stratum lacunosum-moleculare* of CA1 pyramidal cells at 6 months of age. This reduced expression became more pronounced at 12 months of age and was coupled with an increase of the subunit in the intracellular compartment. Further, a reduction of $GABA_B$ receptors was observed in the axon terminal synapsing pyramidal CA1 cells (Martín-Belmonte et al. 2020a). The same group demonstrated a significant decrease of $GABA_B$ receptors in the *stratum moleculare* of the DG, and also in axon terminals synapsing dendritic spines of granule cells, more evident in the outer than in the inner molecular layer (Martín-Belmonte et al. 2020b).

All these data taken together, starting from the earliest indication (Chu et al. 1987a, b) up to the most recent data (Martín-Belmonte et al. 2020a, b), indicate that $GABA_B$ receptors, and particularly $GABA_{B1a}$, have a decreased expression in the hippocampus. The reported reductions in $GABA_B$ expression are specific to the

hippocampal subregions; however, it seems a general trend extended to CA1, CA3, and DG (which make up the trisynaptic circuit). Functionally, due to the decrease of $GABA_B$ receptors, there is an augmented production of Aβ, because the lack of $GABA_B$ receptors promotes the proteolysis of APP. This further supports the conclusions that $GABA_B$-mediated synaptic transmission is a major contributor in AD and $GABA_B$ receptors may be a suitable target for more effective drugs to treat AD. All these studies have focused their attention on $GABA_{B1}$, firstly because its expression was higher in the neuronal bodies and proximal dendrites where NFT accumulates in AD (Iwakiri et al. 2005). Secondly, $GABA_{B1a}$ has been demonstrated to form a complex with APP, whereas $GABA_{B1b}$ does not. Furthermore, the $GABA_{B1a}$ knock-out mice model showed a lack "of $GABA_B$ axonal transport and deficit in GBR-mediated inhibition of glutamate release". This model also showed that secreted APP functions as a $GABA_{B1a}$ ligand to modulate synaptic neurotransmission (Dinamarca et al. 2019; Martín-Belmonte et al. 2020a, b).

4.2 *$GABA_B$ in Parkinson's Disease*

Parkinson's disease (PD) is another neurodegenerative disease, described for the first time by James Parkinson (1817) in "An Essay on the Shaking Palsy". Toodayan (2018) PD is also known as *paralysis agitans*; it was first called Parkinson's disease by Jean-Martin Charcot in 1884. PD affects about 0.1–0.2% of the whole population. The incidence of the disease increases with age affecting 1% of people over 60 years of age. The main symptoms of PD are tremor at rest, muscle rigidity, and bradykinesia. The symptoms observed are due to the specific region of the brain affected by the loss of dopaminergic neurons: the substantia nigra (SN) (Fig. 4).

The main neuropathological features are Lewy bodies, which contain α-synuclein (Fig. 5) in the SN and this is exhibited through impairment of voluntary movement (Braak et al. 2003).

When the disease progresses these features spread to the cortex and neocortex (Tysnes and Storstein 2017). Figure 6 below illustrates the whole circuit and the inhibitory and excitatory connection.

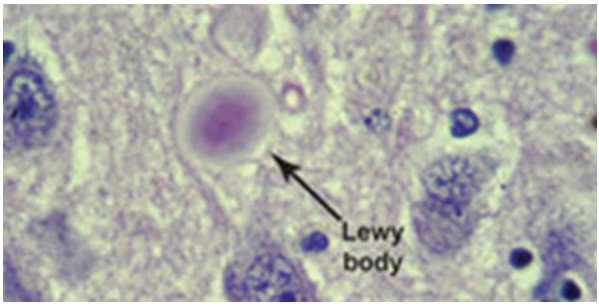

Fig. 5 Photomicrographs showing the presence of Lewy bodies containing α-synuclein. Taken from https://www.alz.org/alzheimers-dementia/what-is-dementia/types-of-dementia/lewy-body-dementia

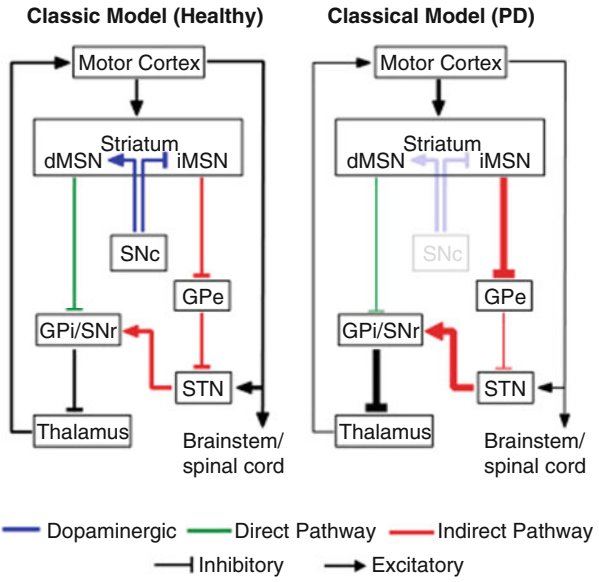

Fig. 6 Schematic representation of the nigrostriatal circuit in the physiology and pathology of Parkinson's disease. Adapted from (McGregor and Nelson 2019; with permission)

When the dopaminergic neurons in the substantia nigra pars compacta (SNpc) start to degenerate, the dopaminergic signal to the caudate and putamen is reduced. The response of the caudate and putamen therefore becomes modified, which results in an overall increase in the output of the interior globus pallidus (GPi). This increases results in the inhibition of the thalamus. The thalamic excitatory signal to the motor cortex is diminished, thus causing reduced motor control. Also, the subthalamic nucleus (STN) plays a critical role in the regulation of movement, and abnormal activity of its neurons is associated with basal ganglia motor symptoms (McGregor and Nelson 2019).

The first evidence of involvement of $GABA_B$ receptors in PD emerged from electrophysiological recordings in neurons isolated from the globus pallidus (GP) in the presence of baclofen. The data showed that the $GABA_B$-mediated effect was present only in one of the subtypes of GP neurons with a small soma, and the activation of $GABA_B$ modulated high-voltage-activated (HVA) calcium currents which may have an impact on the basal ganglia circuit (Stefani et al. 1999). A 40% decrease in the expression of $GABA_B$ receptors in the SNpc and in the GPi was reported in a binding study utilising a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD monkey model (Calon et al. 2000). The same group also demonstrated significant decreases in the mRNAs for $GABA_{B1}$ (−69%) and $GABA_{B2}$ (−66%) in the SNpc, and that the decreased expression of $GABA_{B1}$ mRNA was related to dopamine (DA) concentration (Calon et al. 2001). The same group also analysed, via binding experiments, the expression of $GABA_B$ receptors in human postmortem specimens and found a reduced binding in the putamen and external globus pallidus (GPe) in PD patients compared with controls (Calon et al. 2003). Also, in a rat model of PD with induced lesions of the nigrostriatal pathway, a

reduction in $GABA_B$ mRNA was reported in the SNpc, whereas expression of the $GABA_{B1a}$ subunit was significantly increased in the substantia nigra pars reticulate (SNpr), entopeduncular nucleus, and the STN. Since these brain parts received reduced GABAergic innervation due to the lesion, this could indicate that the increased $GABA_{B1a}$ expression represents a compensatory mechanism (Johnston and Duty 2003). The fine-tuned localization of $GABA_{B1}$ receptors was investigated by Smith et al. (2000), who found an immunopositive signal in the striatopallidal complex in neuronal bodies and dendrites, striatal dendritic spine, axons, and axon terminals. Analysis of $GABA_{B1}$ receptor distribution via immunogold electron microscopy showed extrasynaptic sites on dendrites, spines, and somata in the striatopallidal complex and perisynaptically at the synapses in the GP.

Whole-cell patch-clamp recordings were used to investigate tonic activation of $GABA_B$ receptors at pre- and post-synaptic levels, and the data indicated a major tonic activation of presynaptic $GABA_B$ receptors on the STN terminals compared to postsynaptic $GABA_B$ receptors on STN neurons. Therefore the presynaptic $GABA_B$ receptors could be considered as a new therapeutic target for treating some of the PD symptoms (Chen and Yung 2005).

It was later demonstrated through the frequency-dependent activation of post-synaptic $GABA_B$ receptors that the GP regulates the activity of the STN. These results clarify a novel way in which burst activity can be generated in the STN and suggest that the effect of $GABA_B$ on STN neurons could generate abnormal burst activity in PD (Hallworth and Bevan 2005).

Further proof of the role that the $GABA_B$ receptor plays has come from studies in a rat model whereby the nigrostriatal pathway was depleted by treatment with 6-hydroxydopamine, and the rats were treated with the $GABA_B$ receptor antagonist CGP 56999A. The results showed that the antagonist treatment attenuated the lack of DA in the rat striatum (Enna et al. 2006).

A recently conducted investigation in an MPTP rat model demonstrated that baclofen reversed the effect of PD-like induced symptoms (Tyagi et al. 2015). Another recent study in a mouse model of PD proved that the loss of GABAergic inhibition in the striatonigral connection led to motor impairment (Borgkvist et al. 2015), corroborating once more the role of $GABA_B$ receptors in PD and moreover how it can be used as a potential drug target to treat certain parkinsonian symptoms.

It has long been established that DA plays a pivotal role in action selection and learning in the nigrostriatal pathway. However, any link between DA and $GABA_B$ receptors was not clearly defined until recently. The DA released into the striatum is influenced by local neurons, the majority of which are GABAergic, though it was not clear if it was a direct or indirect modulation via cholinergic innervation. Lopes et al. (2019) established that in the striatum GABA is capable of inhibiting release of DA via both ionotropic and metabotropic GABA receptors and that these actions are not mediated by acetylcholine. These results also demonstrated a tonic inhibition of DA release by striatal GABA, which occurs mainly via $GABA_B$ receptors. However, there is still a lack of evidence of whether GABA receptors are expressed on DA axons (Lopes et al. 2019).

Previously, the main neuropathological features of PD were mentioned: Lewy bodies containing α-synuclein and their accumulation in the intracellular space are major factors in the disease. Emmanouilidou et al. (2016) have examined the molecular pathway of α-synuclein secretion in mouse nucleus striatum and have found a new synaptic network that regulates α-synuclein release. They showed that α-synuclein secretion is a calcium-regulated mechanism depending on the activation of the sulfonylurea receptor 1 (SUR1), which is an inwardly rectifying potassium ion channel Kir6 subunit that senses intracellular levels of the nucleotide ATP. They also demonstrated that modulation of GABA release through SUR1 located on GABAergic neurons controls α-synuclein release through activation of the presynaptic $GABA_B$ receptors. This study suggests that GABA transmission via SUR1 in mouse striatum modulates the α-synuclein secretory pathway, providing new insights for potential therapeutics to treat PD (Emmanouilidou et al. 2016). Also, in a transgenic drosophila model carrying α-synuclein, it was shown that the transgenic flies lacked the capability of climbing, and this action was reversed by providing the drosophila with levodopa (L-DOPA_ or a $GABA_B$ (but not $GABA_A$) agonist in their food (Hillman et al. 2012).

There have been useful studies directed towards clarification of various mechanisms underlying the pathophysiology of PD, of which those involving the role of $GABA_B$ receptors have been summarised above. Taken together, all the evidence available to date not only shows a fundamental role of $GABA_B$ receptors in PD, but also that via more recently described $GABA_B$ receptor innervation and modulation pathways there could be further potentials for better targeted therapies which may treat PD symptoms in a more effective manner.

4.3 $GABA_B$ in Temporal Lobe Epilepsy

Different types of epilepsy are classified according to structural aetiology referring to abnormalities visible on structural neuroimaging such as magnetic resonance imaging (MRI). The structural malformations may be acquired or genetic. The majority of focal seizures originate in the temporal lobes (Zentner et al. 1995).

A well-known form of epilepsy linked with structural malformation is temporal lobe epilepsy associated with hippocampal sclerosis (TLE-HS). Temporal lobe epilepsy (TLE) is the most common form of focal epilepsy. About 6 out of 10 people with focal epilepsy have TLE. Seizures in TLE start in one point (*focus*) and then may involve both temporal lobes in the brain. TLE is subdivided in two types: mesial temporal lobe epilepsy (MTLE) and neocortical temporal lobe epilepsy (NTLE). MTLE encompasses the *medial* or internal structures of the temporal lobe. Seizures often begin in the hippocampus or surrounding area and account for almost 80% of all temporal lobe seizures. NTLE encompasses the outer part of the temporal lobe. About 30–40% of patients affected by MTLE-HS are pharmaco-resistant (Engel 2001). When seizures are prolonged and repeated they produce severe neuronal loss in the temporal lobe, mostly observed in the hippocampus, entorhinal cortex, amygdala, and other brain areas (Van Paesschen et al. 1997; Sutula and Hermann 1999).

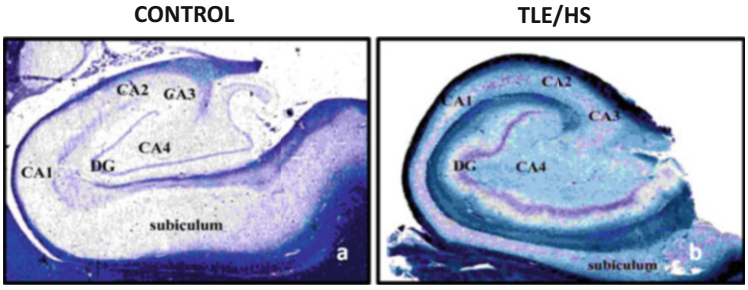

Fig. 7 Cresyl violet/Luxol fast blue stained sections of human hippocampus: (**a**) control specimen; (**b**) sclerotic specimen (not in scale). Taken from Princivalle PhD thesis (2003)

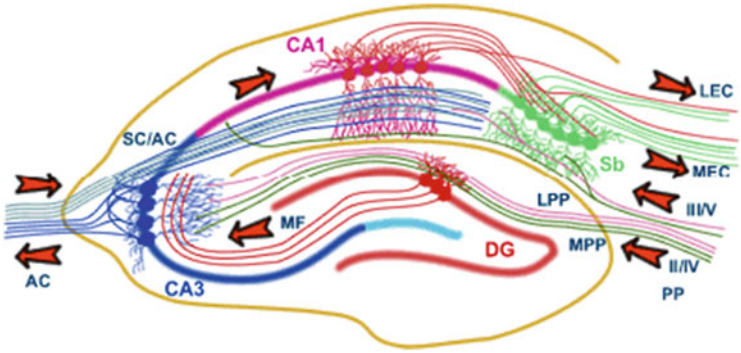

Fig. 8 The hippocampal Network: The hippocampus forms a principally uni-directional network, with input from the entorhinal cortex (EC) that forms connections with the dentate gyrus (DG) and cornu ammonis CA3 pyramidal neurons via the perforant path (PP – split into lateral and medial). CA3 neurons also receive input from the DG via the mossy fibres (MF). They send axons to CA1 pyramidal cells via the Schaffer collateral pathway (SC), as well as to CA1 cells in the contralateral hippocampus via the associational commisural (AC) pathway. CA1 neurons also receive inputs directly from the PP and send axons to the Subiculum (Sb). These neurons in turn send the main hippocampal output back to the EC, forming a loop. Taken from http://www.bristol.ac.uk/synaptic/pathways/

TLE-HS is not always considered or classified among the classical neurodegenerative diseases such as AD or PD. However, neurodegeneration in cornu ammonis (CA) subregions (Fig. 7), aberrant mossy fibre (MF) sprouting (Sutula et al. 1989), granule cell dispersion (Houser 1990), and astrogliosis (Seifert et al. 2010) have been reported in the hippocampus in individuals with TLE-HS. Since the temporal lobe is a major cortical structure involved in learning and memory (Halgren et al. 1991), recurrent spontaneous seizures (which are the primary triggering cause of TLE-HS) result in damage to this structure and therefore memory is impaired (Helmstaedter 2002).

Neurodegeneration associated with TLE-HS has been observed in the human hippocampi (Fig. 7) and subsequently reproduced in rodent models. It is characterised by affecting the so-called trisynaptic circuit (Fig. 8), specifically

CA1 and CA3 subregions of the hippocampus, but not the CA2, DG, or subiculum; the neurodegeneration reported is also associated with MF sprouting (Gloor 1991; Sloviter 1994).

This neurodegeneration is accompanied by loss in GABAergic cells and altered expression of inhibitory receptor subunits in the DG and other parts of the hippocampal formation (Sperk et al. 2004).

Inhibition mediated by GABA has been demonstrated to be reduced in neurons surviving hippocampal sclerosis (HS) associated with TLE (Mangan et al. 1995; Mangan and Lothman 1996). Evoked inhibitory post-synaptic potentials (IPSPs) in neurons from TLE-HS samples have been shown to be reduced compared with samples from patients with different structural lesions (Isokawa et al. 1991; Knowles et al. 1992).

In order to account for this finding, a number of research groups proposed different hypotheses for the decreased synthesis of GABA: (1) impairment in the GABA transporters (GAT) or the glutamate decarboxylase (GAD) enzyme, (2) reduced binding of GABA to the two receptor subtypes, $GABA_A$ and $GABA_B$, (3) reduced production of the receptors at transcriptional or translational level, or (4) post-translational modifications in the hippocampal area. More recent evidence emerged showing impairment in GABA (Thomas et al. 2003, 2005) and GABA transporters (Mathern et al. 1999; Schijns et al. 2015). Also $GABA_A$ receptor subunits were demonstrated to be differentially expressed in both in human TLE-HS hippocampal specimens (Loup et al. 2006) and in animal models (Pirker et al. 2003; Mazzuferi et al. 2010), and in the amygdala and the entorhinal cortex of human patients (Stefanits et al. 2019).

Most interesting for the purpose of this chapter is that anomalies in the expression of $GABA_B$ receptors have been reported both in human TLE-HS and animal models of it.

$GABA_B$ presynaptic receptor function has been demonstrated to be reduced in the DG granule cells of both kindled and kainate rat models of epilepsy (Buhl et al. 1996; Haas et al. 1996). Similar reduction was reported also in CA1 of partially (hippocampus)- or fully (amYgdala)-kindled rats (Asprodini et al. 1992; Wu and Leung 1997); none of these studies reported malfunctions in the $GABA_B$ receptor-mediated post-synaptic potentials. However, Mangan and Lothman (1996) observed a reduction in both pre- and post-synaptic $GABA_B$ receptor function in CA1 neurons in a rat hippocampal-kindling model. The $GABA_{B1}$ and $GABA_{B2}$ transcript expression patterns have been reported in great detail in the rat (Kaupmann et al. 1997, 1998; Muñoz et al. 1998; Bischoff et al. 1999; Benke et al. 1999; Liang et al. 2000; Jones et al. 1998; Kuner et al. 1999; Ng et al. 1999; Clark et al. 2000) and in human hippocampus (Berthele et al. 2001; Princivalle et al. 2003). The binding parameters of agonists and antagonists for $GABA_B$ receptors have also been reported extensively (Billinton et al. 2001; Princivalle et al. 2002; Furtinger et al. 2003). $GABA_B$ receptors have been demonstrated to be differentially expressed in the hippocampus of TLE-HS in rat and mouse models, as well as in human specimens (Princivalle et al. 2001, 2003; Nishimura et al. 2005;Teichgräber et al. 2009; Rocha et al. 2015; Sheilabi et al. 2018).

Electrophysiological evidence indicated that $GABA_B$ receptor expression may be an important factor for the onset of ictogenesis in the rat limbic system and, perhaps, in MTLE patients (Avoli et al. 2004). Lang et al. (2014) demonstrated that $GABA_B$ receptors regulate hippocampal hyperexcitability by inhibiting CA3 glutamatergic synapses. They postulate that positive allosteric modulation of $GABA_B$ receptors may be effective in reducing seizure-related hyperexcitability. All these data demonstrate the loss of $GABA_B$ receptor function in TLE in rodents and humans.

The main common feature emerging from all these pieces of research evidence is that the physiological role of GABA and $GABA_B$ receptors is to induce hyperpolarization. Later on, however, it came up to light that GABA not only has an inhibitory action but could also have a depolarizing action, suggesting that GABA transmission is also involved in promoting epilepsy (Köhling et al. 1998; Cohen et al. 2002). In fact, Kantrowitz and colleagues (2005) demonstrated, by using electrophysiological techniques, that $GABA_B$ receptors regulate the synaptic depolarization to GABA response, and also that blocking of $GABA_B$ receptors with the specific antagonist CGP 55845A caused the depolarizing GABA response to become excitatory and pro-convulsive. Additionally, in very recent years it has been demonstrated in a mouse model of TLE that inhibition of presynaptic $GABA_B$ receptors has a depolarising action on cholecystokinin-positive basket cells [CCK(+) BCs], in the hippocampus, specifically in CA3 (Dugladze et al. 2013).

All this body of evidence highlights the pivotal role that the $GABA_B$ receptor plays in TLE-HS, and the latest data particularly corroborate the importance that the reduced expression of $GABA_B$ receptors has in the pathophysiology of TLE. In the future, studies are needed to design, develop, and test innovative drugs which can target $GABA_B$ receptors, specifically in the trisynaptic circuit.

5 Conclusions

It has long been recognised that GABA is the main inhibitory neurotransmitter in the mammalian brain and that it acts via the $GABA_A$ and $GABA_B$ receptors. This chapter has focused on the review of the role and mechanisms of action of $GABA_B$ receptors in three neurological diseases, which appear similar in some aspects and dissimilar in others. They are similar because they all show neurodegeneration; they are dissimilar because the cerebral circuits involved in their pathophysiology are different in PD versus AD and TLE/HS, and because the main neuropathological features are different.

Altogether, the $GABA_B$ receptor plays a pivotal role in the inhibitory pathway in order to control the balance between excitatory/inhibitory signals in the trisynaptic circuit of the hippocampus, which has been described and demonstrated to have neuronal loss, in both AD and TLE/HS. On the other hand, in PD, $GABA_B$ has been shown to modulate excitatory/inhibitory signals via more newly described pathways different from the trisynaptic circuit.

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$GABA_B$ Receptors and Cognitive Processing in Health and Disease

Styliani Vlachou

Contents

Abstract $GABA_B$ receptors are implicated in numerous central nervous system-based behaviours and mechanisms, including cognitive processing in preclinical animal models. Homeostatic changes in the expression and function of these receptors across brain structures have been found to affect cognitive processing. Numerous preclinical studies have focused on the role of $GABA_B$ receptors in learning, memory and cognition per se with some interesting, although sometimes contradictory, findings. The majority of the existing clinical literature focuses on alterations in

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$GABA_B$ receptor function in conditions and disorders whose main symptomatology includes deficits in cognitive processing. The aim of this chapter is to delineate the role of $GABA_B$ receptors in cognitive processes in health and disease of animal models and human clinical populations. More specifically, this review aims to present literature on the role of $GABA_B$ receptors in animal models with cognitive deficits, especially those of learning and memory. Further, it aims to capture the progress and advances of research studies on the effects of $GABA_B$ receptor compounds in neurodevelopmental and neurodegenerative conditions with cognitive dysfunctions. The neurodevelopmental conditions covered include autism spectrum disorders, fragile X syndrome and Down's syndrome and the neurodegenerative conditions discussed are Alzheimer's disease, epilepsy and autoimmune anti-$GABA_B$ encephalitis. Although some findings are contradictory, results indicate a possible therapeutic role of $GABA_B$ receptor compounds for the treatment of cognitive dysfunction and learning/memory impairments for some of these conditions, especially in neurodegeneration. Moreover, future research efforts should aim to develop selective $GABA_B$ receptor compounds with minimal, if any, side effects.

Keywords Animal models · Cognition · $GABA_B$ receptors · Humans · Learning · Memory · Neurodegeneration · Neurodevelopmental disorders · Pharmacotherapy · Treatment

1 Introduction

Metabotropic $GABA_B$ receptors ($GABA_BRs$) are implicated in numerous central nervous system (CNS)-based behaviours and mechanisms, such as the cognitive processes of learning and memory (Bowery 2006; Heaney and Kinney 2016; Serrats et al. 2017), in preclinical animal models. Homeostatic changes in the levels and functions of these receptors at molecular, cellular or neurochemical levels have been found to affect those cognitive processes. Preclinical studies using a variety of animal models have focused on the role of $GABA_BRs$ in learning, memory and cognition per se. Results from these studies, although sometimes contradictory, are valuable and promising.

Additionally, several clinical conditions and disorders with variable symptomatology that are associated with cognitive deficits also show alterations in $GABA_B$ receptor ($GABA_BR$) function (Heaney and Kinney 2016). These conditions include neurodevelopmental disorders such as Down's syndrome (DS), fragile X syndrome (FXS) and autism spectrum disorders (ASD), neurodegenerative disorders, such as Alzheimer's disease (AD) and epilepsy, and other neuropsychiatric disorders, such as schizophrenia. This correlation has spurred a number of studies that have focused on investigating the role of $GABA_BRs$ in cognitive processing in these clinical conditions. The development and advances in imaging techniques have allowed for a greater understanding of metabotropic GABAergic signalling pathways and

their involvement in cognition (Murrell et al. 2020). An overall extensive account of the role of $GABA_B$Rs in neurodegeneration, with a focus on epilepsy and AD, is presented elsewhere in this book volume (Princivalle 2021). In this chapter the focus will be on cognitive processes identified in animal models of health and disease and in human populations whose cognitive symptomatology is affected or altered by $GABA_B$R mechanisms.

In a broad sense, $GABA_B$R agonists have been shown to impair learning and memory processes, while $GABA_B$R antagonists improve them. These effects largely depend on either the regulation of postsynaptic excitability or on the presynaptic inhibition of neurotransmitter release. These effects also depend on the pre- and/or post-synaptic pathways affected, methodological components of the experiment (such as the tasks implemented, administration method and schedule, and the doses used) and the duration of the effects. It is important to understand the role of $GABA_B$R agonists, antagonists and positive allosteric modulators (PAMs) on cognition in the general population, as well as in clinical populations, and the mechanisms of action underlying their effects. Thus, the aim of this review chapter is to examine these processes in detail and capture all existing literature in this area, in an effort to shed some light on the potential use of $GABA_B$R compounds for the treatment of cognitive impairments in neurodevelopmental, neurodegenerative and/or neuropsychiatric disorders.

2 $GABA_B$ Receptors Mechanisms of Action

A detailed presentation of the $GABA_B$R structure, function, locations and mechanisms of action is presented in this book volume chapters (Fritzius et al. 2020; Rose and Wickman 2020). Briefly, slow sustained neuronal inhibition is mediated by $GABA_B$Rs (Brenowitz et al. 1998), which are heterodimeric G-protein-coupled receptors (GPCRs) constructed from $GABA_B$R1a or $GABA_B$R1b and $GABA_B$R2 subunits present in neurons and glial cells throughout the CNS. These subunits need to co-exist for the receptors to be functional. Through the activation of Gi/o proteins, $GABA_B$Rs limit cAMP accumulation, decrease neurotransmitter release and induce neuronal hyperpolarization.

Taking into account their synaptic loci, $GABA_B$R activation acts in two ways, both ways reducing glutamatergic signalling at excitatory synapses: (1) it reduces presynaptic GABA and glutamate release through inhibition of presynaptic Ca^+ channels in both inhibitory and excitatory synapses and (2) it causes hyperpolarization of postsynaptic neurons by activation of G protein-activated inwardly rectifying potassium (K^+) channels (GIRKs) (Pin and Bettler 2016). Further, in order for the $GABA_B$Rs to be activated, non-synaptic or volume transmission needs to take place, which requires high levels of GABA release. This can be achieved via several mechanisms, including simultaneous discharge of GABAergic interneurons (Holley et al. 2019), very intense discharges in the thalamus, or by the activation of neuroglia interneurons in the cortex (Sanchez-Vives et al. 2020).

3 Effects of $GABA_B$ Receptors on Cognitive Processes: Learning and Memory

Functional activation of $GABA_B$Rs inhibits learning and memory processes, though conflicting results have been reported. As cognitive processes and the effects of $GABA_B$Rs on them are assessed in the context of a vast number of conditions, this section will initially focus on aspects relating to synaptic plasticity and long-term potentiation (LTP) as they relate to cognition, and will then be divided into behavioural procedures used in animal studies for the assessment of cognitive processes in healthy and/or unhealthy populations. The effects of $GABA_B$Rs on cognition will be examined through a variety of procedures, such as: passive and active avoidance tests, Morris Water Maze (MWM), Radial Arm Maze (RAM), working memory tasks, novel object recognition (NOR) and (dis)location (NOL) tasks and the Barnes maze.

3.1 $GABA_B$ Receptors, LTP and Synaptic Plasticity

LTP is a long-lasting increase in synaptic effectiveness after repeated high-frequency stimulation, identified first in the hippocampus in 1973 by Bliss and Lomo (Bliss and Lømo 1973; Lømo 2003, 2018). LTP is a synaptic plasticity mechanism that is important for associative learning and creating memories and well-defined. Moreover, being well-defined, LTP is easy to measure and can be used as a readout for cognitive deficits.

Evidence from numerous studies shows that the GABAergic system is involved in LTP and other synaptic plasticity mechanisms in different areas of the brain, including the hippocampus and the perirhinal cortex. Importantly, oscillatory and synchronous activity is needed to induce synaptic plasticity and GABA seems to play an essential role in controlling these oscillations (Kohl and Paulsen 2010). Specific frequencies, such as gamma and theta, have been linked with memory formation in animals and humans [e.g., (Axmacher et al. 2006; Jutras and Buffalo 2010)], and GABA-initiated synchronous inhibitory postsynaptic potentials (IPSPs) in the hippocampus control these frequencies [e.g., (Gong et al. 2009; Mann and Mody 2010)].

As an example of these processes, there appear to be functional interactions between the GABAergic and cannabinoid (CB) systems in the hippocampus and other brain areas. After LTP induction in the dentate gyrus (DG) of rats, either the cannabinoid 1 receptor (CB1R) antagonist AM251 or the $GABA_B$R antagonist CGP55845 was administered. While AM251 increased LTP, CGP55845 decreased it, while their co-administration had differential effects on the population spike (PS) amplitude and field excitatory postsynaptic potential (fEPSP). Data from this study suggested that $GABA_B$R antagonists modulate cannabinoid outputs that decrease synaptic plasticity, while during co-administration, CB1R antagonists can

alter the release of GABA ultimately resulting in enhancement of LTP induction (Nazari et al. 2016a, b). In a corresponding study by the same research group (Nazari et al. 2016a), where either AM251 or baclofen was administered, results showed that administration of either of the two compounds, AM251 or baclofen, increased both the PS amplitude and the fEPSP slope, while co-administration of AM251 and baclofen induced greater increases in PS amplitude and the fEPSP slope, further strengthening LTP induction through $GABA_BRs$. These findings suggest that CB1R activation in the hippocampus seems to affect synaptic function of GABAergic interneurons located in the DG.

3.2 Active and Passive Avoidance Paradigms

In the active avoidance paradigm, animals (most commonly rodents) learn to avoid an aversive stimulus by initiating a specific locomotor response. In this task, animals are placed in a two-compartment shuttle box and learn the association between a conditioned stimulus (CS), such as light or tone, and an unconditioned stimulus (US), such as footshock. In this active avoidance paradigm, a conditioned avoidance response is defined as when the animal moves to the opposite compartment during the CS presentation (typically within 10 s) so as to avoid the shock. If the animal does not move to the other compartment, footshock is delivered. However, this footshock can be escaped by moving to the opposite compartment. Thus, performance is measured by the latency to avoid (i.e., enter the other chamber before the termination of the CS and the onset of the footshock) or escape (i.e., enter the other chamber after the onset of footshock) the US. There are two primary versions of this task: the one-way and the two-way. The first version always includes a defined safe chamber (i.e., a specific chamber where the animal will never receive the US). The second version does not define a specific chamber associated with the US. Instead, the CS is utilized to signal the onset of the US. The active avoidance paradigm is a cognitive task requiring spatial learning and cognitive coordination. It is useful for assessing associative learning (operant conditioning), short- and long-term memory, and it also provides procedures for testing acquisition, consolidation and retention processes.

The most extensively studied $GABA_BR$-active compound in this paradigm has been the $GABA_BR$ agonist baclofen, with doses of 0.25 to 20 mg/kg. In the active avoidance task, as with other tasks described in this chapter, baclofen has shown contradictory findings, with both improved and impaired performance of mice and rats. Studies in CD1 mice (Farr et al. 2000), Sprague-Dawley (Fogel et al. 2010) and Long Evans rats (Stuchlik and Vales 2009) have shown an impairment in different versions of the active avoidance task, a study using Wistar rats has shown no effect (Kuziemka-Lęska et al. 1999) and other studies have shown memory improvement after administration of baclofen (Georgiev et al. 1988; Sharma and Kulkarni 1993), an effect that is consistently shown with $GABA_BR$ antagonists.

In one of these studies, baclofen dose-dependently disrupted spatial learning and locomotion in the place avoidance task. Spatial learning and memory (i.e., spatial behaviour) refer to the ability of an organism to acquire and maintain, respectively, a mental representation of their environment. Spatial behaviour allows an animal to understand not only their location in relation to their surrounding environment, but also the location and dimension of objects and/or stimuli in the environment and how they are related to them and to each other. Importantly, assessment of the effects of $GABA_BR$ compounds on locomotor activity is a very important measure of possible therapeutic effects (e.g., spasticity, hyperactivity induced by psychostimulants) or locomotor side effects, as sometimes seen with $GABA_BR$ agonists, such as baclofen (e.g., motor impairment, muscle relaxation) in clinical settings and preclinical studies, that can significantly influence aspects of performance in a variety of animal models.

In the place avoidance task, animals are trained to move over a continuously rotating arena, on which an imperceptible to-be-avoided sector is defined, remaining stable with respect to the experimental room (Stuchlik and Vales 2009). In this study, baclofen administered at 3.5 mg/kg 30 min before the task selectively disrupted spatial behaviour in Long Evans rats, while doses of 4 and 6 mg/kg proved to decrease both avoidance efficiency and locomotor activity. Another study in which baclofen was administered 30 min before the task showed the opposite effect. In that study, which used LAKA mice, baclofen was administered at 0.25 and 0.5 mg/kg, and the 0.5 mg/kg dose was effective in improving one-way acquisition in mice (Sharma and Kulkarni 1993). In studies using Wistar rats, baclofen administered either 30 min pre-training (0.75 mg/kg, IP) or post-training (2, 5, 10, 20 mg/kg, IP) either had no effect (Kuziemka-Lęska et al. 1999) on the two-way acquisition or improved the two-way consolidation (Georgiev et al. 1988). Moreover, intra-pedunculopontine nucleus administration of baclofen 17 h post-training impaired two-way consolidation in Sprague-Dawley rats whose REM sleep was disrupted (Fogel et al. 2010). It is worth noting that the pedunculopontine nucleus has been implicated in the generation of REM sleep. Further, as memory consolidation may take place during increased post-learning REM sleep, any disruption of REM sleep can have an impact on the animals' subsequent performance in this task (Fogel et al. 2010). The variability of effects across doses already seen seems to be dependent on not only the exact procedures used, but also the route of administration and the species and strains used. The time of the administration does not seem to differ among studies already mentioned.

A recent study examined the role of dorsal hippocampal (CA1) $GABA_BRs$ on harmaline-induced memory consolidation deficits in mice using the step-down inhibitory avoidance task (Nasehi et al. 2017). Results from this study showed that post-training intra-CA1 injections of the $GABA_BR$ antagonist phaclofen, one of the first $GABA_BR$ antagonists described and the phosphonic acid derivative of baclofen, had no effect, while baclofen (0.1 μg/mouse) impaired animals' performance in this task, suggesting a modulation of storage of information. In further experiments, post-training infusion of harmaline (2 and 5 mg/kg, IP) decreased memory consolidation, while administration of phaclofen at a sub-effective dose (0.001 μg/mouse,

intra-CA1) successfully blocked the deficits on memory consolidation induced by the highest doses of harmaline (2 and 5 mg/kg, IP). Further, a low dose of baclofen (0.001 μg/mouse, intra-CA1) potentiated impairment of memory consolidation induced by harmaline (2 mg/kg, IP). These findings suggest that the CA1 $GABA_BRs$ are involved in memory consolidation and that harmaline interacts with the CA1 $GABA_BRs$ to modulate this memory process (Nasehi et al. 2017).

Considerably more studies using the active avoidance task have used $GABA_BR$ antagonists rather than agonists (Getova et al. 1997; Getova and Bowery 1998; Farr et al. 2000; Getova and Dimitrova 2007). The vast majority of these studies have shown improvement in performance in the active avoidance paradigm. In these studies (Getova et al. 1997; Getova and Bowery 1998; Getova and Dimitrova 2007), Getova and colleagues used the $GABA_BR$ antagonists CGP 36742, CGP 55845, CGP56433, CGP 61344, CGP 62349, and CGP 71982, CGP 63360a, CGP76290a, and CGP 76291a showing different affinity for the $GABA_BR$ and different potency [for a detailed presentation of $GABA_BR$ antagonists, please see Nieto et al. (2021)]. All studies were conducted using the two-way version of the task and tested for both acquisition and retention in Wistar rats. With the exception of CGP36742, which impaired performance in the task, and CGP 76291a, which did not have an effect on acquisition, all other compounds improved performance. Interestingly, in the case of CGP36742, when the one-way version of the task was used, and the $GABA_BR$ antagonist was administered repeatedly for two weeks, prior to the task, it then also improved performance (Yu et al. 1997). Saclofen, another $GABA_BR$ antagonist, also improved performance in this task (Farr et al. 2000). Altogether, these data support the involvement of $GABA_BR$'s in memory processes.

In the passive avoidance paradigm, which is classically used to assess short- or long-term memory in animals (typically rats or mice), animals must react/behave contrary to their innate tendency to avoid light. The apparatus used in this paradigm is composed of two compartments, a black poorly illuminated compartment and a white illuminated compartment. During the acquisition/conditioning phase, the animal is placed in the white compartment. When the animal innately moves to the black compartment, it receives a mild footshock. Thus, during the initial phase the animal learns that moving to the dark compartment has negative consequences. During the test phase the animal is again placed in the white compartment and the passive avoidance response is examined. As opposed to the previous avoidance task discussed, the avoidance of the dark compartment requires the animal to remain in the white compartment and, therefore, the absence of movement (i.e., passive avoidance response).

The passive avoidance task is useful for assessing memory performance, which is positively correlated with the latency to escape from the white compartment; the better the recollection, the greater the latency to move. It is also useful for studying other mechanisms involved in cognition. A number of brain regions have been implicated in mechanisms active during the passive avoidance task [e.g., (Bradley et al. 2004)]; these include the hippocampus, medial prefrontal cortex (mPFC) and basal forebrain for acquisition (Sharma and Kulkarni 1993; Zarrindast et al. 1998; Kuziemka-Lęska et al. 1999; Car and Wiśniewska 2006; Car and Michaluk 2012;

Farahmandfar et al. 2017; Liu et al. 2017; Ebrahimi-Ghiri et al. 2018), and the hippocampus, amygdala, anterior cingulate cortex (ACC), mPFC and nucleus basalis magnocellularis (NBM) for consolidation (Zarrindast et al. 2002; Zarrindast et al. 2008b; Kunisawa et al. 2017).

Most of the studies on active and passive avoidance memory retention tests have used baclofen. Taking into account the species, the rodent strain, the routes of administration and the doses used, the majority of these studies have shown memory impairments, with some showing memory improvement and some showing no effect. Baclofen doses used would range between 0.125 and 20 mg/kg through systemic routes of administration, with variable directions of effect, while central administrations, in the amygdala or intracerebroventricularly (ICV), have shown impairments.

In one of the earliest studies, baclofen at doses of 2, 5 and 10 mg/kg injected intraperitoneally (IP) immediately after training improved retention in both active and passive avoidance tasks in male Wistar rats, while 20 mg/kg had no effect on active avoidance performance (Georgiev et al. 1988). Baclofen also enhanced memory in ICRC mice in the passive avoidance task, an effect that was blocked by the $GABA_BR$ antagonist CGP 35348 at a dose of 10 mg/kg (Saha et al. 1993). Further, both pre-training and post-training administration of CGP 35348 (75, 150 and 300 mg/kg, IP) significantly reduced the amnesic effect induced by scopolamine (1 mg/kg, IP) (Bianchi and Panerai 1993).

In another relevant study assessing cholinergic and GABAergic interactions on learning and memory (Sharma and Kulkarni 1993), (+/−)baclofen (0.25, 0.5 and 1 mg/kg) and (−)baclofen (0.25 and 0.5 mg/kg) also induced memory enhancement (for a discussion on the chirality of baclofen and the differences between the baclofen enantiomers, (R)-(−)-baclofen and (S)-(+)-baclofen, please see Nieto et al. (2021), whereas the $GABA_BR$ antagonist CGP 35348 did not show any effect per se, but reversed the (+/−)baclofen-induced delay in latency, without affecting the retention enhancing action of (+/−)baclofen. When sub-effective doses of GABA (50 mg/kg) and (+/−)baclofen (0.25 mg/kg) were co-administered, they induced a significant improvement in both acquisition and retention. In contrast, a different study assessing the effects of the cholinergic cognitive enhancer NS-105 on memory in the passive avoidance task in Wistar rats showed that NS-105 reversed cognitive impairment induced by baclofen (8 mg/kg, IP) (Ogasawara et al. 1999). Moreover, intra-hippocampal injection of baclofen (0.25, 0.5, 1 and 2 μg) reduced memory retention in rats, while intra-hippocampal injection of lower doses of the $GABA_BR$ antagonist CGP35348 (2.5, 5, 10 μg) did not affect and higher doses (25 and 50 μg) decreased memory retention. The same doses of CGP35348 (2.5, 5 and 10 μg), which did not have an effect per se, reduced the effect of baclofen (Zarrindast et al. 2002). Memory impairment with baclofen has been the primary result of other studies by the same research group in NMRI mice (Zarrindast et al. 2004). Interestingly, baclofen (1, 1.5 and 2 μg) prior to injection of morphine (20 mg/kg per day × 3 days) decreased the reversal of morphine-induced amnesia in morphine sensitized-mice (Zarrindast et al. 2008b). It appears that, at least in some of these studies, post-training administration of different doses of baclofen impairs

memory retention while in some others even acquisition is impaired by administration of baclofen (Car and Wiśniewska 2006). It also appears that animals exhibiting different behavioural stereotypes such as aggressiveness or submissiveness are differentially affected by administration of 1 mg/kg of baclofen. In the passive avoidance test, it was shown that baclofen produced amnesia in aggressive mice and had a stronger amnesic effect in submissive mice (Dubrovina and Loskutova 2007). Moreover, a separate study has shown that $GABA_BR$ activation with baclofen accelerated extinction of fear memory in depressed-like mice (Dubrovina and Zinov'ev 2008).

Contrary to the above findings, numerous studies have shown no effect of baclofen in either of the passive avoidance measures (i.e., retrieval, consolidation, retention) (Swartzwelder et al. 1987; Castellano et al. 1989; Car and Wiśniewski 1998; Zarrindast et al. 1998, 2008a; Kuziemka-Lęska et al. 1999; Car et al. 2000; Cryan et al. 2004; Car and Wiśniewska 2006). The lack of effect of baclofen in the passive avoidance task measures in these studies may be due to differences in experimental condition methodology, drug doses and routes of administration or different rodent strains used.

Not many positive allosteric modulators (PAMs) for the $GABA_BRs$ have been developed, let alone tested for cognitive processes. In one of these few studies, the $GABA_BR$ PAM GS39783 did not show an effect in the passive avoidance task neither in CD1 mice (1, 3, 10, 30, 100 mg/kg) nor in Sprague-Dawley rats (25, 50, 100 mg/kg) at all of the doses tested (Cryan et al. 2004).

In studies utilizing $GABA_BR$ antagonists, administration of either of three $GABA_BR$ antagonists showed differential effects on the passive avoidance task, when the gamma-hydroxybutyrolactone (GHBL) animal model was used. The GHBL is an experimental model of absence seizures in rats and mice. Absence seizures in humans involve brief and sudden lapses of consciousness, as well as mild jerks of facial muscles electrophysiologically depicted as synchronous spike and wave discharges in the EEG (Snead 1991). In a similar manner, the GHBL animal model rapidly and consistently produces a combination of EEG and behavioural changes which resemble the human condition. CGP71982 improved both learning and memory retrieval, whereas CGP55845A and CGP62349 had no effect (Getova and Bowery 2001). Interestingly, administration of the $GABA_BR$ antagonist CGP36742 (SGS742) improved the learning capacity of mice, rats and rhesus monkeys, in a passive avoidance test, a partner recognition test and a conditional spatial colour task, respectively (Mondadori et al. 1993; Froestl et al. 2004). Moreover, treatment with either CGP35348 or CGP36742 significantly blocked acquisition impairment induced by baclofen, consolidation impairment induced by baclofen and NaNO2, and retrieval impairment induced by baclofen and 30% alcohol (Yu et al. 1996). Phaclofen had no effect in the passive avoidance task (Zarrindast et al. 1998; Dubrovina and Zinov'ev 2008).

Finally, some of the studies utilizing the passive avoidance task have examined mutant mice that lack specific $GABA_BR$ subtypes to further elucidate their role in learning and memory processes. In one of these studies, Jacobson and colleagues found that lack of either the $GABA_BR1a$ or $GABA_BR1b$ subunits had no effect on

the passive avoidance task (Jacobson et al. 2007), while other studies have shown memory impairments in $GABA_BR1$ KO (Schuler et al. 2001), at a time when the splice variants of the heterodimeric receptor had not been characterized, or $GABA_BR2$ KO mice (Gassmann et al. 2004) in the same task. Interestingly, in the Jacobson study, latency to enter the dark side of a two-compartment trough-shaped apparatus was greater at retention testing than during training, although there was no effect of genotype on either training or retention latency (Jacobson et al. 2007). This indicated that both mutant strains could remember the shock they received in the dark compartment. The difference between the results of the Jacobson study and that of Schuler and colleagues (Schuler et al. 2001), which showed profound memory deficits in the retention phase of this test, can be attributed to the lack of both functional isoforms of the $GABA_BR1$ in the latter study. This further indicates that heterodimeric $GABA_BR1$ function is essential for the retention of passive shock-avoidance training, and that this may be accomplished with either of the $GABA_BR1a$ or $GABA_BR1b$ isoforms (Jacobson et al. 2007).

3.3 Morris Water Maze (MWM) and Radial Arm Maze (RAM)

Primarily designed to measure spatial learning and recall, the Morris Water Maze (MWM) is the most commonly used test to evaluate cognitive functions utilizing mnemonic mechanisms. By employing a variety of sophisticated protocols or procedures in the MWM, such as the visible or hidden platform test, the transfer test, and the relearning or repeated acquisition test, one can assess the acquisition and spatial location of relevant visual cues. These visual cues are further processed, consolidated, retained and retrieved, in order to successfully navigate, and thus locate, a hidden platform, with the goal of escaping the water and surviving (i.e., implementing memory functions as survival skills). The MWM has recently been used for the evaluation of ageing effects on memory, as well as those of drugs and experimental lesions in rodents, especially as these relate to animal models of neurodegenerative disorders with cognitive decline, such as Alzheimer's and Parkinson's disease. As with other tasks, findings from studies using the MWM are inconsistent. Studies using baclofen in mice or rats have shown impairment or no effect in the maze, while results from studies examining the effects of antagonists show effects in all directions, improvement, impairment or no effect.

More specifically, baclofen administered directly into the entorhinal cortex 15 min before training impaired learning and memory in the MWM in Sprague-Dawley rats (Deng et al. 2009). However, in the same strain of rats, baclofen administration 19 h before training systemically produced no effect (Li et al. 2014). Similar effects were identified in Wistar rats; intra-dorsal hippocampus administration of baclofen impaired performance (Arolfo et al. 1998), while systemic administration had no effect (Car and Michaluk 2012). In contrast, other

studies in Wistar rats showed impairment in performance after systemic administration of baclofen 30 min before training (Nakagawa et al. 1995; Nakagawa and Takashima 1997). These contradictory findings may be due to the different time and doses of administration, taking into account that the same strain of rats was used in both studies, as well as systemic administration. Most recently, differently timed administrations of baclofen (right before pre-test or daily for 14 days, or repeatedly for a month) did not affect performance in the MWM at any of the doses tested in Wistar rats (Holajova and Franek 2018). Effect of $GABA_BR$ blockade by administration of the antagonist CGP35348 directly into the CA1 area of the hippocampus in Wistar rats largely depended on the timing of administration, with post-training and 15 min pre-test administration showing no effect, and 15 min pre-training administration showing an improvement in performance (Shahrzad and Nasser 2015).

In one study using numerous mouse strains to test the effect of CGP36742 on spatial learning and recall, administered systemically 40 min before training, C57BL/6 J and OF1 mice improved their performance compared to the control mice, while CD1 and DBA/2 mice demonstrated impaired task performance (Sunyer et al. 2007, 2009). Further, the performance of RGS7 KO mice indicated that RGS7, which regulates the interaction between $GABA_BRs$ and GIRK channels, is necessary to acquire this task (Ostrovskaya et al. 2014). Most recently, Phf24-null rats were developed by Serikawa and colleagues (Serikawa et al. 2019). Phf24 is known as Gαi-interacting protein (GINIP) and is associated with $GABA_BR$. Interestingly, Phf24 rats showed impairments in the MWM task. Another recent study focused on the effects of exercise and baclofen administration in striatum-lesioned rats and found that mild exercise and baclofen microinjection did not affect spatial learning or motor activity impairments, while the combination of them alleviated spatial learning and motor activity impairments in these rats (Modaberi et al. 2019).

The radial-arm maze (RAM) task is based on the natural survival tendency of food-deprived rodents to learn and remember different spatial locations for food in an eight-arm radial maze. A large number of protocols can be applied in this task which can be very useful in assessing neural bases for learning and memory. In the spatial working memory protocol, each arm of the maze is baited (i.e., food is placed in the arm) and repeat entries into an arm that has already been visited signify a working memory error. In the reference memory protocol, only some arms are baited at the beginning of the session. First entry into a non-baited arm signifies a reference memory error, and repeated entries to baited and non-baited arms are defined as working memory errors. As with some of the tasks previously described, this task can also be used with a large number of delays.

Most of the studies using the RAM to assess performance of animals per se, rather than symptomatology related to other conditions (e.g., memory processes in animal models of epilepsy), have used rats. Results from studies in rats using baclofen are inconsistent which may possibly be due to the different doses used. As an example, systemic administration of baclofen at a dose of 0.25 mg/kg (subcutaneously; SC) in female Sprague-Dawley rats 20 min before the test showed an improvement in performance, while a higher dose (1 mg/kg, SC) showed an impairment and all other doses in between had no significant effect (Levin et al. 2004). Higher doses

(1.25 and 2.5 m/kg, SC) had no effect in male Fisher 344 rats (Sidel et al. 1988). Moreover, intra-septal administration of baclofen in male Sprague-Dawley rats impaired consolidation in this task (Stackman and Walsh 1994). The septum has not been mentioned previously in this chapter. Thus, briefly, it is a subcortical structure in the forebrain that is found near the midline of the brain and its functions are not as well-known as those of other structures (e.g., amygdala, hippocampus, etc.). The septum in humans can be separated into two structures: the septum pellucidum and septum verum. The first seems to be a relay station between the hippocampus and the hypothalamus, and abnormalities in this structure are linked with various neurological conditions. The second is part of the limbic system; it receives afferent projections from the hippocampus, amygdala, and hypothalamus, and the ventral tegmental area (VTA). It also sends projections to the hippocampus, habenula, thalamus, VTA and hypothalamus and is linked with reward processes.

In one study using CD1 mice, either baclofen or the $GABA_BR$ antagonist CGP36742, both administered 15 min before the test, showed impairment and improvement of performance in the RAM, respectively (Carletti et al. 1993). Further, in two different studies, mice overexpressing $GABA_BR1a$ (Wu et al. 2007) or $GABA_BR1b$ (Stewart et al. 2009) showed an impairment in performance in this task, indicating the important role of $GABA_BRs$ in learning and memory.

With the exception of two studies where neither CGP35348 or CGP46381 showed an effect, most studies using CGP35348 (doses ranging from 12.5 to 300 mg/kg, IP) administered 20–30 min before training, or CGP36742 (150 mg/kg, IP) administered either 40 min before training or post-training, in male Long Evans rats, showed an improvement in performance in the RAM (in the 25 to 250 mg/kg range of doses for CGP35348) (Staubli et al. 1999; Helm et al. 2005; Chan et al. 2006).

In summary, these data suggest that $GABA_BR$ activation impairs performance in the MWM and RAM tasks in rodents, while the effects of $GABA_BR$ antagonists depend on the strain of rodents and doses of compounds used. Replication and further testing of these strains with both baclofen and $GABA_BR$ antagonists seems necessary so as to better understand the level of activation or de-activation of $GABA_BRs$ required for specific learning and memory processes to be successfully completed.

3.4 Working Memory Tasks

Working memory is defined as the capability to maintain the representation of a stimulus that is no longer present and perform mental operations on this representation so as to perform a specific task. A number of spontaneous alternation tasks can be used to study working memory in rodents. Some of the most commonly used ones are the T, Y and double-Y mazes. The T-maze in particular is a simple task commonly used for assessing spatial working memory in rats and mice, especially as a delayed alternation task. Similarly to the T-maze, the Y maze spontaneous

alternation test is also commonly used for assessing spatial working memory in rats and mice, by measuring the willingness of rodents to spontaneously explore new environments. As seen with other similar tasks, rats and mice typically prefer to investigate a new arm of the maze rather than returning to one that was previously visited. Briefly, the Y-shaped maze has three white, opaque plastic arms at a 120° angle from each other. After introduction to the centre of the maze, the animal is allowed to freely explore the three arms. Over time and multiple arm entries, the animal naturally shows a tendency to enter a less recently visited arm. Performance is measured in terms of the percentage of alternated arm choices compared to total number of arm entries in order to calculate the percentage of alternations. The Y maze spontaneous alternation test is used to quantify cognitive performance in transgenic mice and identify novel potential therapeutic drugs for their effects on cognition. A higher percentage of alternations indicates better working memory (Miedel et al. 2017).

Another less commonly used set of tasks to assess spatial working memory are the delayed matching to position/non-matching to position tasks (DMTP/DNMTP). Either of these tasks, DMTP or DNMTP, measures short-term spatial memory, which is a component of the working memory construct (Dudchenko 2004; Teutsch and Kätzel 2019). When applied serially, they can also be used to measure reversal learning (Yhnell et al. 2016). In the DMTP task, a water-deprived animal interprets signals from two signal lights (left and right) and responds by pressing one of two levers (left and right). In the initial (sample) phase, one signal light is illuminated followed by a delay period where neither light is illuminated. Following the delay, during the choice phase, both lights are illuminated, and the animal has a limited amount of time to either respond at the lever in the same side to that which was signalled prior to the delay (DMTP) or opposite to that which was signalled prior to the delay (DNMTP), in the two versions of the task, respectively. The animal is given water reward for correct answers. These tests capture spatial working memory because the animal is required to maintain the representation of the stimulus, but it does not need to mentally manipulate the representation.

Experiments utilizing these tasks in rats have only taken place with Sprague-Dawley rats. In doses ranging from 0.03 to 3 nmol administered into the NBM, medial septum, mediodorsal thalamus, baclofen induced impairment, in a dose-dependent manner, although the exact effective doses differed depending on the timing of the administration (DeSousa et al. 1994; Romanides et al. 1999; Erickson et al. 2006). Contrary to these results, Escher and Mittleman showed that baclofen, in the lowest dose tested (2.5 mg/kg, IP), improves performance in female C57BL/6J and DBA/2 mice in the DMTP task when administered 10 min before the test (Escher and Mittleman 2004). The same performance improvement effect has been seen with phaclofen in this study (10, 30 mg/kg, IP), when administered 10 min before the test in both mouse strains.

Results of the effects of antagonists in these tasks have been inconsistent, possibly due to the route of administration, the doses, and the specific memory process captured through the timing of the administrations (i.e., pre-training, post-training, pre-test). Thus, CGP55845 showed no effect in male Fisher 344 rats

when it was administered in the mPFC while it showed an impairment in performance when administered 40 min before training (0.01 mg/kg, IP) (Bañuelos et al. 2014). It also showed no effect in B6/C3H mice (0.5 mg/kg, IP), when administered 2–3 h before training (Kleschevnikov et al. 2012a). In contrast, saclofen administered directly into the DG of Sprague-Dawley rats 15 min before training improved performance (Liu et al. 2014).

Importantly, both $GABA_BR1a$ and $GABA_BR1b$ isoforms are necessary for standard or improved task performance in these mazes, as shown through impaired performance in this task exhibited by $GABA_BR1a$ or $GABA_BR1b$ KO mice (Jacobson et al. 2007). In a more recent study, hippocampal protein levels of both $GABA_BR1$ isoforms were increased in mice trained in this task compared to untrained mice (Falsafi et al. 2015).

3.5 Novel-Object Recognition (NOR) and (Dis)Location (NOL) Tasks

The NOR and NOL tasks are based on the natural tendency of rodents to explore novelty. Both the NOR and NOL tasks measure the ability of animals to discriminate between familiar and novel objects in their environment. In the acquisition/training phase of the task, animals are allowed to freely explore two objects that are identical in an experimental arena. The test phases of these tasks will occur within the same day for the examination of short-term memory or in more than 24 h for the examination of long-term memory. In the test phase of the NOR, two objects, including one of the two previously placed (i.e., familiar) objects (i.e., the objects present during the acquisition/training phase) and a novel one, are presented to the animal. In the NOL, both of the original objects are presented in the test phase. However, one object is placed at a different location. In either of the tasks, time spent investigating the objects is recorded at each phase of testing. If the animal remembers an object, it will not spend as much time investigating the familiar object in the NOR, while in the NOL, the animal should spend less time investigating the object that has not been relocated. The choice to explore the novel object reflects the use of recognition memory processes, which in these two tasks activate both the prefrontal cortex and the hippocampus (Lee et al. 2005; Akirav and Maroun 2006).

Importantly, a number of studies have shown that presynaptic $GABA_BRs$ are essential for NOR and NOL performance (Vigot et al. 2006; Jacobson et al. 2007; Cullen et al. 2014). Or, alternatively, they have shown that if presynaptic $GABA_BRs$ are not present, this has deleterious effects in the NOR and NOL. Another study using RGS7 KO mice showed the same impairments in both males and females. In this study, RGS7 ablation increased GABABR-GIRK coupling sensitivity and slowed GABABR-GIRK deactivation rates, indicating that these mice possibly experience enhanced postsynaptic $GABA_BR$-mediated signalling (Ostrovskaya et al. 2014). In contrast, mice with enhanced postsynaptic $GABA_BR$ signalling did

not change their performance in the NOR, but were impaired in the NOL, suggesting that NOL may be a more sensitive task for this increased signalling than NOR (Terunuma et al. 2014).

In a study by Pitsikas and colleagues (Pitsikas et al. 2003), the role of $GABA_BR$ on recognition memory in Sprague-Dawley rats was assessed by administration of baclofen and the $GABA_BR$ antagonist CGP 35348 using the NOR task. Results showed that baclofen (0.5, 2 and 4 mg/kg, IP) dose-dependently impaired performance in this task, with the highest dose tested showing a significant impairment, suggesting an effect on acquisition and retention of information. Administration of the $GABA_BR$ antagonist CGP 35348 (100 and 300 mg/kg, IP) counteracted the baclofen-induced performance deficits. These results indicated that $GABA_BRs$ are involved in recognition memory. More recent data in Sprague-Dawley rats in which higher doses of baclofen (12.5, 25 mg/kg, IP) were administered at pre-training (19 h before training) had the same impairment effect (Li et al. 2014). Other studies have shown no effects of either baclofen or the $GABA_BR$ antagonist CGP55845, with systemic administration, in Wistar rats or NMRI mice, respectively (Car and Wiśniewski 1998; Khanegheini et al. 2015) in the NOR. Moreover, the $GABA_B$ receptor antagonist CGP35348, administered directly in the DG (1, 10 and 100 μg/μL) was recently identified as a possible therapeutic agent against the progression of acute Aβ toxicity-induced memory impairment through its effects in the NOR in adult male rats (Almasi et al. 2018).

Most recently, the $GABA_BR$ PAMs GS39783 and CGP7930 and mGluR5 antagonist CDPPB were assessed in the NOR in an animal model of schizophrenia (i.e., the MK-801 model) in Wistar rats (Wierońska et al. 2015). MK-801 (i.e., dizocilpine) is a non-competitive antagonist with a high affinity for the N-Methyl-D-aspartate (NMDA) receptor, and a potent anticonvulsant. It binds on NMDA receptors in various brain sites, particularly in the hippocampus. It is a neuroprotective agent in animal models of stroke, trauma and Parkinsonism. However, it can also induce psychotic behaviour and neuronal degeneration. Directly relevant to this content, because of the above-mentioned effects, MK-801 is used as a cognitive impairment/dysfunction associated with schizophrenia (CIAS) animal model (Brown et al. 2014). Both CGP7930 and GS39783, administered IP 30 min before MK-801 administration, dose-dependently reversed MK-801-induced deficits. The effect of CGP7930 was observed at the dose of 1 mg/kg, while lower doses (0.1 and 0.5 mg/kg) and higher doses (2 and 5 mg/kg) were not effective. Further, in the co-administration experiment, CDPPB (1 mg/kg), and GS39783 (0.1 mg/kg) were administered at sub-effective doses, 30 min before acute MK-801 administration. The co-administration of these compounds induced clear antipsychotic-like effect in the NOR task. Interactions of the GABAergic system with mGluRs in the same animal model of cognitive symptoms in schizophrenia (i.e., the MK-801-induced deficits model) were further assessed by the same research group, this time using the mGluR4-selective orthosteric agonist, LSP4–2022 (Woźniak et al. 2016) with no interaction effect in the NOR. Similar promising results have been identified in the interactions between the muscarinic and GABAergic systems in the NOR as they

relate to cognitive impairments in animal models of schizophrenia (Cieślik et al. 2019).

Additionally, in an effort to investigate whether the GABAergic system is involved in the beneficial effects of betaine in cognitive processes through the amelioration of water-immersion restraint stress (WIRS)-induced memory impairments, adult male mice were co-administered betaine and $GABA_BR$ agonists or antagonists after WIRS and were assessed for memory functions using the NOR 3–6 days after WIRS (Kunisawa et al. 2017). The co-administration of the $GABA_A$ receptor antagonist bicuculline (1 mg/kg) or the $GABA_BR$ antagonist phaclofen (10 mg/kg) 1 h after WIRS suppressed the memory-improving effects induced by betaine. Additionally, administration of the $GABA_A$ receptor agonist muscimol (1 mg/kg) or baclofen (10 mg/kg) 1 h after WIRS attenuated memory impairments. These interesting findings indicate that the beneficial effects of betaine may be partly mediated by the GABAergic system. Moreover, intra-hippocampal injection of Cortistatin-14 (CST-14), a neuropeptide related to somatostatin, impaired recognition memory consolidation in mice through activation of $GABA_BRs$, among other receptor systems, an effect that was shown through the reversal of CST-14 impairment by saclofen in the NOR (Jiang et al. 2017).

Specifically for the NOL, except for some of the data presented through other studies above, administration of baclofen or $GABA_BR$ antagonist phaclofen in NMRI mice had the same direction of effects, i.e., impairment in performance (Khanegheini et al. 2015), while CGP55845 in B6/C3H mice, similarly to the result in the NOR, had no effect (Kleschevnikov et al. 2012a).

3.6 Other Cognitive Tasks

Some other tests assessing the role of $GABA_BRs$ in cognitive processes in rodents have also been used, although the literature currently is not extensive. One of these tests is the Barnes maze, a popular test for assessing spatial learning and memory in rats and mice. In this test each animal is placed on top of the Barnes maze cyclic platform, a brightly lit environment consisting of a specific number of holes around its periphery. While on the open platform, rodents naturally seek a dark enclosed space, which is provided in the form of a dark box (goal box) under one of the round holes around the periphery of the platform. Except for examining the learning curve of the animal over consecutive trials, the searching strategy of the animal can also be important in this task. Although some animals randomly search for the correct hole, others may use a certain pattern behaviour and systematically check each hole. When animals move directly to the correct hole irrespective of their starting position, this indicates that spatial memory has formed. The amount of time required for the animal to locate the goal box using visuo-spatial cues surrounding the maze periphery is measured.

In one of the studies using the Barnes maze, Li and colleagues tested the effects of baclofen as well as the $GABA_BR$ PAM BHF177 in C57BL/6 J mice by

administering them either before the training or before the test. Baclofen and BHF177 had no selective effects on spatial learning and memory in the Barnes maze, except at doses that were sedative or pro-convulsant (Li et al. 2013). In addition, in a most recent study (Sahraei et al. 2019), the role of systemic (1, 5 and 10 mg/kg, IP) and intra-nucleus accumbens (NAcc) (1, 5 and 10 μg/rat) injections of baclofen on spatial memory impairments in stress-exposed rats were assessed. Results showed that both the systemic and intra-NAcc administration of baclofen dose-dependently reduced escape latency and total distance and increased velocity in the treatment groups in the training trials. During the test, the rats that had received 5 mg/kg of baclofen had the highest target frequency, but there were no significant differences in velocity, duration or distance to the target between the groups. These findings suggested that baclofen can dose-dependently improve spatial memory, and $GABA_B$Rs in the NAcc play an important role in spatial memory.

Very few studies can also be identified using the conditional space colour task and the social recognition task. In the first case, primates learn to locate a food reward based on the colour and location of beakers. The complexity of the task can be gradually increased, with three conditions implemented and training in between. In the second case, animals are required to recognize a familiar animal by spending less time interacting with it, compared to a novel animal in the same environment, when both are presented at the same time. These tasks seem to require activation of the prefrontal cortex and hippocampus, and the amygdala and hippocampus, respectively. Studies by one research group have shown that either in Rhesus monkeys (0.5 mg/kg) or in Sprague-Dawley rats (0.003 to 300 mg/kg), systemic administration of CGP36742 improved performance in these tasks (Mondadori et al. 1993; Mondadori et al. 1996), indicating that inhibition of $GABA_B$R activation can improve learning and memory in complex tasks and recall in social memory.

4 $GABA_B$R Involvement in Cognitive Performance of Neurodevelopmental Conditions

4.1 $GABA_B$Rs During Embryo-Foetal Development

$GABA_B$Rs have been implicated in glial cell development in the peripheral nervous system (PNS), although the exact function of GABA signalling is not known. Recent studies have shown that GABA and $GABA_B$Rs are expressed in premyelinating and nonmyelinating Schwann cells throughout development and post injury, while GABA through $GABA_B$Rs does not seem to be involved in Schwann cell proliferation (Corell et al. 2015). Further, embryonic $GABA_B$R blockade alters cell migration, adult hypothalamic structure, and anxiety- and depression-like behaviours in mice in a sex-specific manner (Stratton et al. 2014), while GABA also regulates corticotropin releasing hormone levels in the paraventricular nucleus of the hypothalamus in newborn mice (Stratton et al. 2011). Another earlier study showed that

$GABA_BRs$ were present in the ventromedial nucleus of the hypothalamus at all ages examined, from embryonic day 13 to postnatal day 6 (Davis et al. 2002). A very interesting study in rats at about that time, using in situ hybridization and RNase protection assays (RPA) to investigate the early foetal expression of $GABA_BR1$ and $GABA_BR2$ mRNAs on the development of the rat CNS (Kim et al. 2003), showed that there was early and strong $GABA_BR1$ mRNA expression in the spinal cord, medullar and cerebral cortex neuroepithelium of discrete brain regions on gestational day (GD) 11.5. On GD 12.5, $GABA_BR1$ mRNAs were also found in the hippocampal formation, cerebral cortex, intermediate and posterior neuroepithelium, and the pontine neuroepithelium of the whole brain. Further, RPA results showed $GABA_BR1$ mRNA was intensely expressed on GD 11.5 and GD 12.5, when it was first detected in the ganglia, thalamus and cerebellum. Altogether, this data suggested that $GABA_BR1$ might have a role in the early foetal brain and spinal cord during pre- and post-synaptogenesis, neuronal maturation, proliferation and migration in the early development of the rat CNS. Data from the studies presented above indicate that $GABA_BRs$ play an important role in normal neurodevelopment and that, conversely, if $GABA_BR$ expression or function is for some reason (negatively) influenced during different phases of development, this can result in various symptoms and neurodevelopmental conditions, affecting not only development, but also cognition.

Changes in excitation and inhibition mechanisms are associated with neurodevelopmental disorders whose primary symptomatology includes cognitive and intellectual disabilities (Kramvis et al. 2020). Some of these conditions are ASD, FXS and DS, as well as Rett's and Tourette's syndrome, and neurofibromatosis (Deidda et al. 2014; Yamasue et al. 2019). Conversely, reduced GABAergic transmission, resulting from fewer GABA receptors may upset the excitatory/inhibitory balance which could result in both seizure-related disorders and intellectual impairments common to ASD (Fatemi et al. 2014). Delayed synaptic maturation, abnormal synaptic structure and/or function and alterations in intracellular signalling pathways have been linked to the pathogenesis of these conditions (Hampson et al. 2011). However, while DS and FXS are known to result from a specific genetic mutation, the causes of the majority of cases of ASD are unknown.

4.2 Autism Spectrum Disorder (ASD)

Growing evidence suggests a possible role for GABA in the neuropathophysiology of ASD (Blatt and Fatemi 2011; Enticott et al. 2013). Several lines of evidence mainly from animal studies, but also from clinical trials, point to an imbalance between neuronal excitation and inhibition in at least a subgroup of individuals with ASD or corresponding animal models (Gandal et al. 2012; Silverman et al. 2015; Frye et al. 2016; Sinclair et al. 2017; Veenstra-Vanderweele et al. 2017).

At the clinical level, continuously growing evidence suggests that the function of the GABAergic system is abnormally low in ASD (Fatemi et al. 2010). This

indicates that administration of $GABA_BR$ agonists or positive modulators may be useful for the treatment of ASD symptomatology. In an earlier study (Oblak et al. 2010), $GABA_BR$ density in individuals with ASD and controls was quantified in the ACC and posterior cingulate cortex, areas important for socio-emotional and cognitive processing, and the fusiform gyrus, an area important for identification of faces and facial expressions. There were significant reductions in $GABA_BR$ density in all three regions examined, suggesting that alterations in this key inhibitory receptor may contribute to the functional deficits in individuals with ASD.

In a 10-week randomized-controlled study aimed at evaluating the potential of baclofen to enhance the effect of risperidone in children with ASD with moderate-to-severe irritability symptoms (Mahdavinasab et al. 2019), researchers used the Aberrant Behaviour Checklist-Community Edition (ABC-C) for the outcome measures on each of the follow-up visits. Results showed significant improvement for all the ABC subscales used, including that of inappropriate speech, of relevance to this chapter. Importantly, combined administration of baclofen and risperidone exerted a greater effect on improvement of hyperactivity symptoms at both midpoint and endpoint when compared with treatment with placebo plus risperidone, suggesting the possibility of using baclofen as an additional treatment to risperidone for further improvement of variable symptomatology in ASD.

Exploratory clinical trials conducted with STX209 (also known as arbaclofen or R-(−)-baclofen; the R-(−)-enantiomer of baclofen) found improvement on several outcome measures, including the ABC-Irritability (the primary endpoint) and the Lethargy/Social Withdrawal subscales, the Social Responsiveness Scale, the CY-BOCS-PDD and clinical global impression scales (Erickson et al. 2014; Veenstra-Vanderweele et al. 2017). Results from these studies indicated that arbaclofen may have the potential to improve symptoms in some children with ASD.

At the preclinical level, further exploration of the interactions between GABA and glutamate networks in relation to ASD showed that a particular type I transmembrane protein with preferential expression in the mammalian CNS, which is identified as PIANP, is involved in the control of behavioural traits in mammals and interacts with $GABA_BR1$ (Winkler et al. 2019). In a specific strain of global PIANP KO mice, researchers identified decreased size and altered cellular compositions of the DG as well as the cerebellum and decreased the number of cerebellar Purkinje cells. At a functional level, loss of PIANP led to impaired presynaptic $GABA_BR$-mediated inhibition of glutamate release and altered gene expression in the cortex, hippocampus, amygdala and hypothalamus including downregulation of Erdr1, a gene linked to autism-like behaviour. At a behavioural level, PIANP deficiency led to context-dependent enhanced anxiety and spatial learning deficits, an altered stress response, severely impaired social interaction, and enhanced repetitive behaviour, all representative characteristics of ASD phenotypes (Winkler et al. 2019). This study suggested PIANP as a potential new candidate gene involved in ASD, cerebellar and hippocampal pathology, and $GABA_BR$-mediated signalling.

In accordance with these recent findings, earlier results of the role of $GABA_BRs$ in N-methyl-D-aspartate-receptor (NMDAR) hypofunction were examined. NMDAR signalling and, in particular, hypofunction is associated with intellectual

disability and disorders, such as ASD and schizophrenia, with phenotypes of social, cognitive and gamma (30–80 Hz) oscillatory abnormalities. Administration of the $GABA_BR$ agonist baclofen in NMDA-NR1(neo−/−) mice, a strain that shows reduced expression of the necessary NR1 subunit to model disrupted developmental NMDAR function, improved excitatory/inhibitory signalling balance, gamma-signal-to-noise ratio, which predicts deficits in working memory, and broadly reversed behavioural deficits (Gandal et al. 2012). These findings highlight a potential use of $GABA_BR$ agonists for the treatment of common phenotypes in different disorders showing NMDAR hypofunction.

Moreover, a very interesting recent study by Manz and colleagues (Manz et al. 2019) further elucidated the role of the interactions between the GABAergic and glutamatergic systems in reward circuits as they affect goal-directed behaviour, by identifying a new mechanism of feed-forward inhibition within the nucleus accumbens. This is highly relevant to not only autism, but any other disorder affected by reward circuit 'malfunctions', and motivational difficulties, such as major depressive disorder and addiction. In this study (Manz et al. 2019), $GABA_BR$ function at glutamatergic synapses within parvalbumin (PV)-expressing interneurons-embedded microcircuits in the NAcc core of male mice was explored. Results showed that PV-interneurons within feed-forward microcircuits target $GABA_B$ heteroreceptors on glutamate terminals. It is worth noting that activation of presynaptically-expressed $GABA_B$ heteroreceptors decreases glutamatergic synaptic strength by engaging a signalling pathway that interferes with the mechanism of vesicular exocytotic release.

4.3 Fragile X Syndrome (FXS)

Fragile X syndrome (FXS) is a common syndrome within ASD and a monogenetic cause of intellectual disability, co-existing with autism spectrum features, as well as psychiatric and medical problems. FXS is caused by the lack of the fragile X mental retardation protein (FMRP), a translational regulator of specific mRNAs at the postsynaptic level (Tassone and Hagerman 2003; Zafarullah and Tassone 2019) in different brain areas including the cerebellum (Maurin et al. 2015; Zhang et al. 2015). The lack of FMRP leads to atypical synaptic plasticity, potentially triggered by a homeostatic disturbance between excitatory and inhibitory network functioning at the level of the synapse (Zupan and Toth 2008; Pacey et al. 2009; Zeidler et al. 2018).

In the last decade or so, numerous studies have looked at the potential involvement of $GABA_BRs$ in FXS and its symptomatology (Adusei et al. 2010; Yamasue et al. 2019) through the development of a corresponding animal model of the Fragile X Mental Retardation 1 (*Fmr1*) knock out (*Fmr1*-KO) mouse (Kazdoba et al. 2014) and through clinical trials with the $GABA_BR$ agonists baclofen and arbaclofen (Hopkins 2011). *Fmr1*-KO mice share several phenotypes with FXS patients including cognitive deficits – of particular focus to this chapter – altered spine morphology,

hyperactivity, sensory hypersensitivity, repetitive behaviours and macroorchidism (Bakker and Oostra 2003). In regard to the *Fmr1*-KO mouse model, there are divergent opinions in regard to the usefulness of it as a translational preclinical disease model, either because of discrepancies within the *Fmr1*-KO mouse model itself or because of the outcome measures currently used or because of both factors combined making it an over-predictive model of clinical efficacy (Berry-Kravis et al. 2018).

When the expression and presence of $GABA_B$Rs and their subunits was recently examined (Kang et al. 2017), selective deficits in the $GABA_B$R1a subunit expression were identified in *Fmr1*-KO mice, but the levels of the respective mRNAs remained unaltered. Similar trends of $GABA_B$R1a expression were seen in the hippocampus of a subset of FXS patients. Further, corresponding with other studies indicating that $GABA_B$Rs have a strong pre- and postsynaptic inhibitory effect on neurotransmission, with $GABA_B$R1a subunit-containing receptors mediating presynaptic inhibition in particular, deficits in the ability of $GABA_B$Rs to suppress glutamate release were identified in *Fmr1*-KO mice, while their ability to suppress GABA release and induce postsynaptic hyperpolarization was unaltered. In the same study, administration of arbaclofen reversed the imbalance between excitatory and inhibitory neurotransmission in *Fmr1*-KO mice. Altogether, results from this study showed that selective deficits in the activity of presynaptic $GABA_B$Rs contribute to the pathophysiology of FXS.

As seen in the study presented above, in general, administration of $GABA_B$R agonists, such as baclofen or arbaclofen (Henderson et al. 2012), and PAMs, such as CGP7930 (Zhang et al. 2015) or GS39783 (Pacey et al. 2011), corrects exacerbated protein synthesis and multiple phenotypes in *Fmr1*-KO mice (Henderson et al. 2012; Silverman et al. 2015), although in some cases tolerance also develops (Pacey et al. 2011), a side effect commonly seen with $GABA_B$R agonists, but less commonly occurring with $GABA_B$R PAMs for many of their actions (Vlachou et al. 2011). These are the two most commonly used $GABA_B$ compounds used in the FXS preclinical studies. Similarly, acamprosate, which activates both $GABA_A$ and $GABA_B$Rs, also ameliorates several phenotypes in *Fmr1*-KO mice (Schaefer et al. 2017). The focus of this section though is on the effects of these compounds on cognitive processes in these animal models or in the clinical trials.

In one of the recent studies, the *Fmr1*-KO mice showed increased auditory-evoked high-frequency gamma (30–80 Hz) power, indicating sensory hypersensitivity, compared to the C57BL/6 control mice, as measured by electroencephalography, with no sex differences identified. The same study also showed, among other behaviours, decreased T-maze spontaneous alternation, indicating diminished working memory. The $GABA_B$R agonist racemic baclofen (i.e., the racemic mixture of baclofen, containing the S-(+)-enantiomer and the R-(−)-enantiomer of baclofen) normalized auditory-evoked neural oscillations and behavioural deficits in the *Fmr1*-KO mice (Sinclair et al. 2017). In the most recent study by Kramvis and colleagues (Kramvis et al. 2020), GABAergic signalling was investigated in the mPFC of *Fmr1*-KO mice during prepubescence and adolescence. Molecular and functional changes were detected, with the second being most

prominent during early postnatal development and resulting in stronger inhibition, through increased synaptic inhibitory drive and amplitude, and reduction of inhibitory short-term synaptic depression. Further, there was an increased number of receptors opening during peak current in *Fmr1*-KO inhibitory synapses during prepubescence. Changes in amplitudes and plasticity returned to normal during adolescence, although the inhibitory drive was reduced in *Fmr1*-KO, while synaptic kinetics were prolonged. Importantly, $GABA_{B1}R$ expression levels were different in *Fmr1*-KOs compared to their WT littermate controls. These findings further indicated the involvement of synaptic GABAergic changes in the mPFC, an area strongly implicated in cognitive processing, in FXS pathology (Kramvis et al. 2020). Further confirmation of an imbalance between the excitatory and inhibitory synapses in *Fmr1*-KO mice, this time in the cortico-hippocampal feed-forward circuits formed by the temporoammonic (TA) pathway also comes from another study (Wahlstrom-Helgren and Klyachko 2015). The TA is a direct, monosynaptic pathway leading from layer III of the entorhinal cortex to the distal dendritic region of area CA1 of the hippocampus. This projection has been implicated in a number of functions including memory processing (i.e., encoding and retrieval) and spatial navigation, generation of oscillatory activity, and control of hippocampal excitability (Dvorak-Carbone 1999). The above introduced study showed that inhibitory, but not excitatory, synapse dysfunction underlies cortico-hippocampal feed-forward circuit abnormalities in these mice, an effect primarily mediated by presynaptic $GABA_B$ receptor signalling in the TA pathway. The same study also found that GABA release is reduced in TA-associated inhibitory synapses of *Fmr1*-KO mice and this effect is regulated by $GABA_BRs$ (Wahlstrom-Helgren and Klyachko 2015).

Some studies have suggested an overlap in pathophysiology of 16p11.2 microdeletion syndrome and FXS. Human chromosome 16p11.2 microdeletion is among the most common gene copy number variations (CNVs) known to confer risk for intellectual disability and ASD and affects an estimated 3 in 10,000 people. This syndrome is characterized by intellectual disability, impaired language, communication and socialization skills, and ASD. Taking into account improvement in FXS phenotypes observed following chronic treatment with arbaclofen, a recent study aimed to examine the effects of chronic oral arbaclofen administration in two generated mouse models of 16p11.2 microdeletion syndrome. Results showed that chronic activation of $GABA_BRs$ improved performance on a series of cognitive and social tasks known to be impaired in the two different 16p11.2 deletion mouse models, indicating that arbaclofen may be useful in treating some of the core symptoms of human 16p11.2 microdeletion syndrome (Stoppel et al. 2018).

4.4 Down's Syndrome (DS)

DS is a neurodevelopmental disorder caused by the triplication of Chromosome 21 (trisomy 21) and characterized by numerous neurodevelopmental alterations and intellectual disability (Ohira et al. 1997; Hattori et al. 2000). Individuals with DS, as

well as DS animal models, such as the Ts65Dn (Ts) mouse model, exhibit impairments in several cognitive processes, learning and memory domains, including hippocampus-dependent declarative (in humans) or spatial (in rodents) memory and visual recognition memory, the latter of which is largely controlled by the perirhinal cortex (Roncacé et al. 2017). Numerous preclinical research strategies have been examined in the last decade or so, with an aim to identify innovative therapeutic approaches to DS symptomatology, one of which focuses on the modulation of $GABA_BRs$ and the mechanisms or the pathways they influence.

The Ts65Dn (Ts) mouse model of DS is sensitive to an infantile spasms phenotype (Cortez et al. 2009; Blichowski et al. 2015). Further, it contains the core genomic triplication of the DS critical region, which includes 3 copies of the Kcnj6 gene that encodes the $GABA_BR$-coupled GIRK subunit 2 (GIRK2) channel (Best et al. 2007; Cramer et al. 2010; Kleschevnikov et al. 2012a, b; Joshi et al. 2016). GIRK channels hyperpolarize neurons to inhibit synaptic transmission throughout the nervous system (Harashima et al. 2006a, b; Zhou et al. 2012). The $GABA_BR$-coupled GIRK2 channel is necessary for the $GABA_BR$ agonist-induced infantile spasms phenotype in hippocampal neurons and those of the DG, frontal cortex and substantia nigra (Harashima et al. 2006a, b; Fernandez et al. 2007) of the Ts mouse and may represent a novel therapeutic target for the treatment of infantile spasms in DS (Blichowski et al. 2015). Importantly, LTP, a cellular model for learning and memory, is impaired in the CA1 hippocampal area and the DG of Ts and other DS animal models (Siarey et al. 1997; Kleschevnicov et al. 2004; Costa and Grybko 2005; Belichenko et al. 2007; Yu et al. 2010; Kleschevnicov et al. 2017).

Contrary to the $GABA_BR$ agonist-induced spasms in DS and other syndromes, treatment with $GABA_BR$ antagonists seems to improve cognitive performance, especially hippocampus-based cognitive functions, with findings that suggest further exploration of the $GABA_BR$ antagonists' therapeutic potential in DS. In one of these studies using $GABA_BR$ antagonists, CGP55845 restored memory of Ts mice in the novel place recognition, NOR, and contextual fear conditioning tasks, although it did not affect locomotion and performance in the T-maze. Further, CGP55845 increased hippocampal levels of brain-derived neurotrophic factor (BDNF) in Ts mice, while treatment of hippocampal slices with the $GABA_BR$ antagonists CGP55845 or CGP52432 enhanced LTP in the DG of Ts mice (Kleschevnikov et al. 2012a).

Altogether, this data indicate that either $GABA_BR$ agonists or antagonists seem to enhance cognitive processes in neurodevelopmental conditions and may be used therapeutically to treat cognitive deficits, as long as the right $GABA_BR$ location or pathway can be targeted.

5 Neurodegenerative Disorders

A detailed account of the role of $GABA_BRs$ in neurodegeneration, with a special focus on epilepsy and AD, is presented in Princivalle (2021). In this section, a brief summary of relevant research in neurodegenerative disorders as related to cognitive processes will be presented.

5.1 Alzheimer's Disease (AD)

AD pathophysiology is largely known to be based on amyloid-β plagues and neurofibrillary tangles accumulation in the brain tissue, mainly affecting regions of the hippocampus and cortex, but also expanding to other areas of the brain as the disease progresses. The degeneration of these brain areas is responsible for the symptomatology of AD patients which features memory loss (especially short-term memory and expanding to long-term memory) as its main or primary symptom, and includes lack of inhibition and other cognitive functions controlled by the frontal cortex, but also numerous gradual changes in behaviour.

As also discussed extensively in regard to most of the data on the effects of $GABA_BRs$ in cognitive and other processes, it appears that an imbalance between excitatory and inhibitory synapses (Gigout et al. 2015) may be responsible for the changes identified in $GABA_BR$ numbers and/or function or their linked proteins in AD patients or animal models, and these changes may be triggered by amyloid-β in the hippocampus in the case of AD (Nava-Mesa et al. 2013; Mayordomo-Cava et al. 2015; Pilipenko et al. 2018, 2019). Thus, maintaining balance among the GABAergic and other neurotransmitter systems can be considered a beneficial strategy to slow down AD progression (Pilipenko et al. 2019). It is also important to note that these GABAergic and other neurotransmitter changes at cellular and network levels correlate with density of neurofibrillary tangles in the brain tissue.

As indicated previously, most of the recent data point out the role of presynaptic $GABA_BR1$ in AD pathophysiology at cellular level. A most recent review of the role of presynaptic function in AD gives a detailed account of these processes (Barthet and Mulle 2020), including the ones that focus on the presynaptic $GABA_BR1$.

An etiological factor for AD is chronic cerebral hypoperfusion (CCH), a condition that constitutes one of the causes of vascular dementia (VaD). CCH causes cognitive impairment and contributes to Alzheimer's pathology. In an effort to further understand this condition, a mouse model of CCH has been developed by unilateral common carotid artery occlusion (UCCAO). This animal model shows significant short-term memory deficits and mild long-term spatial memory impairment, and selective neurodegeneration in the brain (Zhao et al. 2014), among other effects on protein-related processes. Importantly, $GABA_BR$ activation ameliorates cognitive impairment via restoring the balance of hyperpolarization-activated cyclic-nucleotide-gated cation nonselective (HCN) channels type 1 and 2 (HCN1 and

HCN2, respectively) surface expression in the hippocampal CA1 area in rats with CCH. HCN channels and $GABA_BRs$ mutually co-regulate the function of neurons in many brain areas including the hippocampus [for details on the molecular mechanism of this interaction, please see (Li et al. 2014)].

Extensive preclinical studies have used either $GABA_BR$ agonists or antagonists to test for potential cognitive enhancing effects in AD animal models (Kumar et al. 2017; Almasi et al. 2018; Sahoo et al. 2018). In one of these studies, a non-transgenic rat model of AD obtained by intracerebroventricular streptozotocin (ICV STZ) injection was used (Pilipenko et al. 2018). Single or double ICV STZ injection (s) chronically decrease cerebral glucose uptake and produce multiple effects that resemble molecular, pathological and behavioural features of Alzheimer's disease (AD) (Grieb 2016). The effects of muscimol and baclofen at very low doses (0.01–0.05 mg/kg) on spatial memory were assessed and the expression of cortical and hippocampal proteins related to neuroinflammation. Baclofen enhanced memory and anti-inflammatory effects, strongly indicating an important role of $GABA_BRs$ in AD (Pilipenko et al. 2018). Interestingly, findings from this study indicated that low doses of $GABA_BR$ agonists could be effective because they involve other allosteric sites or cell signalling and regulatory pathways in these processes (Pilipenko et al. 2018). Moreover, the effects of $GABA_BR$ activation on spatial memory and learning ability in the AD rats were measured by the MWM, and results showed that baclofen restored spatial memory and learning ability of AD rats and suppressed the neuronal apoptosis and hippocampal atrophy by activating the PI3K/Akt signalling pathway, once again indicating a therapeutic role for $GABA_BR$ manipulations in AD (Sun et al. 2020).

Importantly, one of the most successful progressions of $GABA_BR$-active compounds into clinical trials and potentially an approval for the treatment of cognitive dysfunction (i.e., memory and attentional performance) in AD was that of the $GABA_BR$ antagonist CGP36742 (SGS742). CGP36742 showed promising results in animal models [e.g., (Brouillette et al. 2007)] and progressed to the first Phase II clinical trial where it showed improvement in attentional performance (i.e., choice reaction time and visual information processing) in mild AD patients. Was, Unfortunately, a second Phase II clinical trial showed no statistically significant improvement, and thus, CGP36742 did not reach Phase III trials (Steulet et al. 1996; Froestl et al. 2004; Davies et al. 2005; Serrats et al. 2017).

However, efforts in this direction continue at preclinical level. In a most recent study, the role of the $GABA_BR$ antagonist CGP35348 was examined on the DG $GABA_BR$ inhibition and its effects on learning and memory impairments that had been induced in adult male Wistar rats by microinjection of β-amyloid (Aβ) (Almasi et al. 2018). Consistent with data from earlier studies on $GABA_BR$ antagonists for AD, results from this study indicated that microinjections of CGP35348 directly into the hippocampus counteract the learning, memory and cognitive impairments induced by Aβ and suggested that CGP35348 could be a possible therapeutic compound against the progression of acute Aβ toxicity-induced memory impairment (Almasi et al. 2018).

Altogether, studies focusing on further understanding the effects of $GABA_BR$ compounds on cognitive impairments characterizing AD and other relevant conditions have been promising and one can only hope that, although the exact targets may vary, the efforts to develop effective $GABA_BR$ drug treatments will continue in this direction.

5.2 Epilepsy

Epilepsy is a disorder occurring in various forms, some of which would be more common, such as the temporal lobe (TLE) or absence epilepsy (AE), and some others would be rare types, such as the Dravet syndrome. The main characteristic symptom of epilepsy is seizures. These seem to stem from an imbalance between excitatory and inhibitory processes, or either of these types of synapses, inhibitory or excitatory, being faulty. Importantly, in some forms of epilepsy, such as the TLE, cognitive disturbances including amnesia commonly occur (Zapata et al. 2017). Moreover, chronic atypical absence seizures characterizing AE are a component of the Lennox-Gastaut syndrome, a disorder invariably associated with severe cognitive impairment in children.

In clinical populations, case reports of specific types of epilepsy symptomatology are very common. In one of these recent case reports (Zeman et al. 2016), researchers focused on a patient in whom long-term, therapeutic administration of baclofen into the cerebrospinal fluid (CSF) induced three distinct varieties of memory impairment: (1) repeated, short periods of severe global amnesia, (2) accelerated long-term forgetting, evident over intervals of days and (3) a loss of established autobiographical memories. The latter of these impairments persisted after discontinuation of baclofen administration. These memory impairments are reported in TLE, specifically in the subtype of transient epileptic amnesia. This case report suggested a role for $GABA_B$ signalling in the modulation of human memory functioning for different duration and implicates it in 'epileptic amnesia' (Zeman et al. 2016).

A number of animal models have been developed in the past few years to resemble the symptomatology of epilepsy. In most recent years, animal models of epilepsy are also developed to resemble rare types of epilepsy or specific symptomatology of them (Löscher 2011; Grone and Baraban 2015; Pitkänen et al. 2017). In these animal models, typically, $GABA_BR$ agonists exacerbate, while $GABA_BR$ antagonists suppress the seizures (Han et al. 2013), suggesting a potential therapeutic role of the antagonists for epilepsy and its cognitive dysfunctions (Enna 1997). Importantly, and as it relates to AE, it appears that hippocampal circuitry in atypical absence seizures is possibly responsible for the cognitive impairment in that disorder (Han et al. 2013). $GABA_BR$ antagonists can reverse both the seizures and the impairment in cognition in experimental atypical absence seizures (Getova et al. 1997), suggesting a therapeutic use of these antagonists in the Lennox-Gastaut syndrome, another type of rare epilepsy. The AY9944 model of chronic atypical absence seizures in rats reliably reproduces the electrographic, behavioural,

pharmacological and cognitive features of AE. Using this model, Chan and colleagues tested the hypothesis that the cognitive impairment associated with this disorder involves a $GABA_BR$-mediated mechanism (Chan et al. 2006). Results showed that CGP35348 blocked atypical absence seizures, restored LTP to normal level and reversed the cognitive deficit in the AY9944-treated animals, suggesting a possible therapeutic role of CGP35348 or other antagonists in this epilepsy type. Importantly, the same research group later showed that overexpression of $GABA_BR1a$ in mice [R1a(+)] resulted in an atypical absence seizure phenotype characterized by 3- to 6-Hz slow spike-and-wave discharges (SSWDs), reduced synaptic plasticity and cognitive impairment, while the overexpressed $GABA_BR1b$ mice (R1b(+)) showed a less pronounced reduction in hippocampal LTP (Stewart et al. 2009; Wang et al. 2009), thus indicating that, although abnormal levels of both $GABA_BR1$ subunits are involved in AE, the abnormal function of $GABA_BR1b$ appears to have less of an involvement in AE symptomatology and cognitive impairments. Acute or chronic administration of vigabatrin, which increases GABA concentration by inhibiting GABA transaminase, increased activation of $GABA_BR$ in the frontal cortex and the reticular thalamic nucleus (Perescis et al. 2020), areas extensively implicated in cognitive functioning directly or indirectly.

5.3 *Autoimmune Anti-$GABA_B$ Encephalitis and Cognitive Impairments*

Autoimmune synaptic encephalitis, although having been described in patients diagnosed with epilepsy (Dubey et al. 2014), can occur as paraneoplastic neurological syndromes, which are dysfunctions of the nervous system occurring in some cancer patients, especially in patients diagnosed with tumours and small cell lung cancer (Golombeck et al. 2016; Qiao et al. 2017; Li et al. 2018; Maureille et al. 2019). One such newly described, rare, but treatable form, is the autoimmune anti-$GABA_B$ limbic encephalitis, which is associated with CSF elevated levels of $GABA_BR$ antibody titre. The main characteristics of this type of encephalitis are cognitive decline, memory impairments, progressive seizures and behavioural disorder for a number of days or weeks (Lancaster et al. 2010; Su et al. 2015; Hui et al. 2016). In some of these patients, abnormalities in the hippocampal region, parahippocampal gyrus, temporal and occipital lobe have been identified using magnetic resonance imaging (MRI) (Cui et al. 2018). As this is a newly identified condition, literature is limited and is mainly based on case reports (Chung et al. 2019; Yao et al. 2019; Qin et al. 2020), some of which suggest that it is most common in middle-aged and elderly men (Zhu et al. 2020).

6 Conclusions

It is clear from all presented research studies on the involvement of $GABA_BRs$ in cognitive processes that GABA as a neurotransmitter and specifically $GABA_BRs$ are involved in different components of cognition. Taking into account the spread of $GABA_BRs$ in the brain as well as their primary role of inhibition of processes, this does not come as a surprise. However, although extensive research has focused on the role of $GABA_BRs$ in health and disease (Bowery 2006), in most cases there still appears to be lack of clarity on the exact mechanisms involved, taking into account the balance between presynaptic and postsynaptic $GABA_BR$ activation and/or inhibition throughout the brain. Yet again, the vast majority of these studies are conducted in male animals, not allowing for identification of potential sex differences in the effects of the drugs tested.

Thus, with the exception of baclofen, which has been approved as a muscle relaxant and anticonvulsant treatment, and not as a cognitive enhancer, no other $GABA_BR$ compound is used clinically. For example, there are no $GABA_BR$ antagonists in the clinic for any population. With all cognitive effect discrepancies taken into account, efforts should continue to help us elucidate the exact role of $GABA_BR$ subtypes in the different conditions affecting cognition, for both males and females, and more compounds, possibly selectively targeting those subtypes, should be developed with an aim to reach the bedside. It is a long effort ahead, but, existing literature suggests, it is worth aiming at.

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