The Receptors

Giancarlo Colombo *Editor*

$GABA_B$ Receptor

Humana Press

The Receptors

Volume 29

Series Editor

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Foreword

Together with all the authors of the different chapters, I would like to introduce this book, entirely devoted to the $GABA_B$ receptor and part of the prestigious series named "The Receptors."

The $GABA_B$ receptor is ubiquitously found in the central and peripheral nervous system, where it contributes to the modulation of neuronal activity. Because of its broad distribution and considerable involvement in neurotransmission, the $GABA_B$ receptor has been implicated in a range of diseases, and several of its ligands constitute well-established or promising pharmacotherapies for spasticity, neuropathic pain, gastroesophageal reflux, epilepsy, autism, anxiety disorder, and substance and alcohol use disorder.

This volume is intended to provide a series of comprehensive reviews on different themes related to the $GABA_B$ receptor, ranging from the medicinal chemistry of its ligands (agonists, antagonists, and the more recently synthesized positive allosteric modulators) (Chaps. 2 and 3) to receptor structure, functioning, distribution, and mode of action (Chaps. 4–8), and the multiple pharmacological effects produced by its activation or blockade (Chaps. 9–19). The pharmacology section of this volume has been organized in such a way that each chapter between 10 and 16 focuses on a specific disorder or disease that may benefit from therapeutic targeting of the $GABA_B$ receptor; conversely, Chaps. 17–19 focus on specific classes of ligands, providing a complementary view of the $GABA_B$ receptor pharmacology. All chapters in the pharmacology section have been developed according to a "lab bench to bedside" translational approach, bridging preclinical animal and clinical data. The book begins with an historical overview of this research field (Chap. 1), written by Norman G. Bowery, a "giant" to whom we owe the discovery of the $GABA_B$ receptor.

The list of colleagues who have generously accepted to contribute to this volume includes the most influential scientists in the field, from both academia and industry. I am indebted to all of them for their enthusiastic and valuable participation in this book.

My colleagues and I wish to dedicate this book to Wolfgang Froestl, a "founding father" of the $GABA_B$ receptor research field and a wonderful friend and colleague for most of us. Wolfgang designed and synthesized several of the $GABA_B$ receptor ligands mentioned in this book, and on which research in the field has largely been based; we are convinced that many of us—chemists, physiologists, and pharmacologists—would have done very little without his fundamental contribution. Wolfgang was the first scientist whom I asked to contribute to this book: he replied immediately (as was his style), accepting enthusiastically (as was his style) to write two chapters. A few months later, we suddenly received the sad news that he had passed away, overcome by a severe disease. This book is our tribute to his masterly contribution and competence.

Wolfgang Froestl (1946–2015)

Giancarlo Colombo

Contents

Part III Pharmacology

Abbreviations

1,4-BD	1,4-Butanediol
5-HT	Serotonin
7TM	Heptahelical transmembrane domain
$(DHQD)_2AQN$	Hydroquinidine (anthraquinone-1,4-diyl) diether
(PDF)-positive neurons	Peptide pigment-dispersing factor-positive neurons
AA rat	Alko Alcohol rat
AAS	Atypical absence seizures
ABC-C_{FX}	Aberrant behavior checklist-community fragile X
ABC-I	Aberrant behavior checklist-irritability
ACTH	Adrenocorticotropic hormone
ADX 71943	*N*-(5-(4-(4-cyano-3-methoxybenzyl)-6-methoxy-3,5-dioxo-4,5-dihydro-1,2,4-triazin-2(3H)-yl)-2-fluorophenyl)acetamide
AKAP	A kinase anchoring protein
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPAR	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
AMPK	5′ Adenosine monophosphate-activated protein kinase
AN	Amygdaloid nuclei
AP2	Adaptor protein 2
APPA	3-Aminopropylphosphinic acid
ARH	Arcuate nucleus of the hypothalamus
ASD	Autism spectrum disorder
ASM	Airway smooth muscle
AUD	Alcohol use disorder
AWS	Alcohol withdrawal syndrome
AY-9944	*trans-N,N'-bis*[2-Chlorophenylmethyl]-1,4-cyclohexanedimethanamine dihydrochloride methanamine;dihydrochloride
BBB	Blood–brain barrier

BDNF	Brain-derived neurotrophic factor
BHF177	N-[(1R,2R,4S)-bicyclo[2.2.1]hept-2-yl]-2-methyl-5-[4-(trifluoromethyl)phenyl]-4-pyrimidinamine
BHFF	5,7-bis(1,1-dimethylethyl)-3-hydroxy-3(trifluoromethyl)-2(3H)-benzofuranone
BHFI	5,7-di-tert-butyl-3-hydroxy-3-trifluoromethyl-1,3-dihydro-indol-2-one
C	Caudate nucleus
CA1	CA1 field of Ammon's horn
CA3	CA3 field of Ammon's horn
CaMKII	Calcium/calmodulin-dependent kinase II
CaMKIIa	Calcium/calmodulin-dependent kinase II α
cAMP (cyclic AMP)	Cyclic 3′,5′-adenosine-monophosphate
CaN	Calmodulin-activated protein phosphatase calcineurin
CaV	Voltage-gated Ca^{2+}
Cb	Cerebellum
CCI	Chronic constriction injury
CCP	Complement control protein
CD-COBS rat	Charles River Sprague–Dawley-derived rat
CDR	Cysteine-rich domain
CFA	Freund's complete adjuvant
CGI-S	Clinical global impression—severity
CGP35348	(3-Aminopropyl)(diethoxymethyl)phosphinic acid
CGP52432	3-[[[((3,4 Dichlorophenyl)methyl]amino]propyl]diethoxy-methyl)phosphinic acid
CGP55845A	(2S)-3-[[(1S)-1-(3,4-dichlorophenyl)ethyl] amino-2-hydroxypropyl](phenylmethyl)phosphinic acid hydrochloride
CGP56999A	[3-[1-(R)-[[3-cyclohexylmethyl)hydroxyphosphinyl]-2-(S)-hydroxypropyl]amino]ethyl]-benzoic acid, mono-lithium salt
CGP62349	3-[(1R)-1-[[(2S)-2-hydroxy-3-[hydroxy-[(4-methoxyphenyl) methyl]phosphoryl]propyl]-methylamino] ethyl]benzoic acid
CGP63360	Cyclohexylmethyl-2-(S)-hydroxy-3-[(6-oxo-1,6-dihydro-pyridin-3-ylmethyl)-amino]propylphosphinic acid, hydrochloride
CGP7930	2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethylpropyl)phenol
CGRP	Calcitonin gene related peptide
CHO	Chinese hamster ovary
CHOP	CCAAT/enhancer-binding protein (C/EBP) homologous protein
CIWA-Ar	Clinical Institute Withdrawal Assessment for Alcohol-revised scale

CMPPE	2-{1-[2-(4-Chlorophenyl)-5-methylpyrazolo[1,5-a]pyrimidin-7-yl]-2-piperidinyl}ethanol
CNS	Central nervous system
COPI	Coat protein complex I
COR627	Methyl 2-(1-adamantanecarboxamido)-4-ethyl-5-methylthiophene-3-carboxylate
COR628	Methyl 2-(cyclohexanecarboxamido)-4-ethyl-5-methylthiophene-3-carboxylate
CPP	Conditioned place preference
CRD	Cysteine-rich domain
CREB	cAMP-responsive element-binding protein
CREB2/ATF4	Binding protein and activating transcription factor 4
CSF	Cerebrospinal fluid
CTEP	2-Chloro-4-[2-[2,5-dimethyl-1-[4-(trifluoromethoxy)phenyl]-1H-imidazol-4-yl]ethynyl]pyridine
C-terminal	Carboxy-terminal
Cx	Cortex
CYP	Cytochrome p450
D1	Dopamine type 1
D2	Dopamine type 2
DA	Dopamine
DARPP-32	Dopamine- and cAMP-regulated phosphoprotein of 32 kDa
DBA/2J	Inbred mouse strain susceptible to audiogenic seizures
DCE	Dichloroethane
DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
%DR	Percentage of drug-appropriate responding
E	Embryonic
EC	Entorhinal cortex
ED	Embryonic day
EDR	Electrodecremental response
EEG	Electroencephalogram
EPM	Elevated plus maze
ER	Endoplasmic reticulum
ERAD	ER-associated protein degradation
ERK	Extracellular signal-regulated kinase
ESCRT	Endosomal sorting complex required for transport
FMR	Fragile X mental retardation
FMR1	Fragile X mental retardation 1 gene
FMRP	Fragile X mental retardation protein
FPS	Fear-potentiated startle

FR	Fixed ratio
FRET	Förster resonance energy transfer (or fluorescence resonance energy transfer)
FST	Forced swim test
FXS	Fragile X syndrome
FXTAS	Fragile X tremor associated ataxias
GABA	γ-Aminobutyric acid
GABA-AT	GABA aminotransferase
$GABA_A$ (or $GABA_AR$)	GABA type A receptor
$GABA_B$ (or $GABA_BR$)	GABA type B receptor
$GABA_BR$ (R1a, R1b)	$GABA_B$ (subunits R1a and R1b)
GAD	Generalized anxiety disorder
GAD	Glutamic acid decarboxylase
GAERS	Genetic absence epilepsy rat of Strasbourg
GAT-1	GABA transporter 1
GBL	γ-Butyrolactone
GER	Gastroesophageal reflux
GERD	Gastroesophageal reflux disease
GHB	γ-Hydroxybutyric acid (or γ-hydroxybutyrate)
GHBL	γ-Hydroxybutyrolactone
GIRK	G-protein-activated inwardly rectifying K^+ channel
GIRK2	G-protein-coupled inwardly rectifying K^+ channel 2
GISP	GPCR interacting scaffold protein
GL	Granular layer
GPCR	G-protein-coupled receptor
GRP78	Glucose-regulated protein 78
GS39783	*N*,*N*-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine
GTC	Generalized tonic-clonic seizure
$GTP\gamma^{35}S$	Guanosine 5-*O*-(3-[^{35}S]thio)triphosphate
HCN channel	Hyperpolarization-activated cyclic nucleotide-gated channel
HEK	Human embryonic kidney
HEK293 cell	Human embryonic kidney 293 cell
Hi	Hippocampus
HIE	Hypoxic ischemic encephalopathy
HLA-F	HLA class I histocompatibility antigen, α chain F
Hrd1 (SYVN1)	E3 ubiquitin-protein ligase synoviolin
Hth	Hypothalamus
IBS	Irritable bowel syndrome
ICSS	Intracranial self-stimulation
ID	Intellectual disability
IGLE	Intraganglionic laminar ending
iGluR	Ionotropic glutamate receptor

i.m.	Intramuscular
INN	International nonproprietary name
i.p.	Intraperitoneal
IPSC	Inhibitory postsynaptic current
IPSP	Inhibitory postsynaptic potential
IR	Immunoreactivity
K2P	Two-pore domain potassium
KA	Kainic acid
KCTD	K^+ Channel tetramerization domain
LAMP1	Lysosomal-associated membrane protein 1
LES	Light-enhanced startle
LES	Lower esophageal sphincter
LIVBP	Leucine/isoleucine/valine binding protein
LNv	Lateral ventral neuron
LSW	Lethargy/social withdrawal
LTD	Long-term depression
LTP	Long-term potentiation
LVA Ca^{+2}	Low voltage-activated T-type calcium channel/current
LVIb	Layer VIb
Marlin-1	RNA-binding protein
MDMA	3,4-Methylenedioxymethamphetamine
MG	Medial geniculate nucleus
mGluR	Metabotropic glutamate receptor
mGluR1/5	Group I, metabotropic glutamate receptor
MM	Mammillary bodies of the hypothalamus
mRNA	Messenger ribonucleic acid
MW	Microwaves
NAc	Nucleus accumbens
nACh	Nicotinic acetylcholine (receptor)
NEC	Nonepileptic control rat
NMDA	*N*-methyl-D-aspartate
NMDAR	*N*-methyl-D-aspartate receptor
NMR	Nuclear magnetic resonance
NO	Nitric oxide
nRe	Nucleus reuniens of the thalamus
nRT	Nucleus reticularis of the thalamus
N-terminal	Amino-terminal
NVP-BHF177	See BHF177
OSN	Olfactory sensory neuron
P	Purkinje cells
PAM	Positive allosteric modulator
PDF	Pigment-dispersing factor
PFC	Prefrontal cortex
PIFA	[bis(trifluoroacetoxy)iodo]benzene
PKA	Protein kinase A

PKC	Protein kinase C
PLP	Pyridoxal 5′-phosphate
PND	Postnatal day
PNS	Peripheral nervous system
p.o.	Per os
PO	Preoptic area
POF	Premature ovarian failure
PP	Protein phosphatase
PP2A	Protein phosphatase 2
PPI	Proton pump inhibitor
PR	Progressive ratio
P rat	Indiana alcohol-preferring rat
PRAF2	Prenylated Rab acceptor family 2
PSA	Polar surface area
PS-DIPEA	Polymer supported *N*,*N*-diisopropylethylamine
PTZ	Pentylenetetrazol
q.i.t.	Four times a day
Rab4, 5, 7, 11	Ras-related protein 4, 5, 7, 11
rac-BHFF	5,7-bis(1,1-dimethylethyl)-3-hydroxy-3-(trifluoromethyl)-2(3H)-benzofuranone
Rap1	Ras-related protein 1
Rap1GAP2	Rap1 GTPase-activating protein 1
RAR	Rapidly adapting receptor
RGS	Regulator of G-protein signaling
RGS4	Regulator of G-protein signaling 4
Rms deviation	Root-mean-square deviation
RNA	Ribonucleic acid
S	Septum
S	Serine
SA	Social avoidance
SAD	Social anxiety disorder
SAR	Slowly adapting receptor
SAR	Structure–activity relationship
SC	Schwann cells
SCH50911	(+)-(2S)-5,5-Dimethyl-2-morpholineacetic acid hydrochloride
SCR	Short consensus repeat
SD	Sushi domain
shRNA	Short-hairpin RNA
SIH	Stress induced hyperthermia
siRNA	Small interfering RNA
SKF97541	3-Aminopropyl(methyl)phosphinic acid
SN	Substantia nigra
SNc	Substantia nigra compacta
SNL	Sciatic nerve ligation
SNP	Single nucleotide polymorphism

SNX27	Sorting nexin 27
sP rat	Sardinian alcohol-preferring rat
SSADH	Succinic semialdehyde dehydrogenase
SSRI	Selective serotonin reuptake inhibitor
SSWD	Slow spike-wave discharge
STAR*D	Sequenced treatment alternatives to relieve depression
SUD	Substance use disorder
SWD	Spike-and-wave discharge
TAS	Typical absence seizure
TBAF	Tetrabutylammonium fluoride
TC	Thalamocortical
TEA	Triethylamine
Th	Thalamus
THF	Tetrahydrofuran
t.i.d.	Three times a day
TLE	Temporal lobe epilepsy
TLESR	Transient lower esophageal sphincter relaxation
TM	Transmembrane helix
TMSCl	Trimethylsilyl chloride
TMS	Transcranial magnetic resonance
TSG101	Tumor-susceptibility gene 101
USP14	Ubiquitin carboxyl-terminal hydrolase 14
VABS	Vineland adaptive behavior socialization
VFT	Venus Flytrap domain
VIP	Vasoactive intestinal peptide
VTA	Ventral tegmental area
Wag/Rij	Wistar Albino Glaxo/Rij rat

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Chapter 1
A Brief History of the $GABA_B$ Receptor

Norman G. Bowery

Abstract Discovery of the novel receptor for GABA, $GABA_B$, arose during an attempt to model central primary afferent GABA receptors using sympathetic ganglia. The presence of chloride-dependent GABA receptors on ganglion cell bodies had been established in the early 1970s and this prompted us to consider the possibility that the receptors were also on the efferent nerve fibres of these cells and on the nerve terminal membranes. To pursue this, we examined the influence of GABA and its analogues on the evoked release of radiolabelled noradrenaline from sympathetic nerve fibres innervating rat isolated atria. A reduction in the release of noradrenaline would provide an indirect measure of receptor activation. As predicted, GABA reduced the evoked release but this effect was not blocked by GABA antagonists such as bicuculline and was not mimicked by muscimol but was mimicked by β-chlorophenyl GABA (baclofen). Further studies on the release of radiolabelled noradrenaline from rat brain cortex slices revealed a similar effect. Subsequently, in 1981, we were able to demonstrate the presence of a baclofen-sensitive ^{3}H-GABA binding site on rat cortical membranes. It was at this stage we were able to designate this novel site '$GABA_B$' to contrast with the classical '$GABA_A$' site. The structure of this novel site only emerged some two decades later enabling a greater understanding of the potential physiological and pharmacological roles of the receptor to be made.

Keywords Presynaptic inhibition • Transmitter release • Receptor binding • ^{3}H-baclofen • Receptor localization • Physiology of $GABA_B$ receptors

Prior to the 1980s, defining receptors was based primarily on pharmacological and biochemical data as molecular biology was in its infancy. So in the late 1970s, the evidence for a second GABA receptor stemmed from functional observations in isolated tissue preparations and subsequently from biochemical studies. Data from molecular biology studies arose some 20 years later supporting the earlier observations and provided important detailed information about the receptor.

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1.1 Modelling Presynaptic Inhibition

At the outset of our studies, we were attempting to obtain an in vitro model for mammalian spinal presynaptic inhibition using tissue obtained from peripheral nervous tissue. We had previously observed that rat sympathetic ganglia possessed GABA receptors which when activated produced neuronal depolarization (Bowery and Brown 1974). The mechanism underlying this effect was due to an increase in neuronal chloride ion conductance (Adams and Brown 1975) in much the same way as occurs in neurons of the CNS except that the reversal potential in the ganglia (−42 mV) produces a net efflux of chloride ions giving rise to neuronal depolarization. Under resting conditions, GABA will normally produce an inward movement of chloride in higher centre cortical neurons where the Cl^- reversal potential is in the region of −75 mV. This produces a hyperpolarization. At presynaptic terminals in the spinal cord, it is believed that GABA depolarizes the terminals to reduce the output of transmitter, hence presynaptic inhibition (Curtis 1977). As this mechanism seemed analogous to the membranal effect we had observed in peripheral ganglia, it seemed appropriate to consider the sympathetic ganglion as a model for spinal presynaptic inhibition. If GABA receptors were located not only on the cell body of ganglia but extended along the postganglionic axon to the nerve terminal then, when activated, they might reduce the evoked release of transmitter. The transmitter in this case is noradrenaline. There is no endogenous action of GABA on this receptor as there is no GABAergic innervation to the site but the receptor could still be activated in the presence of exogenous GABA. The basis of this possible action is illustrated in Fig. 1.1. The question then was how could we test this hypothesis. It had been shown previously by Iversen (1963) (see Trendelenburg et al. 1997) that heart tissue could accumulate ^{3}H-noradrenaline when incubated in vitro and that this material could be released on stimulating the adrenergic fibres to the heart. We pursued this idea using isolated superfused atria from the rat, and after loading the tissue with ^{3}H-noradrenaline, circular electrodes were placed around the tissue to evoke release of the tritiated catecholamine. Repetitive periods of brief electrical transmural stimulation produced repeated regular periods of release into the superfusion medium (Bowery et al. 1981). When GABA was added to the bathing medium, it produced a very small inhibition of the evoked release without any effect on basal release. This effect was too small to quantify but we then superfused the tissue with an inhibitor of noradrenaline reuptake, yohimbine, which enhanced the evoked release of ^{3}H-noradrenaline and enabled the full effect of GABA to be observed (Fig. 1.2). GABA produced a dose-dependent inhibition of the evoked release. This was the effect we were hoping to observe suggesting that GABA receptors could well be located on peripheral nerve terminals to provide a possible model for presynaptic inhibition. But then the surprise came when we attempted to characterize the pharmacology of this effect. The effect appeared to be mediated via a receptor that was different from the classical GABA receptor. The inhibition produced by GABA was unaffected by the competitive antagonist, bicuculline, but was mimicked by the β-chlorophenyl analogue of GABA, baclofen (see Chap. 17 of this book). This compound is inactive at the classical GABA receptor. In

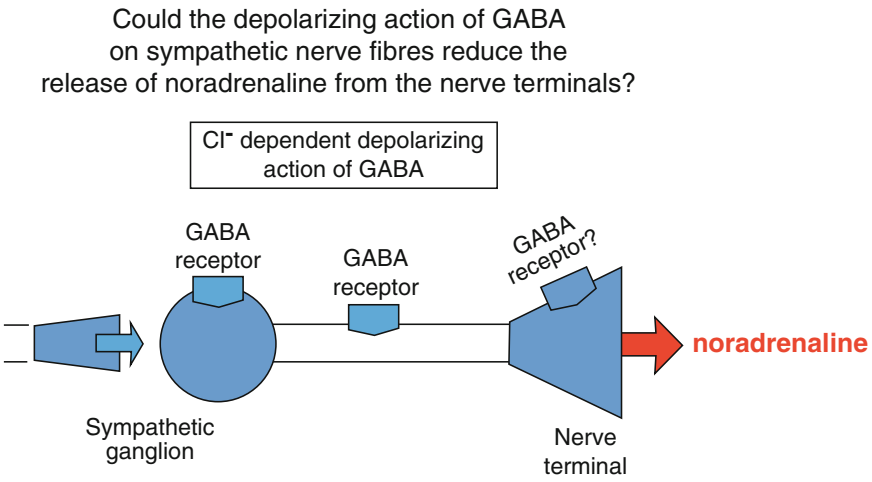

Fig. 1.1 Basis for the original hypothesis that GABA might depolarize the adrenergic nerve terminal in the same way that it depolarizes the ganglion cell body. The chloride-dependent GABA receptor shown to be present on the cell body would also be on the terminal membrane. Depolarization of the terminal could reduce the release of neurotransmitter noradrenaline. In fact, whilst the terminal might be depolarized the overriding effect of GABA was to reduce the evoked release of transmitter by a Cl^--independent mechanism. This provided the first evidence of a distinct receptor for GABA, $GABA_B$

addition isoguvacine and muscimol, which are active GABA-mimetics at the classical receptor, were inactive in our rat atria model. Armed with this information, we proceeded to use cerebrocortical slices of the rat brain to determine whether similar results could be obtained to those in the rat atria (Bowery et al. 1980). We obtained data that supported our previous findings to indicate that this receptor was also present in brain tissue. So our conclusions at this stage were that there exists a receptor for GABA, which is distinct from the classical receptor and does not control Cl^- movement. The action of GABA was not chloride ion dependent.

1.2 Receptor Binding

In an attempt to characterize this receptor further, we needed to demonstrate that a specific binding site for GABA exists which differs from the classical receptor site. Around this time, radiolabelled binding sites for receptors had become an established method for defining their presence in rat brain membranes. The first demonstration of GABA binding to classical receptors was described by Zukin et al. (1974) but no evidence for any additional sites could be demonstrated Enna and Snyder (1975, 1977). The only site that could be detected was sensitive to muscimol and bicuculline. Thus, we would be unlikely to see a novel site under these binding conditions. The answer was to use baclofen as the ligand as we knew that this has no affinity for the

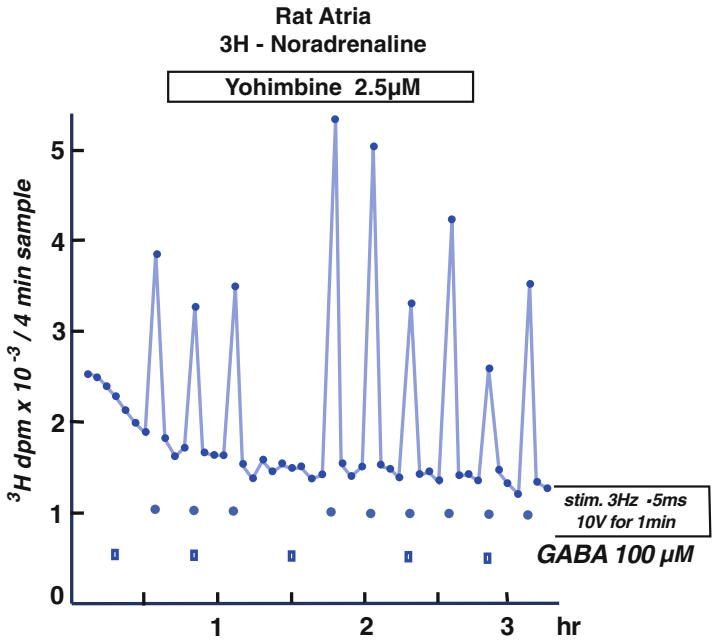

Fig. 1.2 ^{3}H-noradrenaline release from rat isolated atria evoked by transmural stimulation: inhibition by GABA. Release was stimulated as indicated by the *solid dots*. GABA (100 μM) was added to the superfusing medium as indicated by the *small squares* immediately prior to stimulation. The tritium level in each sample of the superfusing medium (Kreb's) was determined by scintillation spectrometry. Yohimbine (2.5 μM) was added to the superfusing medium during the period indicated by the *horizontal bar*

classical receptor. It occurred to us that CIBA-Geigy might have synthesized radiolabelled baclofen, as they were the company who originally produced the compound and marketed it as a muscle relaxant, Lioresal. It was produced as a potential GABA-mimetic, which would be able to cross the blood–brain barrier (Keberle et al. 1968). It transpired that the compound was able to gain access to the brain (Van Bree et al. 1991) and produced muscle relaxation by an action within the spinal cord (Olpe et al. 1978). But there was no evidence that it acted at GABA receptors. We duly contacted individuals at CIBA-Geigy in Basel, namely Helmut Bittiger and his colleagues and asked them, firstly, if ^{3}H-baclofen had been produced and, secondly, if we could obtain a sample for our studies indicating that we wanted to explore the possibility that a binding site exists in cortical membranes. After nearly a year, I received a letter from the group in Switzerland together with a sample of radiolabelled baclofen. The letter informed us that they were aware of our studies and that they had attempted to obtain a binding site without any success. Fortunately, they provided us with information about the experiments that they had performed. They had used a buffer solution that was comparable to that used for classical GABA receptor binding, a non-physiological solution containing TRIS. This type of medium is used to prevent binding to, e.g. any sodium-dependent transport mechanism. We decided that as we had originally

detected the site in a physiological solution, we would attempt to use the same in our binding experiments. In the first experiment that David Hill and I performed we employed the same Krebs' solution as in the rat atria experiments and we observed some saturable binding in the region of 17 % of total. This level increased in subsequent experiments enabling us to characterize the site to show that it was the same as in the atria and cortical slice studies (Hill and Bowery 1981; Bowery et al. 1983). It was at this point we decided that a novel site for GABA exists which was quite distinct from the classical receptor and designated the term '$GABA_B$' for the novel baclofen-sensitive site to distinguish it from classical receptors, which we referred to as '$GABA_A$' (Hill and Bowery 1981). This classification remains to this day (Bowery et al. 2000). The underlying reason for the detection of the binding site was the presence of divalent cations, namely calcium and also magnesium, in the bathing medium. The significance of this enabled us to consider ^{3}H-GABA as a ligand for the receptor. The experiment, which enabled us to observe this, was performed in a Tris–HCl buffer containing Ca^{2+}. Specific binding of ^{3}H-GABA was determined by the addition of isoguvacine but the further addition of unlabeled baclofen produced an increase in the specific binding (Hill and Bowery 1981). Thus, there were two components of binding, one sensitive to isoguvacine and the other to baclofen.

1.3 Does the $GABA_B$ Receptor Have a Physiological Function?

So by 1981 we had a receptor-binding site that was unique and not coupled to chloride ion channels and when activated could reduce the evoked release of transmitter in in vitro models. Moreover, we had demonstrated that the receptor could be labelled with ^{3}H-GABA as well as ^{3}H-baclofen. Subsequent studies showed that $GABA_B$ receptor activation can influence the formation of cyclic AMP and depending on the circumstances produces an inhibition (Xu and Wojcik 1986) or stimulation of the formation of this nucleotide (Karbon et al. 1984; Hill 1985). These effects depend on the nature of the brain tissue preparation. The next major question was does the receptor have an important physiological role. This was surely answered by the elegant work of Roger Nicoll and colleagues. In 1988, Dutar and Nicoll were the first to describe a physiological role for $GABA_B$ receptors in the mammalian central nervous system. They showed that activation of this receptor was responsible for the synaptically mediated late hyperpolarization in rat hippocampal slices (see also Nicoll 2004). This event had been previously noted but the mechanism underlying it was unknown. Now we knew that it was mediated via activation of presynaptic $GABA_B$ receptors to reduce the output of neurotransmitter onto pyramidal cells. The basic mechanism responsible is a decrease in membranal Ca^{2+} conductance (Doze et al. 1995; Isaacson et al. 1993; Isaacson 1998; Wu and Saggau 1995). However, $GABA_B$ receptor activation can also increase K^+ conductance to produce neuronal hyperpolarization but it seems likely that this effect occurs at postsynaptic sites whilst the action on Ca^{2+} conductance is restricted to presynaptic sites (Luscher et al. 1997; Gage 1992; Dunlap 1981).

These two effects produced by $GABA_B$ receptor activation are mediated by G-proteins, which are members of the pertussis toxin-sensitive family (Odagaki and Koyama 2001). The calcium channel associated with $GABA_B$ receptors appears to be predominantly of the 'N' type although 'P' and 'Q' channels have been implicated (Barral et al. 2000; Lambert and Wilson 1996; Santos et al. 1995). More than one type of K^+ channel seems to be associated with the postsynaptic $GABA_B$ receptor (Wagner and Deakin 1993).

1.4 $GABA_B$ Receptor Agonists and Antagonists

β-Chlorophenyl GABA (baclofen) was shown to be stereospecifically active as a receptor agonist and subsequently other compounds, e.g. 3-aminopropyl-phosphinic acid and 3-aminopropyl-methylphosphinic acid were developed which were ~10 fold more potent than the active isomer *R*-(−)-baclofen. Other compounds have been developed which seem to be more able to access the brain after peripheral administration increasing bioavailability (Xu et al. 2011; Abdel-Hafez and Abdel-Wahab 2008). Also licorice (Glycyrrhizae radix) has been shown to act either directly or indirectly as a $GABA_B$ agonist to inhibit cocaine-induced dopamine release in rat brain after oral administration (Jang et al. 2008).

Kerr and colleagues described the first selective antagonist in 1987. These authors showed that phaclofen, and subsequently, saclofen and 2-hydroxy saclofen (Kerr et al. 1988) could block the effects of baclofen and GABA at $GABA_B$ receptors in vitro. Their activity was low so that large concentrations were required. Nevertheless, Dutar and Nicoll (1988) were able to use phaclofen to block the $GABA_B$-mediated effects in hippocampal slices confirming the physiological role on synaptic transmission. Froestl and colleagues achieving the goal of antagonists that can gain ready access to the brain subsequently described selective antagonists with high affinity (Froestl et al. 1995, 1996). (For detailed analysis of agonists and antagonists see Chap. 2 of this book.)

1.5 Where Are $GABA_B$ Receptors Located?

The role of GABA as a neurotransmitter is predominantly within the central nervous system of mammals but this does not exclude the possibility of receptors for this amino acid being outside the brain as well (Erdo and Bowery 1986). What determines whether there is a physiological role for any receptor is the presence of endogenous agonist. In general, the level of GABA outside the brain is very low but there are exceptions. For example, in the intestine and pancreas where GABA may have a functional role mediated by $GABA_B$ and $GABA_A$ receptors (Ong and Kerr 1983; Kleinrok and Kilbinger 1983; Lehmann et al. 2010).

One technique that has been used to detect the distribution of receptors is receptor autoradiography where tissue slices are incubated with radiolabelled ligand and then placed in contact with photographic emulsion to generate an image. The distribution

of $GABA_B$ binding sites (receptors) in rat brain slices was shown in Bowery et al. (1987) and Chu et al. (1990). The highest densities of receptors were observed in the interpeduncular nucleus, the dorsal horn of the spinal cord, the thalamic nuclei, the cerebral cortex, and molecular layer of the cerebellum. This contrasted with the distribution of $GABA_A$ observed in the same tissue. Considerable importance has been attached to these observations, as they appear to concur with functional responses. However, in the hippocampus the density was lower than expected given the importance of $GABA_B$ receptors in neural transmission within this brain region (Nicoll 2004). Distribution of the receptor is considered in detail in Chap. 5 of this book.

1.6 What Do We Know About the Structure of the $GABA_B$ Receptor?

It was some two decades after the $GABA_B$ receptor was first discovered that its structure was defined by three independent groups, Jones et al. (1998), Kaupmann et al. (1998) and White et al. (1998). The receptor exists as a heterodimer with seven membrane-spanning regions in each of the two components, which together form the functional unit (Fig. 1.3. For a full understanding of this see Chap. 4 of this book). Each of the dimers, $GABA_{B1}$ and $GABA_{B2}$, may exist naturally in multiple forms and in general, the distributions of each dimer, detected by immunocytochemistry, concur such that they match each other across the brain (Durkin et al. 1999; Charles et al. 2001; Margeta-Metrovic et al. 1999). However, in the caudate putamen $GABA_{B2}$ is not detectable even though $GABA_{B1}$ is present (Durkin et al. 1999).

Although the subunits of the heterodimer exhibit 35 % homology, they have quite distinct characteristics. Whereas $GABA_{B1}$ contains the binding domain for GABA in its extracellular N-terminal, the $GABA_{B2}$ subunit appears to be responsible for engaging and binding to the other subunit. However, no evidence of the formation of a functional receptor from any protein–protein interaction has been reported. Thus, if the interaction is not producing a receptor, what purpose(s) does this protein-coupling serve? It might be to provide a directional mechanism to enable the correct site insertion of the dimer into the plasma membrane or merely to act as a scaffold or anchoring device to support the dimer when inserted. Alternatively, because of the high affinity of these proteins, they might regulate the formation of the dimer, limiting the level of functional receptor, or by interacting intracellularly with the formed dimer, they may reduce its cell surface expression. The exact role of these interacting proteins, including CREB2/ATF4, fibulin-2, CHOP, and Marlin-1, has yet to be established.

Despite the presence of structural variants of the $GABA_{B1}$ subunits and the possibility of functional modifications of the receptor heterodimer by interacting proteins, there is still no unequivocal evidence for the existence of functional $GABA_B$ receptor subtypes.

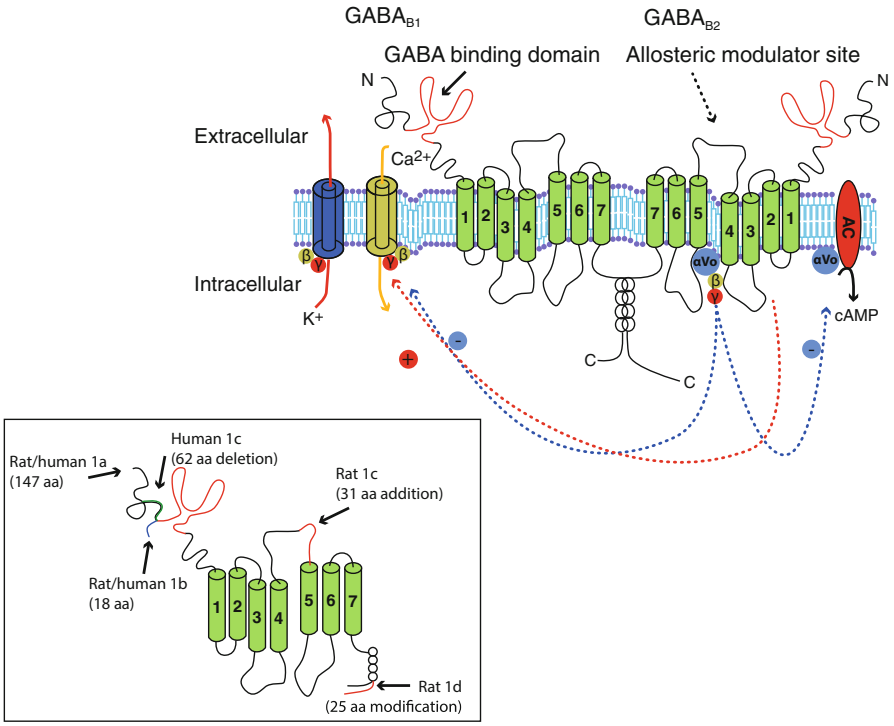

Fig. 1.3 Diagrammatic representation of the $GABA_B$ receptor indicating the two components, $GABA_{B1}$ and $GABA_{B2}$. The *inset* shows the variations in structure that have been described. For a fuller description of the receptor structure see Chap. 4 by Dietmar Benke et al. in this volume

Individual research groups have suggested pharmacological differences between auto- and hetero-receptors as well as between the dual actions of GABA agonists on adenylyl cyclase activity. However, both a lack of reproducibility and sufficient distinction between the effects and the failure to emulate observations made by other groups have cast doubt on the existence of functional receptor subtypes. In general, the available receptor agonists and antagonists do not distinguish between potential subtypes of the $GABA_B$ receptor (Cunningham and Enna 1997; Knight and Bowery 1996).

$GABA_B$ receptors appear to be predominantly located on presynaptic terminals where they function as auto- and hetero-receptors to limit the release of neurotransmitters. Whilst the physiological function of autoreceptors is not difficult to accept because of the local availability of neurotransmitter, the possible functional role of heteroreceptors could be more difficult to accept. However, Isaacson et al. (1993) have shown that GABA released from GABAergic terminals appears to access adjacent non-GABAergic terminals on which $GABA_B$ receptors exist. The extracellular concentration of GABA following its release is in the millimolar range whilst the affinity of $GABA_B$ receptors for GABA is in the nanomolar region. Activation of adjacent 'non-synaptic' sites is, therefore, very likely to occur.

1.7 Pharmacological and Physiological Considerations

The pharmacological actions of the prototypic $GABA_B$ receptor agonist, baclofen, are covered in Chap. 17 of this book but as the clinical use of the drug preceded the discovery of the receptor by about 10 years, it seems appropriate that an outline of the early history of baclofen should be included here. The compound was first synthesized by Keberle in 1962 after he had listened to a lecture given by Wilhelm Feldberg at the Royal Society in London. The lecture had focused on the potential role of GABA in the brain. This made Keberle consider the possibility of making a GABA-mimetic that would gain access to the brain as GABA being a zwitter ion would not cross the blood–brain barrier (Keberle et al. 1968). When injected systemically in vivo in animals, baclofen produced a neuronal depressant effect within the spinal cord but no evidence was obtained to support the idea that it was acting at receptors for GABA. Nevertheless, the effect was sufficient to prompt its introduction to clinical medicine as a muscle relaxant in spasticity in 1972. The mechanism of action was not defined and attempts to implicate a GABA-like action were not successful. Its effect in animal models was unaffected by administration of GABA antagonists such as picrotoxin. Later studies using bicuculline, following its discovery as a potent GABA antagonist, failed to reduce the response to baclofen.

1.8 Positive Allosteric Modulation of $GABA_B$ Receptors

In 2001, Urwyler and colleagues introduced the concept of positive allosteric modulation of the $GABA_B$ receptor. This is fully described in Chaps. 18 and 3 of this book but as it forms part of the history of the receptor I felt that it should also be included here as an important milestone. The first compound shown to be a modulator was CGP7930 (2,6-di-tert-butyl-4-{3-hydroxy-2,2-dimethyl-propyl}-phenol). This compound had no direct effect on the $GABA_B$ recognition site but accentuated responses to GABA and baclofen. This was shown initially in vitro but the effect was subsequently observed in vivo (Carai et al. 2004; Smith et al. 2004) where positive modulators reduced the self-administration of cocaine. This is also observed with the directly acting agonist, baclofen but the advantage of receptor modulation is avoidance of the adverse effects of the agonist where high doses are required and the possible receptor desensitization that often occurs when using directly acting agonists, including baclofen.

1.9 Route of Administration in Clinical Medicine

One of the major hurdles in the use of baclofen as a muscle relaxant is the need for high doses due primarily to the relatively poor brain penetration by passive diffusion (Leisen et al. 2003) although it is transported by a neutral amino acid transporter

(Van Bree et al. 1991) but this counteracted by an efficient efflux from the brain via an organic anion transporter (Deguchi et al. 1995). High doses can produce several side effects including drowsiness, weakness, dizziness, tiredness, headache, seizures, nausea, vomiting, low blood pressure, constipation, confusion, respiratory depression, insomnia, and increased urinary frequency or urinary retention. Whilst positive modulators may well provide answers to this problem they have yet to be used clinically. Meanwhile, a major advance was made during the late 1980s and 1990s with the introduction of implanted mini-pumps to administer baclofen intrathecally (Penn and Kroin 1984, 1985). This enabled much smaller doses to be administered. Leisen et al. (2003) showed that whereas 100 mg baclofen given systemically only produced CSF levels less than 3 ng/g, the intrathecal injection of 0.6 mg produced levels of 5–20 ng/g. The clinical outcome was excellent without the side effects previously observed. However, the possibility of receptor desensitization remains which makes allosteric modulation an attractive proposition.

1.10 Current Status of the $GABA_B$ Receptor

The $GABA_B$ receptor is now firmly established as a metabotropic receptor that is unrelated to the ionotropic $GABA_A$ receptor. The only common characteristic is that they are both activated by the amino acid, GABA. As it will be seen in the subsequent chapters of this volume, the potential application of agonists, antagonists as well as allosteric modulators in clinical medicine is quite extensive and it will be exciting to see their introduction.

References

Abdel-Hafez, A. A., & Abdel-Wahab, B. A. (2008). 5-(4-Chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4H)-one derivatives as lipophilic cyclic analogues of baclofen: Design, synthesis, and neuropharmacological evaluation. *Bioorganic & Medicinal Chemistry, 16*(17), 7983–7991.

Adams, P. R., & Brown, D. A. (1975). Actions of γ-aminobutyric acid on sympathetic ganglion cells. *Journal of Physiology (London), 250*, 85–120.

Barral, J., Toro, S., Galarraga, E., & Bargas, J. (2000). GABAergic presynaptic inhibition of rat neostriatal afferents is mediated by Q-type Ca^{++} channels. *Neuroscience Letters, 283*, 33–36.

Bowery, N. G., Bettler, B., Froestl, W., Gallagher, J. P., Marshall, F., Raiteri, M., et al. (2002). Mammalian gamma-aminobutyric acid (B) receptor: Structure and function. *Pharmacological Reviews, 54*(2), 247–264.

Bowery, N. G., & Brown, D. A. (1974). Depolarising actions of γ-aminobutyric acid and related compounds on rat superior cervical ganglia in vitro. *British Journal of Pharmacology, 50*, 205–218.

Bowery, N. G., Doble, A., Hill, D. R., Hudson, A. L., Shaw, J. S., Turnbull, M. J., et al. (1981). Bicuculline-insensitive GABA receptors on peripheral autonomic nerve terminals. *European Journal of Pharmacology, 73*, 53–70.

Bowery, N. G., Hill, D. R., Hudson, A. L., Doble, A., Middlemiss, D. N., Shaw, J., et al. (1980). (−) Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature, 283*, 92–94.

Bowery, N. G., Hill, D. R., & Hudson, A. L. (1983). Characteristics of $GABA_B$ receptor binding sites on rat whole brain synaptic membranes. *British Journal of Pharmacology, 78*, 191–206.

Bowery, N. G., Price, G. W. P., & Hudson, A. L. (1987). $GABA_A$ and $GABA_B$ receptor site distribution in rat central nervous system. *Neuroscience, 20*, 365–385.

Carai, M. A. M., Colombo, G., Froestl, W., & Gessa, G. L. (2004). In vivo effectiveness of CGP7930, a positive allosteric modulator of the $GABA_B$ receptor. *European Journal of Pharmacology, 504*, 213–216.

Charles, K. J., Evans, M. L., Robbins, M. J., Calver, A. R., Leslie, R. A., & Pangalos, M. N. (2001). Comparative immunohistochemical localization of GABAB1a, GABAB1b, GABAB2 subunits in rat brain, spinal cord, and dorsal root ganglion. *Neuroscience, 106*, 447–467.

Chu, D. C. M., Albin, R. L., Young, A. B., & Penney, J. B. (1990). Distribution and kinetics of $GABA_B$ binding sites in rat central nervous system: A quantitative autoradiographic study. *Neuroscience, 34*, 341–357.

Cunningham, M., & Enna, S. J. (1997). Cellular and biochemical responses to GABAB receptor activation. In S. J. Enna & N. G. Bowery (Eds.), *The GABA receptors* (pp. 237–258). Totowa, NJ: Humana Press.

Curtis, D. R. (1977). Pre- and non-synaptic activities of GABA and related amino acids in the mammalian nervous system. In F. Fonnum (Ed.), *Amino acids as chemical transmitters* (pp. 55–86). New York: Plenum Press.

Deguchi, Y., Inabe, K., Tomiyasu, K., Nozawa, K., Yamada, S., & Kimura, R. (1995). Study on brain interstitial fluid distribution and blood-brain barrier transport of baclofen in rats by microdialysis. *Pharmaceutical Research, 12*, 1838–1844.

Doze, V. A., Cohen, G. A., & Madison, D. V. (1995). Calcium channel involvement in $GABA_B$ receptor-mediated inhibition of GABA release in area CA1 of the rat hippocampus. *Journal of Neurophysiology, 74*, 43–53.

Dunlap, K. (1981). Two types of γ-aminobutyric acid receptor on embryonic sensory neurons. *British Journal of Pharmacology, 74*, 579–585.

Durkin, M. M., Gunwaldsen, C. A., Borowsky, B., Jones, K. A., & Branchek, T. A. (1999). An in situ hybridization study of the distribution of the GABA(B2) protein mRNA in the rat CNS. *Molecular Brain Research, 71*, 185–200.

Dutar, P., & Nicoll, R. A. (1988). A physiological role for $GABA_B$ receptors in the central nervous system. *Nature, 332*, 156–158.

Enna, S. J., & Snyder, S. (1975). Properties of γ-aminobutyric acid (GABA) receptor binding in rat brain membrane fractions. *Brain Research, 100*, 81–97.

Enna, S. J., & Snyder, S. (1977). Influence of ions, enzymes detergents on γ-aminobutyric acid receptor binding in synaptic membranes of rat brain. *Molecular Pharmacology, 13*, 442–453.

Erdo, S. L., & Bowery, N. G. (Eds.). (1986). *GABAergic mechanisms in the mammalian periphery*. New York: Raven.

Froestl, W., Mickel, S. J., Schmutz, M., et al. (1996). Potent, orally active $GABA_B$ receptor antagonists. *Pharmacology Reviews and Communications, 8*, 127–133.

Froestl, W., Mickel, S. J., von Sprecher, G., Diel, P. J., Hall, R. G., Maier, L., et al. (1995). Phosphinic acid analogues of GABA 2. Selective orally active GABAB antagonists. *Journal of Medicinal Chemistry, 38*, 3313–3331.

Gage, P. W. (1992). Activation and modulation of neuronal K^+ channels by GABA. *Trends in Neurosciences, 15*, 46–51.

Hill, D. R. (1985). $GABA_B$ receptor modulation of adenylatecyclase activity in rat brain slices. *British Journal of Pharmacology, 84*, 249–257.

Hill, D. R., & Bowery, N. G. (1981). ^{3}H-Baclofen and ^{3}H-GABA bind to bicuculline-insensitive $GABA_B$ sites in rat brain. *Nature, 290*, 149–152.

Isaacson, J. S. (1998). $GABA_B$ receptor-mediated modulation of presynaptic currents and excitatory transmission at a fast central synapse. *Journal of Neurophysiology, 80*, 1571–1576.

Isaacson, J. S., Solis, J. M., & Nicoll, R. A. (1993). Local and diffuse synaptic actions of GABA in the hippocampus. *Neuron, 10*, 165–175.

Iversen, L. L. (1963). The uptake of noradrenaline by the isolated perfused rat heart. *British Journal of Pharmacology and Chemotherapy, 21*, 523–537.

Jang, E. Y., Choe, E. S., Hwang, M., Kim, S. C., Lee, J. R., Kim, S. G., et al. (2008). Isoliquiritigenin suppresses cocaine induced extracellulardopamine release in rat brain through GABA(B) receptor. *European Journal of Pharmacology, 587*, 124–128.

Jones, K. A., Borowsky, B., Tamm, J. A., Craig, D. A., Durkin, M. M., Dai, M., et al. (1998). $GABA_B$ receptors function as a heteromeric assembly of the subunits $GABA_BR1$ and $GABA_BR2$. *Nature, 396*, 674–679.

Karbon, E. W., Duman, R. S., & Enna, S. J. (1984). $GABA_B$ receptors and norepinephrine-stimulated cAMP production in rat brain cortex. *Brain Research, 306*, 327–332.

Kaupmann, K., Malitschek, B., Schuler, V., Heid, J., Froestl, W., Beck, P., et al. (1998). $GABA_B$-receptor subtypes assemble into functional heteromeric complexes. *Nature, 396*, 683–687.

Keberle H. Faigle J.W. and Wilhelm M. (1968, April 11) *Procedure for the preparation of new aminoacids. Swiss Patent 449046* (Priority 9 July 1963).

Kerr, D. I. B., Ong, J., Johnson, G. A. R., Abbenante, J., & Prager, R. H. (1988). 2-Hydroxysaclofen: An improved antagonist at central and peripheral $GABA_B$ receptors. *Neuroscience Letters, 92*, 92–96.

Kerr, D. I. B., Ong, J., Prager, R. H., Gynther, B. D., & Curtis, D. R. (1987). Phaclofen: A peripheral and central baclofen antagonist. *Brain Research, 405*, 150–154.

Kleinrok, A., & Kilbinger, H. (1983). γ-Aminobutyric acid and cholinergic transmission in the guinea-pig ileum. *Naunyn-Schmiedeberg's Archives of Pharmacology, 322*, 216–220.

Knight, A. R., & Bowery, N. G. (1996). The pharmacology of adenylyl cyclase modulation by $GABA_B$ in rat brain slices. *Neuropharmacology, 35*, 703–712.

Lambert, N. A., & Wilson, W. A. (1996). High-threshold Ca^{2+} currents in rat hippocampal interneurons and their selective inhibition by activation of $GABA_B$ receptors. *Journal of Physiology, 492*, 115–127.

Lehmann, A., Jensen, J. M., & Boeckxstaens, G. E. (2010). $GABA_B$ receptor agonism as a novel therapeutic modality in the treatment of gastroesophageal reflux disease. *Advances in Pharmacology, 58*, 287–313.

Leisen, C., Langguth, P., & Herbert, B. (2003). Lipophilicities of baclofen ester prodrugs correlate with affinities to the ATP-dependent efflux pump P-glycoprotein: Relevance for their permeation across the blood-brain barrier? *Pharmaceutical Research, 20*, 772–778.

Luscher, C., Jan, I. Y., & Stoffel, M. (1997). (1997) G-protein coupled inwardly rectifying K+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron, 19*(3), 687–695.

Margeta-Metrovic, M., Mitrovic, I., Riley, R. C., Jan, L. Y., & Basbaum, A. I. (1999). Immunohistochemical localization of GABA(B) receptors in the rat central nervous system. *Journal of Comparative Neurology, 405*, 299–321.

Nicoll, R. A. (2004). My close encounter with $GABA_B$ receptors. *Biochemical Pharmacology, 68*, 1667–1674.

Odagaki, Y., & Koyama, T. (2001). Identification of G alpha subtype(s) involved in gamma-aminobutyric acid (B) receptor-mediated high-affinity guanosine triphosphate activity in rat cerebral cortical membranes. *Neuroscience Letters, 297*, 137–141.

Olpe, H.-R., Demieville, H., Baltzer, V., Bencze, W. L., Koella, W. P., Wolf, P., et al. (1978). The biological activity of D- and L-baclofen (Lioresal). *European Journal of Pharmacology, 52*, 133–136.

Ong, J., & Kerr, D. I. B. (1983). $GABA_A$- and $GABA_B$-receptor-mediated modification of intestinal motility. *European Journal of Pharmacology, 86*, 9–17.

Penn, R. D., & Kroin, J. S. (1984). Intrathecal baclofen alleviates spinal cord spasticity. *Lancet, 323*(8385), 1078.

Penn, R. D., & Kroin, J. S. (1985). Continuous intrathecal baclofen for severe spasticity. *Lancet, 326*(8447), 125–127.

Santos, A. E., Carvalho, C. M., Macedo, T. A., & Carvalho, A. P. (1995). Regulation of intracellular [Ca^{2+}] GABA release by presynaptic $GABA_B$ receptors in rat cerebrocortical synaptosomes. *Neurochemistry International, 27*, 397–406.

Smith, M. A., Yancey, D. I., Morgan, D., Liu, Y., Froestl, W., & Roberts, D. C. (2004). Effects of positive allosteric modulators of the $GABA_B$ receptor on cocaine self-administration in rats. *Psychopharmacology, 173*, 105–111.

Trendelenburg, A. U., Sutel, I., Wahl, C. A., Molderings, G. J., Rump, L. C., & Starke, K. (1997). A re-investigation of Questionable subclassifications of presynaptic alpha2-autoreceptors: Rat vena cava, rat atria, human kidney and guinea-pig urethra. *Naunyn-Schmiedeberg's Archives of Pharmacology, 356*, 721–737.

Urwyler, S., Mosbacher, J., Lingenhoehl, K., Heid, J., Hofstetter, K., Froestl, W., et al. (2001). Positive allosteric modulation of native and recombinant γ-aminobutyric $acid_B$ receptors by 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and its aldehyde analog CGP13501. *Molecular Pharmacology, 60*, 963–971.

Van Bree, J. B., Heijligers-Feijen, C. D., De Boer, A. G., Danhof, M., & Breimer, D. D. (1991). Stereoselective transport of baclofen across the blood-brain barrier in rats as determined by the unit impulse response methodology. *Pharmaceutical Research, 8*, 259–262.

Wagner, P. G., & Deakin, M. S. (1993). $GABA_B$ receptors are coupled to a barium-insensitive outward rectifying potassium conductance in premotor respiratory neurons. *Journal of Neurophysiology, 69*, 286–289.

White, J. H., Wise, A., Main, M., Green, A., Fraser, N. J., Disney, G. H., et al. (1998). Heterodimerization is required for the formation of a functional $GABA_B$ receptor. *Nature, 396*, 679–682.

Wu, I. G., & Saggau, P. (1995). $GABA_B$ receptor-mediated presynaptic inhibition in guinea-pig hippocampus is caused by reduction of presynaptic Ca^{++} influx. *Journal of Physiology, 485*, 649–657.

Xu, F., Peng, G., Phan, T., Dilip, U., Chen, J. L., Chernov-Rogan, T., et al. (2011). Discovery of novel potent GABA(B) receptor agonist. *Bioorganic & Medicinal Chemistry Letters, 21*(21), 6582–6585.

Xu, J., & Wojcik, W. J. (1986). Gamma aminobutyric acid B receptor-mediated inhibition of adenylatecyclase in cultured cerebellar granule cells. Blockade by islet-activating protein. *Journal of Pharmacology and Experimental Therapeutics, 239*, 568–573.

Zukin, S., Young, A., & Snyder, S. (1974). Gamma-aminobutyric acid binding to receptor sites in the rat central nervous system. *Proceedings of the National Academy of Sciences of the United States of America, 71*, 4802–4807.

Part I
Chemistry

Chapter 2
Chemistry of $GABA_B$ Receptor Ligands: Focus on Agonists and Antagonists

Federico Corelli and Claudia Mugnaini

Abstract Since the discovery of $GABA_B$ receptor by Norman G. Bowery and coworkers in 1980, a striking endeavour was made by industrial and academic researchers to develop $GABA_B$ receptor ligands for therapeutic application in a variety of diseases associated with dysfunctions of the gabaergic system. Although baclofen (Lioresal) is still the only approved $GABA_B$ receptor agonist, this sustained research effort has produced many new compounds which are able to exert GABAB agonist, partial agonist or antagonist activity. This chapter presents an overview of the outcomes in this field, with a special focus on the chemistry, structure–activity relationship and mechanism of action of several GABA and baclofen analogues, derivatives and bioisosteres.

Keywords $GABA_B$ receptor agonists • $GABA_B$ receptor antagonists • GABA analogues • Baclofen analogues • Bioisosteres • SAR

2.1 Introduction

γ-Aminobutyric acid (GABA, Fig. 2.1) has long been known before its target receptors were discovered. It is a neutral amino acid of low molecular weight (MW = 103) and $pK_1 = 4.23$ and $pK_2 = 10.43$, showing very high hydrophilicity (Log$P$ = –3.17, PSA = 63.3 Å^2) and high aqueous solubility (1.3 g/mL), that was first synthesized in 1883 (Froestl 2011). In 1950 three different research groups demonstrated that GABA is present in the mammalian brain (Awapara et al. 1950; Roberts and Frankel 1950; Udenfriend 1950), where it is produced in situ by decarboxylation of glutamic acid, as it is unable to penetrate into the central nervous system (CNS) by crossing the blood–brain barrier (BBB). In 1967, GABA was recognized as the most important and most abundant inhibitory neurotransmitter in the mammalian brain (Krnjevic and Schwartz 1967), where it acts by

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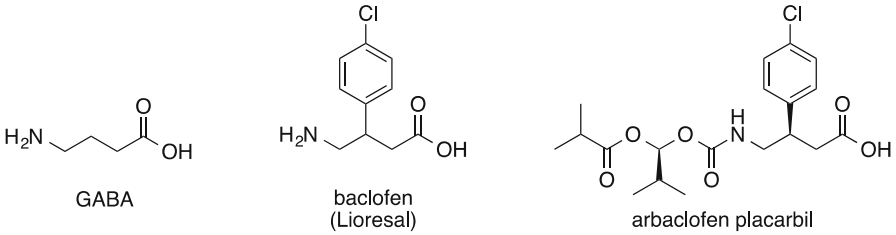


Fig. 2.1 Structure of GABA, baclofen and arbaclofen placarbil

regulating glutamatergic activity and preventing hyperexcitation by activation of both ionotropic ($GABA_A$ and $GABA_C$) and metabotropic ($GABA_B$) receptors.

$GABA_B$ receptors can interfere with the release of many neurotransmitters, such as dopamine, acetylcholine, 5-hydroxytryptamine (5-HT) via different mechanisms (see Chap. 7 of this book) and are implicated in severe neurological and psychiatric disorders, such as epilepsy, schizophrenia, anxiety, depression, autism spectrum disorder, stroke, drug addiction and neurodegenerative disorders (Parkinson's disease, Huntington's disease and Alzheimer's disease) (see Chaps. 10–15 of this book). $GABA_B$ receptors have also been implicated in the treatment of muscle spasticity disorders, pain and gastroesophageal reflux disease (GERD) [(Froestl 2010, 2011); see also Chap. 16 of this book].

Considering the wide range of pathophysiological conditions associated with $GABA_B$ receptors, and that GABA itself cannot be used as a drug because of its poor physicochemical and pharmacokinetic properties, extensive drug development programmes were undertaken to find drug-like analogues for therapeutical application.

In contrast to the plethora of drugs targeting $GABA_A$ receptor that have been approved since the discovery of chlordiazepoxide, only few $GABA_B$ drugs have been introduced into the market and are currently employed for the treatment of various pathological conditions. Medicinal chemistry efforts during the last decades have indeed provided many GABA analogues; but, despite all of them sharing similar structures to that of GABA, their targets may be different and some do not even act on the GABA pathways (Brown et al. 2015).

2.2 $GABA_B$ Receptor Ligands

2.2.1 Baclofen

Currently, the only FDA-approved $GABA_B$ receptor agonist is baclofen (Lioresal) (Fig. 2.1), which was synthesized in 1962 by Keberle et al. (1968) at Ciba, Switzerland, in order to obtain a more lipophilic analogue of GABA which might be able to reach the CNS by penetration of the BBB (see Chap. 17 of this book). Baclofen does not accumulate in the brain by passive diffusion, because its lipophilicity is still too low (Log$D$ = –0.96). However, the large neutral amino acid

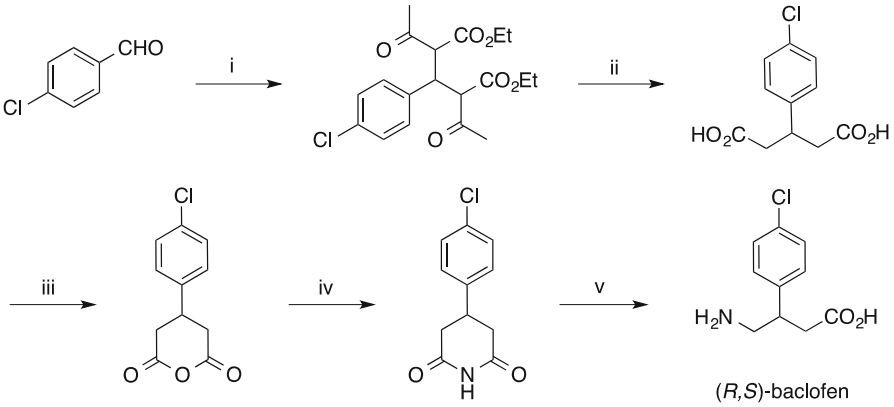
transporter brings baclofen into the brain (van Bree et al. 1988), where its distribution in the interstitial fluid (ISF) is restricted owing to an efficient efflux from the brain via the BBB. This is regulated by a probenecid-sensitive organic anion transport system (Deguchi et al. 1995). Studies using oral and intravenous administration of radiolabeled racemic baclofen demonstrated that the drug is rapidly absorbed after oral administration and is eliminated mostly unchanged by renal excretion. Pharmacokinetic limitations of baclofen include limited absorption in the large intestine (it is only absorbed in the upper small intestine by saturable active transport mechanisms), which has hampered the development of sustained release formulation. Its short half-life of 3–4 h, and rapid clearance from the blood have also contributed. As a result, in order to maintain therapeutically effective blood levels, baclofen must be administered 3–4 times per day, a dose regimen that reduces patient compliance and causes rapid tolerance and significant adverse effects (e.g., somnolence, dizziness, fatigue, motor weakness).

In spite of these pharmacokinetic and toxicological drawbacks, that make baclofen far from being an optimal drug, Lioresal has been utilized since 1972 either orally or intrathecally as a centrally acting muscle relaxant in spasticity associated with diverse pathological conditions, such as hemi- and tetra-plegia, multiple sclerosis, stroke, cerebral palsy in children and adults [(Froestl 2010; Brown et al. 2015); see also Chap. 17 of this book]. Administration of doses up to 600 μg of baclofen directly into the cerebrospinal fluid (CSF) by means of an implanted minipump allows higher plasma levels to be reached compared to oral baclofen at the high dose of 100 mg. A concurrent reduction in the unwanted side effects following repeated systemic administrations also occurs.

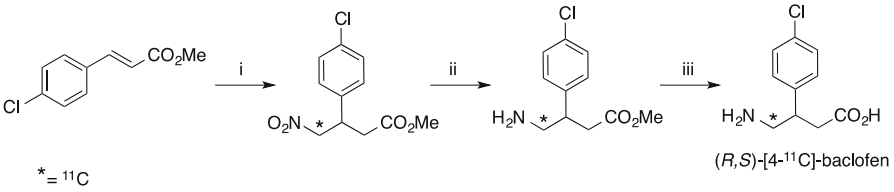
After resolution of baclofen into the two enantiomers, it could be demonstrated that its pharmacological action is enantioselective, as (*R*)-(–)-baclofen inhibits the binding of [^{3}H]-baclofen to $GABA_B$ receptors in cat cerebellum with $IC_{50} = 15$ nM, while (*S*)-(+)-baclofen and racemic baclofen display IC_{50} values 120 and 3 times higher, respectively (Froestl et al. 1995a). Nevertheless, the pharmaceutical Lioresal is sold as a racemic mixture.

Very recently, Qin and coworkers demonstrated that acute administration of (*R*)-baclofen corrected elevated protein synthesis and reduced deficits on a test of social behaviour in adult *Fmr1* KO mice. These findings suggest that treatment via activation of the $GABA_B$ system warrants further study in patients with fragile X syndrome [(Qin et al. 2015); see also Chap. 13 of this book].

A prodrug of (*R*)-baclofen, arbaclofen placarbil (Fig. 2.1), was recently developed by XenoPort with the aim of improving both the pharmacokinetic and pharmacodynamic properties of the parent drug. Thus, the more potent isomer of the drug, (*R*)-baclofen, was converted into a carbamate derivative designed to be efficiently absorbed throughout the gastrointestinal tract, including the colon, and rapidly metabolized by carboxylesterases to release (*R*)-baclofen after absorption (Lal et al. 2009). Despite arbaclofen placarbil showing more favourable pharmacokinetic properties than baclofen, its development was discontinued in 2013 because of unsuccessful results in phase III clinical trials.



Scheme 2.1 Reagents and conditions: *i*, ethyl acetoacetate, NaOEt; *ii*, NaOH, H_2O; *iii*, acetic anhydride; *iv*, NH_3; *v*, Br_2, NaOH

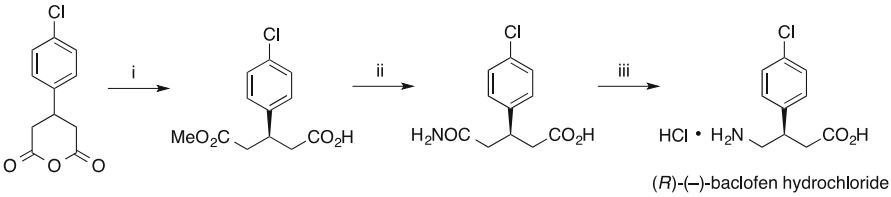


Scheme 2.2 Reagents and conditions: *i*, $[^{11}C]H_3NO_2$, TBAF, THF, rt, 3 min; *ii*, $NaBH_4$, $NiCl_2$, MeOH, H_2O, rt, 3 min; *iii*, NaOH, H_2O, 80 °C, 3 min

After the first synthesis of baclofen was reported in the Ciba patent by Keberle in 1962 (Scheme 2.1), no other syntheses of baclofen were reported in the open literature until 1978, when Swahn and coworkers described the preparation of the deuterium-labelled analogue 4-amino-3-*p*-chlorophenylbutyric acid-2,2,4,4-$^2H_4$ following basically the same approach developed at Ciba (Swahn et al. 1978). Subsequently, a number of alternative synthetic approaches to this drug have been developed; some of them have been discussed in a comprehensive review in 1994 (Tilinsky and Gammill 1994), but other syntheses have followed (Varala and Adapa 2006).

The synthesis of (*R,S*)-[4-^{11}C]baclofen, the first ^{11}C-labeled $GABA_B$ receptor agonist, was performed via Michael addition of nitro[^{11}C]methane as a key step. A tetrabutylammonium fluoride promoted Michael addition of nitro[^{11}C]methane to methyl 4-chlorocinnamate, followed by reduction of the nitro group by $NiCl_2$ and $NaBH_4$ in aqueous MeOH and alkaline hydrolysis yielded (*R,S*)-[4-^{11}C] baclofen in 36.4 ± 1.8 % radiochemical conversion in three steps within 20 min (Scheme 2.2) (Kato et al. 2009).

Substantial efforts have been devoted to obtaining the single isomers of baclofen, particularly the eutomer (*R*)-baclofen, by exploiting diverse methodologies, such as resolution and chemoenzymatic and enantioselective synthesis (Thakur et al. 2007). The last report on this topic goes back to 2009, when a short and highly enantioselective



Scheme 2.3 Reagents and conditions: *i*, $(DHQD)_2AQN$, MeOH, –40 °C, 120 h, 95 % ee; *ii*, NH_3-H_2O, rt, 5 days; *iii*, (a) PIFA, CH_3CN, H_2O, rt, 24 h; (b) conc. HCl, rt, 1 h

synthesis of both enantiomers of baclofen was described involving desymmetrization reaction of cyclic anhydride, leading to (*R*)- and (*S*)-baclofen in 35.1 % and 32.8 % yield, respectively, and high enantiomeric excess (Scheme 2.3) (Ji et al. 2009).

2.2.2 Baclofen Analogues

A good deal of work has been devoted to the modification of the aromatic ring in the search of potential substitutes for baclofen. These studies, mostly over the last 30 years, have provided substantial information on the structure–activity relationship in this field, but led to the identification of only very few compounds comparable to baclofen in terms of potency and selectivity. An obvious modification of baclofen, that is the removal of the chlorine atom to give the des-chloro analogue, led to the identification of phenibut (Fig. 2.2), which was introduced into clinical practice in Russia in the 1960s under the trade name Citrocard for its nootropic and anxiolytic properties (Lapin 2001). Similarly to baclofen, the (*R*)-(–)-enantiomer is the eutomer showing a full agonist profile at $GABA_B$ receptors (Ong et al. 1993).

Early studies focusing on the replacement of the chlorine atom with alkyloxy and aryloxy substituents suggested that lipophilicity and steric hindrance were not the only crucial issues, as electronic characteristics of the substituents played a role as well (Berthelot et al. 1987). The replacement of the *p*-chlorophenyl moiety with furan, thiophene, benzo[a]furan, benzo[a]thiophene, or a quinoline ring afforded a series of compounds (exemplified by structures **1–5** in Fig. 2.2) with different affinities for the $GABA_B$ receptors depending on the nature of the heteroaromatic nucleus as well as on the position and electronic properties of its substituent (Pirard et al. 1995).

Through an integrated QSAR/molecular modelling study, Costantino et al. were able to infer that the *p*-chlorophenyl group and its heteroaromatic replacements are instrumental in the binding of baclofen and its analogues to the $GABA_B$ receptor, according to their ability to establish aromatic–aromatic π-interactions inside the pocket formed by Tyr366 and Tyr395 (Costantino et al. 2001). More recently, a novel potent analogue **6** (Fig. 2.2) was discovered that showed 25- to 50-fold greater activity than (*R*)-baclofen at human and rodent $GABA_B$ receptors in vitro. This compound proved to be active in vivo in a mouse hypothermia assay, to cross the BBB, and to be approximately 50-fold more potent than (*R*)-baclofen after systemic administration (Xu et al. 2011).

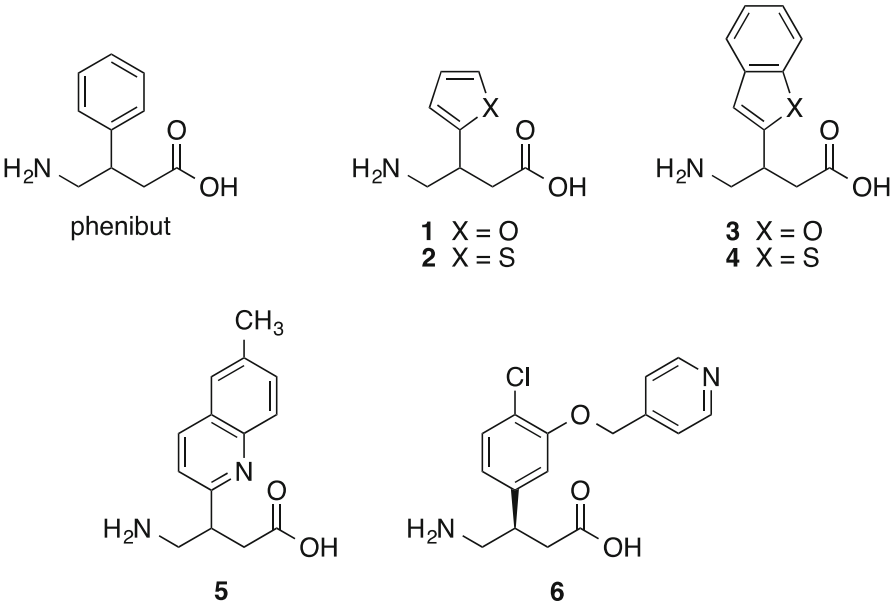


Fig. 2.2 Structure of some analogues of baclofen

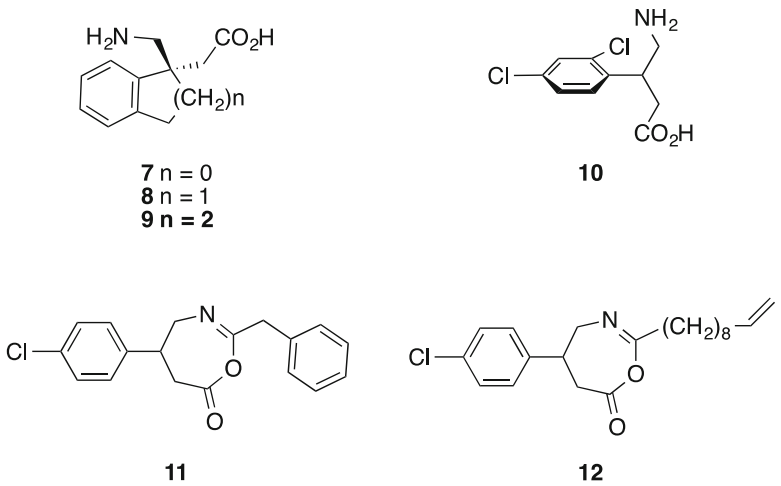


Fig. 2.3 Some conformationally constrained analogues of baclofen

The design of conformationally blocked analogues of baclofen was carried out in an attempt to obtain novel compounds mimicking the solid-state conformation of the parent drug, starting from the working hypothesis that this conformation might correspond to the bioactive conformation of baclofen. However, compounds **7–10** (Fig. 2.3) obtained by rigidifying the baclofen structure by means of methylene, ethylene, or propylene bridges, or by inserting a chlorine atom at the *ortho* position of the

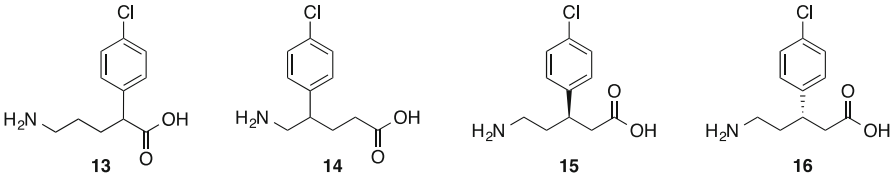


Fig. 2.4 Homologues of baclofen

p-chlorophenyl ring, were basically inactive (Mann et al. 1991). A recent report described the neuropharmacological effects of 5-(4-chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4*H*)-one derivatives as conformationally restricted and more lipophilic analogues of baclofen.

Although some of the new compounds (particularly **11** and **12**, Fig. 2.3) elicited potencies comparable to that of the reference drug baclofen in an array of assays—loss of righting reflex, locomotor activity, rotarod test, traction test, hot-plate test and effect on passive avoidance behaviour—it remains to be definitely assessed whether these compounds act per se or are baclofen prodrugs acting after metabolic activation (Abdel-Hafez and Abdel-Wahab 2008).

A number of 5-aminopentanoic acid (δ-aminovaleric acid, DAVA) derivatives were studied as GABA homologues (Fig. 2.4). DAVA itself has shown antagonism at $GABA_B$ receptors (Muhyaddin et al. 1982), while among analogues **13–16** only **15** inhibited $GABA_B$ binding ($IC_{50} = 0.14 \pm 0.01$ μM) and exhibited weak agonist activity [approximately 13 times lower than that of (*R*)-baclofen] as an inhibitor of guinea pig ileum contractions (Karla et al. 1999). However, this effect was insensitive to the $GABA_B$ receptor antagonist, CGP35348, and the actual mechanism of action of **15** remains unclear. Further investigations on GABA homologues confirmed a very weak agonistic activity of racemic **15** (Attia et al. 2013).

2.2.3 GABA Analogues and Derivatives

The clinical success of baclofen, together with its unsatisfactory profile, have spurred an intensive research activity aimed at discovering baclofen analogues possessing more favourable toxicological and pharmacokinetic characteristics while retaining potency and receptor selectivity. These efforts have been successful in launching into the market some new drugs that are reminiscent of GABA in terms of both chemical structure and international non-proprietary name (INN), such as *gaba*pentin, pre*gaba*lin, vi*gaba*trin (Fig. 2.5). However, these drugs are not GABA analogues from a pharmacological viewpoint, in that they act via different mechanism(s) of action from that of GABA, and are able to cross the BBB as substrates of the L-type amino acid transporters.

When gabapentin was introduced into clinical use for the treatment of epilepsy, the mechanism of action of this "GABA analogue" had not yet been clearly

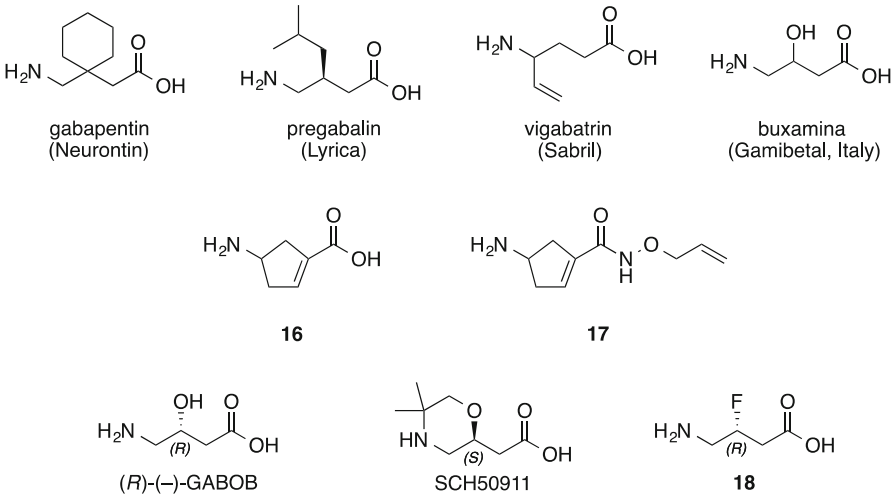
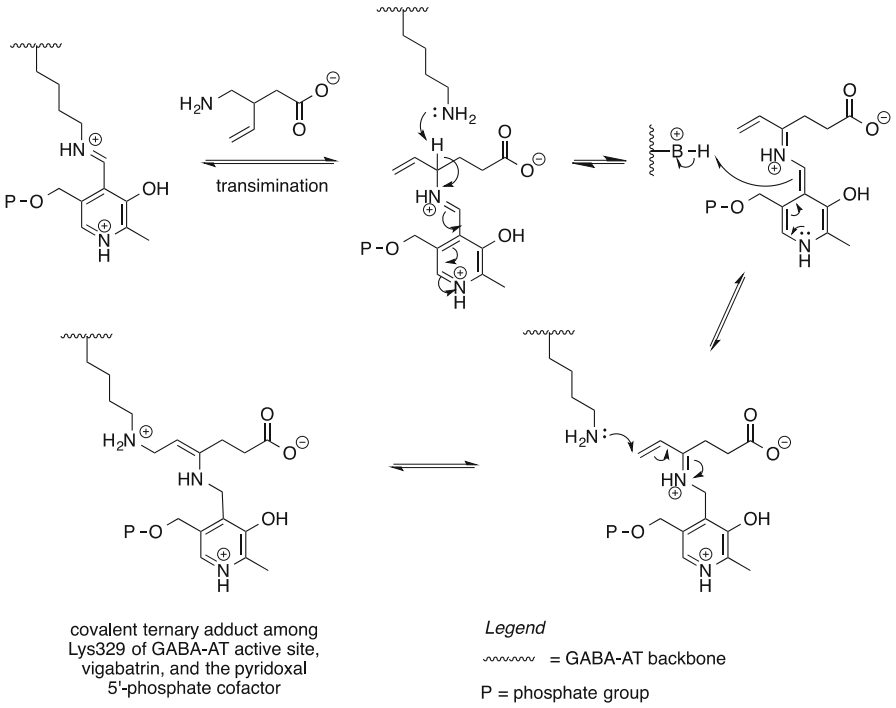


Fig. 2.5 GABA analogues and derivatives

understood. Subsequent investigations, along with the concurrent development of pregabalin, indicated that the multiple cellular effects of these drugs are mediated by a single predominant mechanism able to account for their efficacy as antiepileptic and analgesic drugs. A body of evidence demonstrated that the only high-affinity molecular target for gabapentin and pregabalin is the auxiliary alpha2-delta (α2-δ) protein subunit of voltage-gated calcium channels, or Ca_Vα2-δ (Sills 2006). These findings contributed to the contemporary pharmacological view that calcium channels and their control of neurotransmitter release are validated targets for analgesic drugs (Taylor 2009).

Vigabatrin (γ-vinyl-GABA) is currently used in over 60 countries as an effective therapy for drug-resistant epilepsy and against infantile spasms, either as an adjunctive treatment or for monotherapy. Although the pharmacologically active isomer is (*R*)-vigabatrin, the drug is sold as a racemic mixture. Vigabatrin exerts its antiepileptic effects by increasing the GABA concentration in the brain, thanks to its capability of crossing the BBB and non-competitively inhibiting GABA aminotransferase (GABA-AT), the enzyme that catabolizes the neurotransmitter. In particular, vigabatrin is a suicide inhibitor that forms a covalent ternary adduct with the active site Lys329 and the pyridoxal 5′-phosphate (PLP) cofactor of GABA-AT (Storici et al. 2004), owing to the presence in its structure of the vinyl group, which provides the opportunity of producing a strongly electrophilic (Michael acceptor) intermediate (Scheme 2.4).

The design and synthesis of cyclic compounds as conformationally restricted analogues of GABA (Fig. 2.5) did not lead to the expected results. Thus, compound **16**, which formally can be regarded as a dehydro-vigabatrin, elicited antagonist activity at the $GABA_C$ receptor and agonist activity at the $GABA_A$ receptor. Some agonist activity at the $GABA_B$ receptor could be finally obtained by modification of



Scheme 2.4 Mechanism of action of vigabatrin

the carboxylic acid group into a hydroxamate, such as **17**, but selectivity over the $GABA_A$ receptor was not achieved (Locock et al. 2013).

γ-Amino-β-hydroxybutyric acid is sold in Italy as a racemic mixture under the INN buxamina and the proprietary name Gamibetal (Fig. 2.5) as a treatment for idiophatic epilepsy and convulsive diseases in children. It is a hydroxy derivative of GABA that is physiologically present in the brain and is able to reach the CNS after oral administration. The stereochemistry of the hydroxy group deeply influences the activity of the single enantiomers, as the (*R*)-(–)-enantiomer [also referred to as (*R*)-(–)-GABOB, from **g**amma-**a**mino-**b**eta-hydr**o**xy**b**utyric acid] is a moderate potency $GABA_B$ receptor agonist, while the (*S*)-(+)-enantiomer [(*S*)-(+)-GABOB] is a $GABA_B$ partial agonist and an agonist of the $GABA_A$ receptor. That even subtle changes in the chemical structure of GABA and GABA analogues may have a pronounced impact on their pharmacological properties is demonstrated by the peculiar profile exhibited by SCH50911, where the amino and hydroxy groups of (*R*)-(–)-GABOB have been embodied in a morpholine ring with an (*S*)-stereochemistry, and (*R*)-3-fluoro-GABA (**18**), where the hydroxy group has been replaced by a fluoro atom, though retaining the same stereochemistry (Fig. 2.5). These compounds are active respectively as a $GABA_B$ antagonist and an agonist of the $GABA_A$ receptor (Froestl 2010; Brown et al. 2015).

2.2.4 GABA Bioisosteres

A major boost to the development of novel analogues of GABA and baclofen was given by the bioisosteric replacement of the carboxy group with sulfonic, phosphonic and phosphinic moieties. Although this approach mainly produced $GABA_B$ receptor antagonists, it offered the opportunity of enhancing the chemical diversity, to improve SAR knowledge, and to obtain a finer tuning of the pharmacological properties of GABA analogues. The first $GABA_B$ receptor antagonists, phaclofen (Kerr et al. 1987) and 2-hydroxysaclofen (Kerr et al. 1988), were discovered at the end of the 1980s by substituting a phosphonic acid or a sulfonic acid, respectively, for the carboxy group of baclofen and 2-hydroxybaclofen.

A few years later, it was shown that the (most) active isomers were (*R*)-(–)-phaclofen (Frydenvang et al. 1994) and (*S*)-(+)-2-hydroxysaclofen (Kerr et al. 1995) (Fig. 2.6). As for the latter compound, it should be noted that in spite of its (*S*)-(+) absolute stereochemistry, the spatial arrangements of hydroxy and *p*-chlorophenyl groups on the 3-carbon atom of the eutomer are the same as in (*R*)-(–)-GABOB and (*R*)-(–)-baclofen, which are both $GABA_B$ receptor agonists. Therefore, the shift from agonist to antagonist activity must be attributed to the replacement of the carboxy group with the phosphonic and sulfonic counterparts. Conversely, the phosphinic acid analogue of GABA, known as CGP27492, was a very potent and selective $GABA_B$ receptor agonist, with an $IC_{50} = 2$ nM (Froestl et al. 1995a).

Unfortunately, this compound proved to be inactive in in vivo assays and could not be developed into a marketed drug. Its tritiated derivative, [^{3}H]CGP27492 (APPA), has replaced [^{3}H]baclofen as a pharmacological tool.

These findings prompted further investigation of GABA analogues bearing the phosphinic acid group. Lesogaberan (AZD3355) is a fluorinated derivative of CGP27492 developed by AstraZeneca and characterized by potent agonist activity at *peripheral* $GABA_B$ receptors. Lesogaberan reduces transient lower oesophageal sphincter (LES) relaxations and reflux episodes in patients who have persistent GERD despite concomitant therapy with proton pump inhibitors (PPI). Accordingly, it has been proposed as a treatment for GERD not linked with the adverse CNS effects that characterize other $GABA_B$ receptor agonists, such as baclofen. However, different clinical trials have not yet definitely assessed the actual superiority of lesogaberan over placebo in patients with GERD symptoms partially responsive to PPI therapy (Shaheen et al. 2013; Miner et al. 2014).

In the search for new GABA analogues, the replacement of the carboxylic acid with a phosphinic acid offered the possibility of enhancing the chemical diversity by introducing alkyl substituents at the phosphorus atom. This approach enabled the effects of rising bulkiness of the ligands on affinity and/or functional activity to be examined. The methylphosphinic acid derivative CGP35024 (also known as SK&F97541) is a $GABA_B$ receptor agonist showing in vivo seven times more potent activity than (*R*)-(–)-baclofen against neuropathic pain at doses that do not cause sedation (Patel et al. 2001). Interestingly, it is also a micromolar antagonist at $GABA_C$ receptors. Its 2-hydroxy analogue, CGP44532, is still an agonist at the $GABA_B$ receptor, exhibiting muscle relaxant and antihyperalgesic activities in rats

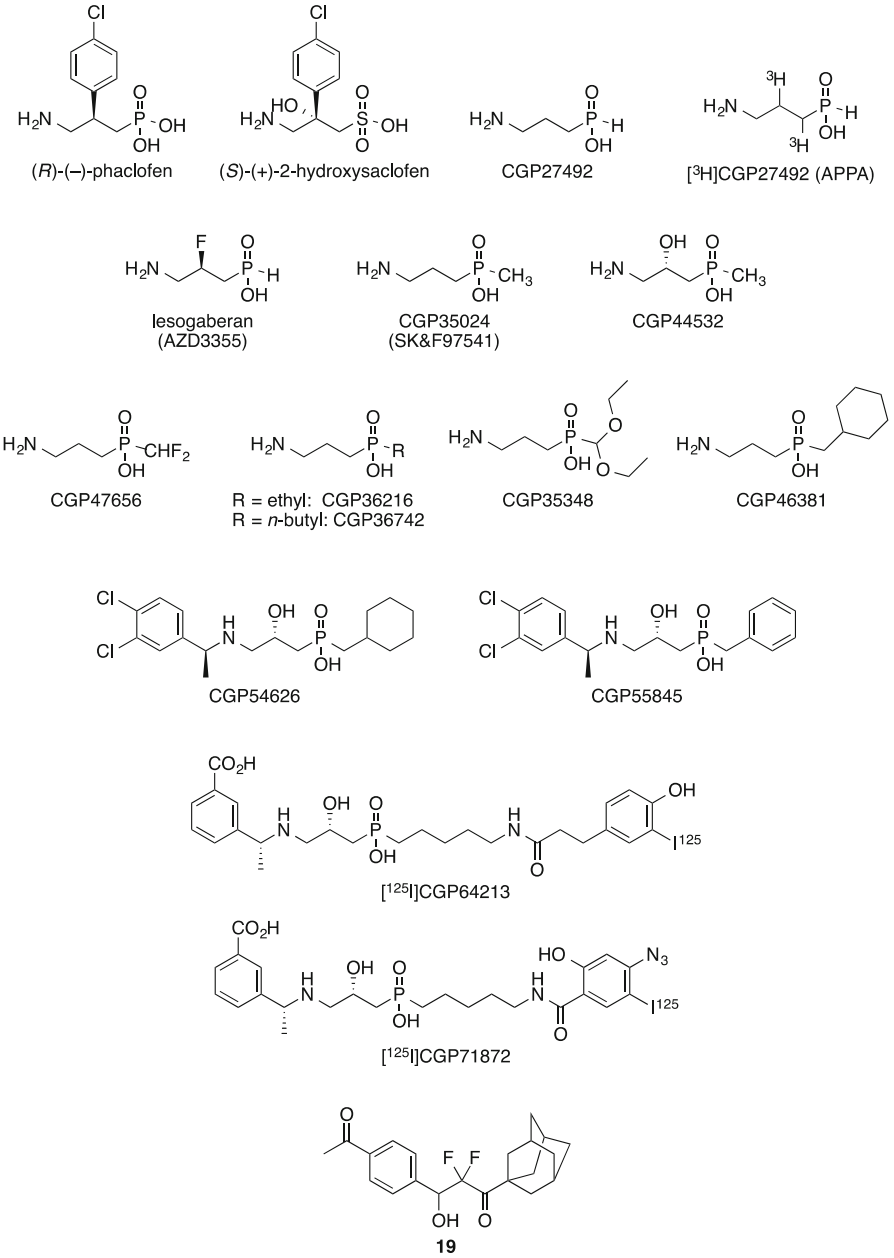
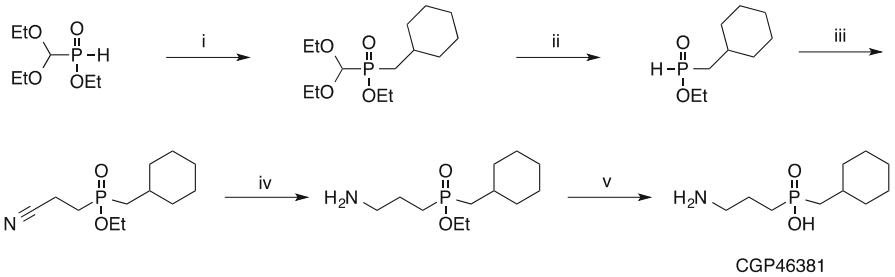


Fig. 2.6 Bioisosteric analogues of GABA and baclofen

after s.c. or oral administration (Enna et al. 1998). It has a longer duration of action and lower incidence of gastrointestinal and CNS side effects than baclofen. This very polar molecule (Log $P = -2.76$) is not subjected to liver metabolism in rat, dog and man, and shows 72 % bioavailability after p.o. administration in rats. Both of



Scheme 2.5 Reagents and conditions: *i*, (a) NaH, THF, rt, 2 h; (b) cyclohexylmethyl halide, reflux, 24 h; *ii*. (a) 4 M HCl, reflux, 24 h; (b) $ClCO_2Et$, Et_3N, DCM, 10 °C to rt, 2 h; *iii*. Na, EtOH, acrylonitrile, 10 °C to rt, 3 h; *iv*. H_2, Ra-Ni, NH_3, EtOH, 70–75 °C, 100 bar, 2 h; *v*. (a) 5 M HCl, reflux, 24 h; (b) propylene oxide, EtOH

its enantiomers are able to act as $GABA_C$ receptor antagonists at micromolar concentrations (Hinton et al. 2008).

A gradual increase in the dimension of the phosphinic acid substituent caused a rapid shift of the ligand profile from agonist to partial agonist, in the case of the difluoromethyl derivative CGP47656, to antagonist, as for the more sterically demanding homologue CGP36216, bearing an ethyl group on the phosphorus atom. Ligands with an acid moiety of still higher dimensions, such as CGP36742 (also known as SGS742), CGP35348 and CGP46381, possess micromolar antagonist activity at $GABA_B$ receptors together with the ability to cross the BBB (Froestl et al. 1995a). Although these results are not unexpected per se, because the practice of medicinal chemistry has taught us that increasing molecular complexity of a receptor ligand may result in agonist to antagonist inversion, what was surprising was the rapidity of this transition going from the methyl derivative to the ethyl homologue. As an example, the synthesis of CGP46381 (Froestl et al. 1995b) is highlighted in Scheme 2.5.

Additional structural modifications, involving the amino portion of the GABA molecule, led to the identification of nanomolar $GABA_B$ receptor antagonists. In particular, a representative group of benzyl derivatives, like compounds CGP54626 and CGP55845, are nanomolar affinity $GABA_B$ receptor antagonists (Asay and Boyd 2006). Some compounds were also developed as radioligands. The fluorescent radioligand [I^{125}]CGP64213 and the photoaffinity ligand [I^{125}]CGP71872 allowed the identification of $GABA_{B1a}$ and $GABA_{B1b}$ receptors in expression cloning studies (Froestl 2010).

Recently, the difluoromethyl ketone scaffold was identified as a novel surrogate for carboxylic acids and phosphinic acids in diverse classes of enzyme inhibitors (Baskakis et al. 2008; Reiter et al. 2000). When extended to the design of $GABA_B$ receptor ligands, this bioisosteric approach resulted in the discovery of a new family of micromolar $GABA_B$ receptor agonists, exemplified by compound **19**. This compound demonstrated activity in vitro as a $GABA_B$ receptor agonist selective over $GABA_A$ receptors, with major activity residing in the (+)-enantiomer. In rodent startle models, including plain acoustic startle and fear-potentiated startle, racemic **19** (36 mg/kg ip) exhibited anxiolytic effects at concentrations less than one order of magnitude greater than baclofen (Han et al. 2013).

2.3 Conclusions

Since the first synthesis of baclofen by Heinrich Keberle in 1962 and the identification by Norman Bowery of the baclofen-sensitive, bicuculline-insensitive $GABA_B$ receptor in 1980 (see Chap. 1 of this book), impressive research efforts in the field of the $GABA_B$ receptor have been carried out by several chemists and pharmacologists, manly working in mutual cooperation in an industrial setting. As outlined by Wolfgang Froestl in his seminal review (Froestl 2010): "There are more than 3000 entries in Scifinder on publications on nonmarketed ligands for $GABA_B$ receptors". This is the unequivocal proof of the substantial interest in this area, in view of the potential use of $GABA_B$ receptor ligands in CNS diseases, such as depression, cognition deficits and Down's syndrome ($GABA_B$ receptor antagonists), and schizophrenia ($GABA_B$ receptor agonists). In spite of this, the actual outcome in terms of drug development has been modest so far, considering the limited number of clinically useful drugs that have reached the market. To our knowledge, the most advanced $GABA_B$ receptor agonist Lesogaberan is currently in Phase 2 clinical trials for the treatment of GERD. On the other hand, a tremendous advance has been made in understanding the structure and the function of GABA and its receptors. The recent elucidation of the X-ray structure of the $GABA_B$ receptor, along with cocrystallized agonists and antagonists, represents a significant step forward in this area and a sound basis for the structure-based drug design of new ligands for this type of receptor. Thus, "the stage is set for future investigations on this receptor to determine if it can be as pharmacologically lucrative as the extensively exploited $GABA_A$ receptor" (Brown et al. 2015).

References

Abdel-Hafez, A. A., & Abdel-Wahab, B. A. (2008). 5-(4-Chlorophenyl)-5,6-dihydro-1,3-oxazepin-7(4H)-one derivatives as lipophilic cyclic analogues of baclofen: Design, synthesis, and neuropharmacological evaluation. *Bioorganic and Medicinal Chemistry, 16*, 7983–7991.

Asay, M. J., & Boyd, S. K. (2006). Characterization of the binding of [^{3}H]CGP54626 to GABABreceptors in the male bullfrog (*Rana catesbeiana*). *Brain Research, 1094*, 76–85.

Attia, M. I., Herdeis, C., & Bräuner-Osborne, H. (2013). $GABA_B$-agonistic activity of certain baclofen homologues. *Molecules, 18*, 10266–10284.

Awapara, J., Landua, A. J., Fuerst, R., & Seale, B. (1950). Free γ-aminobutyric acid in brain. *Journal of Biological Chemistry, 187*(1), 35–39.

Baskakis, C., Magrioti, V., Cotton, N., Stephens, D., Constantinou-Kokotou, V., Dennis, E. A., et al. (2008). Synthesis of polyfluoro ketones for selective inhibition of human phospholipase A2 enzymes. *Journal of Medicinal Chemistry, 51*, 8027–8037.

Berthelot, P., Vaccher, C., Musadad, A., Flouquet, N., Debaert, M., & Luyckx, M. (1987). Synthesis and pharmacological evaluation of γ-aminobutyric acid analogues. New ligands for $GABA_B$ sites. *Journal of Medicinal Chemistry, 30*, 743–746.

Brown, K. M., Roy, K. K., Hockerman, G. H., Doerksen, R. J., & Colby, D. A. (2015). Activation of the γ-aminobutyric acid type B ($GABA_B$) receptor by agonists and positive allosteric modulators. *Journal of Medicinal Chemistry, 58*(16), 6336–6347.

Costantino, G., Macchiarulo, A., Guadix, A. E., & Pellicciari, R. (2001). QSAR and molecular modeling studies of baclofen analogues as $gaba_b$ agonists. Insights into the role of the aromatic moiety in GABAB binding and activation. *Journal of Medicinal Chemistry, 44*, 1827–1832.

Deguchi, Y., Inabe, K., Tomiyasu, K., Nozawa, K., Yamada, S., & Kimura, R. (1995). Study on brain interstitial fluid distribution and blood-brain barrier transport of baclofen in rats by microdialysis. *Pharmaceutical Research, 12*, 1838–1844.

Enna, S. J., Harstad, E. B., & McCarson, K. E. (1998). Regulation of neurokinin-1 receptor expression by GABAB receptor agonists. *Life Sciences, 62*, 1525–1530.

Froestl, W. (2010). Chemistry and pharmacology of $GABA_B$ receptor ligands. In T. P. Blackburn & S. J. Enna (Eds.), *$GABA_B$ receptor pharmacology: a tribute to Norman Bowery* (Vol. 58, pp. 19–62). New York: Academic.

Froestl, W. (2011). An historical perspective on GABAergic drugs. *Future Medicinal Chemistry, 3*(2), 163–175.

Froestl, W., Mickel, S. J., Hall, R. G., von Sprechler, G., Strub, D., Baumann, P. A., et al. (1995a). Phosphinic acid analogues of GABA. 1. New potent and selective $GABA_B$ agonists. *Journal of Medicinal Chemistry, 38*(17), 3297–3312.

Froestl, W., Mickel, S. J., von Sprecher, G., Diel, P. J., Hall, R. G., Maier, L., et al. (1995b). Phosphinic acid analogues of GABA. 1. Selective, orally active $GABA_B$ antagonists. *Journal of Medicinal Chemistry, 38*, 3313–3331.

Frydenvang, K., Hansen, J. J., Krogsgaard-Larsen, P., Mitrovic, A., Tran, H., Drew, C. A., et al. (1994). $GABA_B$ antagonists:resolution, absolute stereochemistry, and pharmacology of (*R*)- and (*S*)-phaclofen. *Chirality, 6*, 583–589.

Han, C., Salyer, A. E., Kim, E. H., Jiang, X., Jarrard, R. E., Powers, M. S., et al. (2013). Evaluation of difluoromethylketones as agonists of the γ-aminobutyric acid type B (GABAB) receptor. *Journal of Medicinal Chemistry, 56*, 2456–2465.

Hinton, T., Chebib, M., & Johnston, G. A. R. (2008). Enantioselective actions of 4-amino-3-hydroxybutanoicacid and (3-amino-2-hydroxypropyl)methylphosphinic acid at recombinant $GABA_C$ receptors. *Bioorganic and Medicinal Chemistry Letters, 18*, 402–404.

Ji, L., Ma, Y., Li, J., Zhang, L., & Zhang, L. (2009). An efficient synthesis of (*R*)- and (*S*)-baclofen via desymmetrization. *Tetrahedron Letters, 50*, 6166–6168.

Karla, R., Ebert, B., Thorkildsen, C., Herdeis, C., Johansen, T. N., Nielsen, B., et al. (1999). Synthesis and pharmacology of the baclofen homologues 5-amino-4-(4-chlorophenyl)pentanoic acid and the *R*- and *S*-enantiomers of 5-amino-3-(4-chlorophenyl)pentanoic acid. *Journal of Medicinal Chemistry, 42*, 2053–2059.

Kato, K., Zhang, M.-R., & Suzuki, K. (2009). Synthesis of (R, S)-[4-11C]baclofen via Michael addition of nitromethane labeled with short-lived 11C. *Bioorganic and Medicinal Chemistry Letters, 19*, 6222–6224.

Keberle, H., Faigle, J. W., & Wilhelm, M. (1968, April 11). Procedure for the preparation of new aminoacids. Swiss Patent 449046. Priority: 09 Jul 1963, *Chemical Abstract, 69*, 106273f.

Kerr, D. I., Ong, J., Doolette, D. J., Schafer, K., & Prager, R. H. (1995). The (*S*)-enantiomer of 2-hydroxysaclofen is the active GABAB receptor antagonist in central and peripheral preparations. *European Journal of Pharmacology, 287*, 185–189.

Kerr, D. I. B., Ong, J., Johnston, G. A. R., Abbenante, J., & Prager, R. H. (1988). 2-Hydroxysaclofen: An improved antagonist at central and peripheral GABAB receptors. *Neuroscience Letters, 92*, 92–96.

Kerr, D. I. B., Ong, J., Prager, R. H., Gynther, B. D., & Curtis, D. R. (1987). Phaclofen: A peripheral and central baclofen antagonist. *Brain Research, 405*, 150–154.

Krnjevic, K., & Schwartz, S. (1967). The action of gamma-aminobutyric acid on cortical neurons. *Experimental Brain Research, 3*(4), 320–336.

Lal, R., Sukbuntherng, J., Tai, E. H. L., Upadhyay, S., Yao, F., Warren, M. S., et al. (2009). Arbaclofen placarbil, a novel R-baclofen prodrug: Improved absorption, distribution, metabolism, and elimination properties compared with R-baclofen. *Journal of Pharmacology and Experimental Therapeutics, 330*(3), 911–921.

Lapin, I. (2001). Phenibut (beta-phenyl-GABA): A tranquilizer and nootropic drug. *CNS Drug Reviews, 7*, 471–481.

Locock, K. E. S., Yamamoto, I., Tran, P., Hanrahan, J. R., Chebib, M., Johnston, G. A. R., et al. (2013). γ-Aminobutyric acid(C) (GABAC)selective antagonists derived from the bioisosteric modification of 4-aminocyclopent-1-enecarboxylic acid: Amides and hydroxamates. *Journal of Medicinal Chemistry, 56*, 5626–5630.

Mann, A., Boulanger, T., Brandau, B., Durant, F., Evrard, G., Heaulme, M., et al. (1991). Synthesis and biochemical evaluation of baclofen analogues locked in the baclofen solid-state conformation. *Journal of Medicinal Chemistry, 34*, 1307–1313.

Miner, P. B., Jr., Silberg, D. G., Ruth, M., Miller, F., & Pandolfino, J. (2014). Dose-dependent effects of lesogaberan on reflux measures in patients with refractory gastroesophageal reflux disease:Arandomized,placebo-controlledstudy.*BMCGastroenterology,14*,188.doi:10.1186/1471-230X-14-188.

Muhyaddin, M., Roberts, P. J., & Woodruff, G. N. (1982). Presynaptic γ-aminobutyric acid receptors in the rat anococcygeus muscle and their antagonism by 5-aminovaleric acid. *British Journal of Pharmacology, 77*, 163–168.

Ong, J., Kerr, D. I., Doolette, D. J., Duke, R. K., Mewett, K. N., Allen, R. D., et al. (1993). (*R*)-(–)-beta-phenyl-GABA is a full agonist at GABAB receptors in brain slices but a partial agonist in the ileum. *European Journal of Pharmacology, 233*, 169–172.

Patel, S., Naeem, S., Kesingland, A., Froestl, W., Capogna, M., Urban, L., et al. (2001). The effects of GABAB agonists and gabapentin on mechanical hyperalgesia in models of neuropathic and inflammatory pain in rats. *Pain, 90*, 217–226.

Pirard, B., Carrupt, P.-A., Testa, B., Tsai, R.-S., Berthelot, P., Vaccher, C., et al. (1995). Structure-affinity relationships of baclofen and 3-heteroaromatic analogues. *Bioorganic and Medicinal Chemistry, 3*, 1537–1545.

Qin, M., Huang, T., Kader, M., Krych, L., Xia, Z., Burlin, T., et al. (2015). R-Baclofen reverses a social behavior deficit and elevated protein synthesis in a mouse model of fragile X syndrome. *International Journal of Neuropsychopharmacology, 18*, 1–13.

Reiter, L. A., Martinelli, G. J., Reeves, L. A., & Mitchell, P. G. (2000). Difluoroketones as inhibitors of matrix metalloprotease-13. *Bioorganic and Medicinal Chemistry Letters, 10*, 1581–1584.

Roberts, E., & Frankel, S. (1950). γ-Aminobutyric acid inbrain: Its formation from glutamic acid. *Journal of Biological Chemistry, 187*(1), 55–63.

Shaheen, N. J., Denison, H., Björck, K., Karlsson, M., & Silberg, D. G. (2013). Efficacy and safety of lesogaberan in gastro-oesophageal reflux disease: A randomised controlled trial. *Gut, 62*, 1248–1255.

Sills, G. J. (2006). The mechanisms of action of gabapentin and pregabalin. *Current Opinion in Pharmacology, 6*, 108–113.

Storici, P., De Biase, D., Bossa, F., Bruno, S., Mozzarelli, A., Peneff, C., et al. (2004). Structures of γ-aminobutyric acid (GABA) aminotransferase, a pyridoxal 5'-phosphate, and [2Fe-2S] cluster-containing enzyme, complexed with γ-ethynyl-GABA and with the antiepilepsy drug vigabatrin. *Journal of Biochemistry, 279*, 363–373.

Swahn, C.-G., Beving, H., & Sedvall, G. J. (1978). Synthesis of 4-amino-3-p-chlorophenyl-butyric acid-2, 2, 4, 4-2H4 (baclofen). *Journal of Labelled Compounds and Radiopharmaceuticals, 15*(Suppl. S1), 739–745.

Taylor, C. P. (2009). Mechanisms of analgesia by gabapentin and pregabalin – calcium channel α2-δ[Ca_Vα2-δ] ligands. *Pain, 142*, 13–16.

Thakur, V. V., Paraskar, A. S., & Sudalai, A. (2007). Asymmetric synthesis of (*R*)-baclofen *via* asymmetric dihydroxylation. *Indian Journal of Chemistry B*, *46B*, 326–330 and references cited therein.

Tilinsky, J., & Gammill, R. B. (1994). The chemistry and pharmacology of $GABA_A$ and $GABA_B$ ligands. *Current Medicinal Chemistry, 3*, 226–253.

Udenfriend, S. (1950). Identification of γ-aminobutyric acid in brain by the isotopederivative method. *Journal of Biological Chemistry, 187*(1), 65–69.

van Bree, J. B., Audus, K. L., & Borchardt, R. T. (1988). Carrier-mediated transport of baclofen across monolayers of bovine brain endothelial cells in primary culture. *Pharmaceutical Research, 5*, 369–371.

Varala, R., & Adapa, S. R. (2006). Novel approach to the synthesis of (*R,S*)-baclofen via Pd(II)-bipyridine–catalyzed conjugative addition. *Synthetic Communications*, *36*, 3743–3747 and references cited therein.

Xu, F., Peng, G., Phan, T., Dilip, U., Chen, J. L., Chernov-Rogan, T., et al. (2011). Discovery of a novel potent $GABA_B$ receptor agonist. *Bioorganic and Medicinal Chemistry Letters, 21*, 6582–6585.

Chapter 3
The Allosteric Modulation of the $GABA_B$ Receptor: A Medicinal Chemistry Perspective

Claudia Mugnaini and Federico Corelli

Abstract Since its cloning, the $GABA_B$ receptor has progressively become a target for potential drugs to be used in the treatment of a wide range of pathological conditions such as spasticity, pain, drug addiction, epilepsy, anxiety, mood disorders. Baclofen, the only $GABA_B$ receptor agonist currently approved for the treatment of muscle rigidity and spasm associated with multiple sclerosis or spinal cord injury, suffers from a number of side effects which hamper its clinical use. As a result, there has been a strong impetus for the development of positive allosteric modulators that modulate the physiological mechanisms of GABAergic regulation and are expected to have a much lower side effect potential than orthosteric ligands. Herein, the major structural classes of $GABA_B$ allosteric modulators are described with an emphasis on structure–activity relationships (SAR) and synthesis of the main representatives of each class. Medicinal chemistry strategies to overcome issues related to allosteric modulators development are also discussed.

Keywords $GABA_B$ positive allosteric modulators • $GABA_B$ NAMs • SAR • Baclofen • Spasticity • Drug addiction

3.1 Introduction

γ-Aminobutyric acid (GABA) (Fig. 3.1) is the most important and abundant inhibitory neurotransmitter in the mammalian brain (Krnjević and Schwartz 1967). It is a small, neutral amino acid characterized by high hydrophilicity and water solubility which exerts its function via ionotropic ($GABA_A$ and $GABA_C$) and metabotropic ($GABA_B$) receptors.

The $GABA_B$ receptor was cloned in 1997 (Kaupmann et al. 1997), 17 years after its identification (Bowery et al. 1980) and 35 years after the first synthesis of baclofen (Keberle et al. 1969) (Fig. 3.1), a lipophilic GABA derivative endowed

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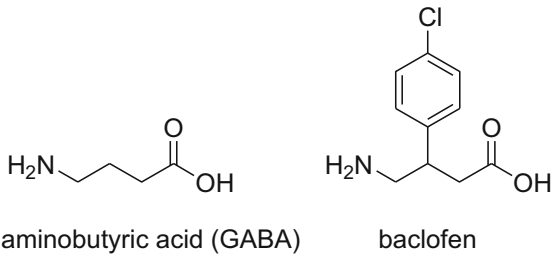


Fig. 3.1 Chemical structure of γ-aminobutyric acid (GABA) and baclofen

with high affinity and strong intrinsic activity for this receptor (see Chaps. 1 and 17 of this book). Since then, $GABA_B$ receptor has become an intriguing target for developing orthosteric as well as allosteric ligands as potential drugs for the treatment of a wide range of pathological conditions.

The structure and function of $GABA_B$ receptors are covered extensively in Chaps. 4 and 6 of this book. $GABA_B$ receptors have been identified not only in the central nervous system but also in the peripheral nervous system and organs (see Chap. 5 of this book). They are involved in several physiological and pathophysiological events, such as spasticity, pain, cognitive function, anxiety, mood disorders, epilepsy, and drug addiction (Benarroch 2012; Froestl 2010a; Balerio and Rubio 2002; Kumar et al. 2013; Philips and Reed 2014). A growing body of evidence showed that they are also involved in tumor development and tumor cell proliferation and migration, in accordance to the observation that neurotransmitters have modulatory roles in tumor cells (Jiang et al. 2012).

Baclofen (Fig. 3.1) is the only $GABA_B$ receptor agonist currently approved for clinical use in the treatment of muscle rigidity and spasm associated with multiple sclerosis or spinal cord injury (Sachais et al. 1977; for details about the clinical use of baclofen, see Froestl 2010) and it has also shown efficacy toward overactive bladder (Abraham and Goldman 2015), gastroesophageal reflux disease (Lehmann et al. 2010), addiction disorders (Agabio and Colombo 2014; Haney et al. 2006), and anxiety (Cryan and Kaupmann 2005) (see also Chap. 2 of this book). Some data are also available describing baclofen able to reduce the incidence of some carcinogen-induced gastrointestinal cancers in rats (Tatsuta et al. 1990) as well as human hepatocarcinoma cell growth (Wang et al. 2008) (see also Chaps. 10–17 of this book). However, the clinical use of baclofen is hampered by a number of side effects including sedation, dizziness, nausea, muscle weakness, and mental confusion which appear when the drug, owing to its poor brain penetration, is administered in high doses. This has been largely overcome, in the treatment of spasticity, by the introduction of intrathecal administration. Nevertheless, the focal injection of the agonist cannot be used in the treatment of pathological states such as drug addiction, pain, depression, and anxiety.

This consciousness has provided a strong impetus for the development of positive allosteric modulators that potentiate $GABA_B$ receptor-mediated actions. In contrast to baclofen and other $GABA_B$ agonists, that activate constantly and everywhere the receptor, positive allosteric modulators are indeed expected to modulate the

physiological mechanisms of GABAergic regulation, enhancing receptor activity only when and where needed, that is when and where GABA is released to act on the $GABA_B$ receptor. On the basis of their mechanism of action, positive allosteric modulators are expected to have a much lower side effect potential than orthosteric ligands (see Chap. 18 of this book).

This chapter will focus exclusively on allosteric modulators of $GABA_B$ receptor with an effort to group known ligands according to their chemical structure. This approach is expected to help medicinal chemists working in the field to explore the chemical space around *old* scaffolds and eventually to design *new* ones on the basis of established structure–activity relationships (SAR).

3.2 Positive Allosteric Modulators of $GABA_B$ Receptors

3.2.1 CGP7930 and CGP13501

The first $GABA_B$ receptor positive allosteric modulators were discovered by Urwyler and colleagues at the Novartis Institute for BioMedical Research in Basel in 2001, by means of a high-throughput screening (Urwyler et al. 2001). Compound CGP7930, structurally close to the general anesthetic agent propofol, and its aldehyde analog CGP13501 (Fig. 3.2) potentiated GABA-induced signals at low micromolar concentrations without stimulating [^{35}S]GTPγS binding in the absence of GABA (Urwyler et al. 2001; Adams and Lawrence 2007).

CGP7930 increased both agonist potency and maximal effect, which was shown to be dose dependent. Only in 2004, Pin and colleagues, using a more sensitive assay, demonstrated that CGP7930 acts as a partial $GABA_B$ receptor agonist, and thus can be classified as an ago-allosteric modulator. Moreover, they were able to localize its binding site on the heptahelical domain of the $GABA_{B2}$ subunit (Binet et al. 2004; Pin and Prézeau 2007). Besides the ability to lower the drug-seeking behavior with substances such as alcohol, nicotine, and cocaine, CGP7930 showed in vivo antidepressant and anxiolytic properties.

The synthesis of CGP13501 and CGP7930 was accomplished starting from propofol (Scheme 3.1), which on treatment with formaldehyde in the presence of base

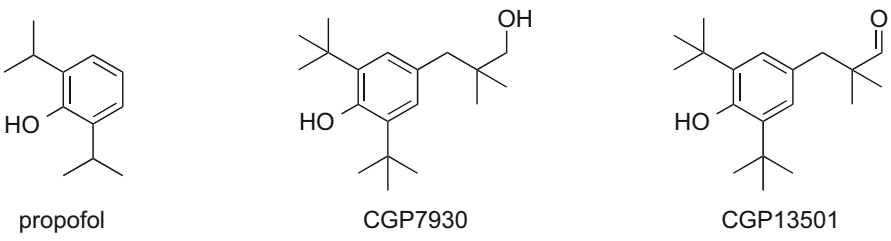
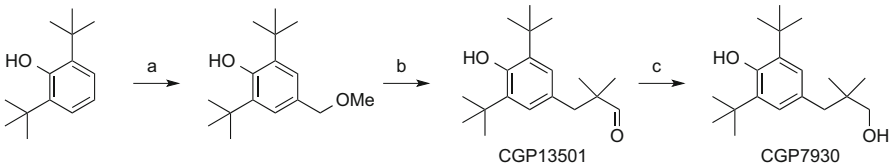


Fig. 3.2 Chemical structure of CGP7930 and CGP13501



Scheme 3.1 Reagents and conditions: (*a*) CH_2O, KOH, MeOH, reflux; (*b*) 2-methylpropanal, KOH, MeOH, 65 °C; (*c*) $NaBH_4$, EtOH, reflux

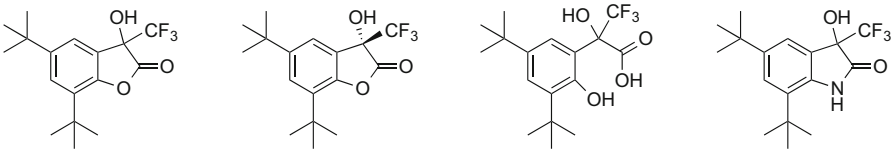


Fig. 3.3 Chemical structure of *rac*-BHFF and (+)-BHFF

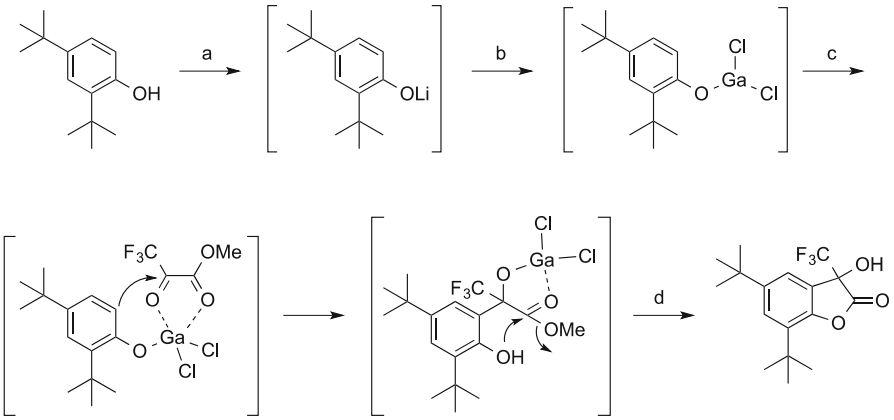
was converted into the corresponding 4-methoxymethyl derivative (Kharasch and Joshi 1957). Subsequent reaction with 2-methylpropanal gave CGP13501, which was then reduced in almost quantitative yield to CGP7930 (Kerr et al. 2006).

3.2.2 rac-*BHFF*

Scientists at Hoffmann-La Roche took up the structure of CGP7930 and elaborated it further, synthesizing novel fluorinated 2-hydroxypropionic acid derivatives and their lactone analogs 3-hydroxybenzofuran-2-ones *rac*-BHFF and (+)-BHFF (Fig. 3.3) (Malherbe et al. 2007; Alker et al. 2008).

rac-BHFF increased the potency and the efficacy with which GABA stimulated [^{35}S]GTPγS binding to membrane preparations from cells enhancing, at 0.3 μM, the EC_{50} of GABA in recombinant cells more than 15-fold. The most potent compound (+)-BHFF, which represents the first example of stereoselectivity at the $GABA_B$ allosteric binding site, increased this value by a factor of 87-fold. During pharmacokinetic studies in mouse plasma after p.o. administration of *rac*-BHFF, only the hydrolyzed form of BHFHP could be measured because quantitative hydrolysis of *rac*-BHFF under the analytical conditions occurred. As *rac*-BHFF and its hydroxy-acid BHFHP proved to be equipotent as enhancers at $GABA_B$ receptor, it was assumed that both compounds may be present in vivo because of rapid interconversion and may contribute to the observed effects in vivo. Isosteric replacement of oxygen atom by a NH group led to a hydrolytically stable lactam analog of BHFF, encoded BHFI (Malherbe et al. 2008).

In spite of the challenging structure of *rac*-BHFF, showing a benzofuran ring crowded with diverse functional groups, its synthesis could be efficiently performed



Scheme 3.2 Reagents and conditions: (*a*) *n*-BuLi, THF, −70 to 20 °C; (*b*) $GaCl_3$, DCE, −10 to 80 °C; (*c*) CF_3COCO_2Me, DCE, 0–20 °C; (*d*) DCE, 28–80 °C

through a gallium(III) chloride-mediated one-pot procedure (Scheme 3.2). Thus, the gallium phenolate obtained from 2,4-di-*tert*-butylphenol was reacted with the highly electrophilic methyl trifluoropyruvate to give regiospecifically an aldol-type intermediate, which subsequently underwent lactonization to *rac*-BHFF. The racemic compound was resolved into the two enantiomers by chiral-phase HPLC and the *R* and *S* configuration could be assigned to the dextrorotatory and levorotatory isomers, respectively, on the basis of X-ray crystallographic analysis of a suitable derivative (Alker et al. 2008).

3.2.3 Arylalkylamines

A variety of phenylalkylamines, including fendiline, a coronary vasodilator, are potent allosteric modulators at extracellular Ca^{2+}-sensing receptors. In 2002 three arylalkylamines (fendiline, prenylamine, and F551) were reported by Kerr and Ong as new $GABA_B$ receptor positive allosteric modulators (Kerr et al. 2002). These compounds showed little or no hyperpolarizing response in the absence of baclofen, but potentiated responses to baclofen and produced a leftward shift of the baclofen concentration–response curve, with a marked increase in the maximal hyperpolarization obtained with baclofen alone, indicative of positive allosteric modulation at $GABA_B$ receptors. Nevertheless, in 2004 Urwyler et al. demonstrated that these compounds are not allosteric modulators of $GABA_B$ receptors. They proposed, instead, that these phenylalkylamines could act at distinct sites in the complex circuitry in brain tissue slice preparations, possibly even at different cells ("downstream effects") or, alternatively, at the effector level. In fact, many examples of "receptor crosstalk" have been documented (Urwyler et al. 2004).

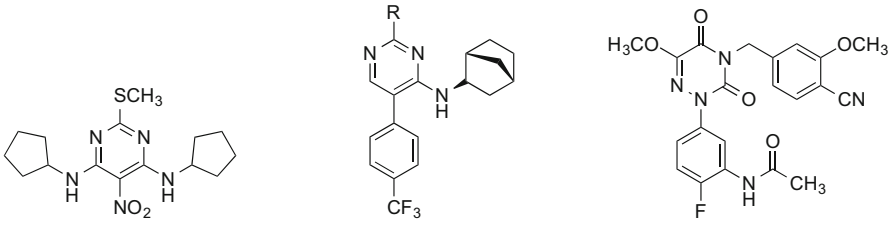
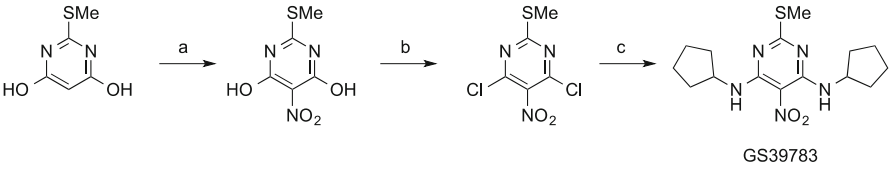


Fig. 3.4 Chemical structure of pyrimidines and related six-membered heterocyclic analogs



Scheme 3.3 Reagents and conditions: (*a*) HNO_3, H_2SO_4, 0–5 °C; (*b*) $POCl_3$, *N*,*N*-diethylaniline, reflux; (*c*) cyclopentylamine, EtOH, reflux

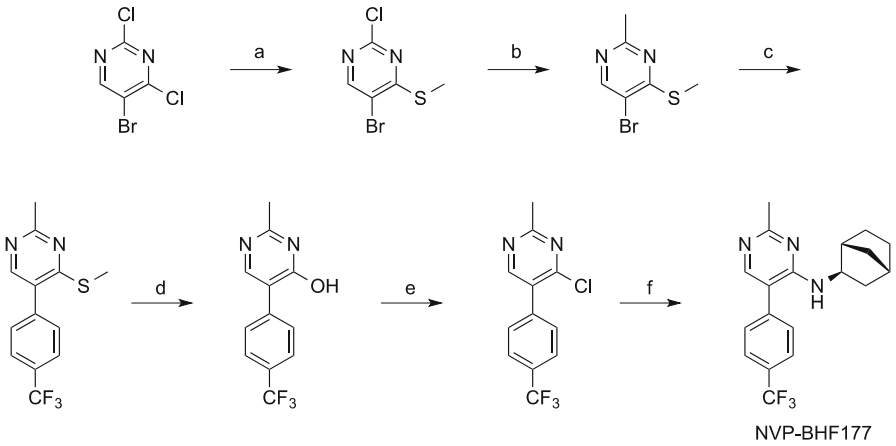
3.2.4 Pyrimidines and Related Six-Membered Heterocyclic Analogs

In 2003, GS39783 (Fig. 3.4) and structurally related compounds were described as novel and more potent positive allosteric modulators of the $GABA_B$ receptor (Urwyler et al. 2003).

GS39783 was synthesized in a straightforward manner (Scheme 3.3) by nitration of 2-methylthio-4,6-pyrimidinedione, followed by chlorination and displacement of both chlorine atoms with an excess of cyclopentylamine (Fisher 1972).

Like CGP7930, GS39783 acted through a dual mechanism, by enhancing at the same time the affinity and the maximal efficacy of GABA by approximately eight-fold and twofold, respectively. It showed anxiolytic-like effects in the elevated plus maze in rats and the elevated zero maze in mice and rats (Cryan et al. 2004) and decreased anxiety in the light–dark box, but it did not show any effects in the forced swim test (Mombereau et al. 2004) (see also Chaps. 12 and 18 of this book). Moreover, it reduced alcohol self-administration in alcohol-preferring rats (Orrù et al. 2005; Maccioni et al. 2012) and attenuated the reward facilitating effects of cocaine (Slattery et al. 2005) and nicotine (Paterson et al. 2008) in rats (see also Chaps. 14 and 15 of this book). Through point mutations, Novartis scientists were able to locate precisely its binding site in the sixth transmembrane domain of the $GABA_{B2}$ receptor (Dupuis et al. 2006).

In order to obtain molecules devoid of genotoxicity, the chemical structure of GS39783 was further elaborated by synthesizing a number of trisubstituted



Scheme 3.4 Reagents and conditions: (*a*) MeSNa, THF, rt; (*b*) i. HI 57 %, rt; ii. MeZnCl, $Pd(PPh_3)_4$, THF, rt to 60 °C; (*c*) 4-(trifluoromethyl)benzeneboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, EtOH, toluene, water, 110 °C; (*d*) HCl 37 %, MeOH, reflux; (*e*) $POCl_3$, cat. DMF, 80 °C; (*f*) *exo*-2-aminonorbornane, THF, 80 °C

pyrimidine derivatives in which the nitro group was replaced by a 4-trifluoromethylphenyl group and one of the two cyclopentylamino moieties was removed (Fig. 3.3) (Floersheim et al. 2006). As a means to introduce molecular diversity at position 4 of the pyrimidine ring at a late stage of the synthesis, a seven-step procedure was developed, starting from the commercially available 5-bromo-2,4-dichloropyrimidine (Scheme 3.4).

Treatment of this trihalogenated pyrimidine with sodium methanethiolate in THF at room temperature resulted in the regioselective displacement of the chlorine atom in position 4, leading to 5-bromo-2-chloro-4-methylthiopyrimidine. This was subjected to halogen exchange to give the corresponding 2-iodo derivative that in turn underwent a Negishi cross coupling with methylzinc chloride to afford the 2-methylpyrimidine intermediate, followed by reaction with 4-trifluoromethylbenzeneboronic acid under the classical conditions of the Suzuki–Miyaura reaction. In order to enhance its reactivity toward nucleophiles, this intermediate was first oxidized to the methanesulfonyl analog, which, however, reacted cleanly with only few amines. Therefore, the methylthio derivative was hydrolyzed to the 4-hydroxypyrimidine (4-pyrimidinone) derivative, which was then transformed into the corresponding 4-chloro compound. This key intermediate was reacted with a variety of amines to yield a small library of 4-aminosubstituted compounds (Guery et al. 2007). Among them, NVP-BHF177, bearing a methyl group at the 2-position and the *exo*-2-norbornanylamino group at the 4-position of the pyrimidine ring, proved to be devoid of in vitro genotoxicity and showed antinicotine and antialcohol effects as well as anxiolytic properties in the mouse stress-induced hyperthermia test (Paterson et al. 2008; Maccioni et al. 2009; Vlachou et al. 2011; Li et al. 2015).

In 2008, Addex Therapeutics patented more than 300 novel triazinedione derivatives identified from a high-throughput screening campaign of their corporate chemical library followed by lead optimization process (Riguet et al. 2008).

Among all the compounds, which were evaluated in a [^{35}S]GTPγS binding assay in rat cortical membranes, 23 compounds displaying $EC_{50} < 100$ nM were selected for additional evaluation in different animal models of anxiety and pain. ADX71943 (Fig. 3.4), a potent and selective $GABA_B$ receptor positive allosteric modulator endowed with a peripheral mode of action, was initially chosen for preclinical development, but due to its inadequate safety profile, it was further characterized as a pharmacological tool compound. ADX71943 showed consistent and target-related efficacy in tests of disorders that have a significant peripheral component (acute and chronic pain), while having no effect in those associated with centrally mediated anxiety-like reactivity and side effects (Kalinichev et al. 2014a). ADX71441, a triazinedione whose exact structure has not been disclosed so far, demonstrated excellent preclinical efficacy and tolerability in several rodent models of pain, anxiety, addiction (Hwa et al. 2014), and overactive bladder (Kalinichev et al. 2014b) and has also proven efficacy in a genetic model of Charcot–Marie–Tooth Type 1A disease (see also Chap. 18 of this book). It is therefore in the Addex pipeline as a phase 1 clinical candidate.

3.2.5 Quinolines and Bicyclic Congeners

Quinolines **1** (Fig. 3.5) (Malherbe et al. 2006a), 3-methanesulfonylquinolines **2** (Malherbe et al. 2006b), and thienopyridine **3** (Malherbe et al. 2006c) are representative molecules of new structural classes presented by Hoffmann-La Roche in 2006 as $GABA_B$ receptor positive allosteric modulators potentially useful in the treatment of CNS disorders.

Three years later, AstraZeneca presented a number of substituted quinolines, structurally correlated to those described by Hoffmann-La Roche (i.e., compound **4**, Fig. 3.5) (Cheng and Karle 2009), more than 100 substituted xanthines (i.e., compound **5**) (Cheng et al. 2008), and some examples of substituted pteridines (i.e., compound **6**) (Cheng et al. 2009). All of these compounds showed EC_{50}values in the submicromolar range in the [^{35}S]GTPγS binding test and were potentially useful in the treatment of gastroesophageal disease (GERD), irritable bowel syndrome (IBS), and other gastrointestinal pathological states.

The synthesis of representative compounds **1**, **5**, and **6** is described in Schemes 3.5, 3.6, and 3.7, respectively. Starting from 4-bromoaniline, the suitably substituted benzophenone derivative was prepared and submitted to a Friedländer reaction for the construction of the quinoline ring. Substitution of the bromine atom of the quinoline by piperidine in a Pd-catalyzed amination reaction gave compound **1** (Scheme 3.5).

For the synthesis of **5**, the key intermediate ethyl 4-amino-2-ethyl-1-methylimidazole-5-carboxylate was prepared through a five-step procedure from propionitrile and then converted into a ureido derivative by acylation of its amino group with 3,4-dichlorobenzylisocyanate. Sodium methoxide-catalyzed intramolecular amidation allowed to install the xanthine ring, which was in turn alkylated with 1-bromopinacolone to yield **5** (Scheme 3.6).

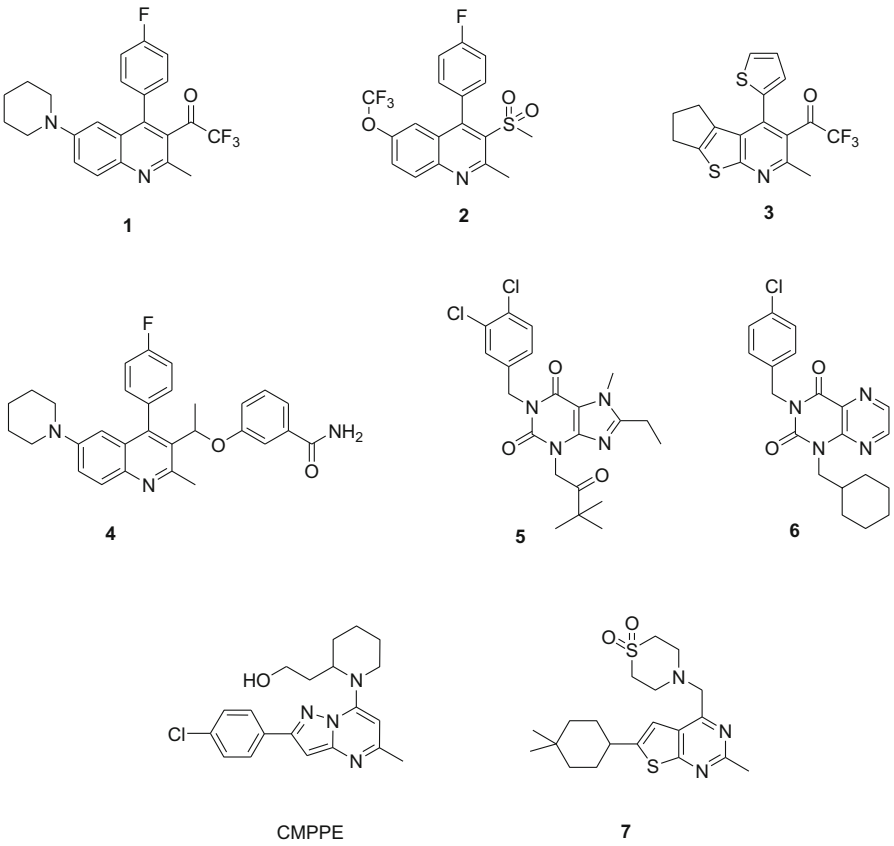
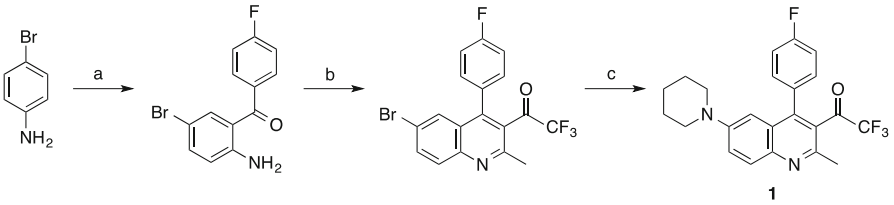


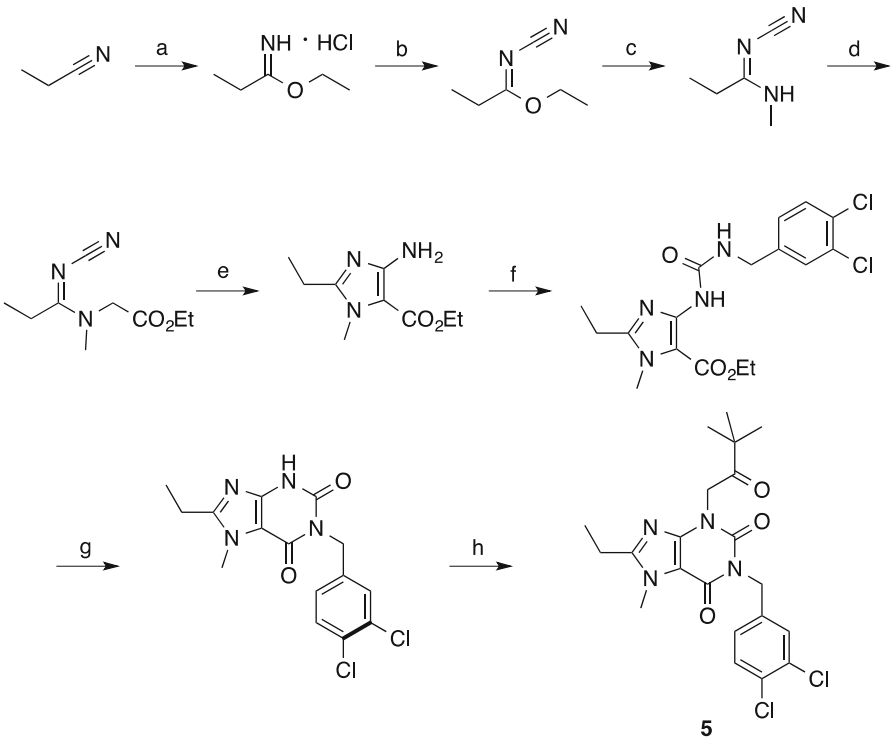
Fig. 3.5 Chemical structures of quinolines and bicylic congeners



Scheme 3.5 Reagents and conditions: (*a*) 4-fluorobenzonitrile, $AlCl_3$, BCl_3, DCE, reflux; (*b*) 1,1,1-trifluoro-2,4-pentanedione, $NaAuCl_4$, 2-propanol, reflux; (*c*) $Pd_2(dba)_3$, BINAP, Cs_2CO_3, dioxane, *t*-BuOH, 120 °C

The pteridine derivative **6** was synthesized from methyl 2-aminopyrazine-3-carboxylate (Scheme 3.7) according to a procedure set up to enhance molecular diversity in position 1.

To this end, protection/deprotection steps were necessary in order to control the regiochemistry of the *N*-alkylation reactions. Thus, the starting compound was

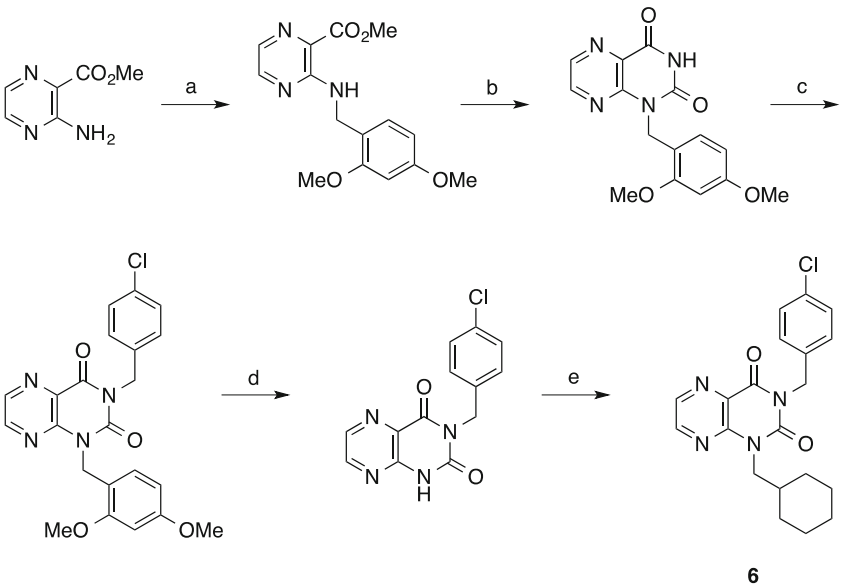


Scheme 3.6 Reagents and conditions: (*a*) HCl(g), EtOH; (*b*) cyanamide, K_2HPO_4, H_2O, 0 °C; (*c*) methylamine, EtOH, reflux; (*d*) ethyl bromoacetate, TBAI, DMF, rt; (*e*) MeONa, EtOH, reflux; (*f*) 3,4-dichlorobenzyl isocyanate, toluene, MW, 120 °C; (*g*) MeONa, MeOH, 100 °C; (*h*) 1-bromopinacolone, K_2CO_3, DMF, rt

subjected to reductive alkylation with 2,4-dimethoxybenzaldehyde and the product obtained was cyclized by treatment with trichloroacetylisocyanate, followed by cleavage of the trichloroacetyl group by sodium methoxide. Removal of the dimethoxybenzyl protecting group with trifluoroacetic acid led to a pteridine intermediate with a free NH group at 1-position, which could be alkylated with a variety of electrophiles to generate a library of pteridine derivatives. In particular, reaction with (bromomethyl)cyclohexane provided compound **6**.

In 2011, GlaxoSmithKline reported on the discovery of the new $GABA_B$ receptor positive allosteric modulator, CMPPE (Fig. 3.5), a pyrazolo[1,5-*a*]pyrimidine derivative identified by screening the GSK compound collection using the [^{35}S] GTPγS-binding assay (Perdonà et al. 2011). CMPPE was fully profiled in vitro and in rat models of food intake and locomotor activity, demonstrating its involvement in the regulation of food consumption without impairment on the animal locomotor activity.

In 2015 Astellas Pharma patented novel thieno[2,3-*d*]pyridine derivatives (i.e. compound **7**) for the prevention/treatment of diseases such as schizophrenia, cognitive disorder, pain (Shiraishi et al. 2015).



Scheme 3.7 Reagents and conditions: (*a*) 2,4-dimethoxybenzaldehyde, $NaBH(OAc)_3$, DCE, rt; (*b*) i. trichloroacetylisocyanate, DCM, rt; ii. MeONa, MeOH, 60 °C; (*c*) 4-chlorobenzyl chloride, K_2CO_3, DMF, rt; (*d*) trifluoroacetic acid, DCM, 100 °C; (*e*) (bromomethyl)cyclohexane, K_2CO_3, DMF, rt

3.2.6 Five-Membered Heterocyclic Amides

In seven patents between 2006 and 2008, AstraZeneca described approximately 200 molecules characterized by a five-membered heterocyclic scaffold functionalized with amide and ester groups. Examples of these compounds are reported in Fig. 3.6: imidazoles (i.e., compounds **8** and **9**) (Bauer et al. 2006, 2007a, b, c, 2008), pyrazoles (i.e., compound **10**) (Bauer 2007), oxazoles (i.e., compound **11**), thiazoles (i.e., compound **12**) (Bauer et al. 2007d).

The compounds were characterized by the [^{35}S]GTPγS-binding test and were proposed as new drug candidates for the treatment of different pathologies, such as GERD and IBS.

The 2-methoxyimidazole compound **9** (Scheme 3.8) was prepared by acylation with 2,4,6-trifluorobenzoyl chloride of the key intermediate *tert*-butyl 4-amino-2-methoxy-1-(4-fluorophenyl)-1*H*-imidazole-5-carboxylate. This was synthesized from dimethylcyanodithioimidocarbonate in three steps entailing: (i) the substitution of one methylthio group by 4-fluoroaniline, (ii) alkylation of the NH group by *tert*-butyl bromoacetate, (iii) substitution of the remaining methylthio group with a methoxy group and concurrent intramolecular *C*-acylation to generate the imidazole ring.

The synthesis of compound **10** was accomplished in an easy and fast way (Scheme 3.9) by alkylation with ethyl iodide of the commercially available ethyl

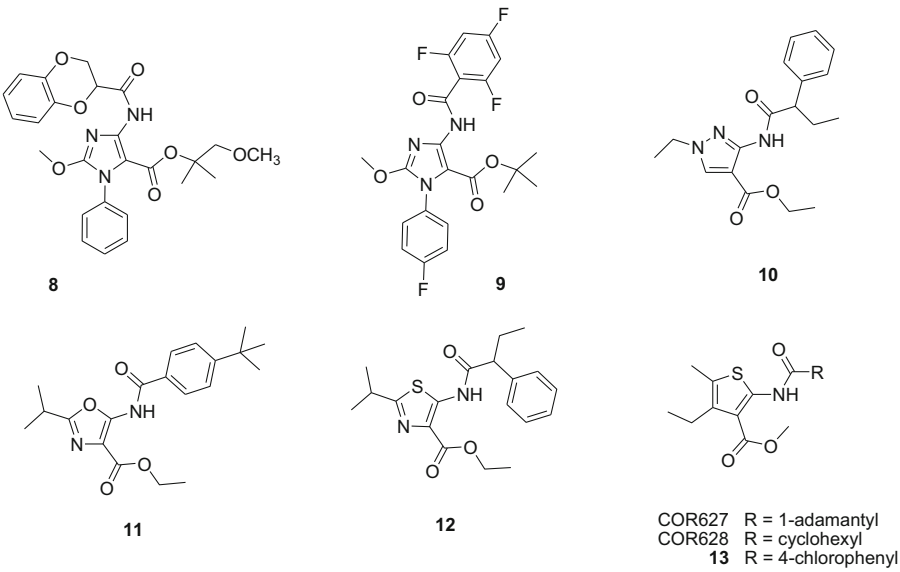
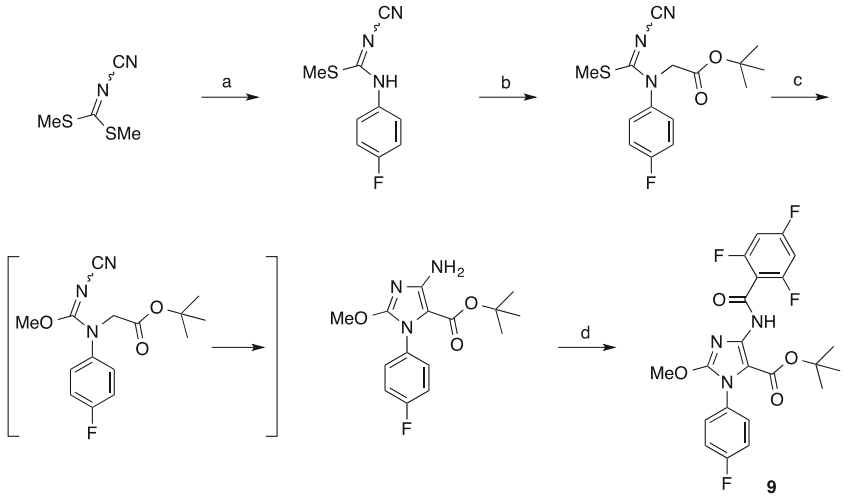


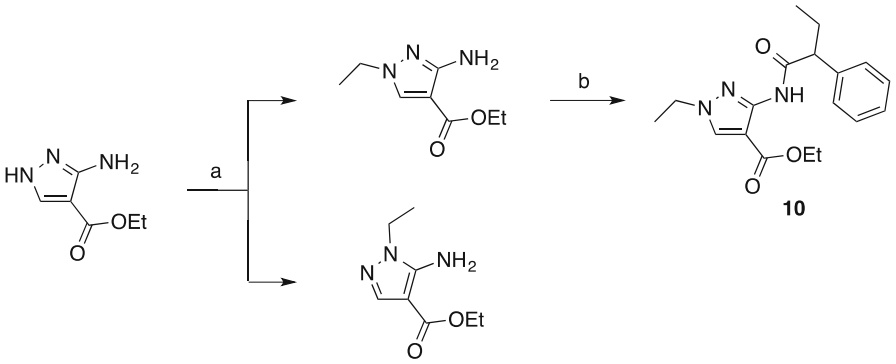
Fig. 3.6 Chemical structure of five-membered heterocyclic amides



Scheme 3.8 Reagents and conditions: (*a*) 4-fluoroaniline, EtOH, reflux; (*b*) *tert*-butyl bromoacetate, K_2CO_3; (*c*) MeONa; (*d*) 2,4,6-trifluorobenzoyl chloride, PS-DIPEA, rt to 50 °C

3-amino-1*H*-pyrazole-4-carboxylate to give the alkylated derivative as a mixture of isomers, which was used without separation in the subsequent acylation step with 2-phenylbutyryl chloride. Purification of the reaction mixture afforded the expected amido derivative **10**.

Compounds **11** and **12** were prepared from the common intermediate ethyl 2-[(2-methyl)propionylamino]cyanoacetate (Scheme 3.10) in turn obtained by



Scheme 3.9 Reagents and conditions: (*a*) EtI, NaH, acetonitrile, rt; (*b*) 2-phenylbutyryl chloride, TEA, THF, rt

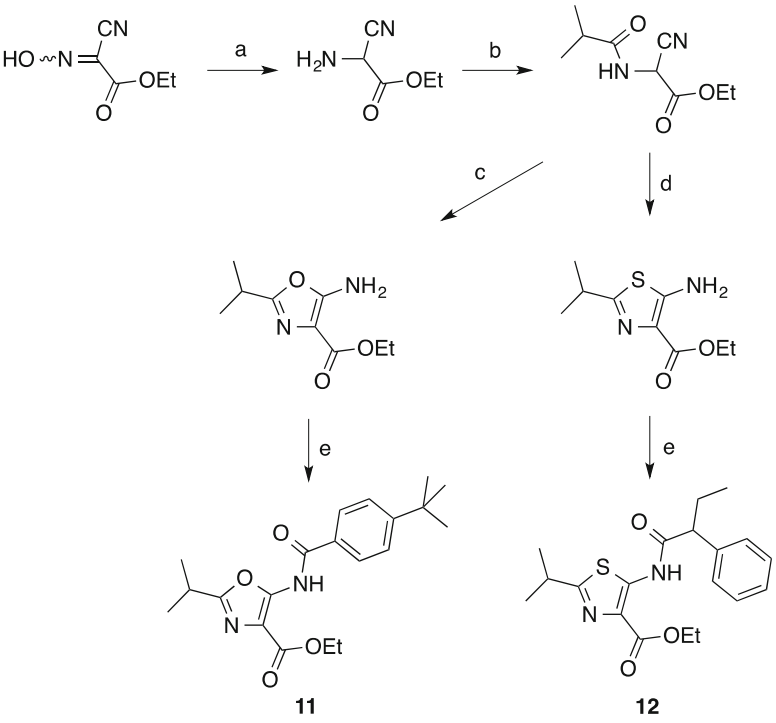
reduction and subsequent acylation of easily available ethyl 2-(hydroxyimino)cyanoacetate. Treatment of the common intermediate with hydrogen chloride or Lawesson's reagent led to ethyl 5-amino-4-ethoxycarbonyl-2-isopropyl-oxazole or thiazole, respectively, which were then converted into the desired amides by acylation of the amino group with the appropriate acyl chloride.

An extensive survey of 19 patents of the different scaffolds for positive allosteric modulators of $GABA_B$ receptors and of the major players in the field was published by Froestl (2010b). This paper gives a relevant overview of several indications that can be addressed with $GABA_B$ positive allosteric modulators.

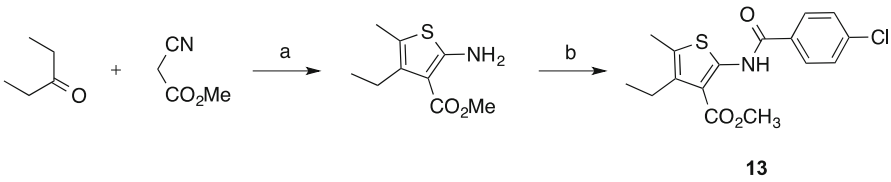
In 2012, Corelli and coworkers identified, by means of a virtual screening protocol, two new 2-(acylamino)thiophene derivatives, referred to as COR627 and COR628 (Fig. 3.6), as novel $GABA_B$ receptor positive allosteric modulators (Castelli et al. 2012). In an attempt to optimize this structural motif and obtain SAR information, a number of congeners were synthesized by acylation of methyl 2-amino-4-ethyl-5-methylthiophene-3-carboxylate, in turn obtained by Gewald synthesis from methyl cyanoacetate and 3-pentanone in the presence of sulfur and a base (Scheme 3.11). These compounds showed an interesting positive allosteric modulator profile (Mugnaini et al. 2013). Although less potent than GS39783, used as a reference compound, some of them, such as compound **13**, were found to be more active in vivo, even after intragastric administration, and displayed low cytotoxicity.

3.3 Negative Allosteric Modulators of $GABA_B$ Receptors

In an attempt to find novel and more potent $GABA_B$ receptor positive allosteric modulators starting from the structure of CGP7930, Nan and coworkers reported, in 2014, the discovery of the first negative allosteric modulator (NAM) of the $GABA_B$ receptor (compound **14**, later on named CLH304a, Fig. 3.7) (Chen et al. 2014).



Scheme 3.10 Reagents and conditions: (*a*) $Na_2S_2O_3$, H_2O, $NaHCO_3$; (*b*) isobutyryl chloride, base; (*c*) HCl, dioxane, reflux; (*d*) Lawesson's reagent, toluene, reflux; (*e*) appropriate acyl chloride, TEA or PS-DIPEA, THF, rt to 50 °C



Scheme 3.11 Reagents and conditions: (*a*) S_8, morpholine, EtOH, reflux; (*b*) 4-chlorobenzoyl chloride, dioxane, 100 °C

Compound **14**, characterized by a di-(*tert*-butyl)phenol scaffold reminiscent of CGP7930 and *rac*-BHFF, was synthesized according to the procedure depicted in Scheme 3.12. 3,5-Bis-(*tert*-butyl)-4-hydroxybenzaldehyde was converted into the corresponding dimethyl acetal and this was reacted, in the presence of boron trifluoride etherate, with the silyl enol ether obtained from ethyl 2-oxopropanoate.

Compound **14** decreased GABA-induced IP3 production with an IC_{50} of 37.9 μM and had no effect on other GPCR Class C members, such as mGluR1, mGluR2, and

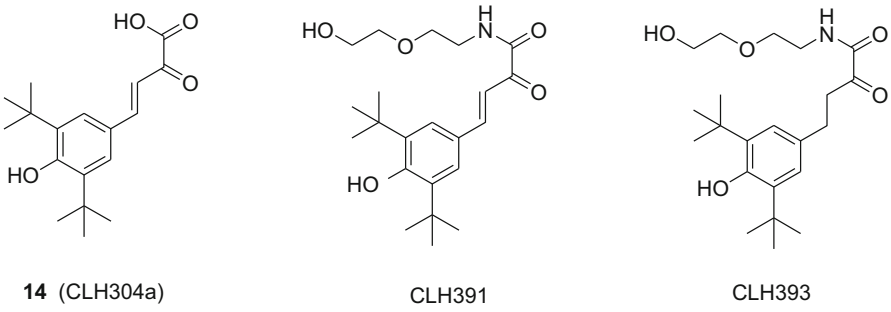
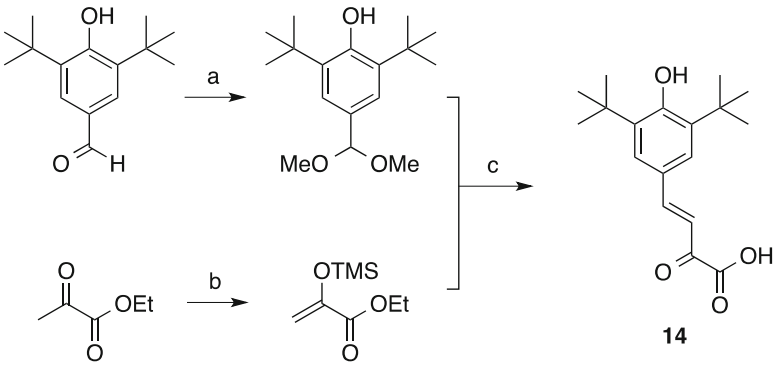


Fig. 3.7 Chemical structure of the first $GABA_B$ NAMs



Scheme 3.12 Reagents and conditions: (*a*) $HC(OEt)_3$, NH_4Cl, MeOH, reflux; (*b*) TMSCl, DMAP, Et_3N, toluene, reflux; (*c*) $BF_3{\cdot}Et_2O$, DCM, –78 to 0 °C

mGluR5. Moreover, it was shown that **14** does not bind to the orthosteric binding site of the receptor, demonstrating that it negatively modulates $GABA_B$ receptors activity as a NAM through the heptahelical domain of the $GABA_{B2}$ receptor subunits (Sun et al. 2016).

Two amide derivatives of CLH304a, namely, CLH391 and CLH393 (Fig. 3.7), showed comparable activity to that of the parent compound (Sun et al. 2016). However, these NAMs are not very attractive from the medicinal chemistry viewpoint. They were designed as derivatives of CGP7930, which was initially developed in 2001 as a pharmacological tool but then structurally elaborated by removing the phenol function in order to obtain more promising $GABA_B$ modulators, such as *rac*-BHFF. Conversely, the CLH compounds may show limitations in terms of pharmaceutical development, as they still possess an OH group on the aromatic ring, which might limit their pharmacokinetic properties, as well as a quite electrophilic alpha,beta-unsaturated ketone, which may be responsible for toxicological liability. Therefore, the efficacy and safety of these CHL compounds in vivo must be still carefully evaluated.

3.4 Challenges in the Discovery of New GPCRS Allosteric Modulators

The allosteric approach for GPCRs was impracticable until the late 1990s because of the lack of high-throughput screening technologies and functional assays able to identify molecules affecting target function irrespective of the site of binding. Nowadays, it is still very challenging considering that neither the binding modes nor the molecular mechanism of the drugs are known (Conn et al. 2012, 2014). Indeed, allosteric modulators show the phenomenon of functional (or mode) switching with a relatively high frequency, eliciting the propensity of structurally similar PAMs, NAMs and silent allosteric modulators (SAMs) to be generated by subtle structural changes of a given allosteric chemotype. Thus, during the lead-optimization process particular attention is required to avoid chemical series with a strong tendency to molecular switches because this can definitely complicate the SAR.

In addition, allosteric modulators very often suffer from extremely shallow or flat SAR. Very small electronic or steric modifications can have a dramatic impact on the biological response of the compound leading, in most of the cases, to a complete loss of activity. This behavior translates in a rate of success of around 10% during the chemical optimization phase. It is therefore necessary, when trying a chemical optimization of allosteric ligands, to use a focused iterative library synthesis based on the identification of regions of the hit that can tolerate modifications. To identify these particular sites of the molecule, the strategy of "walking" fluorine atoms has showed some potentiality (Conn et al. 2014).

Finally, CYP-mediated molecular switches are always possible. To study this phenomenon, major metabolites of allosteric ligands should be prepared and evaluated and, if necessary, metabolically labile sites should be blocked with deuterium. This strategy has emerged as an attractive one in order to improve metabolic stability without affecting potency and functional activity (Conn et al. 2014).

Functional switches and flat SAR have been widely observed for $GABA_B$ allosteric modulators. Since the discovery of the first $GABA_B$ PAM, the medicinal chemistry effort has allowed the identification of new structural motifs able to positively modulate the receptor with a significant expansion of our knowledge about SAR. Nevertheless, the attempt to rationalize biological outcomes on the basis of targeted structural modifications within a molecular class has often failed (Paterson et al. 2008; Mugnaini et al. 2013). Moreover, slight chemical manipulation of a PAM without affecting the central structural nucleus has led to the identification of a $GABA_B$ NAM (Chen et al. 2014).

If we add to this scenario the lack of structural information related to $GABA_B$ receptor, whose X-ray structure has been reported only in 2013 (Geng et al. 2013), it will come as no surprise that research in the area of allosteric modulators of the $GABA_B$ receptor has involved almost exclusively big pharma, with four companies (Novartis, Roche, AstraZeneca, and Addex) having invested substantial resources, and academia playing only a marginal role.

3.5 Conclusions

Since the discovery of the first $GABA_B$ positive allosteric modulator CGP7930 in 2001, a number of new scaffolds have been identified and, in some cases a lead optimization process has been undertaken with the aim of finding more potent ligands and getting new insight in the SAR of specific families of compounds. Because of the absence of structural knowledge about the allosteric site/sites on the receptor, this effort has not led to precise SAR information; on the contrary, besides shallow SAR, functional switches have been observed, driving to the discovery in 2014 of the first $GABA_B$ NAM, a molecule structurally related to CGP7930.

References

Abraham, N., & Goldman, H. B. (2015). An update on the pharmacology for the lower urinary tract dysfunction. *Expert Opinion on Pharmacotherapy, 16*, 79–93.

Adams, C. L., & Lawrence, A. J. (2007). CGP7930: a positive allosteric modulator of the GABAB receptor. *CNS Drug Reviews, 13*, 308–316.

Agabio, R., & Colombo, G. (2014). $GABA_B$ receptor ligands for the treatment of alcohol use disorder: Preclinical and clinical evidence. *Frontiers in Neuroscience, 8*, 140.

Alker, A. M., Grillet, F., Malherbe, P., Norcross, R. D., Thomas, A. W., & Masciadri, R. (2008). Efficient one-pot synthesis of the GABAB positive allosteric modulator (R, S)-5,7-di-tert-butyl-3-hydroxy-3-trifluoromethyl-3H-benzofuran-2-one. *Synthetic Communications, 38*, 3398–3405.

Balerio, G. N., & Rubio, M. C. (2002). Baclofen analgesia: Involvement of the GABAergic system. *Pharmacological Research, 46*, 281–286.

Bauer, U. (2007, June 28). Pyrazoles for the treatment of GERD and IBS. WO073297.

Bauer, U., Brailsford, W., Cheng, L., Jonforsen, M., Raubacher, F., Schell, P., et al. (2008, October 30). Imidazole derivatives as modulators of the GABA receptor for the treatment of gastrointestinal disorders. WO130313.

Bauer, U., Brailsford, W., Chhajlani, V., Egner, B., Fjellstrom, O., Gustafsson, L., et al. (2006, January 5). Imidazole variants as modulators of GABA receptor for the treatment of GI disorders. WO001750.

Bauer, U., Brailsford, W., Gustafsson, L., Saxin, M., & Svensson, T. (2007, June 28). Imidazole derivatives for the treatment of gastrointestinal disorders. WO073298.

Bauer, U., Brailsford, W., Gustafsson, L., & Svensson, T. (2007). GABA-B receptor modulators. WO073300.

Bauer, U., Gustafsson, L., Saxin, M., & Svensson, T. (2007, June 28). Heterocyclic GABA-B modulators.WO073296.

Bauer, U., Gustafsson, L., & Saxin, M. (2007, June 28). Imidazoles as GABA-B receptor modulators. WO073299.

Benarroch, E. E. (2012). $GABA_B$ receptors, structure, functions, and clinical implications. *Neurology, 78*, 578–584.

Binet, V., Brajon, C., Le Corre, L., Acher, F., Pin, J.-P., & Prézeau, L. (2004). The heptahelical domain of $GABA_{B2}$ is activated directly byCGP7930, a positive allosteric modulator of the $GABA_B$ receptor. *Journal of Biological Chemistry, 279*, 29085–29091.

Bowery, N. G., Hill, D. R., Hudson, A. L., Doble, A., Middlemiss, D. N., Shaw, J., et al. (1980). (–)-Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature, 283*, 92–94.

Castelli, M. P., Casu, A., Casti, P., Lobina, C., Carai, M. A., Colombo, G., et al. (2012). Characterization of methyl 2-(1-adamantanecarboxamido)-4-ethyl-5-methylthiophene-3-carboxylate (COR627) and methyl 2-(cyclohexanecarboxamido)-4-ethyl-5-methylthiophene-3-carboxylate (COR628),

two novel positive allosteric modulators of the $GABA_B$ receptor. *Journal of Pharmacology and Experimental Therapeutics, 340*, 529–538.

Chen, L.-H., Sun, B., Zhang, Y., Xu, T.-J., Xia, Z.-X., Liu, J.-F., et al. (2014). Discovery of a negative allosteric modulator of $GABA_B$ receptors. *ACS Medicinal Chemistry Letters, 5*, 742–747.

Cheng, L., Holmquist, S., Raubacher, F., & Schell, P. (2008, October 30). Xanthine compounds having a positive allosteric GABA-B receptor modulator effect. WO130314.

Cheng, L., Jonforsen, M., & Schell, P. (2009, April 2). Pteridine compounds having activity on the GABA-B receptors. WO041905.

Cheng, L., & Karle, M. (2009, April 2). Quinoline compounds having an activity against the GABA-B receptor. WO041904.

Conn, P. J., Lindsley, C. W., Meiler, J., & Niswender, C. M. (2014). Opportunities and challenges in the discovery of allosteric modulators of GPCRs for treating CNS disorders. *Nature Reviews Drug Discovery*, *13*, 692–708 and references cited therein.

Conn, P. J., Scott, D. K., & Doller, D. (2012). In: M. C. Desai (Ed.), *Drug design strategies for GPCR allosteric modulators* (Vol. 47, pp. 441–457). San Diego, CA: Academic Press and references cited therein.

Cryan, J. F., & Kaupmann, K. (2005). Don't worry 'B' happy!: a role for GABA(B) receptors in anxiety and depression. *Trends in Pharmacological Sciences, 26*, 36–43.

Cryan, J. F., Kelly, P. H., Chaperon, F., Gentsch, C., Mombereau, C., Lingenhoehl, K., et al. (2004). Behavioral characterization of the novel GABAB receptor-positive modulator GS39783 (N, N'-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine): Anxiolytic-like activity without side effects associated with baclofen or benzodiazepines. *Journal of Pharmacology and Experimental Therapeutics, 310*, 952–963.

Dupuis, D. S., Relkovic, D., Lhuillier, L., Mosbacher, J., & Kaupmann, K. (2006). Point mutations in the transmembrane region of GABA-B2 facilitate activation by the positive modulator N, N'-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) in the absence of the GABA-B1 subunit. *Molecular Pharmacology, 70*, 2027–2036.

Fisher, H. (1972). Herbicidal and plant growth regulating 2-(alkylthio)-4,6-diamino-5-nitropyrimidine derivatives (original title: Mittel zur Beeinflussung des Pflanzenwachstums). German Patent for CIBA-GEIGY AG DE2223644.

Floersheim, P., Froestl, W., Guery, S., Kaupmann, K., & Koller, M. (2006, December 28). Pyrimidine derivatives for the treatment of GABA-B mediated nervous system disorders. WO136442.

For details about the clinical use of baclofen see Froestl, W. (2010). In: T. P. Blackburn & S. J. Enna (Eds.), *GABAB receptor pharmacology: A tribute to Norman Bowery* (Vol. 58, pp. 19–62). New York: Academic Press and references cited therein.

Froestl, W. (2010a). Chemistry and pharmacology of GABAB receptor ligands. *Advances in Pharmacology, 58*, 19–62.

Froestl, W. (2010b). Novel GABAB receptor positive modulators: A patent survey. *Expert Opinion on Therapeutic Patents, 20*, 1007–1017.

Geng, Y., Bush, M., Mosyak, L., Wang, F., & Fan, Q. R. (2013). Structural mechanism of ligand activation in human $GABA_B$ receptor. *Nature, 504*, 254–259.

Guery, S., Floersheim, P., Kaupmann, K., & Froestl, W. (2007). Syntheses and optimization of new GS39783 analogues as positive allosteric modulators of GABA-B receptors. *Bioorganic and Medicinal Chemistry Letters, 17*, 6206–6211.

Haney, M., Hart, C. L., & Foltin, R. W. (2006). Effects of baclofen on cocaine self-administration: Opioid-and nonopioid-dependent volunteers. *Neuropsychopharmacology, 31*, 1814–1821.

Hwa, L. S., Kalinichev, M., Haddouk, H., Poli, S., & Miczek, K. A. (2014). Reduction of excessive alcohol drinking by a novel GABAB receptor positive allosteric modulator ADX71441 in mice. *Psychopharmacology (Heidelberg, Germany), 231*, 333–343.

Jiang, X., Su, L., Zhang, Q., He, C., Zhang, Z., Yi, P., et al. (2012). $GABA_B$ receptor complex as a potential target for tumor therapy. *Journal of Histochemistry and Cytochemistry, 60*, 269–279.

Kalinichev, M., Donovan-Rodriguez, T., Girard, F., Riguet, E., Rouillier, M., Bournique, B., et al. (2014a). Evaluation of peripheral versus central effects of GABAB receptor activation using a novel, positive allosteric modulator of the GABAB receptor ADX71943, a pharmacological tool compound with a fully peripheral activity profile. *British Journal of Pharmacology, 171*, 4941–4954.

Kalinichev, M., Palea, S., Haddouk, H., Royer-Urios, I., Guilloteau, V., Lluel, P., et al. (2014b). ADX71441, a novel, potent and selective positive allosteric modulator of the GABAB receptor, shows efficacy in rodent models of overactive bladder. *British Journal of Pharmacology, 171*, 995–1006.

Kaupmann, K., Huggel, K., Heid, J., Flor, P. J., Bischoff, S., Mickel, S. J., et al. (1997). Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. *Nature, 386*, 239–246.

Keberle, H., Faigle, J. W., & Wilhelm, M. (1969, October 7). Gamma-amino-beta-(para-halophenyl)-butyric acids and their esters. U.S. Patent 3,471,548.

Kerr, D. I. B., Khalafy, J., Ong, J., Perkins, M. V., Prager, R. H., Puspawati, N. M., et al. (2006). Synthesis and biological activity of allosteric modulators of $GABA_B$ receptors, Part 2. 3-(2,6-Bis-*tert*-butyl-4-hydroxyphenyl)propanols. *Australian Journal of Chemistry, 59*, 457–462.

Kerr, D. I. B., Ong, J., Puspawati, N. M., & Prager, R. H. (2002). Arylalkylamines are a novel class of positive allosteric modulators at $GABA_B$ receptors in rat neocortex. *European Journal of Pharmacology, 451*, 69–77.

Kharasch, M. S., & Joshi, B. S. (1957). Reactions of hindered phenols. I. Reactions of 4,4'-dihydroxy-3,5,3',5'-tetra-*tert*-butyl diphenylmethane. *Journal of Organic Chemistry, 22*, 1435–1438.

Krnjević, K., & Schwartz, S. (1967). The action of gamma-aminobutyric acid on cortical neurons. *Experimental Brain Research, 3*, 320–336.

Kumar, K., Sharma, S., Kumar, P., & Deshmukh, R. (2013). Therapeutic potential of $GABA_B$ receptor ligands in drug addiction, anxiety, depression and other CNS disorders. *Pharmacology, Biochemistry, and Behavior, 110*, 174–184.

Lehmann, A., Jensen, J. M., & Boeckxstaens, G. E. (2010). $GABA_B$ receptor agonism as a novel therapeutic modality in the treatment of gastroesophageal reflux disease. *Advances in Pharmacology, 58*, 287–313.

Li, X., Kaczanowska, K., Finn, M. G., Markou, A., & Risbrough, V. B. (2015). The GABA(B) receptor positive modulator BHF177 attenuated anxiety, but not conditioned fear, in rats. *Neuropharmacology, 97*, 357–364.

Maccioni, P., Carai, M. A. M., Kaupmann, K., Guery, S., Froestl, W., Leite-Morris, K. A., et al. (2009). Reduction of alcohol's reinforcing and motivational properties by the positive allosteric modulator of the GABA(B) receptor, BHF177, in alcohol-preferring rats. *Alcoholism: Clinical and Experimental Research, 33*, 1749–1756.

Maccioni, P., Zaru, A., Loi, B., Lobina, C., Carai, M. A. M., Gessa, G. L., et al. (2012). Comparison of the effect of the positive allosteric modulator of the GABAb receptor, GS39783, on alcohol self-administration in three different lines of alcohol-preferring rats. *Alcoholism: Clinical and Experimental Research, 36*, 1748–1766.

Malherbe, P., Masciadri, R., Norcross, R. D., Knoflach, F., Kratzeisen, C., Zenner, M. T., et al. (2008). Characterization of (R, S)-5,7-di-tert-butyl-3-hydroxy-3-trifluoromethyl-3H-benzofuran-2-one as a positive allosteric modulator of GABAB receptors. *British Journal of Pharmacology, 154*, 797–811.

Malherbe, P., Masciadri, R., Norcross, R. D., Ratni, H., & Thomas, A. W. (2006, May 5). Quinoline as allosteric enhancers of the GABA-B receptors. WO048146.

Malherbe, P., Masciadri, R., Norcross, R. D., Ratni, H., & Thomas, A. W. (2006, June 22). Thieno-pyridine derivatives as GABA-B allosteric enhancers. WO063732.

Malherbe, P., Masciadri, R., Norcross, R. D., & Prinssen, E. (2006, April 12). 3-Methanesulfonylquinolines as GABA-B enhancers. WO128802.

Malherbe, P., Masciadri, R., Norcross, R. D., & Prinssen, E. (2007, February 8). 2-Hydroxypropionic acid derivatives and 3-hydroxy-benzofuran-2-one derivatives with affinity for the GABA B receptor. WO014843.

Mombereau, C., Kaupmann, K., Froestl, W., Sansig, G., van der Putten, H., & Cryan, J. F. (2004). Genetic and pharmacological evidence of a role for $GABA_B$ receptors in the modulation of anxiety- and antidepressant-like behavior. *Neuropsychopharmacology, 29*, 1050–1062.

Mugnaini, C., Pedani, V., Casu, A., Lobina, C., Casti, A., Maccioni, P., et al. (2013). Synthesis and pharmacological characterization of 2-(acylamino)thiophene derivatives as metabolically stable, orally effective, positive allosteric modulators of the GABAB receptor. *Journal of Medicinal Chemistry, 56*, 3620–3635.

Orrù, A., Lai, P., Lobina, C., Maccioni, P., Piras, P., Scanu, L., et al. (2005). Reducing effect of positive allosteric modulators of the GABAB receptor, CGP7930 and GS39783, on alcohol intake in alcohol preferring rats. *European Journal of Pharmacology, 525*, 105–111.

Paterson, N. E., Vlachou, S., Guery, S., Kaupmann, K., Froestl, W., & Markou, A. (2008). Positive modulation of GABA-B receptors decreased nicotine self-administration and counteracted nicotine-induced enhancement of brain reward function in rats. *Journal of Pharmacology and Experimental Therapeutics, 326*, 306–314.

Perdonà, E., Costantini, V. J. A., Tessari, M., Martinelli, P., Carignani, C., Valerio, E., et al. (2011). In vitro and in vivo characterization of the novel GABAB receptor positive allosteric modulator, 2-{1-[2-(4-chlorophenyl)-5-methylpyrazolo[1,5-a]pyrimidin-7-yl]-2-piperidinyl}ethanol (CMPPE). *Neuropharmacology, 61*, 957–966.

Philips, T. J., & Reed, C. (2014). Targeting $GABA_B$ receptors for anti-abuse drug discovery. *Expert Opinion on Drug Discovery, 9*, 1–11.

Pin, J.-P., & Prézeau, L. (2007). Allosteric modulators of $GABA_B$ receptors: mechanism of action and therapeutic perspective. *Current Neuropharmacology, 5*, 195–201.

Riguet, E., Campo, B., Gibelin, A., & Mhalla, K. (2008, May 15). Novel triazinedione derivatives as GABA-B receptor modulators. WO056257.

Sachais, B. A., Logue, J. N., & Carey, M. S. (1977). Baclofen, a new antispastic drug. A controlled, multicenter trial in patients with multiple sclerosis. *Archives of Neurology, 34*, 422–428.

Shiraishi, N., Hoshii, H., Hamaguchi, W., Honjo, E., Takuwa, T., Kondo, Y., et al. (2015, April 23). Sulfur-containing bicyclic compound. WO 2015056771.

Slattery, D. A., Markou, A., Froestl, W., & Cryan, J. F. (2005). The GABA-B receptor-positive modulator GS39783 and the GABA-B receptor agonist baclofen attenuate the reward-facilitating effects of cocaine:intercranial self-stimulation studies in the rat. *Neuropsychopharmacology, 30*, 2065–2072.

Sun, B., Chen, L., Liu, L., Xia, Z., Pin, J.-P., Nan, F., et al. (2016). A negative allosteric modulator modulates $GABA_B$-receptor signaling through GB2 subunits. *Biochemical Journal, 473*, 779–787.

Tatsuta, M., Iishi, H., Baba, M., Nakaizumi, A., Ichii, M., & Taniguchi, H. (1990). Inhibition by gamma-amino-n-butyric acid and baclofen of gastric carcinogenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine in Wistar rats. *Cancer Research, 50*, 4931–4934.

Urwyler, S., Gjoni, T., Kaupmann, K., Pozza, M. F., & Mosbacher, J. (2004). Selected amino acids, dipeptides and arylalkylamine derivatives do not act as allosteric modulators of GABAB receptors. *European Journal of Pharmacology, 483*, 147–153.

Urwyler, S., Mosbacher, J., Lingenhoehl, K., Heid, J., Hofstetter, K., Froestl, W., et al. (2001). Positive allosteric modulation of native and recombinant γ-aminobutyric $acid_B$ receptors by 2,6-di-*tert*-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and its aldehyde analog CGP13501. *Molecular Pharmacology, 60*, 963–971.

Urwyler, S., Pozza, M. F., Lingenhoehl, K., Mosbacher, J., Lampert, C., Froestl, W., et al. (2003). N, N'-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) and structurally related compounds: Novel allosteric enhancers of gamma-aminobutyric acid B receptor function. *Journal of Pharmacology and Experimental Therapeutics, 307*, 322–330.

Vlachou, S., Guery, S., Froestl, W., Banerjee, D., Benedict, J., Finn, M. G., et al. (2011). Repeated administration of the GABAB receptor positive modulator BHF177 decreased nicotine self-administration, and acute administration decreased cue-induced reinstatement of nicotine seeking in rats. *Psychopharmacology, 215*, 117–128.

Wang, T., Huang, W., & Chen, F. (2008). Baclofen, a GABAB receptor agonist, inhibits human hepatocellular carcinoma cell growth in vitro and in vivo. *Life Sciences, 82*, 536–541.

Part II
Molecular Biology, Biochemistry, & Physiology

Chapter 4
Molecular Organization, Trafficking, and Degradation of the $GABA_B$ Receptor

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Abstract $GABA_B$ receptors are heterodimeric G protein-coupled receptors composed of the two seven transmembrane spanning proteins $GABA_{B1}$ and $GABA_{B2}$. They are expressed in the vast majority of neurons and primarily regulate neuronal excitability via several distinct effector systems. There is evidence that $GABA_B$ receptors are organized in large macromolecular complexes composed of accessory and effector proteins to ensure efficient signaling. Communication through and regulation of $GABA_B$ receptors is determined by a constantly growing list of interacting proteins. In particular, trafficking events that regulate the cell surface availability of the receptors and thereby their signaling strength are controlled by protein–protein interactions that often convey posttranslational modifications such as phosphorylation or ubiquitination. Understanding the mechanisms regulating $GABA_B$ receptor availability is of major importance since it is increasingly recognized that aberrant regulation of $GABA_B$ receptor cell surface expression contributes to disease states including addiction, cerebral ischemia, and chronic pain. Here we briefly review our current understanding of the macromolecular structural organization of $GABA_B$ receptor complexes and the regulation of cell surface receptor availability by trafficking events and controlled receptor degradation.

Keywords $GABA_B$ receptor • Trafficking • Interacting proteins • Lysosomal degradation • Proteasomal degradation

4.1 Molecular Organization of $GABA_B$ Receptors

Unlike other G protein-coupled receptors, functional $GABA_B$ receptors are obligatory heterodimers constituted from the two subunits $GABA_{B1}$ and $GABA_{B2}$ (Jones et al. 1998; Kaupmann et al. 1998; White et al. 1998). Both subunits are structurally composed in a similar way: a large extracellular domain forming a

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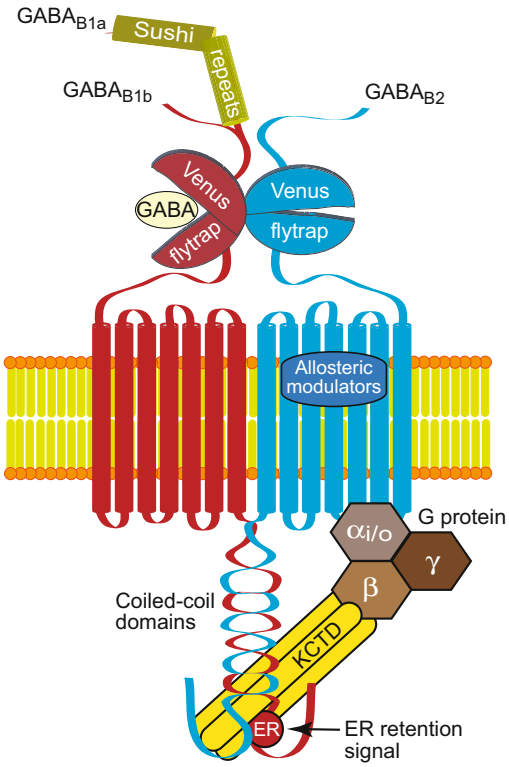

Fig. 4.1 Minimum core structure of $GABA_B$ receptor complexes. Functional $GABA_B$ receptors consist of the two distinct subunits $GABA_{B1}$ and $GABA_{B2}$. $GABA_{B1}$ exists in two isoforms, $GABA_{B1a}$ and $GABA_{B1b}$, which differ by the additional inclusion of two sushi domains (protein–protein interaction domains) in $GABA_{B1a}$. Each subunit contains a large N-terminal domain building a Venus flytrap structure, seven transmembrane domains, and a large intracellular located C-terminal domain comprising a coiled-coil structure (protein–protein interaction domain). Heterodimerization involves the coiled-coil domains and sterically inactivates an adjacent located ER retention/retrieval signal (ER) in $GABA_{B1}$, permitting ER exit and forward trafficking of the receptor complex. The Venus flytrap in $GABA_{B1}$ constitutes the binding site for orthosteric ligands (GABA binding site), whereas the same structure in $GABA_{B2}$ is inactive and does not bind ligands. However, $GABA_{B2}$ binds allosteric modulators at a site located within the transmembrane domain. The G protein is bound to $GABA_{B2}$ and stabilized via interaction with KCTD proteins associated with the C-terminal domain of $GABA_{B2}$

Venus flytrap structure is followed by a seven-transmembrane domain and an intracellular located C-terminal domain (Fig. 4.1). Despite their similar structural organization, both subunits serve distinct functions.

The Venus flytrap in $GABA_{B1}$ constitutes the binding site for orthosteric ligands (Bernard et al. 2001; Galvez et al. 1999, 2000; Geng et al. 2012, 2013), whereas the same structure in $GABA_{B2}$ does not bind ligands. However, $GABA_{B2}$ indirectly contributes to ligand binding by allosterically enhancing the affinity of the $GABA_{B1}$-binding site for agonists (Galvez et al. 2001; Geng et al. 2012; Kniazeff et al. 2002;

Liu et al. 2004). In addition, $GABA_{B2}$ harbors a binding site for allosteric modulators located in the transmembrane domain (Binet et al. 2004; Dupuis et al. 2006). Binding of positive allosteric modulators, such as CGP7930, GS39783, or rac-BHFF, to this site do not activate the receptor but increase the affinity as well as efficacy for agonists binding to $GABA_{B1}$ (Malherbe et al. 2008; Urwyler et al. 2001, 2003; see also Chaps. 6 and 18 of this book).

While $GABA_{B1}$ is indispensable for receptor activation, $GABA_{B2}$ recruits the G protein (Galvez et al. 2001). Like in other G protein-coupled receptors, the second and third intracellular loop connecting transmembrane sequence 3/4 and 5/6 in $GABA_{B2}$ is involved in G protein binding and activation, as revealed by domain swapping experiments and mutation of single amino acids (Duthey et al. 2002; Havlickova et al. 2002; Margeta-Mitrovic et al. 2001; Robbins et al. 2001).

An additional striking role of $GABA_{B2}$ is its absolute requirement for cell surface trafficking of the heterodimeric receptor complex. When $GABA_{B1}$ is ectopically expressed in the absence of $GABA_{B2}$ in diverse cell lines, it is retained in the endoplasmic reticulum (ER) and fails to traffic to the cell surface (Couve et al. 1998). This is due to an arginine-rich ER retention signal (RSRR) in the intracellular located C-terminal domain of $GABA_{B1}$. Heterodimerization of $GABA_{B1}$ with $GABA_{B2}$ inactivates the ER retention signal and permits trafficking of the receptor complex to the cell surface (Calver et al. 2001; Gassmann et al. 2005; Margeta-Mitrovic et al. 2000; Pagano et al. 2001). This mechanism ensures that predominantly fully functional heterodimeric $GABA_B$ receptors reach the plasma membrane.

Finally, a further important structure present in the C-terminal domains of $GABA_{B1}$ and $GABA_{B2}$ is a stretch of about 30 amino acids, which form a coiled-coil domain. Coiled-coil domains are protein–protein interaction sites (Burkhard et al. 2001) and are important determinants for $GABA_B$ receptors heterodimerization as well as for cell surface targeting of the receptors (Burmakina et al. 2014; Kammerer et al. 1999). The coiled-coil domains of $GABA_{B1}$ and $GABA_{B2}$ bind with high affinity to each other and sterically inactivate the ER retention signal in $GABA_{B1}$ located at the end of the coiled-coil domain (Burmakina et al. 2014). Mutation of amino acids in the core of the coiled-coil domains of $GABA_{B1}$ as well as $GABA_{B2}$ retained $GABA_{B1}$ in the ER. In addition, the coiled-coil domains are binding sites for a number of $GABA_B$ receptor interacting proteins.

4.1.1 $GABA_B$ Receptor Isoforms

There are two well-characterized isoforms of $GABA_{B1}$, termed $GABA_{B1a}$ and $GABA_{B1b}$ (Kaupmann et al. 1997), which are expressed in virtually all neurons in the brain (Bischoff et al. 1999). They are generated by alternative promoter usage (Steiger et al. 2004) and are independently regulated during development (Fritschy et al. 1999; Malitschek et al. 1998). $GABA_{B1a}$ and $GABA_{B1b}$ differ solely in their very N-terminal extracellular domain by the inclusion of a pair of sushi domains in $GABA_{B1a}$, which

are absent in $GABA_{B1b}$ (Kaupmann et al. 1997). Sushi domains are protein–protein interaction domains and play an important role in axonal targeting of $GABA_{B1a}$ in glutamatergic neurons (Biermann et al. 2010). $GABA_{B1a}$ and $GABA_{B1b}$ display distinct, though overlapping, subcellular distributions in neurons. While $GABA_{B1a}$ and $GABA_{B1b}$ are both found on dendritic shafts, $GABA_{B1b}$ is predominant in spines and $GABA_{B1a}$ is specifically found in glutamatergic axon terminals (Vigot et al. 2006).

Apart from $GABA_{B1a}$ and $GABA_{B1b}$, a number of additional $GABA_{B1}$ isoforms had been described: $GABA_{B1c,d,e,f,g,h,i,j,k,l,m,n}$ (Holter et al. 2005; Isomoto et al. 1998; Lee et al. 2010; Pfaff et al. 1999; Schwarz et al. 2000; Tiao et al. 2008; Wei et al. 2001a, b). Interestingly, some of those variants ($GABA_{B1e,g,h,i,j,l,m,n}$) lack the transmembrane as well as C-terminal domains and thus consist of, probably secreted, N-terminal domains of variable length. Two of those isoforms, $GABA_{B1e}$ and $GABA_{B1j}$, had been analyzed in more detail. $GABA_{B1e}$is highly expressed in peripheral tissues and disrupts $GABA_B$ receptor function by interfering with $GABA_{B1}$/$GABA_{B2}$ heterodimerization (Schwarz et al. 2000). In addition, $GABA_{B1j}$, which consist primarily of the two $GABA_{B1a}$ sushi domains, interferes selectively with $GABA_{B1a,2}$ heteroreceptor function, most likely by scavenging an extracellular $GABA_{B1a,2}$ receptor interacting protein that ensures correct subcellular location of the receptors (Tiao et al. 2008). Detailed information on the other $GABA_B$ receptor isoforms is mostly lacking. They appear to be expressed at considerably lower levels in brain than $GABA_{B1a}$ and $GABA_{B1b}$ and their in vivo relevance is not clear yet.

4.1.2 Macromolecular Organization of $GABA_B$ Receptor Complexes

Neuronal $GABA_B$ receptors exist in large macromolecular complexes as revealed by density gradient centrifugation (Benke et al. 1999) and nondenaturating blue native polyacrylamide gel electrophoresis followed by Western blotting (Schwenk et al. 2010). This indicates that $GABA_B$ receptors are associated with a variety of distinct interacting proteins organized in preformed signaling complexes to ensure optimal signal transduction. Because $GABA_B$ receptors activate several distinct downstream effector systems, such as Kir 3 potassium channels, voltage-gated calcium channels, or adenylyl cyclases, the existence of numerous distinct $GABA_B$ receptor signaling complexes is expected. For instance, $GABA_B$ receptors colocalize and copurify with G proteins and voltage-gated calcium channels (Fernandez-Alacid et al. 2009; Laviv et al. 2011; Muller et al. 2010; Park et al. 2011), suggesting that they are part of such a signaling complex.

There are, in principle, three categories of protein–protein interactions that most likely contribute to the formation of macromolecular $GABA_B$ receptor signaling complexes. First, $GABA_B$ receptor heterodimers can form tetramers or even higher order oligomers via interaction of the $GABA_{B1}$ subunits (Calebiro et al. 2013; Comps-Agrar et al. 2011, 2012; Maurel et al. 2008; Villemure et al. 2005) (see Chap. 6 of this book for details). Because $GABA_B$ receptor oligomers show a reduced efficiency to activate G proteins (Comps-Agrar et al. 2011), oligomerization may be

one way to rapidly regulate $GABA_B$ receptor signaling. Second, a number of proteins robustly associated with $GABA_B$ receptors had been identified that most likely build the core complexes (Schwenk et al. 2010, 2015). Third, numerous additional $GABA_B$ receptor interacting proteins may join the core complexes depending on the physiological or pathological state of the neurons.

4.1.2.1 $GABA_B$ Receptor Core Complexes

Recent high stringency proteomic studies revealed a number of robustly interacting proteins that are most likely members of persistent $GABA_B$ receptor core complexes. Most importantly, four members of the K^+channel tetramerization domain (KCTD) proteins (KCTD 8, 12, 12b, 16) had been identified as auxiliary subunits of $GABA_B$ receptors (Schwenk et al. 2010). Association of KCTD proteins with the C-terminal domain of $GABA_{B2}$ (Fig. 4.1) increases the affinity of the receptor for GABA and accelerates the activation kinetics of $GABA_B$ receptor responses (Schwenk et al. 2010). The constitutive association of KCTD proteins with native $GABA_B$ receptors therefore explains why ectopically expressed $GABA_B$ receptors, which are not associated with KCTD proteins, do not resemble exactly the properties of native receptors in neurons. Beside of this, the kind of KCTD isoform endows the $GABA_B$ receptors with distinct properties. The presence of KCTD 12, for example, increases cell surface stability of $GABA_B$ receptors by decreasing constitutive internalization of the receptors (Ivankova et al. 2013). Furthermore, the presence of KCTD 8 and 16 gives rise to long-lasting $GABA_B$ receptors responses, whereas KCTD 12 and 12b mediates fast desensitization by uncoupling the G protein βγ subunits from their effectors, which temporally restricts the signaling responses (Schwenk et al. 2010; Seddik et al. 2012; Turecek et al. 2014).

In addition to the KCTD proteins, a recent multiepitope knockout controlled proteomic study identified additional proteins that robustly associate with $GABA_B$ receptors (Schwenk et al. 2015). Differential association with these proteins may thus build distinct $GABA_B$ receptor core complexes, which give rise to the heterogeneity of $GABA_B$ receptor function observed in the brain. According to the currently available data, the minimum functional units of $GABA_B$ receptor complexes comprise the receptor heterodimers ($GABA_{B1a,2}$ and $GABA_{B1b,2}$), the G proteins, and the KCTD proteins (Fig. 4.1). The function of the KCTD proteins in this complex is to stabilize the binding of the G proteins to the receptor by interacting with the Gβγ subunit and to control the signaling kinetics (see earlier) (Schwenk et al. 2010; Turecek et al. 2014).

The next layer of proteins attached to this core unit is the effector proteins. Surprisingly, only voltage-gated calcium channels and HCN channels are robustly associated with this complex via interaction with KCTD 16 (Schwenk et al. 2015). The other well-known effectors, Kir 3 channels and adenylyl cyclases, were not found in the isolated, detergent solubilized, complexes. However, Kir 3 channels colocalize with $GABA_B$ receptors, had been reported to interact with $GABA_{B1}$, and their levels are often coregulated (David et al. 2006; Fernandez-Alacid et al. 2009; Hearing et al. 2013; Kulik et al. 2006; Padgett et al. 2012). This indicates that Kir 3

channels are likely associated with the receptor core via weaker interaction forces, which do not withstand the isolation procedure used by Schwenk et al. (2015).

A further layer of core proteins constitute the cytoplasmic proteins reticulocalbin-2 as well as several members of the 14-3-3 protein family, the transmembrane proteins calnexin, synaptotagmin-11, neuroligin 3, and inactive dipeptidyl peptidase 6 and 10. All these proteins were found to be strongly associated with $GABA_B$ receptors. The function of most of these proteins in relation to $GABA_B$ receptors remains to be established. However, there is evidence that 14-3-3η decouples $GABA_B$ receptors from Kir3 channels (Workman et al. 2015).

Interestingly, a number of proteins were identified that specifically associate with the extracellular located sushi domains present solely in $GABA_{B1a}$ (adherens junction-associated protein 1, amyloid beta A4 protein, amyloid-like protein 2, integral membrane protein 2B and 2C, neuron-specific gene family member 1 and 2, JNK-interacting protein 3, Charcot–Leyden crystal protein, calsyntenin-3, see Schwenk et al. (2015)). The function of these proteins in the physiology of $GABA_B$ receptors is currently unclear. They are most likely involved in the mechanisms that anchor and target $GABA_{B1a,2}$ receptors to specific cellular compartments, such as presynaptic terminals (Biermann et al. 2010; Tiao et al. 2008).

4.1.2.2 Additional $GABA_B$ Receptor Interacting Proteins

The earlier described stable $GABA_B$ receptor interactors likely constitute a variety of core complexes that give rise to the functional heterogeneity of native $GABA_B$ receptors in the brain. In addition to those proteins, a steadily increasing number of $GABA_B$ receptor interacting proteins are detected (for a review, see Lujan and Ciruela (2012)). Most of those proteins obviously are more weakly associated with the receptors, interact with the receptor transiently, or only under specific conditions. For instance, the stress-induced transcription factor CHOP interacts with $GABA_B$ receptors specifically in the ER only after its stress-induced upregulation. CHOP interferes with heterodimerization of the receptors thereby preventing ER export and forward trafficking of the receptors (Maier et al. 2014). Another example is the scaffolding protein 14-3-3ζ. 14-3-3ζ is upregulated under conditions of neuropathic pain, binds to $GABA_{B1}$ at the cell surface, and disrupts the heterodimer by a so far unknown mechanism. This inactivates the receptors and reduces $GABA_B$ receptor-mediated signaling (Laffray et al. 2012).

4.2 Trafficking and Degradation of $GABA_B$ Receptors

The lifecycle of $GABA_B$ receptors encompasses their synthesis at the ER, their maturation and forward trafficking to the plasma membrane, their internalization, recycling, and finally their degradation. All these processes need to be tightly regulated to ensure the precise number of receptors at the cell surface required for signaling

under a given physiological state. Changes in the rates of any of these parameters will affect the cell surface number of the receptors and thus the strength of $GABA_B$ receptor-mediated signaling. In addition, aberrant regulation of trafficking mechanisms under pathological conditions severely affects $GABA_B$ receptor-mediated neuronal inhibition (Doly et al. 2015; Guetg et al. 2010; Hearing et al. 2013; Lecca et al. 2016; Maier et al. 2010, 2014; Padgett et al. 2012; Terunuma et al. 2010). In the following, we describe our current knowledge on trafficking and degradation mechanisms of $GABA_B$ receptors.

4.2.1 *$GABA_B$ Receptors are Transported to Dendritic and Axonal Sites Within the ER*

As membrane proteins, $GABA_B$ receptor subunits are synthesized at the ER and are incorporated into the ER membrane. It is presently unclear whether $GABA_{B1}$ and $GABA_{B2}$ subunits already cotranslationally assemble into heterodimeric complexes or whether they first exist as individual subunits, which would require stabilization via chaperone proteins as well as a mechanism that regulates the assembly of the receptor heterodimers. Quantitative immunoprecipitation experiments using solubilized rat brain membranes did not detect unassembled subunits, indicating that the majority of subunits assemble into heterodimers (Benke et al. 1999). However, since total brain membranes were used, it could not be excluded that a significant pool of unassembled subunits may specifically exist in ER membranes. There is indeed evidence from cultured neurons that a substantial fraction of intracellular $GABA_{B1}$ and $GABA_{B2}$ subunits do not colocalize and are individually transported over long distances within the ER from the soma into dendrites and axons where they assemble and exit the ER (Ramirez et al. 2009; Valdes et al. 2012; Valenzuela et al. 2014). Movement of $GABA_{B1a}$ along the axonal ER involves microtubules and kinesin I (a molecular motor protein) (Valdes et al. 2012). Likewise, dendritic $GABA_{B1}$ ER transport has been reported to be microtuble and dynein dependent (Valenzuela et al. 2014). However, it should be noted that for efficient axonal targeting of $GABA_{B2}$ the presence of $GABA_{B1a}$ seems to be required (Biermann et al. 2010). In addition, targeting of $GABA_{B1}$ into neurites was shown to depend on intact trafficking signals present in the C-terminal domain of $GABA_{B2}$ (Pooler et al. 2009). Therefore, it is presently not resolved whether always unassembled $GABA_B$ receptor subunits are individually transported within the ER to dendritic and axonal sites.

The currently available data strongly indicate that $GABA_B$ receptor subunits can reside unassembled in ER membranes and can be transported within the ER over long distances into dendrites and axons where they assemble with $GABA_{B2}$ and exit the ER for cell surface trafficking. In glutamatergic neurons, the receptors seem to be sorted to dendrites by default and are only targeted to axons in the presence of $GABA_{B1a}$, which solely relies on the presence on the sushi domains in $GABA_{B1a}$ (Biermann et al. 2010).

4.2.2 ER Exit and Cell Surface Targeting of $GABA_B$ Receptors

Due to the presence of the ER retention motif (RSRR) in the C-terminal domain, $GABA_{B1}$ is retained in the ER as long as it is not assembled with $GABA_{B2}$ (Calver et al. 2001; Gassmann et al. 2005; Margeta-Mitrovic et al. 2000; Pagano et al. 2001). High-resolution crystal structure analysis indicated that heterodimerization via the $GABA_{B1}$ and $GABA_{B2}$ coiled-coil domains inactivates the ER retention signal by steric shielding (Burmakina et al. 2014), which permits ER exit of the assembled receptor.

If $GABA_{B1}$ and $GABA_{B2}$ can exist as unassembled subunits in the ER (see earlier), then there must be a mechanism that prevents or restricts their association. Interestingly, an ER resident transmembrane protein (PRAF2, prenylated Rab acceptor family) had been identified, which interacts with $GABA_{B1}$ via a sequence containing the ER retention signal and a proximal—within the coiled-coil domain located—di-leucine motif (Doly et al. 2015). PRAF2 competes with $GABA_{B2}$ for association with $GABA_{B1}$ and retains unassembled $GABA_{B1}$ in the ER. The relative expression levels of PRAF2 and $GABA_{B2}$ appear to determine the amount of heteromeric receptors trafficked to the cell surface (Doly et al. 2015). This is thus one mechanism that controls cell surface levels of functional $GABA_B$ receptors. Since PRAF2 is not equally well expressed in all brain areas (basal ganglia, thalamus, and hypothalamus contain low or no detectable levels of PRAF2) (Cifuentes-Diaz et al. 2015), there might be either other ER proteins substituting PRAF2 or heterodimerization and ER exit in these brain areas depends solely on the presence of $GABA_{B2}$.

In this regard, another protein that interacts via the coiled-coil domains with $GABA_B$ receptors in the ER and prevents their forward trafficking is the CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP) (Sauter et al. 2005). CHOP is a stress-induced transcription factor, which is only marginally expressed under normal physiological conditions but is highly upregulated in the nucleus and ER upon ER stress (Oyadomari and Mori 2004). Interaction of CHOP with $GABA_B$ receptors in the ER upon stress, such as ischemic conditions, disrupts or prevents heterodimerization of $GABA_{B1}$ and $GABA_{B2}$ in the ER and thereby blocks forward trafficking of the receptors (Maier et al. 2014). Because the supply of newly synthesized receptors is inhibited but normal degradation remains unaffected, this mechanism results in the downregulation of functional $GABA_B$ receptors from the cell surface. It had been speculated that CHOP-induced downregulation of $GABA_B$ receptors contributes to neuronal death following stroke by fostering excitotoxicity (Maier et al. 2014). This mechanism is clearly one example for aberrant regulation of $GABA_B$ receptor ER exit under pathological condition and is apparently not operative under normal physiological conditions.

The ER retention of unassembled $GABA_{B1}$ subunits with an exposed ER retention signal appears not to be totally strict. Individual $GABA_{B1}$ subunits can escape the ER and travel to the cis-Golgi (Brock et al. 2005; Villemure et al. 2005). However, they are retranslocated to the ER by a mechanism involving the coat protein complex I (COPI) (Brock et al. 2005). COPI is a multiprotein complex involved in retrograde protein trafficking from the Golgi to the ER (Beck et al. 2009). COPI binds to the ER

retention signal of $GABA_{B1}$ and is thought to shuttle it from the cis-Golgi back to the ER. Indeed, life cell imaging of $GABA_{B1a}$ traveling along the ER in axons of cultured hippocampal neurons revealed that $GABA_{B1a}$ positive puncta occasionally became separated from the ER and soon rejoined it again (Valdes et al. 2012).

The currently available data support the view that at least a fraction $GABA_B$ receptor subunits are individually transported within the ER into dendrites and axons where they are locally assembled into the heterodimeric complex, which permits forward trafficking of the receptors via the Golgi apparatus to the plasma membrane (Ramirez et al. 2009; Valdes et al. 2012; Valenzuela et al. 2014). ER exit of $GABA_B$ receptors does not require specific exit points. They appear to be able leaving the ER throughout the soma, dendrites, and axons.

4.2.3 Endocytosis and Recycling of $GABA_B$ Receptors

$GABA_B$ receptors undergo fast constitutive endocytosis and recycling irrespective whether they are ectopically expressed in cell lines or natively in neurons (Grampp et al. 2007, 2008; Pooler et al. 2009; Vargas et al. 2008; Wilkins et al. 2008). However, their internalization kinetics differs for currently unknown reasons (half maximum internalization of ectopic receptors ~20–40 min, native receptors ~2–15 min). $GABA_B$ receptors internalize via the classical clathrin and dynamin-dependent route (Grampp et al. 2007, 2008; Laffray et al. 2007; Vargas et al. 2008). They are recruited to clathrin-coated vesicles via the adaptor protein 2 (AP2) and fission of the $GABA_B$ receptor-containing vesicles from the plasma membrane is mediated by the small GTPase dynamin. Colocalization immunofluorescence studies indicate that endocytosed $GABA_B$ receptors first enter Rab5 positive early endosomes from which they are sorted to either Rab4 (fast recycling), Rab11 (slow recycling), or Rab7/Lamp1 positive late endosomes (lysosomal degradation) (Grampp et al. 2008; Hannan et al. 2011; Laffray et al. 2007; Vargas et al. 2008). The extent of receptor recycling had been studied using cell-surface biotinylation and immunofluorescence methods. It was estimated that about half of the internalized receptors are recycled to the cell surface within 15 min (Maier et al. 2010; Grampp et al. 2008; Vargas et al. 2008). The data also suggest that only a minor fraction of yet undetermined size of internalized receptors is constitutively degraded in lysosomes.

4.2.3.1 Structural Determinants Influencing $GABA_B$ Receptor Endocytosis

According to the current data, $GABA_B$ receptors internalize as heterodimers and are not dissociated into single subunits prior endocytosis (Grampp et al. 2008; Hannan et al. 2011; Laffray et al. 2007; Vargas et al. 2008). When expressed alone, $GABA_{B1}$ (with inactivated ER retention signal to permit cell surface expression) exhibits a significantly faster internalization rate than $GABA_{B2}$ and

heterodimeric $GABA_{B1,2}$ receptors (Hannan et al. 2011, 2012). This is due to a di-leucine motive in the C-terminal domain of $GABA_{B1}$, which represents a dominant positive internalization signal. After heterodimerization, the di-leucine motive is buried inside the receptor complex and is no longer accessible from the outside (Burmakina et al. 2014).

Other important structural determinants for the rate of internalization are the N-terminal located sushi repeats present in $GABA_{B1a}$. The internalization rate of $GABA_{B1a,2}$ receptors is considerably slower than that of $GABA_{B1b,2}$ receptors lacking the sushi repeats (Hannan et al. 2012). Deleting the sushi repeats from $GABA_{B1a}$ increased the internalization of $GABA_{B1a,2}$ receptors to the rate of $GABA_{B1b,2}$ receptors. Thus, $GABA_{B1a,2}$ receptors are more resistant to internalization and more stably expressed at the cell surface. The greater cell surface stability of $GABA_{B1a,2}$ receptors might result in more robust $GABA_B$ receptor-mediated signaling. However, the precise physiological implications of this difference in internalization rates among receptor isoforms are currently not understood.

A further regulator of internalization and cell surface stability is the auxiliary protein KCTD12 (Ivankova et al. 2013). Among the KCTD proteins that assemble with $GABA_B$ receptors only KCTD12 reduces $GABA_B$ receptor internalization and thereby increases cell surface levels of the receptors. KCTD12 associates with $GABA_B$ receptors already in the ER, travels bound to the receptors to the plasma membrane without affecting the forward trafficking rate, and stays associated with the receptors during their activation and internalization (Ivankova et al. 2013). Knockdown of KCTD12 significantly reduced the level of cell surface $GABA_B$ receptors in cultured neurons. It is therefore conceivable that regulating the expression of KCTD12 under physiological or pathological conditions will significantly affect $GABA_B$ receptor cell surface expression in neurons expressing KCTD12.

4.2.3.2 The Effect of Receptor Activation on Endocytosis and Recycling

Most G protein-coupled receptors undergo agonist-induced phosphorylation and arrestin-dependent internalization to terminate signaling. Subsequently, the receptors are dephosphorylated and recycled to the plasma membrane ready for further signaling (Gainetdinov et al. 2004; Marchese et al. 2003; Tian et al. 2014). However, $GABA_B$ receptors do not follow this classic scheme. Activation of $GABA_B$ receptors does not lead to a downregulation of cell surface receptors as observed in the classical pathway (Fairfax et al. 2004; Grampp et al. 2007, 2008; Mutneja et al. 2005; Perroy et al. 2003; Sudo et al. 2012; Vargas et al. 2008). Live cell imaging and cell surface biotinylation experiments revealed that agonist stimulation increases the rate of $GABA_B$ receptor endocytosis (Wilkins et al. 2008) but on the same time also accelerates the rate of their recycling (Grampp et al. 2008), keeping the number of cell surface receptors stable. The underlying mechanism had been delineated recently: activation of $GABA_B$ receptors activates the associated Gi/o protein, resulting in the phosphorylation of the GTPase activating protein Rap1GAP2,

which controls the activity of the small GTPase Rap1. Activated Rap1 in turn binds to the C-terminus of $GABA_{B1}$ and promotes recycling of the receptors (Zhang et al. 2015). Preventing this interaction led to the baclofen-induced downregulation of cell surface $GABA_B$ receptors.

4.2.3.3 Regulation of Postendocytic Sorting of $GABA_B$ Receptors

How constitutive endocytosis and recycling is regulated and how the decision is made to sort the receptors to lysosomes for degradation is currently not understood. First data touching this issue came from studies analyzing the role of prolonged excitation, mimicking ischemic conditions, on the regulation of $GABA_B$ receptors. Sustained activation of AMPA or NMDA receptors led to the rapid Ca^{2+}-dependent downregulation of $GABA_B$ receptors via lysosomal degradation (Guetg et al. 2010; Kantamneni et al. 2014; Maier et al. 2010; Terunuma et al. 2010). Downregulation of the receptors was caused by a reduced rate of recycling and an increased rate of lysosomal degradation. These events were triggered by phosphorylation of serine 867 in $GABA_{B1}$ by CaMKII (Guetg et al. 2010) as well as the initial phosphorylation of serine 783 in $GABA_{B2}$ by AMPK followed by dephosphorylation by PP2A (Terunuma et al. 2010). Because phosphorylation of $GABA_{B2}$ by AMPK stabilizes cell surface $GABA_B$ receptors (Kuramoto et al. 2007) this appears to be a compensatory but unsuccessful reaction of the neurons to counteract downregulation of the receptors. Both, CaMKII-mediated phosphorylation of $GABA_{B1}$ and PP2A-mediated dephosphorylation of $GABA_{B2}$ are essential for downregulation of the receptors since inhibition of either pathway prevented degradation of the receptors. It is thus evident that CaMKII and PP2A regulate the balance between recycling and lysosomal sorting of the receptors. However, it is currently unclear, which steps in this mechanism are specifically addressed by CaMKII and PP2A.

In particular, downregulation of $GABA_B$ receptors by PP2A diminish $GABA_B$ receptor signaling under pathological conditions. For instance, mice exposed to foot-shocks develop depression-like symptoms accompanied by PP2A-mediated downregulation of $GABA_B$ receptors in the lateral habenula (Lecca et al. 2016). Inhibition of PP2A restored $GABA_B$ receptor signaling and ameliorated the depression-like behaviors. Similarly, cocaine and methamphetamine treatment of mice led to a PP2A-mediated downregulation of $GABA_B$ receptors in GABAergic neurons of the VTA (ventral tegmental area) (Padgett et al. 2012) and layer 5/6 pyramidal neurons in the medial prefrontal cortex (Hearing et al. 2013).

4.2.4 Degradation of $GABA_B$ Receptors

Protein degradation is an essential part of intracellular metabolism to maintain cellular stability and is involved in the regulation of many cellular processes (Ciechanover 2006). Protein degradation is mediated via two main pathways

involving lysosomes and proteasomes, respectively. Endocytosed plasma membrane proteins are predominantly degraded by lysosomes, which are vesicular structures containing proteolytic enzymes (Saftig and Klumperman 2009). Soluble proteins as well as membrane proteins in the ER are usually degraded via proteasomes. Proteasomes are multiprotein complexes with proteolytic activity and are located throughout the cell (Grabbe et al. 2011; Weissman et al. 2011). Degradation of membrane proteins in the ER is mediated by the ubiquitin and proteasome-dependent ER-associated protein degradation (ERAD) machinery. ERAD primarily degrades incorrectly folded or assembled membrane proteins in the ER. It is regarded as a quality control mechanism that ensures that only intact proteins leave the ER and are transported to their final destination (Vembar and Brodsky 2008).

4.2.4.1 Lysosomal Degradation of $GABA_B$ Receptors

At the end of their life cycle, internalized $GABA_B$ receptors are sorted to late endosomes from which they are targeted toward lysosomal degradation (Grampp et al. 2007, 2008; Kantamneni et al. 2008). This became evident by the colocalization of $GABA_B$ receptors with lysosomal marker proteins (Grampp et al. 2008; Hannan et al. 2011; Laffray et al. 2007) and by the observation that inhibition of lysosomal proteases led to their intracellular accumulation (Grampp et al. 2007, 2008; Maier et al. 2010). The endosomal sorting complex required for transport (ESCRT) appears to play a major role in sorting $GABA_B$ receptors to lysosomal degradation. ESCRT is a multiprotein and ubiquitin-dependent machinery that guides ubiquitinated membrane proteins through the endosomal pathway to lysosomes (Raiborg and Stenmark 2009). It had been shown that a subcomplex of the ESCRT machinery, tumor-susceptibility gene 101 (TSG101), is involved in the degradation of $GABA_B$ receptors (Kantamneni et al. 2008). Knockdown of TSG101 inhibited lysosomal degradation of $GABA_B$ receptors.

There is indirect evidence for ubiquitination being involved in lysosomal degradation of the receptors. Blocking global ubiquitination by inhibiting the ubiquitin-activating enzyme 1 (E1) decreased lysosome-dependent degradation of $GABA_B$ receptors (Maier et al. 2010). In addition, the deubiquitinating enzyme USP14 was shown to regulate the postendocytic ubiquitination state and degradation of $GABA_B$ receptors (Lahaie et al. 2016). However, the precise sorting signals that target $GABA_B$ receptors to lysosomes are so far elusive.

4.2.4.2 Proteasomal Degradation

It is increasingly recognized that proteasomal degradation of proteins via ERAD not only removes defective newly synthesized proteins from the ER but can also regulate the available quantity of membrane proteins. Recent studies have shown that the amount of $GABA_B$ receptors in the ER available for forward trafficking to the

cell surface is controlled by proteasome-mediated degradation via the ERAD machinery (Zemoura and Benke 2014; Zemoura et al. 2013).

For proteasomal degradation, $GABA_B$ receptors are K48-linked ubiquitinated at lysine residues 767/771 in the C-terminal domain of $GABA_{B2}$ and interact with major components of the ERAD machinery such as Hrd1 (an ERAD E3 ligase (Smith et al. 2011)) and p97 (mediates the retrotranslocation of the proteins from the ER membrane to the cytoplasmic proteasomes (Wang et al. 2004; Zemoura et al. 2013)). Finally, degradation is triggered by the interaction of the C-terminus of $GABA_{B2}$ with the proteasomal AAA-ATPase Rpt6 (Zemoura and Benke 2014). This mechanism determines the amount of $GABA_B$ receptors in the ER available for forward trafficking to the plasma membrane and is, most importantly, controlled by the activity state of the neuron (Zemoura and Benke 2014). In this respect, the ERAD machinery acts as a gatekeeper regulating the number of functional receptors at the cell surface in response to neuronal activity and may thus represent a mechanism involved in homeostatic neuronal plasticity.

4.3 Conclusions

Recent work clearly demonstrates that $GABA_B$ receptors are organized in large multiprotein complexes. The minimum core complex appears to consist of the heterodimeric receptor protein, the G protein attached to $GABA_{B2}$, and the KCTD proteins associated with $GABA_{B2}$ and the G protein (Fig. 4.1). Several other proteins are robustly associated with $GABA_B$ receptors whose function in $GABA_B$ receptor physiology needs to be established. Regarding effector systems, only Ca^{2+}channels and HCN channels appear to be strongly attached to $GABA_B$ receptors and can be regarded as members of the core complex. Other effectors such as K^+ channels, adenylyl cyclases, and MAP kinases appear to be more loosely associated with the receptors. However, it should be noted that this classification largely depends on the ability of the core complex proteins to withstand the solubilization procedure required for immunoprecipitation and mass spectroscopy. Whether the strength of interaction reflects a stable long-lasting interaction is currently unclear. Analyzing the dynamics of those proteins in relation to the $GABA_B$ receptor core complex will be an important and challenging task in the future.

As outlined in this review, trafficking events and controlled degradation are increasingly recognized as important mechanisms that regulate the availability of cell surface receptors and thereby determine the level of possible $GABA_B$ receptor-mediated neuronal inhibition. Both, lysosomal degradation of internalized receptors and proteasomal degradation of newly synthesized receptors appear to be tightly regulated by neuronal activity, suggesting that they play an important role in synaptic plasticity. Interestingly, aberrant regulation of trafficking/degradation mechanisms is associated with neurological diseases such as cerebral ischemia, drug addiction, and neuropathic pain. In this regard a deep insight into the regulation of $GABA_B$ receptor trafficking and degradation

mechanisms may lead in the future to ideas for the development of novel therapeutic treatments.

References

Beck, R., Rawet, M., Wieland, F. T., & Cassel, D. (2009). The COPI system: Molecular mechanisms and function. *FEBS Letters, 583*, 2701–2709.

Benke, D., Honer, M., Michel, C., Bettler, B., & Mohler, H. (1999). γ-Aminobutyric acid type B receptor splice variant proteins GBR1a and GBR1b are both associated with GBR2 *in situ* and display differential regional and subcellular distribution. *Journal of Biological Chemistry, 274*, 27323–27330.

Bernard, P., Guedin, D., & Hibert, M. (2001). Molecular modeling of the GABA/$GABA_B$ receptor complex. *Journal of Medicinal Chemistry, 44*, 27–35.

Biermann, B., Ivankova-Susankova, K., Bradaia, A., Abdel Aziz, S., Besseyrias, V., Kapfhammer, J. P., et al. (2010). The Sushi domains of $GABA_B$ receptors function as axonal targeting signals. *Journal of Neuroscience, 30*, 1385–1394.

Binet, V., Brajon, C., Le Corre, L., Acher, F., Pin, J. P., & Prezeau, L. (2004). The heptahelical domain of $GABA_{B2}$ is activated directly by CGP7930, a positive allosteric modulator of the $GABA_B$ receptor. *Journal of Biological Chemistry, 279*, 29085–29091.

Bischoff, S., Leonhard, S., Reymann, N., Schuler, V., Shigemoto, R., Kaupmann, K., et al. (1999). Spatial distribution of $GABA_BR1$ receptor mRNA and binding sites in the rat brain. *Journal of Comparative Neurology, 412*, 1–16.

Brock, C., Boudier, L., Maurel, D., Blahos, J., & Pin, J. P. (2005). Assembly-dependent surface targeting of the heterodimeric $GABA_B$ receptor is controlled by COPI but not 14-3-3. *Molecular Biology of the Cell, 16*, 5572–5578.

Burkhard, P., Stetefeld, J., & Strelkov, S. V. (2001). Coiled coils: A highly versatile protein folding motif. *Trends in Cell Biology, 11*, 82–88.

Burmakina, S., Geng, Y., Chen, Y., & Fan, Q. R. (2014). Heterodimeric coiled-coil interactions of human $GABA_B$ receptor. *Proceedings of the National Academy of Sciences of the United States of America, 111*, 6958–6963.

Calebiro, D., Rieken, F., Wagner, J., Sungkaworn, T., Zabel, U., Borzi, A., et al. (2013). Single-molecule analysis of fluorescently labeled G-protein-coupled receptors reveals complexes with distinct dynamics and organization. *Proceedings of the National Academy of Sciences of the United States of America, 110*, 743–748.

Calver, A. R., Robbins, M. J., Cosio, C., Rice, S. Q. J., Babbs, A. J., Hirst, W. D., et al. (2001). The C-terminal domains of the $GABA_B$ receptor subunits mediate intracellular trafficking but are not required for receptor signaling. *Journal of Neuroscience, 21*, 1203–1210.

Ciechanover, A. (2006). Intracellular protein degradation: From a vague idea thru the lysosome and the ubiquitin-proteasome system and onto human diseases and drug targeting. *Hematology American Society of Hematology. Education Program, 2006*, 1–12.

Cifuentes-Diaz, C., Marullo, S., & Doly, S. (2015). Anatomical and ultrastructural study of PRAF2 expression in the mouse central nervous system. *Brain Structure and Function*, doi:10.1007/s00429-015-1159-8.

Comps-Agrar, L., Kniazeff, J., Brock, C., Trinquet, E., & Pin, J. P. (2012). Stability of $GABA_B$ receptor oligomers revealed by dual TR-FRET and drug-induced cell surface targeting. *The FASEB Journal, 26*, 3430–3439.

Comps-Agrar, L., Kniazeff, J., Norskov-Lauritsen, L., Maurel, D., Gassmann, M., Gregor, N., et al. (2011). The oligomeric state sets $GABA_B$ receptor signalling efficacy. *The EMBO Journal, 30*, 2336–2349.

Couve, A., Filippov, A. K., Connolly, C. N., Bettler, B., Brown, D. A., & Moss, S. J. (1998). Intracellular retention of recombinant $GABA_B$ receptors. *Journal of Biological Chemistry, 273*, 26361–26367.

David, M., Richer, M., Mamarbachi, A. M., Villeneuve, L. R., Dupre, D. J., & Hebert, T. E. (2006). Interactions between $GABA_{B1}$ receptors and Kir 3 inwardly rectifying potassium channels. *Cellular Signalling, 18*, 2172–2181.

Doly, S., Shirvani, H., Gata, G., Meye, F. J., Emerit, M. B., Enslen, H., et al. (2015). $GABA_B$ receptor cell-surface export is controlled by an endoplasmic reticulum gatekeeper. *Molecular Psychiatry, 21*, 480–490.

Dupuis, D. S., Relkovic, D., Lhuillier, L., Mosbacher, J., & Kaupmann, K. (2006). Point mutations in the transmembrane region of $GABA_{B2}$ facilitate activation by the positive modulator N, N'-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) in the absence of the $GABA_{B1}$ subunit. *Molecular Pharmacology, 70*, 2027–2036.

Duthey, B., Caudron, S., Perroy, J., Bettler, B., Fagni, L., Pin, J. P., et al. (2002). A single subunit (GB2) is required for G-protein activation by the heterodimeric $GABA_B$ receptor. *Journal of Biological Chemistry, 277*, 3236–3241.

Fairfax, B. P., Pitcher, J. A., Scott, M. G., Calver, A. R., Pangalos, M. N., Moss, S. J., et al. (2004). Phosphorylation and chronic agonist treatment atypically modulate $GABA_B$ receptor cell surface stability. *Journal of Biological Chemistry, 279*, 12565–12573.

Fernandez-Alacid, L., Aguado, C., Ciruela, F., Martin, R., Colon, J., Cabanero, M. J., et al. (2009). Subcellular compartment-specific molecular diversity of pre- and post-synaptic GABA-activated GIRK channels in Purkinje cells. *Journal of Neurochemistry, 110*, 1363–1376.

Fritschy, J. M., Meskenaite, V., Weinmann, O., Honer, M., Benke, D., & Mohler, H. (1999). $GABA_B$ receptor splice variants GB1a and GB1b in rat brain: Developmental regulation, cellular distribution and extrasynaptic localization. *The European Journal of Neuroscience, 11*, 761–768.

Gainetdinov, R. R., Premont, R. T., Bohn, L. M., Lefkowitz, R. J., & Caron, M. G. (2004). Desensitization of G protein-coupled receptors and neuronal functions. *Annual Review of Neuroscience, 27*, 107–144.

Galvez, T., Duthey, B., Kniazeff, J., Blahos, J., Rovelli, G., Bettler, B., et al. (2001). Allosteric interactions between GB1 and GB2 subunits are required for optimal $GABA_B$ receptor function. *The EMBO Journal, 20*, 2152–2159.

Galvez, T., Parmentier, M. L., Joly, C., Malitschek, B., Kaupmann, K., Kuhn, R., et al. (1999). Mutagenesis and modeling of the $GABA_B$ receptor extracellular domain support a Venus flytrap mechanism for ligand binding. *Journal of Biological Chemistry, 274*, 13362–13369.

Galvez, T., Prezeau, L., Milioti, G., Franek, M., Joly, C., Froestl, W., et al. (2000). Mapping the agonist-binding site of $GABA_B$ type 1 subunit sheds light on the activation process of $GABA_B$ receptors. *Journal of Biological Chemistry, 275*, 41166–41174.

Gassmann, M., Haller, C., Stoll, Y., Aziz, S. A., Biermann, B., Mosbacher, J., et al. (2005). The RXR-type endoplasmic reticulum-retention/retrieval signal of $GABA_{B1}$ requires distant spacing from the membrane to function. *Molecular Pharmacology, 68*, 137–144.

Geng, Y., Bush, M., Mosyak, L., Wang, F., & Fan, Q. R. (2013). Structural mechanism of ligand activation in human $GABA_B$ receptor. *Nature, 504*, 254–259.

Geng, Y., Xiong, D., Mosyak, L., Malito, D. L., Kniazeff, J., Chen, Y., et al. (2012). Structure and functional interaction of the extracellular domain of human $GABA_B$ receptor GBR2. *Nature Neuroscience, 15*, 970–978.

Grabbe, C., Husnjak, K., & Dikic, I. (2011). The spatial and temporal organization of ubiquitin networks. *Nature Reviews. Molecular Cell Biology, 12*, 295–307.

Grampp, T., Notz, V., Broll, I., Fischer, N., & Benke, D. (2008). Constitutive, agonist-accelerated, recycling and lysosomal degradation of $GABA_B$ receptors in cortical neurons. *Molecular and Cellular Neurosciences, 39*, 628–637.

Grampp, T., Sauter, K., Markovic, B., & Benke, D. (2007). γ-Aminobutyric acid type B receptors are constitutively internalized via the clathrin-dependent pathway and targeted to lysosomes for degradation. *Journal of Biological Chemistry, 282*, 24157–24165.

Guetg, N., Aziz, S. A., Holbro, N., Turecek, R., Rose, T., Seddik, R., et al. (2010). NMDA receptor-dependent $GABA_B$ receptor internalization via CaMKII phosphorylation of serine 867 in $GABA_{B1}$. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 13924–13929.

Hannan, S., Wilkins, M. E., Dehghani-Tafti, E., Thomas, P., Baddeley, S. M., & Smart, T. G. (2011). $GABA_B$ receptor internalisation is regulated by the R2 subunit. *Journal of Biological Chemistry, 286*, 24324–24335.

Hannan, S., Wilkins, M. E., & Smart, T. G. (2012). Sushi domains confer distinct trafficking profiles on $GABA_B$ receptors. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 12171–12176.

Havlickova, M., Prezeau, L., Duthey, B., Bettler, B., Pin, J. P., & Blahos, J. (2002). The intracellular loops of the GB2 subunit are crucial for G-protein coupling of the heteromeric γ-aminobutyrate$_B$ receptor. *Molecular Pharmacology, 62*, 343–350.

Hearing, M., Kotecki, L., Fernandez de Velasco, E., Fajardo-Serrano, A., Chung, H. J., Lujan, R., et al. (2013). Repeated cocaine weakens GABA-Girk signaling in layer 5/6 pyramidal neurons in the prelimbic cortex. *Neuron, 80*, 159–170.

Holter, J., Davies, J., Leresche, N., Crunelli, V., & Carter, D. A. (2005). Identification of two further splice variants of *GABABR1* characterizes the conserved micro-exon 4 as a hot spot for regulated splicing in the rat brain. *Journal of Molecular Neuroscience, 26*, 99–108.

Isomoto, S., Kaibara, M., Sakurai-Yamashita, Y., Nagayama, Y., Uezono, Y., Yano, K., et al. (1998). Cloning and tissue distribution of novel splice variants of the rat $GABA_B$ receptor. *Biochemical and Biophysical Research Communications, 253*, 10–15.

Ivankova, K., Turecek, R., Fritzius, T., Seddik, R., Prezeau, L., Comps-Agrar, L., et al. (2013). Up-regulation of $GABA_B$ receptor signaling by constitutive assembly with the K^+ channel tetramerization domain-containing protein 12 (KCTD12). *Journal of Biological Chemistry, 288*, 24848–24856.

Jones, K. A., Borowsky, B., Tamm, J. A., Craig, D. A., Durkin, M. M., Dai, M., et al. (1998). $GABA_B$ receptor function as a heteromeric assembly of the subunits $GABA_{BR1}$ and $GABA_{BR2}$. *Nature, 396*, 674–679.

Kammerer, R. A., Frank, S., Schulthess, T., Landwehr, R., Lustig, A., & Engel, J. (1999). Heterodimerization of a functional $GABA_B$ receptor is mediated by parallel coiled-coil alpha-helices. *Biochemistry, 38*, 13263–13269.

Kantamneni, S., Gonzalez-Gonzalez, I. M., Luo, J., Cimarosti, H., Jacobs, S. C., Jaafari, N., et al. (2014). Differential regulation of $GABA_B$ receptor trafficking by different modes of N-methyl-D-aspartate (NMDA) receptor signaling. *Journal of Biological Chemistry, 289*, 6681–6694.

Kantamneni, S., Holman, D., Wilkinson, K. A., Correa, S. A., Feligioni, M., Ogden, S., et al. (2008). GISP binding to TSG101 increases $GABA_B$ receptor stability by down-regulating ESCRT-mediated lysosomal degradation. *Journal of Neurochemistry, 107*, 86–95.

Kaupmann, K., Huggel, K., Heid, J., Flor, P. J., Bischoff, S., Mickel, S. J., et al. (1997). Expression cloning of $GABA_B$ receptors uncovers similarity to metabotropic glutamate receptors. *Nature, 386*, 239–246.

Kaupmann, K., Malitschek, B., Schuler, V., Heid, J., Froestl, W., Beck, P., et al. (1998). $GABA_B$ receptor subtypes assemble into functional heteromeric complexes. *Nature, 396*, 683–687.

Kniazeff, J., Galvez, T., Labesse, G., & Pin, J. P. (2002). No ligand binding in the GB2 subunit of the $GABA_B$ receptor is required for activation and allosteric interaction between the subunits. *Journal of Neuroscience, 22*, 7352–7361.

Kulik, A., Vida, I., Fukazawa, Y., Guetg, N., Kasugai, Y., Marker, C. L., et al. (2006). Compartment-dependent colocalization of Kir3.2-containing K^+ channels and $GABA_B$ receptors in hippocampal pyramidal cells. *Journal of Neuroscience, 26*, 4289–4297.

Kuramoto, N., Wilkins, M. E., Fairfax, B. P., Revilla-Sanchez, R., Terunuma, M., Tamaki, K., et al. (2007). Phospho-dependent functional modulation of $GABA_B$ receptors by the metabolic sensor AMP-dependent protein kinase. *Neuron, 53*, 233–247.

Laffray, S., Bouali-Benazzouz, R., Papon, M. A., Favereaux, A., Jiang, Y., Holm, T., et al. (2012). Impairment of $GABA_B$ receptor dimer by endogenous 14-3-3ζ in chronic pain conditions. *The EMBO Journal, 31*, 3239–3251.

Laffray, S., Tan, K., Dulluc, J., Bouali-Benazzouz, R., Calver, A. R., Nagy, F., et al. (2007). Dissociation and trafficking of rat $GABA_B$ receptor heterodimer upon chronic capsaicin stimulation. *The European Journal of Neuroscience, 25*, 1402–1416.

Lahaie, N., Kralikova, M., Pr Eacutezeau, L., Blahos, J., & Bouvier, M. (2016). Post-endocytotic deubiquitination and degradation of the metabotropic γ-aminobutyric acid receptor by the ubiquitin specific protease 14. *Journal of Biological Chemistry, 291*, 7156–7170.

Laviv, T., Vertkin, I., Berdichevsky, Y., Fogel, H., Riven, I., Bettler, B., et al. (2011). Compartmentalization of the $GABA_B$receptor signaling complex is required for presynaptic inhibition at hippocampal synapses. *Journal of Neuroscience, 31*, 12523–12532.

Lecca, S., Pelosi, A., Tchenio, A., Moutkine, I., Lujan, R., Herve, D., et al. (2016). Rescue of $GABA_B$ and GIRK function in the lateral habenula by protein phosphatase 2A inhibition ameliorates depression-like phenotypes in mice. *Nature Medicine, 22*, 254–261.

Lee, C., Mayfield, R. D., & Harris, R. A. (2010). Intron 4 containing novel $GABA_{B1}$ isoforms impair $GABA_B$ receptor function. *PLoS One, 5*, e14044.

Liu, J., Maurel, D., Etzol, S., Brabet, I., Ansanay, H., Pin, J. P., et al. (2004). Molecular determinants involved in the allosteric control of agonist affinity in the $GABA_B$ receptor by the $GABA_{B2}$ subunit. *Journal of Biological Chemistry, 279*, 15824–15830.

Lujan, R., & Ciruela, F. (2012). $GABA_B$ receptors-associated proteins: Potential drug targets in neurological disorders? *Current Drug Targets, 13*, 129–144.

Maier, P. J., Marin, I., Grampp, T., Sommer, A., & Benke, D. (2010). Sustained glutamate receptor activation down-regulates $GABA_B$ receptors by shifting the balance from recycling to lysosomal degradation. *Journal of Biological Chemistry, 285*, 35606–35614.

Maier, P. J., Zemoura, K., Acuna, M. A., Yevenes, G. E., Zeilhofer, H. U., & Benke, D. (2014). Ischemia-like oxygen and glucose deprivation mediates down-regulation of cell surface γ-aminobutyric $acid_B$ receptors via the endoplasmic reticulum (ER) stress-induced transcription factor CCAAT/enhancer-binding protein (C/EBP)-homologous protein (CHOP). *Journal of Biological Chemistry, 289*, 12896–12907.

Malherbe, P., Masciadri, R., Norcross, R. D., Knoflach, F., Kratzeisen, C., Zenner, M. T., et al. (2008). Characterization of (R, S)-5,7-di-tert-butyl-3-hydroxy-3-trifluoromethyl-3H-benzofuran-2-one as a positive allosteric modulator of $GABA_B$ receptors. *British Journal of Pharmacology, 154*, 797–811.

Malitschek, B., Ruegg, D., Heid, J., Kaupmann, K., Bittiger, H., Frostl, W., et al. (1998). Developmental changes of agonist affinity at $GABA_B$R1 receptor variants in rat brain. *Molecular and Cellular Neurosciences, 12*, 56–64.

Marchese, A., Chen, C., Kim, Y. M., & Benovic, J. L. (2003). The ins and outs of G protein-coupled receptor trafficking. *Trends in Biochemical Sciences, 28*, 369–376.

Margeta-Mitrovic, M., Jan, Y. N., & Jan, L. Y. (2000). A trafficking checkpoint controls $GABA_B$ receptor heterodimerization. *Neuron, 27*, 97–106.

Margeta-Mitrovic, M., Jan, Y. N., & Jan, L. Y. (2001). Function of GB1 and GB2 subunits in G protein coupling of $GABA_B$ receptors. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 14649–14654.

Maurel, D., Comps-Agrar, L., Brock, C., Rives, M. L., Bourrier, E., Ayoub, M. A., et al. (2008). Cell-surface protein-protein interaction analysis with time-resolved FRET and snap-tag technologies: Application to GPCR oligomerization. *Nature Methods, 5*, 561–567.

Muller, C. S., Haupt, A., Bildl, W., Schindler, J., Knaus, H. G., Meissner, M., et al. (2010). Quantitative proteomics of the Cav2 channel nano-environments in the mammalian brain. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 14950–14957.

Mutneja, M., Berton, F., Suen, K. F., Lüscher, C., & Slesinger, P. A. (2005). Endogenous RGS proteins enhance acute desensitization of $GABA_B$ receptor-activated GIRK currents in HEK-293T cells. *Pflügers Archiv, 450*, 61–73.

Oyadomari, S., & Mori, M. (2004). Roles of CHOP/GADD153 in endoplasmic reticulum stress. *Cell Death and Differentiation, 11*, 381–389.

Padgett, C. L., Lalive, A. L., Tan, K. R., Terunuma, M., Munoz, M. B., Pangalos, M. N., et al. (2012). Methamphetamine-evoked depression of $GABA_B$ receptor signaling in GABA neurons of the VTA. *Neuron, 73*, 978–989.

Pagano, A., Rovelli, G., Mosbacher, J., Lohmann, T., Duthey, B., Stauffer, D., et al. (2001). C-terminal interaction is essential for surface trafficking but not for heteromeric assembly of $GABA_B$ receptors. *Journal of Neuroscience, 21*, 1189–1202.

Park, H. W., Jung, H., Choi, K. H., Baik, J. H., & Rhim, H. (2011). Direct interaction and functional coupling between voltage-gated CaV1.3 Ca^{2+} channel and $GABA_B$ receptor subunit 2. *FEBS Letters, 584*, 3317–3322.

Perroy, J., Adam, L., Qanbar, R., Chénier, S., & Bouvier, M. (2003). Phosphorylation-independent desensitization of $GABA_B$ receptor by GRK4. *The EMBO Journal, 22*, 3816–3824.

Pfaff, T., Malitschek, B., Kaupmann, K., Prezeau, L., Pin, J. P., Bettler, B., et al. (1999). Alternative splicing generates a novel isoform of the rat metabotropic $GABA_BR1$ receptor. *The European Journal of Neuroscience, 11*, 2874–2882.

Pooler, A. M., Gray, A. G., & McIlhinney, R. A. (2009). Identification of a novel region of the $GABA_{B2}$ C-terminus that regulates surface expression and neuronal targeting of the $GABA_B$ receptor. *The European Journal of Neuroscience, 29*, 869–878.

Raiborg, C., & Stenmark, H. (2009). The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins. *Nature, 458*, 445–452.

Ramirez, O. A., Vidal, R. L., Tello, J. A., Vargas, K. J., Kindler, S., Härtel, S., et al. (2009). Dendritic assembly of heteromeric γ-aminobutyric acid type B receptor subunits in hippocampal neurons. *Journal of Biological Chemistry, 284*, 13077–13085.

Robbins, M. J., Calver, A. R., Filippov, A. K., Hirst, W. D., Russell, R. B., Wood, M. D., et al. (2001). $GABA_{B2}$ is essential for G-protein coupling of the $GABA_B$ receptor heterodimer. *Journal of Neuroscience, 21*, 8043–8052.

Saftig, P., & Klumperman, J. (2009). Lysosome biogenesis and lysosomal membrane proteins: Trafficking meets function. *Nature Reviews. Molecular Cell Biology, 10*, 623–635.

Sauter, K., Grampp, T., Fritschy, J. M., Kaupmann, K., Bettler, B., Mohler, H., et al. (2005). Subtype-selective interaction with the transcription factor CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP) regulates cell surface expression of $GABA_B$ receptors. *Journal of Biological Chemistry, 280*, 33566–33572.

Schwarz, D. A., Barry, G., Eliasof, S. D., Petroski, R. E., Conlon, P. J., & Maki, R. A. (2000). Characterization of γ-aminobutyric acid receptor $GABA_{B(1e)}$, a $GABA_{B(1)}$ splice variant encoding a truncated receptor. *Journal of Biological Chemistry, 275*, 32174–32181.

Schwenk, J., Metz, M., Zolles, G., Turecek, R., Fritzius, T., Bildl, W., et al. (2010). Native $GABA_B$ receptors are heteromultimers with a family of auxiliary subunits. *Nature, 465*, 231–235.

Schwenk, J., Perez-Garci, E., Schneider, A., Kollewe, A., Gauthier-Kemper, A., Fritzius, T., et al. (2015). Modular composition and dynamics of native $GABA_B$ receptors identified by high-resolution proteomics. *Nature Neuroscience, 19*, 233–242.

Seddik, R., Jungblut, S. P., Silander, O. K., Rajalu, M., Fritzius, T., Besseyrias, V., et al. (2012). Opposite effects of KCTD subunit domains on $GABA_B$ receptor-mediated desensitization. *Journal of Biological Chemistry, 287*, 39869–39877.

Smith, M. H., Ploegh, H. L., & Weissman, J. S. (2011). Road to ruin: Targeting proteins for degradation in the endoplasmic reticulum. *Science, 334*, 1086–1090.

Steiger, J. L., Bandyopadhyay, S., Farb, D. H., & Russek, S. J. (2004). cAMP response element-binding protein, activating transcription factor-4, and upstream stimulatory factor differentially control hippocampal $GABA_{BR1a}$ and $GABA_{BR1b}$ subunit gene expression through alternative promoters. *Journal of Neuroscience, 24*, 6115–6126.

Sudo, Y., Hojo, M., Ando, Y., Takada, M., Murata, H., Kurata, S., et al. (2012). $GABA_B$ receptors do not internalize after baclofen treatment, possibly due to a lack of β-arrestin association: Study with a real-time visualizing assay. *Synapse, 66*, 759–769.

Terunuma, M., Vargas, K. J., Wilkins, M. E., Ramirez, O. A., Jaureguiberry-Bravo, M., Pangalos, M. N., et al. (2010). Prolonged activation of NMDA receptors promotes dephosphorylation and

alters postendocytic sorting of $GABA_B$ receptors. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 13918–13923.

Tian, X., Kang, D. S., & Benovic, J. L. (2014). β-Arrestins and G protein-coupled receptor trafficking. *Handbook of Experimental Pharmacology, 219*, 173–186.

Tiao, J. Y., Bradaia, A., Biermann, B., Kaupmann, K., Metz, M., Haller, C., et al. (2008). The sushi domains of secreted $GABA_{B1}$ isoforms selectively impair $GABA_B$ heteroreceptor function. *Journal of Biological Chemistry, 283*, 31005–31011.

Turecek, R., Schwenk, J., Fritzius, T., Ivankova, K., Zolles, G., Adelfinger, L., et al. (2014). Auxiliary $GABA_B$ receptor subunits uncouple G protein βγ subunits from effector channels to induce desensitization. *Neuron, 82*, 1032–1044.

Urwyler, S., Mosbacher, J., Lingenhoehl, K., Heid, J., Hofstetter, K., Froestl, W., et al. (2001). Positive allosteric modulation of native and recombinant γ-aminobutyric $acid_B$ receptors by 2,6-Di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and its aldehyde analog CGP13501. *Molecular Pharmacology, 60*, 963–971.

Urwyler, S., Pozza, M. F., Lingenhoehl, K., Mosbacher, J., Lampert, C., Froestl, W., et al. (2003). N, N'-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) and structurally related compounds: novel allosteric enhancers of γ-aminobutyric acid B receptor function. *Journal of Pharmacology and Experimental Therapeutics, 307*, 322–330.

Valdes, V., Valenzuela, J. I., Salas, D. A., Jaureguiberry-Bravo, M., Otero, C., Thiede, C., et al. (2012). Endoplasmic reticulum sorting and kinesin-1 command the targeting of axonal $GABA_B$ receptors. *PLoS One, 7*, e44168.

Valenzuela, J. I., Jaureguiberry-Bravo, M., Salas, D. A., Ramirez, O. A., Cornejo, V. H., Lu, H. E., et al. (2014). Transport along the dendritic endoplasmic reticulum mediates the trafficking of $GABA_B$ receptors. *Journal of Cell Science, 127*, 3382–3395.

Vargas, K. J., Terunuma, M., Tello, J. A., Pangalos, M. N., Moss, S. J., & Couve, A. (2008). The availability of surface $GABA_B$ receptors is independent of γ-aminobutyric acid but controlled by glutamate in central neurons. *Journal of Biological Chemistry, 283*, 24641–24648.

Vembar, S. S., & Brodsky, J. L. (2008). One step at a time: Endoplasmic reticulum-associated degradation. *Nature Reviews. Molecular Cell Biology, 9*, 944–957.

Vigot, R., Barbieri, S., Bräuner-Osborne, H., Turecek, R., Shigemoto, R., Zhang, Y. P., et al. (2006). Differential compartmentalization and distinct functions of $GABA_B$ receptor variants. *Neuron, 50*, 589–601.

Villemure, J. F., Adam, L., Bevan, N. J., Gearing, K., Chenier, S., & Bouvier, M. (2005). Subcellular distribution of $GABA_B$ receptor homo- and hetero-dimers. *Biochemical Journal, 388*, 47–55.

Wang, Q., Song, C., & Li, C. C. (2004). Molecular perspectives on p97-VCP: Progress in understanding its structure and diverse biological functions. *Journal of Structural Biology, 146*, 44–57.

Wei, K. R., Eubanks, J. H., Francis, J., Jia, Z. P., & Snead, O. C. (2001a). Cloning and tissue distribution of a novel isoform of the rat $GABA_BR1$ receptor subunit. *Neuroreport, 12*, 833–837.

Wei, K., Jia, Z., Wang, Y. T., Yang, J., Liu, C. C., & Snead, O. C., 3rd. (2001b). Cloning and characterization of a novel variant of rat $GABA_BR1$ with a truncated C-terminus. *Brain Research. Molecular Brain Research, 89*, 103–110.

Weissman, A. M., Shabek, N., & Ciechanover, A. (2011). The predator becomes the prey: Regulating the ubiquitin system by ubiquitylation and degradation. *Nature Reviews. Molecular Cell Biology, 12*, 605–620.

White, J. H., Wise, A., Main, M. J., Green, A., Fraser, N. J., Disney, G. H., et al. (1998). Heterodimerization is required for the formation of functional $GABA_B$ receptors. *Nature, 396*, 679–682.

Wilkins, M. E., Li, X., & Smart, T. G. (2008). Tracking cell surface $GABA_B$ receptors using an α-bungarotoxin tag. *Journal of Biological Chemistry, 283*, 34745–34752.

Workman, E. R., Haddick, P. C., Bush, K., Dilly, G. A., Niere, F., Zemelman, B. V., et al. (2015). Rapid antidepressants stimulate the decoupling of $GABA_B$ receptors from GIRK/Kir3 channels through increased protein stability of 14-3-3η. *Molecular Psychiatry, 20*, 298–310.

Zemoura, K., & Benke, D. (2014). Proteasomal degradation of γ-aminobutyric acid$_B$ receptors is mediated by the interaction of the GABA$_{B2}$ C terminus with the proteasomal ATPase Rtp6 and regulated by neuronal activity. *Journal of Biological Chemistry, 289*, 7738–7746.

Zemoura, K., Schenkel, M., Acuna, M. A., Yevenes, G. E., Zeilhofer, H. U., & Benke, D. (2013). Endoplasmic reticulum-associated degradation (ERAD) controls cell surface expression of γ-aminobutyric acid, type B receptors. *Journal of Biological Chemistry, 288*, 34897–34905.

Zhang, Z., Zhang, W., Huang, S., Sun, Q., Wang, Y., Hu, Y., et al. (2015). GABA$_B$ receptor promotes its own surface expression by recruiting a Rap1-dependent signaling cascade. *Journal of Cell Science, 128*, 2302–2313.

Chapter 5
Distribution and Localization of the $GABA_B$ Receptor

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Abstract The functional $GABA_B$ receptors ($GABA_BRs$) are formed by obligate heteromers composed of two principal subunits named $GABA_{B1}$ and $GABA_{B2}$. In *Drosophila melanogaster* three $GABA_B$ subunits have been identified: GB1, GB2, and GB3. The GB1 and GB2 subunits need to be coexpressed in Xenopus oocytes or in mammalian cell lines to produce functional $GABAB_Rs$. A subfamily of potassium channel tetramerization domain-containing (KCTD8, 12, 12b, and 16) proteins that are constituents of native $GABA_BRs$ were recently identified. KCTDs show a temporal and spatial distribution pattern that may contribute to the heterogeneity of native $GABA_BRs$ and their pharmacological properties.

Of several isoforms of the $GABA_{B1}$ subunit identified to date, the most abundant in the brain are the isoforms 1a and 1b; they are coexpressed with the subunit $GABA_{B2}$ and their expression differs across brain and neuronal populations. $GABA_{B1a}$ localizes to glutamatergic terminals and is necessary for hetero-receptor function. Both isoforms 1a and 1b are detected in dendrites, but only $GABA_{B1b}$ in spine heads. Electron microscopy studies show that, in the central nervous system (CNS), $GABA_{B1}$ and $GABA_{B2}$ are both pre- and postsynaptic, but mostly localize to postsynaptic sites. The $GABA_{B1(a/b)}$ and $GABA_{B2}$ subunits show an overlapping pattern of distribution throughout the CNS with certain exceptions (i.e., caudate–putamen and cerebellum). $GABA_BRs$ are also detected in Schwann cells, in several peripheral tissues, and in nonneuronal cells (cardiomyocytes and airway smooth muscle). The widespread distribution of $GABA_BRs$ in the CNS and the periphery reflects their physiological, pathophysiological, and pharmacological relevance.

Keywords $GABA_{B1}$ and $GABA_{B2}$ receptor subunits • Central nervous system • Peripheral nervous system • mRNA distribution • Immunohistochemical distribution

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5.1 Introduction

The $GABA_B$ receptors ($GABA_BRs$), which are members of the class C of G protein-coupled receptors (GCPRs), are the metabotropic target of γ-amino butyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system (CNS). The $GABA_BRs$ mediate GABAergic transmission by interacting with G-proteins, mainly the Gαi/o subunits, and modulating the second messenger system (Gassmann and Bettler 2012).

$GABA_BRs$ were first identified by Bowery and colleagues (1980) (see Chap. 1 of this book) in the periphery and later shown to be present through the CNS of various vertebrate species, where they are expressed by almost all neurons and nonneuronal brain cells (Bettler et al. 2004; Gassmann and Bettler 2012). Cloning of the receptor in the late 1990s revealed that functional $GABA_BR$ is formed by obligate heteromers composed of two principal subunits named $GABA_{B1}$ and $GABA_{B2}$ (see Chap. 4 of this book). Both subunits have the prototypical seven transmembrane domains of GPCRs; subunit B1 has the Venus flytrap domain containing the binding sites for agonists and antagonists (Galvez et al. 2000), while the B2 subunit is required for cell membrane expression, G-protein coupling and activation, and high-affinity binding of $GABA_{B1}$ agonists (Galvez et al. 2001). Several isoforms of the $GABA_{B1}$ subunit have been identified (Bettler et al. 2004; Pfaff et al. 1999; Billinton et al. 2001); the most abundant in the brain and highly conserved among species are the isoforms 1a and 1b. The difference between these isoforms is the presence of a pair of sushi domains in the N-terminal domain of 1a compared with the 1b isoform (Blein et al. 2004).

The functional diversity of native $GABA_B$ receptors suggests the existence of multiple receptors subtypes; however, until recently, the only $GABA_BR$ functional unit was composed of the heteromeric assembly of $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$. Several factors might influence the heterogeneity of native $GABA_BR$ responses, such as receptor phosphorylation (Couve et al. 2002), regulator of G protein signaling proteins (RGS proteins) (Labouèbe et al. 2007; Mutneja et al. 2005), and a plethora of $GABA_BR$ interacting proteins important for the activation of signaling pathways, modulation of receptor function, and for targeting receptors to the surface membrane or to subcellular compartments (Xu et al. 2014; Lujan and Ciruela 2012).

Recently, Schwenk et al. (2010) used proteomic functional approaches and identified a subfamily of potassium channel tetramerization domain-containing (KCTD) proteins that appear to be constituents of native $GABA_BRs$. Specifically, four cytoplasmic proteins termed KCTD 8, 12, 12b, and 16 are tightly associated with the C-terminal domain of the $GABA_{B2}$ subunit and influence agonist potency and the kinetics of the receptor response (Turecek et al. 2014).

In the following paragraphs, we will briefly review studies that used different experimental approaches (i.e., in situ hybridization, autoradiography, and immunohistochemical analysis) to describe the cellular and subcellular distribution of $GABA_BRs$ in mammalian and nonmammalian CNS and periphery.

5.2 Distribution and Expression of the $GABA_B$ Receptor in the CNS: Mammalian and Nonmammalian Studies

5.2.1 Regional Distribution of $GABA_B$ Receptor mRNAs, Binding Sites, and Immunohistochemical Localization in Mammals

The mRNAs encoding the subunits $GABA_{B1}$ and $GABA_{B2}$ are widely expressed throughout the brain of vertebrate species (Bettler et al. 2004).

The highest levels of mRNA expression are found in the hippocampus, thalamic nuclei, cerebellum, and cortex for the $GABA_{B1}$ subunit, and piriform cortex, hippocampus, and medial habenula for the $GABA_{B2}$ subunit (Bischoff et al. 1999; Durkin et al. 1999). $GABA_{B1}$ and $GABA_{B2}$ transcripts overlap in the CNS, indicating that heteromeric $GABA_{B1,2}$ constitutes the majority of native $GABA_B$Rs present in the brain.

In situ hybridization studies (Kaupmann et al. 1997; Bischoff et al. 1999; Liang et al. 2000) examining the tissue brain distribution of $GABA_{B1}$R and its isoforms B1a and B1b demonstrated that these two isoforms of $GABA_{B1}$R are present in all brain areas analyzed, although differences in their regional patterns were detected (Fig. 5.1). Specifically, Bischoff et al. (1999) mapped and quantified the $GABA_B$R1a and $GABA_B$R1b transcripts in rat brain by means of in situ hybridization using both specific riboprobes for either isoform B1a or B1b and a pan-riboprobe that detected both subunits. The quantitative measurements of transcript levels were represented by the density of silver grains within a given cell that was visualized by counterstained. The levels of expression in individual brain regions, nuclei, or layers were expressed as the percentage of the surface of the cells covered by the silver grains. They demonstrated that $GABA_B$R expression levels vary among different brain structures, ranging from 8 % in the molecular layer of the cerebellum to 70 % in the dorsal lateral geniculate nucleus. In particular, $GABA_{B1}$ is heterogeneously expressed at low levels in the olfactory bulb, showing the highest levels of the B1a isoform in the mitral cells, while both B1a and B1b are barely detectable in granular cells. Both transcripts are present in all layers of the cortex, exhibiting high to moderate expression in the frontal and parietal cortex; the B1a isoform is more abundant in parietal layers than in the frontal cortex, while B1b levels are similar between the two regions, with low expression in the middle layers (III–IV) and increasing levels in the deeper layer VI. In basal ganglia, both dorsal (caudate–putamen and globus pallidus) and ventral (nucleus accumbens and ventral pallidum) circuits show low to modest $GABA_{B1}$R mRNA expression. Comparison of B1a and B1b transcript levels shows a low to moderate $GABA_{B1a}$ hybridization signal in the dorsal structures and a very low signal for $GABA_{B1b}$ (Liang et al. 2000). By contrast, the hippocampal formation shows the highest mRNA levels for $GABA_{B1}$R in the pyramidal cell layer CA1, high levels in the CA3 layer, and moderate levels in the granular layer of the dentate gyrus. Specifically, in the pyramidal

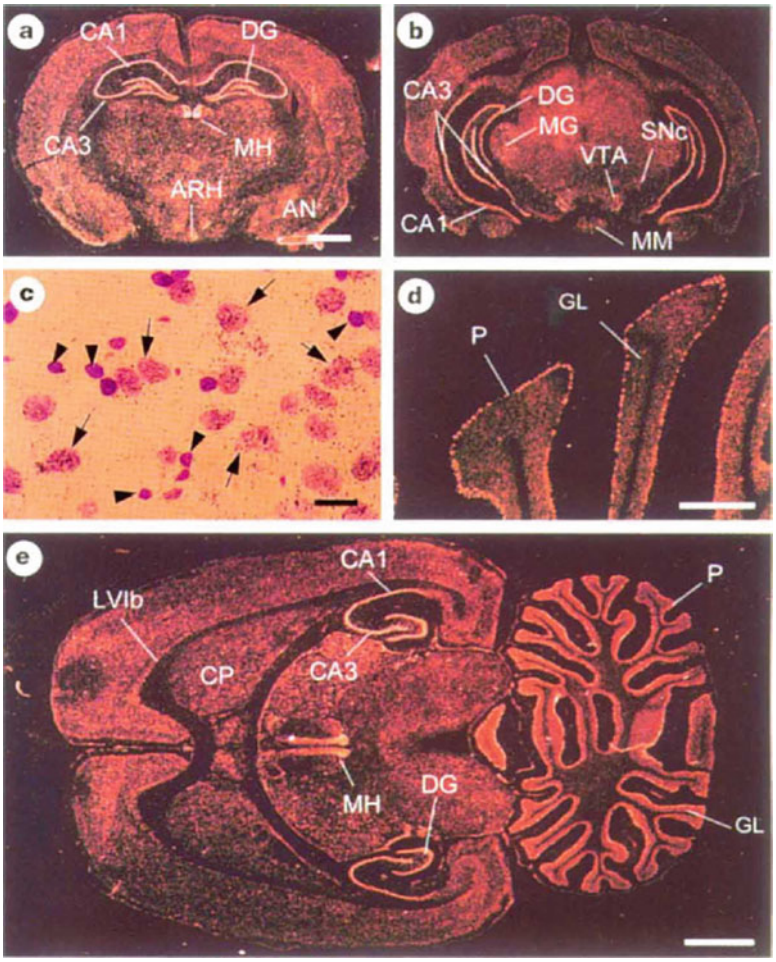

Fig. 5.1 In situ hybridization analysis of $GABA_BR1$ transcripts in rat brain. Tissue sections were hybridized to ^{35}S-labelled antisense probes. Darkfield illuminations of representative autoradiograms of coronal (**a**, **b**, **d**) and horizontal sections (**e**), and a brightfield illumination (**c**) are shown. (**a**) Dorsal hippocampus plane, (**b**) cerebellar cortex, (**e**) dorsal tier of the brain. Transcripts are abundant in all cerebral cortical layers, especially in the layer VIb (LVI6), in the pyramidal cell layers of the CA1–CA3 subfields of the hippocampus as well as in the granular layer of the dentate gyrus (DG) and in the medial habenula (MH). $GABA_BR1$ mRNA is detected in the medial geniculate nucleus (MG); in the substantia nigra, pars compacta (SNc), in the ventral tegmental area (VTA); and in several thalamic, amygdaloid (AN), and hypothalamic nuclei, such as the arcuate nucleus of the hypothalamus (ARH) and mammillary bodies of the hypothalamus (MM). In the cerebellum, high levels of transcripts are found in the Purkinje cells (P) and moderate levels in the granular layer (GL). *Arrows* indicate neuronal, *arrowheads* glial cells. The sections were exposed to nuclear emulsion for 12 days. No hybridization signal was observed with radiolabeled sense probes. Scale bars, 2 mm (**a**, **b**), 10 μm (**c**), 30 μm (**d**), 1.3 mm (**e**). Reprinted from Kaupmann et al. (1997), with permission from Nature Publishing Group

neurons of CA1/CA3, the B1a isoform is strongly expressed, while B1b is lower in CA3 than in CA1; granule cells of the dentate gyrus show medium B1a and low B1b levels. The thalamus is another region with a strong expression of $GABA_BR$ mRNA, with high to very high B1b transcript levels detected in medial and dorsolateral geniculate nuclei, as well as in most dorsal and medial thalamic nuclei. B1a transcript levels are generally low to moderate except in medial and ventrolateral geniculate nuclei, which show high levels. The medial habenula expresses high and low amounts of B1b and B1a transcripts, respectively. In the amygdaloid nuclei, without reflecting the anatomical subdivision of corticomedial and basolateral amygdala (McDonald 1992), $GABA_{B1}R$ mRNA expression levels are moderate in 50 % of nuclei and high in the remaining nuclei, with high and low to moderate levels of B1a and B1b isoform transcripts, respectively. In other brain structures such as the hypothalamus, $GABA_{B1}R$ expression is detected in nuclei, with moderate and low expression of isoforms B1a and B1b, respectively.

$GABA_{B1}R$ expression differs significantly between the substantia nigra (SN) and ventral tegmental area (VTA): neurons in the SN pars compacta and reticulata and those in the VTA display maximal levels of hybridization signals for isoform B1a, while B1b is expressed at moderate levels in the SN pars compacta and at low levels in the SN pars reticulata and the VTA. Similarly, B1a is the predominant isoform in raphe nuclei and in the locus coeruleus. In the cerebellum different cell types show unique patterns of expression of $GABA_{B1}$ isoforms; Purkinje cells have a strong signal for B1b and a weak signal for B1a mRNA; granule cells are weakly positive for both transcripts and positive for the B1b isoformin the deep cerebellar nuclei. Towers et al. (2000) demonstrated that $GABA_{B1a}$ and $GABA_{B1b}$ receptor mRNAs are distributed throughout the spinal cord and dorsal root ganglia, where B1a is predominant (90 % versus 10 % of B1b). The $GABA_{B2}$ transcript is present both in the spinal cord and dorsal root ganglia.

As previously reported (see Introduction), other isoforms besides the two principal B1a and B1b forms have been identified (Isomoto et al. 1998; Pfaff et al. 1999; Calver et al. 2000; Bettler et al. 2004; Xu et al. 2014); however, their distribution has been exclusively analyzed using Northern blotting, PCR, or in situ hybridization. $GABA_B1c$ is heterogeneously expressed throughout the nervous system, whereas the 1d isoform is expressed in the forebrain, cerebellum, and peripheral tissues.

To the best of our knowledge, the proteins encoded by these $GABA_B$ splice isoforms (c, d, e, g, h, j, l, m, and n for rat $GABA_{B1}$ and c and e for human $GABA_{B1}$) have not been identified to date.

The $GABA_{B2}R$ subunit was cloned and its mRNA distribution was investigated in detail and compared to that of the $GABA_{B1}R$ subunit in the rat brain (Durkin et al. 1999; Clark et al. 2000). Although both $GABA_{B2}R$ and $GABA_{B1}R$ mRNAs are heterogeneously expressed in the rat brain, the mRNA corresponding to the B2 subunit is less abundant than that of the B1 subunit. Specifically, in brain regions such as the cortex, thalamus, medial and lateral geniculate nuclei, habenula, and cerebellum, both transcripts are abundantly expressed and show an overlapping pattern; however, in contrast to the B1 subunit, the B2 subunit is expressed at low

to very low levels in certain areas. For instance, $GABA_{B2}R$ mRNA levels in the caudate–putamen, septum, preoptic area, and hypothalamus are significantly lower than those of $GABA_{B1}R$ (Fig. 5.2). In catecholaminergic brain areas (i.e., SN and locus coeruleus), $GABA_{B2}R$ is expressed at lower levels than $GABA_{B1}R$. In the hippocampus both transcripts are present at high levels, although they show a different expression pattern: $GABA_{B2}$ shows a gradual pattern of expression with low, high, and very high levels in the CA1, CA2, and CA3 areas, respectively, while $GABA_{B1}R$ is homogenously and abundantly expressed throughout all hippocampal formations. In addition, while the B1 subunit is present in glial cells of areas of white matter, the B2 subunit is expressed only in neurons.

Northern blot analysis of the distribution of the B2 receptor subunit in the human brain revealed the presence of a single mRNA species of 6.2 kb that was expressed at different levels in the areas investigated, with the highest expression in the cortex and thalamus, and the lowest in the caudate nucleus and medulla (Clark et al. 2000). In situ hybridization with $GABA_B$ subtype specific probes detected both B2 and B1 subunit transcripts with no apparent mismatch in most regions of the human brain, such as the prefrontal cortex, hippocampus, and cerebellum (Berthele et al. 2001). Similar to findings in rodents, the most striking difference in the expression of B2/B1 mRNA in the human brain is observed in basal ganglia; macroscopic analysis revealed moderate and low signals for the B1 subunit in the caudate–putamen and globus pallidus, respectively, while the signal for B2 was not distinguishable from the background in the caudate–putamen and globus pallidus. Microscopically, moderate amounts of B1 mRNA are visible in large and medium-sized neurons in the caudate and globus pallidus. By contrast, B2 hybridization signals are detected at low levels in large sized cells of the globus pallidus and absent or at best faint in medium and large sized cells of caudate–putamen nuclei.

Concerning the distribution of $GABA_{B1}$ and $GABA_{B2}$ receptor proteins, the availability of specific antibodies directed against the COOH- and NH2- termini has enabled the analysis of $GABA_{B2}$ receptor localization (Margeta-Mitrovic et al. 1999; Charles et al. 2001; Billinton et al. 2000; Princivalle et al. 2001; Towers et al. 2000). The distribution of $GABA_BRs$ was originally investigated using radioligands and autoradiography (Bowery et al. 1987; Price et al. 1987; Chu et al. 1990) and later using the more potent radioligands [^{3}H]CGP54626 and [^{3}H]CGP64239 or the photoaffinity ligand [^{125}I]CGP71872, which localized the $GABA_{B1}$ subunit (Bischoff et al. 1999; Kaupmann et al. 1997; Billinton et al. 1999). Generally, autoradiographic data are well correlated with both immunohistochemical and mRNA localization of native $GABA_BRs$. However, there are discrepancies between autoradiographic, immunohistochemical, and mRNA data. Specifically, a mismatch between the cellular localization of $GABA_{B1a}$, $GABA_{B1b}$, and $GABA_{B2}$ transcripts, protein, and [^{3}H]CGP54626 binding sites was reported in the cerebellum. Strong expression of $GABA_{B1b}$ is detected in Purkinje cells, and high binding levels are detected in the molecular layer, suggesting that $GABA_{B1b}$ proteins are located mainly on dendritic arborizations of Purkinje cells in the molecular layer (considered postsynaptic). On the other hand, in the granular layer, the weak [^{3}H]CGP54626 binding coupled to a

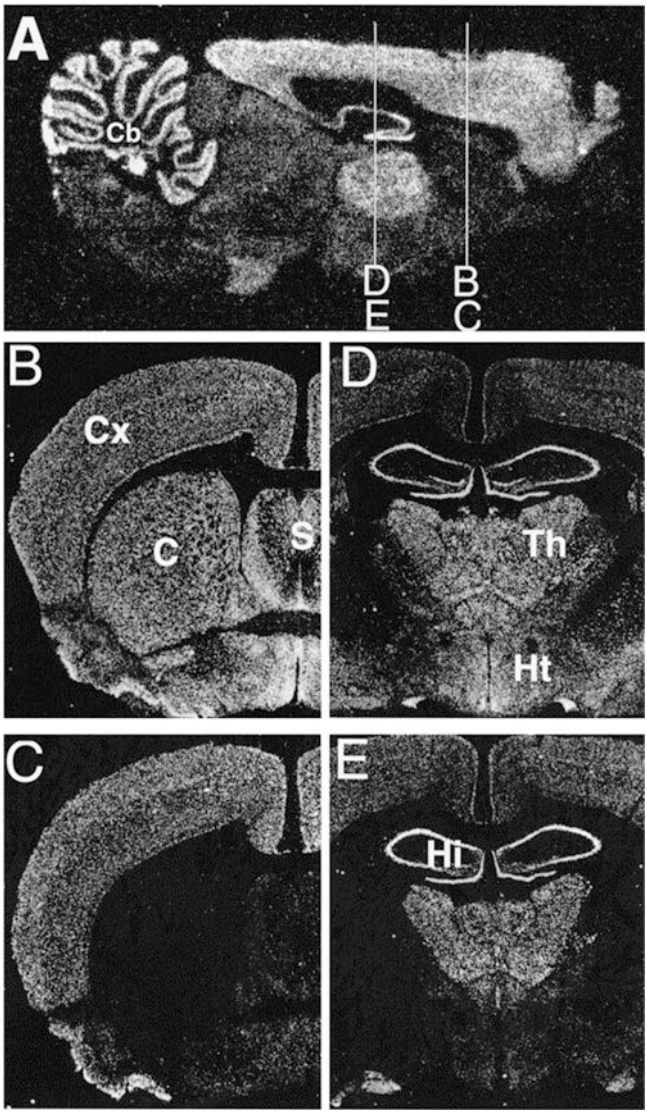

Fig 5.2 Comparison of the distribution of gb2 and gb1 receptor mRNA in the rat brain by in situ hybridization histochemistry. (**a**) shows an autoradiographic image of a parasagittal rat brain section hybridized with the rat gb2 probe. The perpendicular lines depict the levels at which 12 μm thin coronal sections (**b**–**e**) are shown. (**b**) and (**c**), and (**d**) and (**e**) show the same levels (adjacent sections) hybridized with rat gb1 (**b** and **d**) and gb2 (**c** and **e**) receptor probes. Note the lack of grains over the caudate nucleus, septum, and preoptic area in (**c**) as compared with (**b**) and the lack of grains over the hypothalamus in (**e**) as compared with (**d**). Abbreviations: *C* caudate nucleus; *Hi* hippocampus; *Hth* hypothalamus; *PO* preoptic area; *S* septum; *Th* thalamus. Scale bar = 1 mm. Reprinted from Clark et al. (2000), with permission from Elsevier

moderate expression of $GABA_{B1a}$ transcripts suggests that the $GABA_{B1a}$ protein is localized in granule cell parallel fiber terminals of the cerebellum (considered presynaptic) (Bischoff et al. 1999; Billinton et al. 1999; Liang et al. 2000).

In human and rat brains, the distribution profile of each $GABA_BR$ subunit (i.e., $GABA_{B1a}$, $GABA_{B1b}$, $GABA_{B2}$) identified using specific antibodies is in agreement with the mRNA distribution revealed by in situ hybridization (Charles et al. 2001; Billinton et al. 2000). The regions showing the highest immunoreactivity (IR) for the $GABA_{B1a}$, $GABA_{B1b}$, and $GABA_{B2}$ subunits include the neocortex, hippocampus, most thalamic nuclei, habenula, interpeduncular nucleus, nucleus accumbens shell, hypothalamic median eminence, and cerebellum.

However, in the caudate–putamen, $GABA_{B1}$ and $GABA_{B2}$ show moderate IR, which is inconsistent with their mRNA expression, which shows low and almost undetectable transcript levels, respectively (Billinton et al. 2000; Charles et al. 2001). $GABA_{B1}$ and $GABA_{B2}$ immunostaining is weakly present in the neuropil, which could be arising from cortical projection neurons, whereas only certain neurons are positive for the B1 and B2 subunits. These results are consistent with the fact that mRNA may be present in cells positioned outside the caudate, and the protein is transported to dendrites and axon terminals within the caudate. Accordingly, lesion studies in rodents showed that direct striatal lesions do not reduce $GABA_B$ receptor binding in the striatum, while lesions of cortical and nigral inputs decrease binding (Kuner et al. 1999; Kilpatrick et al. 1983).

5.2.2 *Nonmammalian $GABA_B$ Receptors*

$GABA_BR$ was identified in *Caenorhabditis elegans* (Schultheis et al. 2011; Dittman and Kaplan 2008) and cloned in *Drosophila melanogaster* (Mezler et al. 2001) and in the cockroach (Blankenburg et al. 2015). A partial cDNA encoding an incomplete subunit 1 of $GABA_BR$ was cloned from the tobacco budworm *Heliothis virescens*. Whole-mount in situ hybridization experiments and sectioning of the male antenna of the moth *Heliothis virescens* demonstrated the expression of the B1 subunit in olfactory sensory neurons (OSNs) of *Sensilla trichodea* containing pheromone-responsive OSNs. These data suggest an important role of $GABA_BRs$ in pheromone cognition by male moths (Pregitzer et al. 2013).

In *Drosophila* three subunits have been identified and named GB1, GB2, and GB3; both GB1 and GB2 show high sequence identity with the mammalian subunits, whereas GB3 may be insect specific and its function remains unknown. As in its mammalian counterpart, $GABA_BR$ of *Drosophila* couples to Gai-subtype G proteins, and the GB1 and GB2 subunits need to be coexpressed in Xenopus oocytes or in mammalian cell lines to produce functional $GABAB_Rs$ (Mezler et al. 2001; Manev and Dzitoyeva 2010). These data indicate that $GABA_B$ heteromerization is necessary for its function both in vertebrates and invertebrates. An in situ hybridization distribution study of the GB1, GB2, and GB3 subunits showed that all three subunits are widely expressed in the embryonic CNS and that GB1/GB2 has an

overlapping pattern. Accordingly, in the *Drosophila melanogaster* adult brain, all three subunits show the same expression pattern; the GB2-IR is displayed in the neuropils of the antennal lobe, mushroom body calyces, ellipsoid body, and in specific layers of the optic lobes (Enell et al. 2007). In the antennal lobe of adult *Drosophila*, staining for $GABA_BR2$ is mainly present on axonal terminals of olfactory receptor neurons, and most of the staining is eliminated after surgical removal of olfactory appendages (Root et al. 2008). In addition, consistent with the role of $GABA_BRs$ in regulating sleep in humans and flies, $GABA_{B2}$-IR is present in *Drosophila melanogaster* in the dendritic regions of lateral ventral neurons (LNvs), which express different clock genes, and in neuro-peptide pigment-dispersing factor (PDF)-positive neurons (Gmeiner et al. 2013; Hamasaka et al. 2005).

Recently, molecular characterization, pharmacological properties, and tissue distribution have been reported for the arthropod, American cockroach, *Periplaneta Americana* (Blankenburg et al. 2015). Two clones encoding $GABA_BR$ subtypes, namely, PeaGB1 and PeaGB2, were identified and their distribution was investigated by western blotting and immunohistochemistry. Both subunits are expressed in the brain, and PeaGB1 is also present in salivary and male accessory glands. Specifically, PeaGB1-IR is present in mushroom body calyces, in the neuropil of optic lobes, and in antennal lobes. In addition, PeaGB1 staining is observed in the GABAergic salivary neuron 2, where it could act as an autoreceptor. The widespread distribution of PeaGB1 in the cockroach brain suggests a key role for $GABA_BR$ in olfactory and visual processes as well as in other brain functions such as learning.

5.2.3 *Subcellular Localization of $GABA_B$ Receptors*

The use of high-resolution immunohistochemical techniques, such as preembedding and postembedding immunogold methods and electron microscopy, has allowed the subcellular neuronal localization of $GABA_BRs$. Based on the heterodimeric structure of this receptor, the two principal subunits have been shown to colocalize at the plasma membrane. Indeed, electron microscopy studies show that in the CNS, $GABA_{B1}$ and $GABA_{B2}$ are both pre- and postsynaptic, although they are predominantly located at postsynaptic sites (Kulik et al. 2003; López-Bendito et al. 2002; Lujan and Ciruela 2012). These anatomic findings corroborate electrophysiological studies demonstrating the functional presence of these receptors both at presynaptic (Chen and van den Pol 1998; Iyadomi et al. 2000; Takahashi et al. 1998) and postsynaptic sites (Deisz et al. 1997; Li and Stern 2004). In particular, presynaptic $GABA_{B(1,2)}Rs$ mainly localize to the extrasynaptic membrane, over the presynaptic membrane specialization of excitatory glutamatergic terminals, and, to a lesser extent, to GABAergic axon terminals (Kulik et al. 2002; López-Bendito et al. 2002; Luján et al. 2004). Postsynaptically, they are detected on the extrasynaptic membrane of the dendritic shaft and in the neck of dendritic spines. Specifically, they are positioned on dendritic spines postsynaptic to glutamatergic terminals mainly at

extrasynaptic and presynaptic sites (Lujan and Shigemoto 2006; Luján et al. 2004). Concerning the synaptic localization of the B1a and B1b subunits, the lack of specific antibodies for the detection of isoforms hinders their exact localization. Vigot et al. (2006) used $GABA_{B1a}$−/− and $GABA_{B1b}$−/− deficient mice and electron microscopy and showed that these isoforms localize to distinct synaptic sites and have separate functions in vivo. $GABA_{B1a}$, which is detected at the Schaffer collateral to the CA1 pyramidal synapse, is mostly present in the glutamatergic terminals, whereas $GABA_{B1b}$ is in dendritic spines juxtaposed to glutamate terminals. Comparisons of mice deficient for each subunit isoform showed that $GABA_{B1a}$ is localized to glutamatergic terminals and is necessary for hetero-receptor function. The $GABA_{B1a}$ subunit, which contains a sushi domain, is preferentially targeted to the axon terminals of excitatory synapses (Biermann et al. 2010; Gassmann and Bettler 2012). Both isoforms are present in dendrites and expressed along the dendritic shaft, but only $GABA_{B1b}$ localizes to the spine head (Vigot et al. 2006; Biermann et al. 2010). This different distribution of $GABA_{B1a}$ and $GABA_{B1b}$ in axonal and dendritic sites may underlie the heterogeneous pharmacological properties of native $GABA_B$Rs.

5.2.4 Distribution of the Auxiliary $GABA_B$ Receptor Subunits

The recently identified auxiliary $GABA_B$R subunits KCTD8, 12, 12b, and 16, which show a distinct spatial distribution, might be one of the factors contributing to the functional differences observed in native $GABA_B$Rs.

The expression profile investigated by Northern blot analysis, in situ hybridization, and immunohistochemistry revealed a unique and overlapping distribution of KCTD transcripts and proteins (Metz et al. 2011). In particular, Northern blot analysis showed that the mRNAs for KCTD8, 12, and 16 are mainly expressed in the brain, whereas that for 12b is below detection levels in the brain and spinal cord. KCTD16 and 12b are detected in the intestine, kidney, heart, testis, bone marrow, ovary, and adipose tissues, whereas KCTD8 is not detectable in any peripheral tissues. In situ hybridization revealed that KCTD12 is primarily expressed in the septum, cerebellum, and hippocampal formation, whereas KCTD16 is present in the cortex, thalamus, and hippocampal formation. KCTD8 is detectable in the medial habenula, some brainstem nuclei, and the granular cell layer of the cerebellum, while KCTD12b mRNA is only detected in the medial habenula. Certain brain regions (i.e., globus pallidus, claustrum, mammillary bodies, and anterior dorsal thalamic nuclei) express only one KCTD subtype, whereas more than one transcript is expressed in specific neuronal populations of some brain areas. For example, granule and pyramidal cells in hippocampal CA1/CA3 areas coexpress KCTD12 and 16; however, in cerebellar granules, Purkinje and Golgi cells express only one auxiliary protein (Metz et al. 2011). These anatomical findings substantiate biochemical data showing that both $GABA_{B1a,2}$ and $GABA_{B1b,2}$ receptors are assembled with KCTD subunits, although some $GABA_B$Rs are associated with KCTD12 and others with KCTD16.

Preembedding immunohistochemistry techniques demonstrated that both the KCTD12 and KCTD16 proteins localize to the membrane of presynaptic and postsynaptic sites, where $GABA_B$Rs are also detected (Schwenk et al. 2010). Immunohistochemistry experiments revealed that these proteins have distinct axonal or dendritic localization in neuronal populations; strong KCTD12-IR is observed in the outer molecular layer of the hippocampus, representing the KCTD protein in distal dendrites of granule cells, while KCTD16 is detected in the soma and neuropil of dentate granule cells (Metz et al. 2011).

Biochemical, electrophysiological, and anatomical findings suggest that these auxiliary proteins confer subtype specificity to $GABA_B$Rs and contribute to the heterogenous responses of native $GABA_B$Rs.

5.2.5 *$GABA_B$ Receptor Expression and Localization During Development*

In situ hybridization studies revealed that both $GABA_{B1}$ and $GABA_{B2}$ subunits are developmentally regulated, with $GABA_{B1}$ being predominantly expressed during the embryonic period in the rat brain (Gaiarsa et al. 2011). Specifically, $GABA_{B1}$ transcripts are present at embryonic day (ED) 11 (Kim et al. 2003), while $GABA_{B2}$ mRNAs are detectable only at ED14, except in the olfactory bulb and striatum, where $GABA_{B2}$ is poorly detectable until ED17 (Martin et al. 2004).

Regarding $GABA_B$R protein levels, early pioneer binding studies using [^{3}H] GABA binding to $GABA_B$ binding sites or receptor autoradiography demonstrated that $GABA_B$ binding sites increase after birth, peaking at regionally specific times during the first 2–3 postnatal weeks, and then decreasing to adult levels (Turgeon and Albin 1993, 1994). Recent studies used Western blotting and immunohistochemical analysis confirming the presence of both $GABA_{B1}$ and $GABA_{B2}$ subunits in the rat brain during development (Malitschek et al. 1998; Princivalle et al. 2000; Bianchi et al. 2005). The B1a and B1b isoforms show different temporal and spatial expression patterns: the levels of B1a are highest at birth, and decrease within 2 weeks, whereas the levels of the B1b subunit increase after postnatal day 5 and reach a peak at postnatal day 10. At the end of the third postnatal week, both isoforms reach adult levels, although subunit B1a is more abundant than B1b. The distribution of the two subunits overlaps in many brain regions with some exceptions; at birth, $GABA_{B1}$-IR is uniform across the cortex layers, while $GABA_{B2}$-IR is stronger in layers I and V–VI. In the hippocampus, $GABA_{B2}$-IR is localized mostly in the dendritic layers and GABAB1-IR in the pyramidal layer (Fritschy et al. 2004).

A selective $GABA_{B2}$-IR is present in the axonal tract, while transient $GABA_{B1}$ staining is observed on glial cells in the hippocampus (Lopez-Bendito et al. 2004) and in the cerebellum (Fritschy et al. 2004; Lujan and Shigemoto 2006).

At the electron microscopic level, the distribution in the developing brain is similar to that in the adult, with both subunits present at pre- and postsynaptic

sites in the cerebellum (Lujan and Shigemoto 2006), neocortex, and hippocampus (López-Bendito et al. 2002, 2004). These anatomical studies support the important role of $GABA_BRs$ in brain development and in the control of neuronal network activity, neurite outgrowth, axon guidance, and synaptogenesis (Gaiarsa and Porcher 2013).

5.3 Distribution and Expression of the $GABA_B$ Receptor in the Peripheral Nervous System and Tissues: Mammalian Studies

5.3.1 $GABA_B$ Receptor mRNAs, Binding Sites, and Immunohistochemical Localization

More than 30 years ago, Bowery and colleagues (1980) demonstrated the presence of $GABA_BRs$ in the peripheral nervous system (PNS); however, because of methodological difficulties such as the lack of high-affinity $GABA_B$ receptor-selective radioligands, their presence in the peripheral organs and tissues remained elusive for many years. The later use of reverse-transcriptase-PCR analysis resulted in the detection of $GABA_{B1}$ and $GABA_{B2}$ mRNAs in several peripheral organs, as well as the heart, lungs, intestine, liver, ovary, testis, kidney, gallbladder, and urinary bladder (Castelli et al. 1999; Isomoto et al. 1998; Schwarz et al. 2000; Wei et al. 2001). The presence of $GABA_{B1}$ isoforms (1a, 1b, 1c, and 1d) was revealed in different rat and human peripheral tissues (Isomoto et al. 1998; Calver et al. 2000), whereas $GABA_{B2}$ isoforms (2a, 2b, and 2c) were found to beat undetectable levels in peripheral tissues (Calver et al. 2000). At the protein level, the presence of $GABA_B1a/b$ subunits in peripheral tissues was confirmed using [^{125}I]CGP7187 photoaffinity labeling and immunoblot analysis (Belley et al. 1999; Calver et al. 2000). The $GABA_B1$ subunit is expressed at high levels in the spleen and uterus, whereas the $GABA_B2$ subunit is expressed at low levels in these tissues (Calver et al. 2000).

Studies in $GABA_{B1}(-/-)$ deficient-mice provided evidence that the $GABA_B1$ subunit is necessary for the function of $GABA_BRs$ in the enteric nervous system and the PNS, similar to its role in the CNS (Sanger et al. 2002). The presence of $GABA_BRs$ was also demonstrated in Schwann cells (SCs) (Magnaghi et al. 2004, 2006; Magnaghi 2007), where they are expressed in the membrane-enriched fraction. Immunofluorescence and confocal laser microscopy studies demonstrated that both $GABA_BR$ subunits (B1 and B2) are expressed on SC membranes and colocalize with f-actin at the cell border surface (Procacci et al. 2013).

$GABA_BRs$ in the PNS are expressed not only in neurons but also in nonneuronal cells; both subunits have been identified in cardiomyocytes (Lorente et al. 2000) and in native human and guinea pig airway smooth muscle (ASM) cells (Osawa et al. 2006). In the heart both $GABA_BR$ subunits are distributed within the sarcolemma and in the traverse tubular system of cardiomyocytes, while $GABA_{B1}R$-IR in the trachea is found in

the ASM, airway epithelium, and tracheal cartilage chondrocytes. In both cardiomyocytes and ASM, $GABA_B$Rs are functional, as demonstrated by the baclofen-induced inward rectifying K+ channel and activation of GTPγS binding, respectively.

5.4 Conclusions

The functional $GABA_B$Rs, which comprise two principal subunits, $GABA_{B1(a/b)}$ and $GABA_{B2}$, and the auxiliary KCTD subunits are unevenly distributed in the mammalian brain, in the PNS, and in peripheral tissues.

Different experimental approaches (morphological, biochemical, and genetic) showed that the $GABA_{B1(a/b)}$ and $GABA_{B2}$ subunits are present in an overlapping distribution pattern throughout the CNS. Despite the fact that $GABA_{B1a,b}$ and $GABA_{B2}$ are expressed at different levels in native brain tissues, the expression of a single $GABA_B$ subunit has not been detected, providing evidence that $GABA_{B1,2}$ represent the functional $GABA_B$Rs (Charles et al. 2001). Similarly, for $GABA_B$R in *Drosophila melanogaster*, the GB1 and GB2 subunits need to be coexpressed in Xenopus oocytes or in mammalian cell lines to produce functional $GABAB_R$s (Mezler et al. 2001; Manev and Dzitoyeva 2010), providing additional evidence that $GABA_B$ heteromerization is a prerequisite for its function both in vertebrates and invertebrates.

$GABA_B$Rs are localized presynaptically on glutamate (hetero-receptors) and GABA (autoreceptors) terminals, and postsynaptically on the dendritic shaft and spines.

The isoforms B1a and B1b are coexpressed with the subunit $GABA_{B2}$, and their expression differs across the brain and neuronal populations. $GABA_{B1a}$ is mainly localized to glutamatergic terminals and is necessary for hetero-receptor function; both isoforms 1a and 1b are detected in dendrites, but only $GABA_{B1b}$ is localized on the spine head. This differential distribution between axonal and dendritic sites may underlie the heterogeneity of the pharmacological properties of native $GABA_B$Rs. The recent identification of the KCTD auxiliary subunits, which are present only in vertebrates in contrast to $GABA_{B1}$ and $GABA_{B2}$, provides a further tool to understand the physiology and pharmacology of these receptors. Moreover, accumulating evidence suggests that the KCTD subunits are involved in the mechanism(s) underlying the functional heterogeneity of native $GABA_B$Rs.

In conclusion, the wide distribution of $GABA_B$R in the brain reflects its physiological and pharmacological relevance. $GABA_B$Rs are present in virtually all neurons in the brain and control both inhibitory and excitatory pathways; they are therefore implicated in all main brain functions, such as the regulation of neuronal network activity (Kohl and Paulsen 2010; Craig and McBain 2014), synaptic plasticity (Pinard et al. 2010), and neuronal development (Gaiarsa et al. 2011; Gaiarsa and Porcher 2013). $GABA_B$Rs play a key role in numerous physiopathological conditions, including pain, drug addiction, epilepsy, cognitive function, anxiety, and depression (see Chaps. 10–15 of this book).

In addition to their involvement in CNS disorders, the discovery of $GABA_BRs$ in tissues such as the heart, lung, intestine, liver, ovary, testis, kidney, and gallbladder confirms their functional presence in the periphery and suggests their role in the physiological regulation of these organs. In particular, $GABA_BRs$ show a widespread distribution in the gut of several species, as well as in the enteric nervous system, muscle, epithelial layers, and endocrine cells, which is consistent with their modulatory role in intestinal motility (i.e., gastric empting, gastric secretion, and transient lower esophageal sphincter relaxation) (see Chap. 16 of this book). $GABA_BR$ ligands could be useful in the treatment of gastroenteric disorders, asthma, or as possible antitussive agents. Finally, in the PNS, $GABA_BRs$ are detected in SC cells, where they may contribute to cell fate regulation, maturation, and plasticity (Procacci et al. 2013).

References

Belley, M., Sullivan, R., Reeves, A., Evans, J., O'Neill, G., & Ng, G. Y. (1999). Synthesis of the nanomolar photoaffinity GABA(B) receptor ligand CGP 71872 reveals diversity in the tissue distribution of GABA(B) receptor forms. *Bioorganic and Medicinal Chemistry, 7*, 2697–2704.

Berthele, A., Platzer, S., Weis, S., Conrad, B., & Tölle, T. R. (2001). Expression of GABA(B1) and GABA(B2) mRNA in the human brain. *Neuroreport, 12*, 3269–3275.

Bettler, B., Kaupmann, K., Mosbacher, J., & Gassmann, M. (2004). Molecular structure and physiological functions of GABA(B) receptors. *Physiological Reviews, 84*, 835–867.

Bianchi, M. S., Lux-Lantos, V. A., Bettler, B., & Libertun, C. (2005). Expression of gamma-aminobutyric acid B receptor subunits in hypothalamus of male and female developing rats. *Brain Research. Developmental Brain Research, 160*, 124–129.

Biermann, B., Ivankova-Susankova, K., Bradaia, A., Abdel Aziz, S., Besseyrias, V., Kapfhammer, J. P., et al. (2010). The Sushi domains of GABAB receptors function as axonal targeting signals. *Journal of Neuroscience, 30*, 1385–1394.

Billinton, A., Ige, A. O., Bolam, J. P., White, J. H., Marshall, F. H., & Emson, P. C. (2001). Advances in the molecular understanding of GABA(B) receptors. *Trends in Neurosciences, 24*, 277–282.

Billinton, A., Ige, A. O., Wise, A., White, J. H., Disney, G. H., Marshall, F. H., et al. (2000). GABA(B) receptor heterodimer-component localisation in human Brain. *Brain Research. Molecular Brain Research, 77*, 111–124.

Billinton, A., Upton, N., & Bowery, N. G. (1999). GABA(B) receptor isoforms GBR1a and GBR1b, appear to be associated with pre- and post-synaptic elements respectively in rat and human cerebellum. *British Journal of Pharmacology, 126*, 1387–1392.

Bischoff, S., Leonhard, S., Reymann, N., Schuler, V., Shigemoto, R., Kaupmann, K., et al. (1999). Spatial distribution of GABA(B)R1 receptor mRNA and binding sites in the ratbrain. *Journal of Comparative Neurology, 412*, 1–16.

Blankenburg, S., Balfanz, S., Hayashi, Y., Shigenobu, S., Miura, T., Baumann, O., et al. (2015). Cockroach GABAB receptor subtypes: molecular characterization, pharmacological properties and tissue distribution. *Neuropharmacology, 88*, 134–144.

Blein, S., Ginham, R., Uhrin, D., Smith, B. O., Soares, D. C., Veltel, S., et al. (2004). Structural analysis of the complement control protein (CCP) modules of GABA(B) receptor 1a: only one of the two CCP modules is compactly folded. *Journal of Biological Chemistry, 279*, 48292–48306.

Bowery, N. G., Hill, D. R., Hudson, A. L., Doble, A., Middlemiss, D. N., Shaw, J., et al. (1980). (−) Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature, 283*, 92–94.

Bowery, N. G., Hudson, A. L., & Price, G. W. (1987). GABAA and GABAB receptor site distribution in the rat central nervous system. *Neuroscience, 20*, 365–383.

Calver, A. R., Medhurst, A. D., Robbins, M. J., Charles, K. J., Evans, M. L., Harrison, D. C., et al. (2000). The expression of GABA(B1) and GABA(B2) receptor subunits in the cNS differs from that in peripheral tissues. *Neuroscience, 100*, 155–170.

Castelli, M. P., Ingianni, A., Stefanini, E., & Gessa, G. L. (1999). Distribution of GABA(B) receptor mRNAs in the rat brain and peripheral organs. *Life Sciences, 64*, 1321–1328.

Charles, K. J., Evans, M. L., Robbins, M. J., Calver, A. R., Leslie, R. A., & Pangalos, M. N. (2001). Comparative immunohistochemical localisation of GABA(B1a), GABA(B1b) and GABA(B2) subunits in rat brain, spinal cord and dorsal root ganglion. *Neuroscience, 106*, 447–467.

Chen, G., & van den Pol, A. N. (1998). Presynaptic GABAB autoreceptor modulation of P/Q-type calcium channels and GABA release in rat suprachiasmatic nucleus neurons. *Journal of Neuroscience, 18*, 1913–1922.

Chu, D. C., Albin, R. L., Young, A. B., & Penney, J. B. (1990). Distribution and kinetics of GABAB binding sites in rat central nervous system: A quantitative autoradiographic study. *Neuroscience, 34*, 341–357.

Clark, J. A., Mezey, E., Lam, A. S., & Bonner, T. I. (2000). Distribution of the GABA(B) receptor subunit gb2 in rat CNS. *Brain Research, 860*, 41–52.

Couve, A., Thomas, P., Calver, A. R., Hirst, W. D., Pangalos, M. N., Walsh, F. S., et al. (2002). Cyclic AMP-dependent protein kinase phosphorylation facilitates GABA(B) receptor-effector coupling. *Nature Neuroscience, 5*, 415–424.

Craig, M. T., & McBain, C. J. (2014). The emerging role of GABAB receptors as regulators of network dynamics: Fast actions from a 'slow' receptor? *Current Opinion in Neurobiology, 26*, 15–21.

Deisz, R. A., Billard, J. M., & Zieglgänsberger, W. (1997). Presynaptic and postsynaptic GABAB receptors of neocortical neurons of the rat in vitro: Differences in pharmacology and ionic mechanisms. *Synapse, 25*, 62–72.

Dittman, J. S., & Kaplan, J. M. (2008). Behavioral impact of neurotransmitter-activated G-protein-coupled receptors: Muscarinic and GABAB receptors regulate Caenorhabditis elegans locomotion. *Journal of Neuroscience, 28*, 7104–7112.

Durkin, M. M., Gunwaldsen, C. A., Borowsky, B., Jones, K. A., & Branchek, T. A. (1999). An in situ hybridization study of the distribution of the GABA(B2) protein mRNA in the rat CNS. *Brain Research. Molecular Brain Research, 71*, 185–200.

Enell, L., Hamasaka, Y., Kolodziejczyk, A., & Nässel, D. R. (2007). gamma-Aminobutyric acid(GABA) signaling components in Drosophila: immunocytochemical localization of GABA(B) receptors in relation to the GABA(A) receptor subunit RDL and a vesicular GABA transporter. *Journal of Comparative Neurology, 505*, 18–31.

Fritschy, J. M., Sidler, C., Parpan, F., Gassmann, M., Kaupmann, K., Bettler, B., et al. (2004). Independent maturation of the GABA(B) receptor subunits GABA(B1) and GABA(B2) during postnatal development in rodent brain. *Journal of Comparative Neurology, 477*, 235–252.

Gaiarsa, J. L., Kuczewski, N., & Porcher, C. (2011). Contribution of metabotropic GABA(B) receptors to neuronal network construction. *Pharmacology and Therapeutics, 132*, 170–179.

Gaiarsa, J. L., & Porcher, C. (2013). Emerging neurotrophic role of GABAB receptors in neuronal circuit development. *Frontiers in Cellular Neuroscience, 7*, 206.

Galvez, T., Duthey, B., Kniazeff, J., Blahos, J., Rovelli, G., Bettler, B., et al. (2001). Allosteric interactions between GB1 and GB2 subunits are required for optimal GABA(B) receptor function. *The EMBO Journal, 20*, 2152–2159.

Galvez, T., Prezeau, L., Milioti, G., Franek, M., Joly, C., Froestl, W., et al. (2000). Mapping the agonist-binding site of GABAB type 1 subunit sheds light on the activation process of GABAB receptors. *Journal of Biological Chemistry, 275*, 41166–41174.

Gassmann, M., & Bettler, B. (2012). Regulation of neuronal GABA(B) receptor functions by subunit composition. *Nature Reviews. Neuroscience, 13*, 380–394.

Gmeiner, F., Kołodziejczyk, A., Yoshii, T., Rieger, D., Nässel, D. R., & Helfrich-Förster, C. (2013). GABA(B) receptors play an essential role in maintaining sleep during the second half of the night in Drosophila melanogaster. *Journal of Experimental Biology, 216*, 3837–3843.

Hamasaka, Y., Wegener, C., & Nässel, D. R. (2005). GABA modulates Drosophila circadian clock neurons via GABAB receptors and decreases in calcium. *Journal of Neurobiology, 65*, 225–240.

Isomoto, S., Kaibara, M., Sakurai-Yamashita, Y., Nagayama, Y., Uezono, Y., Yano, K., et al. (1998). Cloning and tissue distribution of novel splice variants of the rat GABAB receptor. *Biochemical and Biophysical Research Communications, 253*, 10–15.

Iyadomi, M., Iyadomi, I., Kumamoto, E., Tomokuni, K., & Yoshimura, M. (2000). Presynaptic inhibition by baclofen of miniature EPSCs and IPSCs in substantia gelatinosa neurons of the adult rat spinal dorsal horn. *Pain, 85*, 385–393.

Kaupmann, K., Huggel, K., Heid, J., Flor, P. J., Bischoff, S., Mickel, S. J., et al. (1997). Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. *Nature, 386*, 239–246.

Kilpatrick, G. J., Muhyaddin, M. S., Roberts, P. J., & Woodruff, G. N. (1983). GABAB binding sites on rat striatal synaptic membranes. *British Journal of Pharmacology, 78*, 6.

Kim, M. O., Li, S., Park, M. S., & Hornung, J. P. (2003). Early fetal expression of GABA(B1) and GABA(B2) receptor mRNAs on the development of the rat central nervous system. *Brain Research. Developmental Brain Research, 143*, 47–55.

Kohl, M. M., & Paulsen, O. (2010). The roles of GABAB receptors in cortical network activity. *Advances in Pharmacology, 58*, 205–229.

Kulik, A., Nakadate, K., Nyíri, G., Notomi, T., Malitschek, B., Bettler, B., et al. (2002). Distinct localization of GABA(B) receptors relative to synaptic sites in the rat cerebellum and ventrobasal thalamus. *European Journal of Neuroscience, 15*, 291–307.

Kulik, A., Vida, I., Luján, R., Haas, C. A., López-Bendito, G., Shigemoto, R., et al. (2003). Subcellular localization of metabotropic GABA(B) receptor subunits GABA(B1a/b) and GABA(B2) in the rat hippocampus. *Journal of Neuroscience, 23*, 11026–11035.

Kuner, R., Köhr, G., Grünewald, S., Eisenhardt, G., Bach, A., & Kornau, H. C. (1999). Role of heteromer formation in GABAB receptor function. *Science, 283*, 74–77.

Labouèbe, G., Lomazzi, M., Cruz, H. G., Creton, C., Luján, R., Li, M., et al. (2007). RGS2 modulates GABAB receptors and GIRK channels in dopamine neurons of the ventral tegmental area. *Nature Neuroscience, 10*, 1559–1568.

Li, Y., & Stern, J. E. (2004). Activation of postsynaptic GABAB receptors modulate the firing activity of supraoptic oxytocin and vasopressin neurones: role of calcium channels. *Journal of Neuroendocrinology, 16*, 119–130.

Liang, F., Hatanaka, Y., Saito, H., Yamamori, T., & Hashikawa, T. (2000). Differential expression of γ-aminobutyric acid type B receptor-1a and -1b mRNA variants in GABA and non-GABAergic neurons of the rat brain. *Journal of Comparative Neurology, 416*, 475–495.

López-Bendito, G., Shigemoto, R., Kulik, A., Paulsen, O., Fairén, A., & Luján, R. (2002). Expression and distribution of metabotropic GABA receptor subtypes GABABR1 and GABABR2 during rat neocortical development. *European Journal of Neuroscience, 15*, 1766–1778.

Lopez-Bendito, G., Shigemoto, R., Kulik, A., Vida, I., Fairen, A., & Lujan, R. (2004). Distribution of metabotropic GABA receptor subunits GABAB1a/b andGABAB2 in the rat hippocampus during prenatal and postnatal development. *Hippocampus, 14*, 836–848.

Lorente, P., Lacampagne, A., Pouzeratte, Y., Richards, S., Malitschek, B., Kuhn, R., et al. (2000). gamma-aminobutyric acid type B receptors are expressed and functional in mammalian cardiomyocytes. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 8664–8669.

Lujan, R., & Ciruela, F. (2012). GABAB receptors-associated proteins: Potential drug targets in neurological disorders? *Current Drug Targets, 13*, 129–144.

Lujan, R., & Shigemoto, R. (2006). Localization of metabotropic GABA receptor subunits GABAB1 and GABAB2 relative to synaptic sites in the rat developing cerebellum. *European Journal of Neuroscience, 23*, 1479–1490.

Luján, R., Shigemoto, R., Kulik, A., & Juiz, J. M. (2004). Localization of the GABAB receptor 1a/b subunit relative to glutamatergic synapses in the dorsal cochlear nucleus of the rat. *Journal of Comparative Neurology, 475*, 36–46.

Magnaghi, V. (2007). GABA and neuroactive steroid interactions in glia: New roles for old players? *Current Neuropharmacology, 5*, 47–64.

Magnaghi, V., Ballabio, M., Cavarretta, I. T., Froestl, W., Lambert, J. J., Zucchi, I., et al. (2004). GABAB receptors in Schwann cells influence proliferation and myelin protein expression. *European Journal of Neuroscience, 19*, 2641–2649.

Magnaghi, V., Ballabio, M., Consoli, A., Lambert, J. J., Roglio, I., & Melcangi, R. C. (2006). GABA receptor-mediated effects in the peripheral nervous system: A cross-interaction with neuroactive steroids. *Journal of Molecular Neuroscience, 28*, 89–102.

Malitschek, B., Rüegg, D., Heid, J., Kaupmann, K., Bittiger, H., Fröstl, W., et al. (1998). Developmental changes of agonist affinity at GABABR1 receptor variants in rat brain. *Molecular and Cellular Neuroscience, 12*, 56–64.

Manev, H., & Dzitoyeva, S. (2010). GABA-B receptors in Drosophila. *Advances in Pharmacology, 58*, 453–464.

Margeta-Mitrovic, M., Mitrovic, I., Riley, R. C., Jan, L. Y., & Basbaum, A. I. (1999). Immunohistochemical localization of GABA(B) receptors in the rat central nervous system. *Journal of Comparative Neurology, 405*, 299–321.

Martin, S. C., Steiger, J. L., Gravielle, M. C., Lyons, H. R., Russek, S. J., & Farb, D. H. (2004). Differential expression of gamma-aminobutyric acid type B receptor subunit mRNAs in the developing nervous system and receptor coupling to adenylyl cyclase in embryonic neurons. *Journal of Comparative Neurology, 473*, 16–29.

McDonald, A. J. (1992). Cell types and intrinsic connections of the amygdala. In J. P. Aggleton (Ed.), *The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction* (pp. 67–96). New York: Wiley-Liss.

Metz, M., Gassmann, M., Fakler, B., Schaeren-Wiemers, N., & Bettler, B. (2011). Distribution of the auxiliary GABAB receptor subunits KCTD8, 12, 12b, and 16 in the mouse brain. *Journal of Comparative Neurology, 519*, 1435–1454.

Mezler, M., Müller, T., & Raming, K. (2001). Cloning and functional expression of GABA(B) receptors from Drosophila. *European Journal of Neuroscience, 13*, 477–486.

Mutneja, M., Berton, F., Suen, K. F., Lüscher, C., & Slesinger, P. A. (2005). Endogenous RGS proteins enhance acute desensitization of GABA(B) receptor-activated GIRK currents in HEK-293T cells. *Pflügers Archiv, 450*, 61–73.

Osawa, Y., Xu, D., Sternberg, D., Sonett, J. R., D'Armiento, J., Panettieri, R. A., et al. (2006). Functional expression of the GABAB receptor in human airway smooth muscle. *American Journal of Physiology. Lung Cellular and Molecular Physiology, 291*, L923–L931.

Pfaff, T., Malitschek, B., Kaupmann, K., Prézeau, L., Pin, J. P., Bettler, B., et al. (1999). Alternative splicing generates a novel isoform of the rat metabotropic GABA(B)R1 receptor. *European Journal of Neuroscience, 11*, 2874–2882.

Pinard, A., Seddik, R., & Bettler, B. (2010). GABAB receptors: Physiological functions and mechanisms of diversity. *Advances in Pharmacology, 58*, 231–255.

Pregitzer, P., Schultze, A., Raming, K., Breer, H., & Krieger, J. (2013). Expression of a GABA(B)-receptor in olfactory sensory neurons of sensilla trichodea on the male antenna of the moth Heliothis virescens. *International Journal of Biological Sciences, 9*, 707–715.

Price, G. W., Kelly, J. S., & Bowery, N. G. (1987). The location of GABAB receptor binding sites in mammalian spinal cord. *Synapse, 1*, 530–538.

Princivalle, A. P., Pangalos, M. N., Bowery, N. G., & Spreafico, R. (2001). Distribution of GABA(B(1a)), GABA(B(1b)) and GABA(B2) receptor protein in cerebral cortex and thalamus of adult rats. *Neuroreport, 12*, 591–595.

Princivalle, A., Regondi, M. C., Frassoni, C., Bowery, N. G., & Spreafico, R. (2000). Distribution of GABA(B) receptor protein in somatosensory cortex and thalamus of adult rats and during postnatal development. *Brain Research Bulletin, 52*, 397–405.

Procacci, P., Ballabio, M., Castelnovo, L. F., Mantovani, C., & Magnaghi, V. (2013). GABA-B receptors in the PNS have a role in Schwann cells differentiation? *Frontiers in Cellular Neuroscience, 6*, 68.

Root, C. M., Masuyama, K., Green, D. S., Enell, L. E., Nässel, D. R., Lee, C. H., et al. (2008). A presynaptic gain control mechanism fine-tunes olfactory behavior. *Neuron, 59*, 311–321.

Sanger, G. J., Munonyara, M. L., Dass, N., Prosser, H., Pangalos, M. N., & Parsons, M. E. (2002). GABA(B) receptor function in the ileum and urinary bladder of wildtype and GABA(B1) subunit null mice. *Autonomic & Autacoid Pharmacology, 22*, 147–154.

Schultheis, C., Brauner, M., Liewald, J. F., & Gottschalk, A. (2011). Optogenetic analysis of GABAB receptor signaling in Caenorhabditis elegans motor neurons. *Journal of Neurophysiology, 106*, 817–827.

Schwarz, D. A., Barry, G., Eliasof, S. D., Petroski, R. E., Conlon, P. J., & Maki, R. A. (2000). Characterization of gamma-aminobutyric acid receptor GABAB(1e), a GABAB(1) splice variant encoding a truncated receptor. *Journal of Biological Chemistry, 275*, 32174–32181.

Schwenk, J., Metz, M., Zolles, G., Turecek, R., Fritzius, T., Bildl, W., et al. (2010). Native GABA(B) receptors are heteromultimers with a family of auxiliary subunits. *Nature, 465*, 231–235.

Takahashi, T., Kajikawa, Y., & Tsujimoto, T. (1998). G-Protein-coupled modulation of presynaptic calcium currents and transmitter release by a GABAB receptor. *Journal of Neuroscience, 18*, 3138–3146.

Towers, S., Princivalle, A., Billinton, A., Edmunds, M., Bettler, B., Urban, L., et al. (2000). GABAB receptor protein and mRNA distribution in rat spinal cord and dorsal root ganglia. *European Journal of Neuroscience, 12*, 3201–3210.

Turecek, R., Schwenk, J., Fritzius, T., Ivankova, K., Zolles, G., Adelfinger, L., et al. (2014). Auxiliary GABAB receptor subunits uncouple G protein βγ subunits from effector channels to induce desensitization. *Neuron, 82*, 1032–1944.

Turgeon, S. M., & Albin, R. L. (1993). Pharmacology, distribution, cellular localization, and development of GABAB binding in rodent cerebellum. *Neuroscience, 55*, 311–323.

Turgeon, S. M., & Albin, R. L. (1994). Postnatal ontogeny of GABAB binding in rat brain. *Neuroscience, 62*, 601–613.

Vigot, R., Barbieri, S., Bräuner-Osborne, H., Turecek, R., Shigemoto, R., Zhang, Y. P., et al. (2006). Differential compartmentalization and distinct functions of GABAB receptor variants. *Neuron, 50*, 589–601.

Wei, K., Eubanks, J. H., Francis, J., Jia, Z., & Snead, O. C., 3rd. (2001). Cloning and tissue distribution of a novel isoform of the rat GABA(B)R1 receptor subunit. *Neuroreport, 12*, 833–837.

Xu, C., Zhang, W., Rondard, P., Pin, J. P., & Liu, J. (2014). Complex GABAB receptor complexes: How to generate multiple functionally distinct units from a single receptor. *Frontiers in Pharmacology, 5*, 12.

Chapter 6
Activation Mechanism and Allosteric Properties of the $GABA_B$ Receptor

Julie Kniazeff, Xavier Rovira, Philippe Rondard, and Jean-Philippe Pin

Abstract The $GABA_B$ receptor is quite original within the large G protein-coupled receptor (GPCR) family. When first identified at the molecular level, it was the only GPCR to require two subunits to form a functional receptor, composed of $GABA_{B1}$ and $GABA_{B2}$. Although part of the mandatory dimeric class C group of GPCRs that also includes the receptors activated by glutamate, calcium, the sweet and umami taste compounds, the $GABA_B$ is unique in that it lacks an essential element, the cysteine-rich domain that interconnects the ligand binding domain to the heptahelical transmembrane domain (7TM) responsible for G protein activation. Here, we will summarize our actual knowledge on the structure, stoichiometry, allosteric properties, and activation mechanism. These reveal some similarities and major differences with the other class C GPCRs and highlight novel possibilities to develop approaches to regulate its activity.

Keywords G protein-coupled receptor • Activation mechanism • Structure • Allostery • Dimer

6.1 Introduction

Since their discovery in the mid 80s as the molecular target of the anti-spasticity drug baclofen, the $GABA_B$ receptors raised much interest with a first goal to elucidate their molecular bases. Based on pharmacological studies, and as already observed for G protein-coupled receptors (GPCRs) activated by other neurotransmitters, several $GABA_B$ receptor genes were expected (Bonanno et al. 1997; Deisz et al. 1997; Zhang et al. 1997). A first clone was identified in 1997 by the Bettler lab (Kaupmann et al. 1997). Although it was found to display the expected pharmacological and brain localization profiles for a $GABA_B$ receptor, agonist affinity was

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lower than expected, and no functional response could be measured in recombinant assays. However, this first clone already revealed a general organization similar to class C GPCRs, with a large extracellular venus flytrap-like domain (VFT) similar to the binding domain of metabotropic glutamate (mGlu) receptors, and where the GABA binding site was rapidly identified (Galvez et al. 1999). However, in contrast to the other class C GPCRs like the mGlu receptors, the VFT was directly linked to the 7TM, such that the cysteine-rich domain (CRD) found in other class C receptors is missing (Kaupmann et al. 1997). This first gene encodes two variants $GABA_{B1a}$ and $GABA_{B1b}$, thanks to an alternative initiation site, adding two sushi domains (SDs) at the N-terminus of the $GABA_{B1a}$ variants (Kaupmann et al. 1997). However, both subunits displayed the same pharmacological properties. Only about 2 years later, a second subunit $GABA_{B2}$ was independently identified by three groups, which was structurally homologous to $GABA_{B1}$ and was absolutely required for agonist high affinity, and for proper coupling to G proteins (Jones et al. 1998; Kaupmann et al. 1998; White et al. 1998). $GABA_{B2}$ was also found to be essential for the proper membrane insertion of the $GABA_B$ receptor. Indeed, when expressed alone, $GABA_{B1}$ remains intracellularly retained between endoplasmic reticulum and Golgi because of an intracellular retention signal in its C-terminal tail (Margeta-Mitrovic et al. 2000; Pagano et al. 2001). Only when interacting with $GABA_{B2}$, the retention signal is masked and the heterodimer reaches the cell surface making this receptor unique among all GPCRs known at that time, being the first mandatory heterodimeric GPCR. In addition, it was early demonstrated that $GABA_{B1}$ was responsible for agonist binding while $GABA_{B2}$ was critical for G protein activation (Galvez et al. 2001; Margeta-Mitrovic et al. 2001a, b). These findings were a major breakthrough not only in the $GABA_B$ receptor field, but also in the large GPCR community, where the notion of GPCR dimerization was still the subject of intense debate. Since then, and despite a number of mammalian genomes sequences, no additional $GABA_B$ receptor subunits were identified, making the $GABA_{B1}$-$GABA_{B2}$ heterodimer the only known $GABA_B$ receptor.

As such, the $GABA_B$ receptor is unique in its general structural organization, showing differences with the other class C receptors, and being the first heterodimeric GPCR. This obviously stimulated much effort to elucidate its activation mechanism and allosteric properties, not only to identify novel possibilities to develop drugs to modulate its activity with potential therapeutic application, but also as a model of GPCR heterodimers, with the hope to better understand the possible roles of the still questioned class A rhodopsin-like GPCR heterodimers.

In this chapter, we will report on our actual knowledge of the structure of the various domains of the $GABA_B$ receptor, and how such a multidomain membrane protein is activated by a small ligand to control G protein activity. We will see that despite some similarities with the other class C GPCRs, a different activation mechanism is observed, and we will show how allosteric transitions between the different domains control receptor activity. Such understanding certainly reveals new ideas on how to develop innovative drugs to control this important brain receptor and sheds light on the possible assembly and allosteric interactions between other GPCRs.

6.2 Structure and Organization of the $GABA_B$ Receptor

Both subunits of the $GABA_B$ receptor, $GABA_{B1}$ and $GABA_{B2}$, belong to class C GPCRs together with the mGlu receptors, the calcium-sensing receptor, the sweet and umami taste receptors, and the basic amino acids GPRC6A receptor (Kniazeff et al. 2011). All these proteins share both sequence and structural homology. They are composed of a transmembrane domain (7TM) made of 7 alpha helices (about 260 residues) and of a large and well-structured extracellular domain referred to as the VFT domain (about 410 residues) (Rondard et al. 2011) (see also Chap. 4 of this book) (Fig. 6.1a). Compared to other class C GPCR, $GABA_B$ receptor subunits have the particularity to lack the CRD connecting the VFT to the 7TM that is replaced by a shorter linker (10–15 amino acids) of unknown structure. The intracellular C-terminal tail of $GABA_{B1}$ and $GABA_{B2}$ is rather long (107 and 200 residues, respectively) and contains a well-structured coiled-coil domain that is important for heterodimerization and to guarantee the correct assembly of the heterodimer before proper targeting to the plasma membrane (Margeta-Mitrovic et al. 2000; Pagano et al. 2001; Kammerer et al. 1999).

The VFT domains in class C GPCRs are known to bear the agonist binding site (Okamoto et al. 1998). They share some structural homology with bacterial periplasmic amino acid binding proteins such as the leucine/isoleucine/valine binding protein (LIVBP) (O'Hara et al. 1993). A general folding of these domains was first proposed based on homology modeling and was later confirmed by the structure resolution of VFTs from different mGlu receptors and more recently from both $GABA_B$ receptor subunits in the presence or not of various ligands, agonists, and antagonists (Geng et al. 2012, 2013; Kunishima et al. 2000; Muto et al. 2007; Tsuchiya et al. 2002). The domain is about 70 Å long and 35 Å wide. Each VFT is composed of two opposite lobes linked by three short loops, with lobe 1 being the N-terminal lobe and lobe 2 the C-terminal one (Fig. 6.1b). Both lobes have an αβ-fold with a central β-sheet being surrounded by α-helices. Overall, in the absence of ligand (apo form), $GABA_{B1}$ and $GABA_{B2}$ VFTs share a good structural homology (rms deviation of 1.48 Å for 356 C_α atoms—pdb code 4MQE) (Geng et al. 2013). In addition, when considering separately each lobe of the VFT, there is also a good superimposition with $mGlu_1$ VFT structure (rms deviation ~1.6 Å).

Besides the general homology between $GABA_{B1}$ and $GABA_{B2}$ VFTs, a major difference exists in the relative orientation of the two lobes of the VFTs. Indeed, while the angle defined by the two lobes remains nearly constant for $GABA_{B2}$ VFT in all available structures, it differs for $GABA_{B1}$ VFT depending on the presence and the identity of the bound-ligand in the crystal (Geng et al. 2013). The angle is larger in the apo form and the antagonist-bound form and smaller in the presence of agonists, defining two conformations for the $GABA_{B1}$ VFT, open and close, respectively (Fig. 6.1b). In comparison, the angle defined by the two lobes in $GABA_{B2}$ VFT is in line with the large angle observed for the apo-form and antagonist-bound conformations of $GABA_{B1}$ VFT. Hence, $GABA_{B2}$ is in an open-like conformation, either alone or when associated with the closed or open $GABA_{B1}$ VFT (Geng et al. 2012,

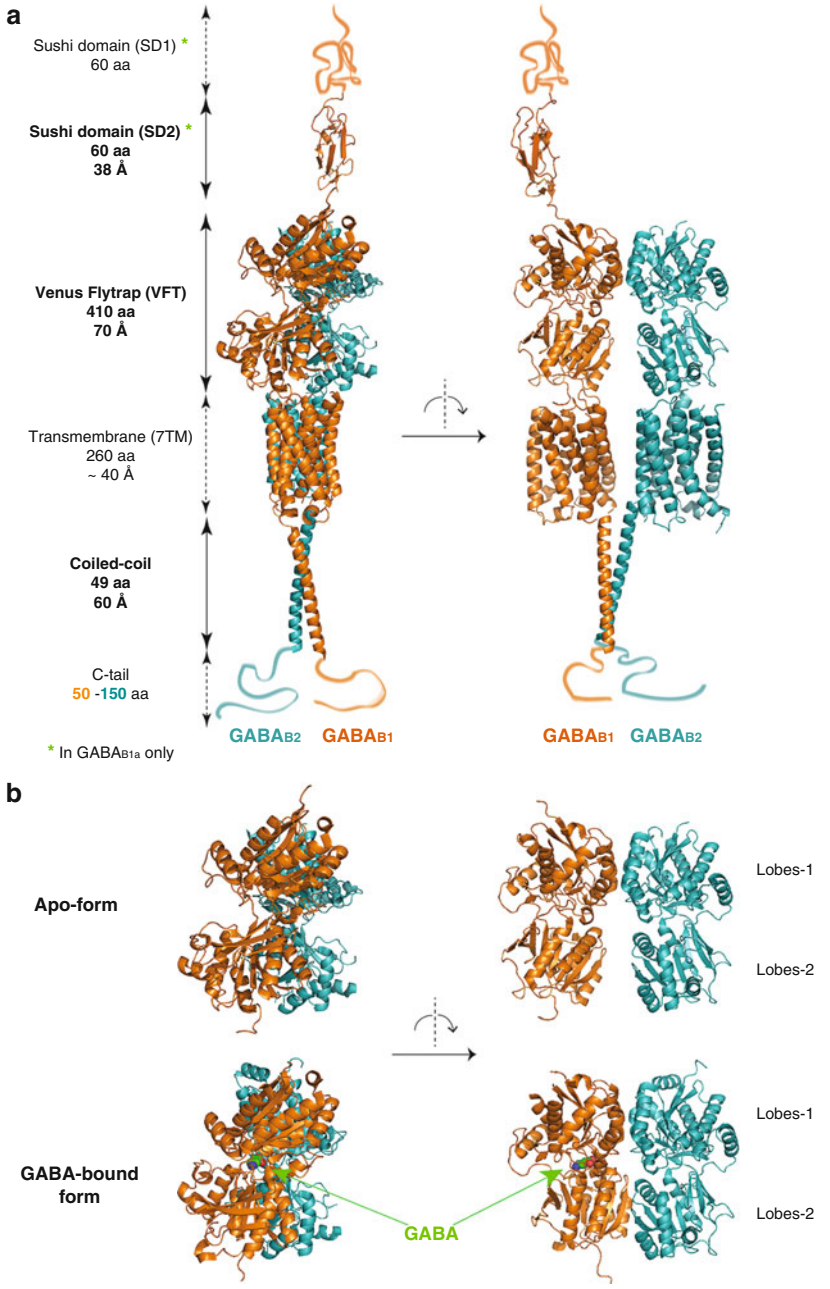

Fig. 6.1 $GABA_B$ receptor structural organization. (**a**) Representation of the structural domains composing the $GABA_B$ receptor. Hypothetical assembly of the receptor heterodimer based on the 3D structures available for the SD2 (pdb code 1SRZ), the VFT dimer (apo form—pdb code 4MQE) and the coiled-coil dimer (pdb code 4PAS), and a model of 7TM dimer. The structures of SD1 and of the C-termini remain unknown and are represented by a cartoon. $GABA_{B1}$ is represented in *orange* and $GABA_{B2}$ in *teal*. SD1 and SD2 are present in the $GABA_{B1a}$ isoform only. The domains for which pdb coordinates are available are noted with *plain-lined arrows* while the others are noted with *dash-lined arrows. Left and right panels* represent the same structure with an approximately

2013). This is in agreement with $GABA_{B1}$ being the only subunit binding agonists in the $GABA_B$ receptor and being responsible for the activation of the entire receptor complex (Kniazeff et al. 2002, 2004).

Thanks to both structural and mutagenesis studies; the binding site of GABA in $GABA_{B1}$ VFT has been described precisely (Galvez et al. 1999, 2000; Geng et al. 2013; Kniazeff et al. 2002). The carboxylate moiety of GABA is at the center of a hydrogen-bound network involving Ser 246 and Ser 269 in lobe 1 and Tyr 366 in lobe 2 (in the whole chapter, indicated residues correspond to $GABA_{B1a}$ numbering). The γ-amino group interacts with His 286 and Glu 465 in lobe 1 and with Trp 394 in lobe 2 through hydrogen-bound and van der Waals contacts (Fig. 6.2). Baclofen, a $GABA_B$ receptor-specific agonist, binds in a similar way than GABA but with a conformational flip of Tyr 366 to accommodate the chlorophenyl moiety of the ligand (Geng et al. 2013; Galvez et al. 2000). Orthosteric antagonists are GABA derivatives that also bind to $GABA_{B1}$ VFT only. Co-crystallization of $GABA_B$ VFTs

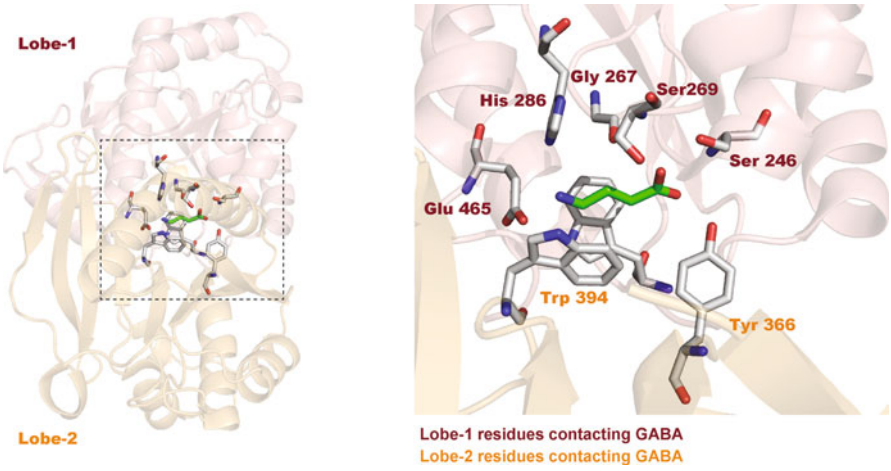

Fig. 6.2 GABA binding site in $GABA_{B1}$ VFT. (*Left panel*) General view of $GABA_{B1}$ VFT in the presence of GABA with lobe-1 colored in *pale red* and lobe-2 colored in *pale orange* (pdb code 4MS3). (*Right panel*) Closer view of the GABA binding site indicated with a *dash-box* in the *left panel*. Residues interacting with GABA (in *green*) are represented by sticks (*gray*). Lobe-1 residues are labeled in *dark red* and lobe-2 residues are labeled in *orange*. For clarity, the residues 171–217 in lobe-2 were removed in the *right panel*. In both panels, the cartoon of secondary structures was set to transparency. Figures were generated using Pymol (Delano Scientific)

Fig. 6.1 (continued) 90° rotation. (**b**) Conformational changes of VFT dimer upon GABA binding. (*Upper line*) Apo-form of $GABA_B$ VFT dimer (pdb code 4MQE); (*Lower line*) GABA-bound form of $GABA_B$ VFT dimer (pdb code 4MS3). The GABA is represented in balls with the carbons in *green*. $GABA_{B1}$ is represented in *orange* and $GABA_{B2}$ in *teal*. *Left and right panels* represent the same structures with an approximately 90° rotation. Figures were generated using Pymol (Delano Scientific)

with the antagonists showed that they bind tightly to the lobe 1 involving similar residues than GABA binding (Ser 246, Ser 269, His 286, Glu 465, and Trp 181). However, compared to agonist-bound conformation, there is only sparse interaction with lobe 2, which is in line with the two lobes being further apart and the stabilization of an "open" conformation (Geng et al. 2013). Of note, the residues involved in GABA binding in $GABA_{B1}$ VFT are not conserved in $GABA_{B2}$ (Kniazeff et al. 2002). In addition, in contrast to the $GABA_{B1}$ VFT cleft where agonists bind, that of $GABA_{B2}$ does no show a specific and high conservation during evolution, strongly suggesting the absence of ligand interaction at this site (Kniazeff et al. 2002).

Crystal structure resolution of the heterodimeric $GABA_B$ VFTs has also shed light on the interaction between $GABA_{B1}$ and $GABA_{B2}$ VFTs (Geng et al. 2013). Lobes 1 of each protomer interact burying a 1400 $Å^2$ surface from solvent accessibility. The interface consists of a central hydrophobic patch surrounded by hydrogen bonds and of a salt-bridge that are well conserved in all available structures. This is in contrast to the equivalent interface in mGlu receptors that is mostly hydrophobic (Kunishima et al. 2000). In the agonist-bound structures, an additional contact between the lobes 2 is present and buries about 1300 $Å^2$ involving mostly polar interactions and showing a lower shape complementarity than lobe 1 interface. Altogether, thanks to structural and mutagenesis studies, we gained a good knowledge of the VFT molecular organization.

Less structural information is available for $GABA_B$ receptor 7TM. Indeed, no crystal structure has been solved so far for this part of the receptor. Only homology models can be obtained based on the crystal structure of $mGlu_1$ and $mGlu_5$ 7TM (Dore et al. 2014; Wu et al. 2014), but not much validation of these models has been obtained so far. The models indicated that $GABA_B$ 7TM is about 40 Å long and 27 Å wide to cross the cell membrane (Fig. 6.1a). Although small molecules identified as positive or negative allosteric modulators were shown to interact in the 7TM domains of the $GABA_B$ receptor (Chen et al. 2014; Malherbe et al. 2008; Urwyler et al. 2001, 2003), their precise binding mode, and the residues involved have not been clearly identified so far. Nonetheless, early models identified an ionic lock stabilizing an interaction between TM3 and TM6 that is important to stabilize the inactive conformation of the $GABA_{B2}$ 7TM (Binet et al. 2007). Such an ionic lock has been confirmed in both $mGlu_1$ and $mGlu_5$ 7TM structures (Dore et al. 2014; Wu et al. 2014). This is consistent with $GABA_{B2}$ 7TM domain undergoing a similar change in conformation leading to G protein activation. Of interest, the ionic lock is absent in $GABA_{B1}$ 7TM (Binet et al. 2007) in agreement with its inability to activate G proteins.

Nothing is known yet on how the 7TM domain of $GABA_{B1}$ interacts with that of $GABA_{B2}$. In mGlu receptors, cysteine cross-linking experiments, associated with functional studies identified TM4 and TM5 as the interface in the inactive form of the dimer, while TM6 appears critical in the active dimer (Xue et al. 2015). However, the heterodimeric nature of the $GABA_B$ receptor, its ability to associate into large complexes in contrast to mGlu receptors (Maurel et al. 2008) and the absence of a large movement between the VFTs (Geng et al. 2013) hence suggesting much smaller movements between $GABA_B$ receptor 7TM indicate a different mode of

subunit interaction in the $GABA_B$ receptor compared to mGlu receptors. More work is then necessary to clarify the general structure, the allosteric interaction, and the modulation of the 7TM domains of each subunit of the $GABA_B$ receptor.

The $GABA_B$ heterodimeric interaction is stabilized by a coiled-coil interaction between the $GABA_{B1}$ and $GABA_{B2}$ C-termini encompassing about 49 residues in each subunit (Ser 772—His 810 in $GABA_{B1}$ and Ser 779 Lys 827 in $GABA_{B2}$) (Kammerer et al. 1999; Burmakina et al. 2014) (Fig. 6.1a). Coiled-coil domains are known structural motives formed of at least two intertwined helices composed of heptad repeats that tightly interact to form a super coil (Mason and Arndt 2004). The structure of $GABA_B$ receptor coiled-coil domain has been solved by X-ray crystallography and highlights the molecular details of the interaction (Burmakina et al. 2014). The two parallel helices form an extended stalk about 60 Å long and 22 Å wide constituted of five complete heptad repeats and additional coiled-coil elements at both ends. The interaction buries a surface of about 2000 $Å^2$. The general packing of the $GABA_B$ coiled-coil domain is in line with the reported interaction of such structural motives. There is a succession of knobs and holes where the knobs of one helix interlock with the holes formed between four residues of the other helix. A particularity of $GABA_B$ coiled-coil interaction is the network of hydrogen-bounds all along the domain, which is favored by the presence of asparagine residues at the center of the coiled-coil interaction. It was proposed to enhance the specificity of the interaction together with the presence of three salt bridges (Burmakina et al. 2014).

An additional structural domain is present on one of the isoforms of the $GABA_B$ receptor. Indeed, two main isoforms, $GABA_{B1a}$ and $GABA_{B1b}$, of the $GABA_{B1}$ subunit are generated through an alternate promoter usage (Steiger et al. 2004). It results in the presence of a repeat of two sushi domains (SD1 and SD2) at the extracellular N-terminus of $GABA_{B1a}$ only (Fig. 6.1a). SDs, which are also named complement control protein (CCP) modules or the short consensus repeats (SCRs), are about 60 residues long and are known to be involved in many recognition processes including that of the complement system (Reid and Day 1989). In the case of the $GABA_B$ receptor, SDs control the specific targeting of the receptor to excitatory terminals most probably through interactions with the extracellular matrix (Vigot et al. 2006). In an attempt to gain a better knowledge on their structural organization, biostructural analyses of the purified $GABA_B$ SDs have been performed (Blein et al. 2004). SD2 is a typical SD with approximately 60 amino acid residues including four cysteines forming two disulphide bridges. Nuclear magnetic resonance (NMR) analysis reveals its 3D structure which is in agreement with previously solved SD structures. It is mostly constituted of five antiparallel β-strands forming part of a barrel-like structure (Fig. 6.1a). An additional two antiparallel β-strands are separated from the other. Compared to other SDs, $GABA_B$ receptor SD2 has a long hypervariable region forming a long loop extending toward its N-terminus. It is suggested that it may interact either with SD1 or with other interacting proteins. In contrast to SD2, SD1 has less sequence homology with typical SDs and is unstable when purified alone or when fused to SD2 (Blein et al. 2004). Mass spectrometry analysis showed, however, the presence of the two typical SD disulphide bridges in SD1 and pull-down

experiments indicated that the purified isolated SD1 maintained its ability to interact with fibulin-2 (an in vitro reported partner for $GABA_{B1a}$). As a consequence of the instability of SD1, the NMR spectra were of poor quality and could not lead to structure determination. Hence, the precise folding of the SD1 remains unknown.

As reported above, precise molecular information is available on the different structural domains composing the $GABA_B$ receptor heterodimers except for the 7TM which is still waiting for structure resolution but which is also rather challenging. A further step would be the resolution of the full-length heterodimeric $GABA_B$ receptor structure that would surely unravel new molecular interactions that remain unknown.

6.3 Activation Mechanism and Allosteric Interaction Between the Various $GABA_B$ Receptor Domains

Having reported the general structure of the $GABA_B$ heterodimeric receptor, we will now describe our current view on how these four main domains can link GABA interaction in the $GABA_{B1}$ VFT, to G protein activation by the 7TM of $GABA_{B2}$ (Galvez et al. 2001).

As well documented for the VFT domains, including the binding domain of the mGlu receptors, GABA interaction in the $GABA_{B1}$ VFT stabilizes its closed state. This is well validated by mutagenesis and modeling studies and confirmed by the crystal structure of the $GABA_{B1}$ VFT (Geng et al. 2013; Galvez et al. 2000). $GABA_{B1}$ VFT closure was indeed found essential and sufficient for $GABA_B$ receptor activation since locking this domain in its closed conformation through an inter-lobes disulfide bridge generates a fully active receptor (Kniazeff et al. 2004). On the opposite, $GABA_{B2}$ VFT could not be observed in a closed conformation, even in the presence of agonist in $GABA_{B1}$ VFT (Geng et al. 2012, 2013). Moreover, any attempt to prevent a putative closure of $GABA_{B2}$ VFT using a glycan wedge approach (insertion of a glycosylation site in the $GABA_{B2}$ VFT cleft) did not affect the properties of the heterodimer, still displaying a high agonist affinity and still being functional with no noticeable differences from the wild-type (Geng et al. 2012). Accordingly, the first effect of agonists on the $GABA_B$ heterodimer is to stabilize the $GABA_{B1}$ VFT in a closed conformation.

In contrast to mGlu VFT dimers, in which domain closure is associated with a major change in the relative orientation of the two VFTs (Kunishima et al. 2000), no such major reorientation is observed in the dimeric $GABA_B$ and instead the relative movement of the VFTs is more subtle (Geng et al. 2013). Indeed, $GABA_{B1}$ VFT closure induces further interactions between the lobes-2 of both VFTs that likely stabilize further the $GABA_{B1}$ closed state promoting an increased agonist affinity (Liu et al. 2004). However, the movement between the lobes-2 was shown to play an important role in the $GABA_B$ receptor activation, since engineering of glycan wedges at this interface prevented G protein activation (Rondard et al. 2008).

At the 7TM level, far less is known, even though a change in conformation in $GABA_{B2}$ 7TM is obviously occurring, as evidenced by the ability of small molecules to activate the isolated 7TM of $GABA_{B2}$ expressed alone (Binet et al. 2004). In addition and as mentioned above, an ionic interaction linking TM3 and TM6 is important to stabilize the inactive conformation of the receptor (Binet et al. 2007). Indeed, removing this lock has been shown to likely stabilize $GABA_{B2}$-7TM in an active state, as indicated by the increased agonist affinity.

But how can the conformational change in the VFT dimer lead to the activation of the $GABA_{B2}$ 7TM? A number of observations revealed an interconnection between all four domains of the $GABA_B$ receptor heterodimer. First, the tighter interaction between the $GABA_{B1}$ and $GABA_{B2}$ VFTs in the presence of agonist stabilizes the closed state of $GABA_{B1}$ increasing agonist potency and revealing a first positive allostery from $GABA_{B2}$ VFT to $GABA_{B1}$ VFT (Geng et al. 2013; Liu et al. 2004) (Fig. 6.3). However, while the interactions between the lobes 2 are strictly required for activation of the wild-type receptor, a $GABA_B$ mutant lacking the $GABA_{B2}$ VFT is still functional although displaying a low agonist potency and a low efficacy (Monnier et al. 2011). These findings highlight a second important allosteric transition between the $GABA_{B1}$ VFT and the 7TM domain of $GABA_{B1}$ leading to an undefined conformational change in this domain that is eventually transmitted to the 7TM of $GABA_{B2}$ through a third allosteric interaction. Of note, this allosteric transition could also be highlighted in full-length receptor since the presence $GABA_{B1}$ 7TM is important to fully activate G proteins (Galvez et al. 2001; Duthey et al. 2002; Havlickova et al. 2002; Robbins et al. 2001). Accordingly, a first activation pathway of the receptor can be defined from the GABA binding site in $GABA_{B1}$ VFT to the G protein coupling site in $GABA_{B2}$ 7TM mediated through $GABA_{B1}$7TM and independent of $GABA_{B2}$VFT (Fig. 6.3). On another end, a second major observation highlighting the conformational transitions of the receptor activation is that a $GABA_B$ receptor mutant lacking the 7TM domain of $GABA_{B1}$ is also functional though displaying a lower coupling efficacy than the wild-type heterodimer (Monnier et al.

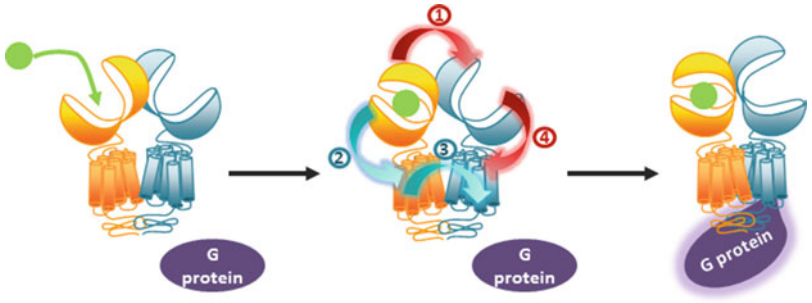

Fig. 6.3 Schematic representation of the allosteric transitions during $GABA_B$ receptor activation. Two independent but concomitant pathways (*one cyan* and *one red*) were defined and are associated with four allosteric transitions (numbered *1–4*) between the four main structural domains of the $GABA_B$ receptor (VFT and 7TM of both $GABA_{B1}$ and $GABA_{B2}$). $GABA_{B1}$ is represented in *orange* and $GABA_{B2}$ in *blue*. The G protein is represented in *purple* and the GABA in *green*

2011). This demonstrates a second activation pathway linking $GABA_{B1}$ VFT to $GABA_{B2}$ VFT and then to the 7TM of $GABA_{B2}$ (fourth allosteric transition), again enabling its coupling to G proteins (Fig. 6.3). This is only when both activation pathways are simultaneously effective that a fully efficient activation is reached.

A model of the possible allosteric coupling between the two 7TM domains of the dimeric mGlu receptors has been proposed. It involves a large relative movement between these domains interacting through TM4-5 in the inactive state and TM6 in the active state (Xue et al. 2015). No such major movement is expected for the $GABA_B$ receptor due to the small conformational changes observed upon activation of the dimer of $GABA_B$ VFTs (Geng et al. 2013). In addition, one has to keep in mind that the $GABA_B$ receptor lacks the rigid CRD linking the VFT to the 7TM in mGlu receptors and that has been shown to also participate in the receptor activation (Huang et al. 2011). This observation indicates further that the precise activation mechanism of $GABA_B$ receptor must differ from that of mGlu receptors. Further studies are required to elucidate the structural bases of the allosteric control of the 7TM of each $GABA_B$ subunit by their respective VFT.

6.4 Higher Order Oligomers of the $GABA_B$ Receptor

As reported above, the $GABA_B$ receptor is an obligatory heterodimer whose heterodimerization plays a critical role in the activation mechanism leading from GABA binding to G protein activation. However, an unexpected property of the $GABA_B$ receptor was reported: the heterodimers assemble to form higher order oligomers. Indeed, two independent studies revealed the oligomerization of the $GABA_B$ receptor. First, a Förster resonance energy transfer (FRET) analysis showed that, at the cell surface, two $GABA_B$ heterodimers are in close enough proximity to promote an inter-heterodimer FRET signal (Maurel et al. 2008). This indicates that the distance between the two heterodimers is below 100 Å, hence that two heterodimers are likely to directly interact. Second, an analysis of fluorescent $GABA_B$ heterodimers diffusion in cell membrane suggested that the $GABA_B$ receptor has a higher propensity to form larger entities than strict heterodimers than any other tested GPCRs starting from tetramers but also even larger complexes (Calebiro et al. 2013).

Several arguments may arise against this occurring in native systems since both studies were performed on transiently transfected mammalian cells. However, most of these could be ruled out. The use of transient transfection is often accounted for leading to expression levels that are way higher than the physiological ones that might favor unspecific interactions. However in both studies, the oligomers were detected already at very low expression levels even though more oligomers or larger ones are present when the expression level was increased as nicely illustrated by the diffusion study (Calebiro et al. 2013). In the FRET study, a comparison of the expression level in the transfected cells relative to the endogenous expression in cortical neurons showed that they were similar in both systems (Maurel et al. 2008;

Comps-Agrar et al. 2011). In addition, one could also exclude that the FRET signal arose from collisional FRET since the site of insertion of the FRET-compatible fluorophores, either on $GABA_{B1}$ or on $GABA_{B2}$, is critical to measure inter-heterodimer FRET: only fluorophore insertion in $GABA_{B1}$ subunit led to a strong and significant FRET signal compared to the low FRET signal obtained when the fluorophores were inserted in $GABA_{B2}$ subunit (Maurel et al. 2008). This furthermore indicates that $GABA_{B1}$ subunit is likely to be at the center of the oligomeric association.

Two additional results obtained with endogenous receptors further support the ability of the $GABA_B$ receptor to form oligomers in the brain. First, the apparent molecular weight of the protein complex pulled down from brain using anti-$GABA_B$ antibodies is compatible with the molecular weight of two $GABA_B$ heterodimers together with their accessory proteins, K^+ channel tetramerization domains (KCTDs) (Schwenk et al. 2010). Second, when performing FRET measurements using fluorescent anti-SD antibodies (i.e., anti $GABA_{B1a}$ antibodies (Tiao et al. 2008)) on membrane prepared from mouse brain, a significant signal was measured indicating that two $GABA_{B1a}$ subunits were in close proximity (Comps-Agrar et al. 2011).

As indicated above, the association of $GABA_B$ receptor heterodimers is mediated by $GABA_{B1}$ subunits. In order to comprehend the molecular determinants of the interaction, the crystal structure of a non-related tetrameric protein that also contains a VFT was taken into account (Sobolevsky et al. 2009). Actually, the N-terminal domain of the ionotropic glutamate receptors subunits has a VFT-fold. In agreement, the crystal structure of the full-length tetrameric α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor GluR2 revealed for the first time the interactions that could take place in a VFT tetramer. A first interface very similar to that between $GABA_{B1}$ and $GABA_{B2}$ VFTs is conserved and a second smaller interface is present and may represent a model for the $GABA_{B1}$/$GABA_{B1}$ VFT interface. This interface involves residues at the "lips" of the lobes 2. Using mutagenesis and FRET measurements, it was shown that a similar region in the lobe 2 of $GABA_{B1}$ VFT was indeed important for the proper interaction between $GABA_B$ heterodimers (Comps-Agrar et al. 2011). However, the mutagenesis of this small area did not fully abolish the interaction suggesting that other molecular determinants of the interface, probably at the 7TM level, remain to be identified.

The discovery of the propensity of $GABA_B$ receptor to form oligomers raised some questions starting with the physiological roles of these complexes. A first effort was made in order to determine the differential G protein coupling profiles of the heterodimers and of the oligomers. Since oligomerization is constitutive, a major challenge was to develop strategies to control the oligomerization level of the receptor in cells. By using a competitor of the $GABA_{B1}$/$GABA_{B1}$ interface (a minimal construct consisting of the 7TM part of $GABA_{B1}$ without the VFT and the C-terminal tail), G protein activation upon GABA stimulation showed a better efficacy than in the absence of the competitor (Maurel et al. 2008; Comps-Agrar et al. 2011). The potency of the GABA response was left unchanged. This indicates first that the oligomerization plays a critical role in controlling the G protein coupling efficacy and that oligomers limit G protein coupling compared to heterodimers. To confirm these results, a mutation in $GABA_{B1}$ VFT at the level of the putative $GABA_{B1}$/$GABA_{B1}$

interaction that was shown to decrease the FRET signal of the oligomers was tested for G protein activation. In a similar way than the use of the competitor, introduction of the mutation increased the G protein coupling efficacy without affecting the potency of GABA stimulation (Comps-Agrar et al. 2011). Altogether these data show that the oligomerization surprisingly decreases G protein coupling efficacy, at least when it comes to the canonical Gi/o protein coupling.

One would then wonder what would be the advantage of oligomer formation for the cells if it only limits G protein activation. A first possibility is that oligomers regulate a unique and still undiscovered downstream signaling compared to isolated heterodimers. Alternatively, some physiological conditions could play a regulatory role on the oligomerization and thus control the extent of the $GABA_B$-mediated Gi/o protein activation. Additional studies are required to understand further this phenomenon. In addition, other parameters like trafficking and internalization could also be assessed in the context of the oligomer versus the heterodimer.

An additional intriguing question is to understand the molecular basis of the limitation of G protein coupling in the oligomer. One of the hypotheses is that it may arise from negative allostery in the complex either for ligand binding or for G protein coupling. It could also come from conformational allostery, where activation of one heterodimer hampers the activation of the others. Further studies are required to highlight this mechanism.

Since the propensity of the $GABA_B$ receptor to form oligomers is rather high, one might question the stability of these oligomers. The study of Calibero et al. shows that at any receptor density, several oligomeric species coexist (Calebiro et al. 2013). Also only at very low density strict heterodimers are found and the higher the density, the larger the complexes. Are heterodimers exchanging from one complex to the other leading to transiently existing heterodimers? To assess this, we have developed a methodology in order to measure the stability of the oligomers at the cell surface of HEK293 cells (Comps-Agrar et al. 2012). It consists on following the FRET signal of the oligomers present at the cell surface and targeting new unlabeled (or differently labeled) receptors in a drug-induced manner. We showed that the FRET signal of the preexisting oligomers remained unchanged, which suggests that the $GABA_B$ receptor oligomers are stable at the surface of HEK293 cells. In addition, when targeting new receptors, we could detect the association of these new receptors to receptors that were already present at the cell surface suggesting the constitution of higher order $GABA_B$ receptor oligomers when targeting new receptors to the cell surface, thus when increasing receptor density. This is in agreement with the study of Calibero et al. (2013).

6.5 Conclusions

The discovery that two distinct subunits were requested to form a functional $GABA_B$ receptor was a real breakthrough in the GPCR field. Since this major discovery, major information highlighting the importance of this heterodimeric assembly for

the proper function of the $GABA_B$ receptor was obtained. Not only such an assembly is required for the proper plasma membrane targeting of the receptor, but it is also essential for the allosteric interaction between the various $GABA_B$ receptor domains to allow agonist binding in $GABA_{B1}$ VFT and to activate the 7TM domain of $GABA_{B2}$ leading to G protein activation. Although fewer details are known compared to the dimeric mGlu receptors, the available information already indicates a different activation mechanism for the $GABA_B$ receptor, likely resulting from the lack of a CRD. However, it is clear that the main four domains are tightly linked by allosteric interactions, enabling the information to efficiently reach one domain when the conformation of another is modified. A better understanding of the molecular details undergoing $GABA_B$ receptor G protein coupling will certainly help designing novel $GABA_B$ ligands, and especially allosteric modulators that may have better therapeutic efficacy, with fewer side effects.

Of interest, the $GABA_B$ heterodimeric complex leads to G protein activation through one single subunit only, an observation that is consistent with what is observed with many other dimeric GPCRs. This highlights the interest of studying the $GABA_B$ receptor, not only for the improvement of $GABA_B$ targeting drugs, but also for the more general purpose of elucidating the role and physiological interest of GPCR dimerization.

Today, they are increasing number of papers indicating that GPCRs may assemble into tetramers or larger oligomers (Calebiro et al. 2013; Patowary et al. 2013; Pisterzi et al. 2010). Again, the $GABA_B$ receptor may help unravel the functional consequence of such a receptor assembly since it is clearly one of the best characterized oligomeric GPCRs, being supported not only in recombinant systems, but also in native neurons. Already, allosteric interaction between dimers within such a large receptor complex provides some indication on the possible roles of such complex assembly.

These observations highlight the need for a better understanding of the structural bases of $GABA_B$ receptor assembly and conformational dynamics, as one of the most exciting example of GPCR complex.

References

Binet, V., Brajon, C., Le Corre, L., Acher, F., Pin, J. P., & Prezeau, L. (2004). The heptahelical domain of GABA(B2) is activated directly by CGP7930, a positive allosteric modulator of the GABA(B) receptor. *Journal of Biological Chemistry, 279*(28), 29085–29091.

Binet, V., Duthey, B., Lecaillon, J., Vol, C., Quoyer, J., Labesse, G., et al. (2007). Common structural requirements for heptahelical domain function in class A and class C G protein-coupled receptors. *Journal of Biological Chemistry, 282*(16), 12154–12163.

Blein, S., Ginham, R., Uhrin, D., Smith, B. O., Soares, D. C., Veltel, S., et al. (2004). Structural analysis of the complement control protein (CCP) modules of GABA(B) receptor 1a: Only one of the two CCP modules is compactly folded. *Journal of Biological Chemistry, 279*(46), 48292–48306.

Bonanno, G., Fassio, A., Schmid, G., Severi, P., Sala, R., & Raiteri, M. (1997). Pharmacologically distinct GABAB receptors that mediate inhibition of GABA and glutamate release in human neocortex. *British Journal of Pharmacology, 120*(1), 60–64.

Burmakina, S., Geng, Y., Chen, Y., & Fan, Q. R. (2014). Heterodimeric coiled-coil interactions of human GABAB receptor. *Proceedings of the National Academy of Sciences of the United States of America, 111*(19), 6958–6963.

Calebiro, D., Rieken, F., Wagner, J., Sungkaworn, T., Zabel, U., Borzi, A., et al. (2013). Single-molecule analysis of fluorescently labeled G-protein-coupled receptors reveals complexes with distinct dynamics and organization. *Proceedings of the National Academy of Sciences of the United States of America, 110*(2), 743–748.

Chen, L. H., Sun, B., Zhang, Y., Xu, T. J., Xia, Z. X., Liu, J. F., et al. (2014). Discovery of a negative allosteric modulator of GABAB receptors. *ACS Medicinal Chemistry Letters, 5*(7), 742–747.

Comps-Agrar, L., Kniazeff, J., Brock, C., Trinquet, E., & Pin, J. P. (2011). The oligomeric state sets GABA(B) receptor signalling efficacy. *EMBO Journal, 30*(12), 2336–2349.

Comps-Agrar, L., Kniazeff, J., Norskov-Lauritsen, L., Maurel, D., Gassmann, M., Gregor, N., et al. (2012). Stability of GABAB receptor oligomers revealed by dual TR-FRET and drug-induced cell surface targeting. *FASEB Journal, 26*(8), 3430–3439.

Deisz, R. A., Billard, J. M., & Zieglgansberger, W. (1997). Presynaptic and postsynaptic GABAB receptors of neocortical neurons of the rat in vitro: Differences in pharmacology and ionic mechanisms. *Synapse, 25*(1), 62–72.

Dore, A. S., Okrasa, K., Patel, J. C., Serrano-Vega, M., Bennett, K., Cooke, R. M., et al. (2014). Structure of class C GPCR metabotropic glutamate receptor 5 transmembrane domain. *Nature, 511*(7511), 557–562.

Duthey, B., Caudron, S., Perroy, J., Bettler, B., Fagni, L., Pin, J. P., et al. (2002). A single subunit (GB2) is required for G-protein activation by the heterodimeric GABA(B) receptor. *Journal of Biological Chemistry, 277*(5), 3236–3241.

Galvez, T., Parmentier, M. L., Joly, C., Malitschek, B., Kaupmann, K., Kuhn, R., et al. (1999). Mutagenesis and modeling of the GABAB receptor extracellular domain support a venus flytrap mechanism for ligand binding. *Journal of Biological Chemistry, 274*(19), 13362–13369.

Galvez, T., Prezeau, L., Milioti, G., Franek, M., Joly, C., Froestl, W., et al. (2000). Mapping the agonist-binding site of GABAB type 1 subunit sheds light on the activation process of GABAB receptors. *Journal of Biological Chemistry, 275*(52), 41166–41174.

Galvez, T., Duthey, B., Kniazeff, J., Blahos, J., Rovelli, G., Bettler, B., et al. (2001). Allosteric interactions between GB1 and GB2 subunits are required for optimal GABA(B) receptor function. *EMBO Journal, 20*(9), 2152–2159.

Geng, Y., Xiong, D., Mosyak, L., Malito, D. L., Kniazeff, J., Chen, Y., et al. (2012). Structure and functional interaction of the extracellular domain of human GABA(B) receptor GBR2. *Nature Neuroscience, 15*(7), 970–978.

Geng, Y., Bush, M., Mosyak, L., Wang, F., & Fan, Q. R. (2013). Structural mechanism of ligand activation in human GABA(B) receptor. *Nature, 504*(7479), 254–259.

Havlickova, M., Prezeau, L., Duthey, B., Bettler, B., Pin, J. P., & Blahos, J. (2002). The intracellular loops of the GB2 subunit are crucial for G-protein coupling of the heteromeric gamma-aminobutyrate B receptor. *Molecular Pharmacology, 62*(2), 343–350.

Huang, S., Cao, J., Jiang, M., Labesse, G., Liu, J., Pin, J. P., et al. (2011). Interdomain movements in metabotropic glutamate receptor activation. *Proceedings of the National Academy of Sciences of the United States of America, 108*(37), 15480–15485.

Jones, K. A., Borowsky, B., Tamm, J. A., Craig, D. A., Durkin, M. M., Dai, M., et al. (1998). GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2. *Nature, 396*(6712), 674–679.

Kammerer, R. A., Frank, S., Schulthess, T., Landwehr, R., Lustig, A., & Engel, J. (1999). Heterodimerization of a functional GABAB receptor is mediated by parallel coiled-coil alpha-helices. *Biochemistry, 38*(40), 13263–13269.

Kaupmann, K., Huggel, K., Heid, J., Flor, P. J., Bischoff, S., Mickel, S. J., et al. (1997). Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. *Nature, 386*(6622), 239–246.

Kaupmann, K., Malitschek, B., Schuler, V., Heid, J., Froestl, W., Beck, P., et al. (1998). GABA(B)-receptor subtypes assemble into functional heteromeric complexes. *Nature, 396*(6712), 683–687.

Kniazeff, J., Galvez, T., Labesse, G., & Pin, J. P. (2002). No ligand binding in the GB2 subunit of the GABA(B) receptor is required for activation and allosteric interaction between the subunits. *Journal of Neuroscience, 22*(17), 7352–7361.

Kniazeff, J., Saintot, P. P., Goudet, C., Liu, J., Charnet, A., Guillon, G., et al. (2004). Locking the dimeric GABA(B) G-protein-coupled receptor in its active state. *Journal of Neuroscience, 24*(2), 370–377.

Kniazeff, J., Prezeau, L., Rondard, P., Pin, J. P., & Goudet, C. (2011). Dimers and beyond: The functional puzzles of class C GPCRs. *Pharmacology & Therapeutics, 130*(1), 9–25.

Kunishima, N., Shimada, Y., Tsuji, Y., Sato, T., Yamamoto, M., Kumasaka, T., et al. (2000). Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. *Nature, 407*(6807), 971–977.

Liu, J., Maurel, D., Etzol, S., Brabet, I., Ansanay, H., Pin, J. P., et al. (2004). Molecular determinants involved in the allosteric control of agonist affinity in the GABAB receptor by the GABAB2 subunit. *Journal of Biological Chemistry, 279*(16), 15824–15830.

Malherbe, P., Masciadri, R., Norcross, R. D., Knoflach, F., Kratzeisen, C., Zenner, M. T., et al. (2008). Characterization of (R, S)-5,7-di-tert-butyl-3-hydroxy-3-trifluoromethyl-3H-benzofuran-2-one as a positive allosteric modulator of GABAB receptors. *British Journal of Pharmacology, 154*(4), 797–811.

Margeta-Mitrovic, M., Jan, Y. N., & Jan, L. Y. (2000). A trafficking checkpoint controls GABA(B) receptor heterodimerization. *Neuron, 27*(1), 97–106.

Margeta-Mitrovic, M., Jan, Y. N., & Jan, L. Y. (2001a). Ligand-induced signal transduction within heterodimeric GABA(B) receptor. *Proceedings of the National Academy of Sciences of the United States of America, 98*(25), 14643–14648.

Margeta-Mitrovic, M., Jan, Y. N., & Jan, L. Y. (2001b). Function of GB1 and GB2 subunits in G protein coupling of GABA(B) receptors. *Proceedings of the National Academy of Sciences of the United States of America, 98*(25), 14649–14654.

Mason, J. M., & Arndt, K. M. (2004). Coiled coil domains: Stability, specificity, and biological implications. *Chembiochem, 5*(2), 170–176.

Maurel, D., Comps-Agrar, L., Brock, C., Rives, M. L., Bourrier, E., Ayoub, M. A., et al. (2008). Cell-surface proteinprotein interaction analysis with time-resolved FRET and snap-tag technologies: Application to GPCR oligomerization. *Nature Methods, 5*(6), 561–567.

Monnier, C., Tu, H., Bourrier, E., Vol, C., Lamarque, L., Trinquet, E., et al. (2011). Trans-activation between 7TM domains: Implication in heterodimeric GABAB receptor activation. *EMBO Journal, 30*(1), 32–42.

Muto, T., Tsuchiya, D., Morikawa, K., & Jingami, H. (2007). Structures of the extracellular regions of the group II/III metabotropic glutamate receptors. *Proceedings of the National Academy of Sciences of the United States of America, 104*(10), 3759–3764.

O'Hara, P. J., Sheppard, P. O., Thogersen, H., Venezia, D., Haldeman, B. A., McGrane, V., et al. (1993). The ligand-binding domain in metabotropic glutamate receptors is related to bacterial periplasmic binding proteins. *Neuron, 11*(1), 41–52.

Okamoto, T., Sekiyama, N., Otsu, M., Shimada, Y., Sato, A., Nakanishi, S., et al. (1998). Expression and purification of the extracellular ligand binding region of metabotropic glutamate receptor subtype 1. *Journal of Biological Chemistry, 273*(21), 13089–13096.

Pagano, A., Rovelli, G., Mosbacher, J., Lohmann, T., Duthey, B., Stauffer, D., et al. (2001). C-terminal interaction is essential for surface trafficking but not for heteromeric assembly of GABA(b) receptors. *Journal of Neuroscience, 21*(4), 1189–1202.

Patowary, S., Alvarez-Curto, E., Xu, T. R., Holz, J. D., Oliver, J. A., Milligan, G., et al. (2013). The muscarinic M3 acetylcholine receptor exists as two differently sized complexes at the plasma membrane. *Biochemical Journal, 452*(2), 303–312.

Pisterzi, L. F., Jansma, D. B., Georgiou, J., Woodside, M. J., Chou, J. T., Angers, S., et al. (2010). Oligomeric size of the m2 muscarinic receptor in live cells as determined by quantitative fluorescence resonance energy transfer. *Journal of Biological Chemistry, 285*(22), 16723–16738.

Reid, K. B., & Day, A. J. (1989). Structure-function relationships of the complement components. *Immunology Today, 10*(6), 177–180.

Robbins, M. J., Calver, A. R., Filippov, A. K., Hirst, W. D., Russell, R. B., Wood, M. D., et al. (2001). GABA(B2) is essential for g-protein coupling of the GABA(B) receptor heterodimer. *Journal of Neuroscience, 21*(20), 8043–8052.

Rondard, P., Huang, S., Monnier, C., Tu, H., Blanchard, B., Oueslati, N., et al. (2008). Functioning of the dimeric GABA(B) receptor extracellular domain revealed by glycan wedge scanning. *EMBO Journal, 27*(9), 1321–1332.

Rondard, P., Goudet, C., Kniazeff, J., Pin, J. P., & Prezeau, L. (2011). The complexity of their activation mechanism opens new possibilities for the modulation of mGlu and GABAB class C G protein-coupled receptors. *Neuropharmacology, 60*(1), 82–92.

Schwenk, J., Metz, M., Zolles, G., Turecek, R., Fritzius, T., Bildl, W., et al. (2010). Native GABA(B) receptors are heteromultimers with a family of auxiliary subunits. *Nature, 465*(7295), 231–235.

Sobolevsky, A. I., Rosconi, M. P., & Gouaux, E. (2009). X-ray structure, symmetry and mechanism of an AMPA-subtype glutamate receptor. *Nature, 462*(7274), 745–756.

Steiger, J. L., Bandyopadhyay, S., Farb, D. H., & Russek, S. J. (2004). cAMP response element-binding protein, activating transcription factor-4, and upstream stimulatory factor differentially control hippocampal GABABR1a and GABABR1b subunit gene expression through alternative promoters. *Journal of Neuroscience, 24*(27), 6115–6126.

Tiao, J. Y., Bradaia, A., Biermann, B., Kaupmann, K., Metz, M., Haller, C., et al. (2008). The sushi domains of secreted GABA(B1) isoforms selectively impair GABA(B) heteroreceptor function. *Journal of Biological Chemistry, 283*(45), 31005–31011.

Tsuchiya, D., Kunishima, N., Kamiya, N., Jingami, H., & Morikawa, K. (2002). Structural views of the ligand-binding cores of a metabotropic glutamate receptor complexed with an antagonist and both glutamate and Gd3+. *Proceedings of the National Academy of Sciences of the United States of America, 99*(5), 2660–2665.

Urwyler, S., Mosbacher, J., Lingenhoehl, K., Heid, J., Hofstetter, K., Froestl, W., et al. (2001). Positive allosteric modulation of native and recombinant gamma-aminobutyric acid(B) receptors by 2,6-Di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and its aldehyde analog CGP13501. *Molecular Pharmacology, 60*(5), 963–971.

Urwyler, S., Pozza, M. F., Lingenhoehl, K., Mosbacher, J., Lampert, C., Froestl, W., et al. (2003). N,N′-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) and structurally related compounds: Novel allosteric enhancers of gamma-aminobutyric acid B receptor function. *Journal of Pharmacology and Experimental Therapeutics, 307*(1), 322–330.

Vigot, R., Barbieri, S., Brauner-Osborne, H., Turecek, R., Shigemoto, R., Zhang, Y. P., et al. (2006). Differential compartmentalization and distinct functions of GABAB receptor variants. *Neuron, 50*(4), 589–601.

White, J. H., Wise, A., Main, M. J., Green, A., Fraser, N. J., Disney, G. H., et al. (1998). Heterodimerization is required for the formation of a functional GABA(B) receptor. *Nature, 396*(6712), 679–682.

Wu, H., Wang, C., Gregory, K. J., Han, G. W., Cho, H. P., Xia, Y., et al. (2014). Structure of a class C GPCR metabotropic glutamate receptor 1 bound to an allosteric modulator. *Science, 344*(6179), 58–64.

Xue, L., Rovira, X., Scholler, P., Zhao, H., Liu, J., Pin, J. P., et al. (2015). Major ligand-induced rearrangement of the heptahelical domain interface in a GPCR dimer. *Nature Chemical Biology, 11*(2), 134–140.

Zhang, J., Shen, W., & Slaughter, M. M. (1997). Two metabotropic gamma-aminobutyric acid receptors differentially modulate calcium currents in retinal ganglion cells. *Journal of General Physiology, 110*(1), 45–58.

Chapter 7
Modulation of Neurotransmission by the $GABA_B$ Receptor

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Abstract Most inhibitory signals are mediated via γ-aminobutyric acid (GABA) receptors whereas glutamate receptors mediate most excitatory signals (Trends Neurosci 14:515–519, 1991; Annu Rev Neurosci 17:31–108, 1994). Many factors influence the regulation of excitatory and inhibitory synaptic inputs on a given neuron. One important factor is the subtype of neurotransmitter receptor present not only at the correct location to receive the appropriate signals but also their abundance at synapses (Pharmacol Rev 51: 7–61, 1999; Cold Spring Harb Perspect Biol 3, 2011). $GABA_B$ receptors are G-protein-coupled receptors and different subunits dimerise to form a functional receptor. $GABA_B$ receptor subunits are widely expressed in the brain and by assembling different isoform combinations and accessory proteins they produce variety of physiological and pharmacological profiles in mediating both inhibitory and excitatory neurotransmission. This chapter will describe the understanding of the molecular mechanisms underlying $GABA_B$ receptor regulation of glutamate and $GABA_A$ receptors and how they modulate excitatory and inhibitory neurotransmission.

Keywords $GABA_B$ receptor • Glutamate receptor • GPCR • Neurotransmission • Cross-talk • AKAP • Excitation • Inhibition

7.1 Introduction

The mammalian brain and central nervous system (CNS) has complex circuitry enabling it to perform many complex processes such as cognition, learning, memory formation and motor function. Through many synaptic connections, a given neuron either inhibits or excites other neurons in the circuit. This synaptic connectivity of the circuits is crucial for normal function and disruption of

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circuit leads to many CNS impairments. The balance of excitation and inhibition is fundamental for brain function and any aberrant formation of either inhibitory or excitatory synapses may cause neurodevelopmental disorders such as epilepsy, mental retardation, and autism spectrum disorders (Zoghbi 2003; Fernandez and Garner 2007; Rubenstein and Merzenich 2003; Tabuchi et al. 2007).

Most synapses in CNS are chemical synapses that are defined by the release of specific neurotransmitter. Usually a given neurotransmitter is released into synaptic cleft between the pre- and postsynaptic neuron, and binds to its specific receptors present on postsynaptic membrane. The binding of neurotransmitter to the receptor will generally cause ion flow through them resulting in either depolarisation or hyperpolarisation of the postsynaptic neuron. Generally, glutamatergic synapses are excitatory and depolarise the postsynaptic neuron, while mature GABAergic synapses are inhibitory, hyperpolarising the postsynaptic neuron (Cherubini et al. 1991; Hollmann and Heinemann, 1994). Glutamatergic transmission is excitatory and critical for fast neuronal communication, whereas neuronal firing is inhibited by inhibitory synapses, which are important for modulating synaptic strength, action potential firing patterns, and threshold gating of postsynaptic neurons (Dingledine et al. 1999; Cobb et al. 1999; Davies et al. 1991; Buhl et al. 1994).

7.1.1 $GABA_B$ Receptors

The metabotropic $GABA_B$ receptors are seven transmembrane G-protein-coupled receptors (GPCRs) containing extracellular amino-terminal (N-terminal) ligand binding domain and intracellular carboxy-terminal (C-terminal) tail. They belong to class C GPCR family similar to metabotropic glutamate (mGluRs) and calcium sensing receptors. $GABA_B$ receptors are generally found at presynaptic terminals of glutamatergic and GABAergic synapses and postsynaptically at GABAergic synapses, especially in the hippocampus. Presynaptic $GABA_B$ receptors at glutamatergic synapses function as hetero-receptors and at GABAergic synapses they function as autoreceptors. Release of glutamate and other neurotransmitters is inhibited by activation of $GABA_B$ hetero-receptors (heterosynaptic inhibition of neurotransmitter release). At GABAergic synapses, release of GABA can be inhibited by activation of $GABA_B$ autoreceptors as a feedback mechanism (autoinhibition of GABA release). Postsynaptic $GABA_B$ receptors are also activated by spillover of GABA due to oscillatory or rhythmic activity (Scanziani 2000). During neuronal circuit activity or communication, spillover of neurotransmitters due to repetitive release plays an important function (Ventura and Harris 1999). Some of the excess/spillover neurotransmitters such as GABA or glutamate are taken up by transporters and rest acts on receptors present at extrasynaptic sites and/or adjacent synapses through diffusion from the cleft where it is released (Scanziani 2000, 2002).

7.1.2 $GABA_B$ Receptor-Mediated Effects

GABA receptors are responsible for most inhibitory responses in the brain. $GABA_B$ receptors mediate slow inhibitory neurotransmission in the CNS, present at pre- and postsynaptic compartments, and changes in their localisation, number and activity can modulate synaptic transmission. Presynaptic $GABA_B$ receptors inhibit release of neurotransmitters by inhibiting voltage-gated Ca^{2+} channels, indirectly limiting calcium influx and vesicular release (Thompson and Gahwiler 1992; Takahashi et al. 1998; Chalifoux and Carter 2010). Activation of postsynaptic $GABA_B$ receptors activates inwardly rectifying K^+ channels (GIRK) and leads to hyperpolarisation of the postsynaptic cell to generate slow inhibitory postsynaptic potentials (IPSPs) that may be necessary for rhythmic activity (Dutar and Nicoll 1988; Nicoll et al. 1990; Scanziani 2000; Luscher et al. 1997; Gahwiler and Brown 1985). Heteromeric $GABA_B$ receptors consisting of $GABA_{B1}$ and $GABA_{B2}$ subunits produce much longer lasting synaptic inhibition compared to $GABA_A$ ion channels (Marshall et al. 1999; Watanabe et al. 2002; Schwenk et al. 2010). The $GABA_{B1}$ subunit binds to ligand and $GABA_{B2}$ regulates adenylate cyclase, GIRK channels and Ca^{2+} channels via interaction with G-proteins (Xu and Wojcik 1986; Gahwiler and Brown 1985; Malitschek et al. 1999; Robbins et al. 2001).

7.1.3 $GABA_B$ Receptor's Functional Diversity and Auxiliary Subunits

$GABA_B$ receptors play an important role in brain function and signal through G_o and G_i proteins, which lead to multiple downstream consequences such as a decrease in protein kinase A (PKA) activity via inhibition of adenylyl cyclase. Functional $GABA_B$ receptors are made up of two subunits $GABA_{B1}$ and $GABA_{B2}$ (see Chap. 4 of this book) compared to 19 different subunits identified for $GABA_A$ receptors (Baumann et al. 2001). However, the functional diversity observed with native $GABA_B$ receptors could not be reproduced with heterodimers of cloned $GABA_{B1/B2}$ receptors (Deisz et al. 1997; Cruz et al. 2004). Bettler and Fakler groups first showed that $GABA_B$ receptors form functional complexes with a subfamily of the potassium channel tetramerisation domain-containing (KCTD) proteins using a functional proteomics approach (Schwenk et al. 2010). Specifically KCTD-8, -12, -12b and -16 proteins were shown to bind the $GABA_{B2}$ receptor subunit carboxy terminus as tetramers (Schwenk et al. 2010). Similarly, Kornau group demonstrated that KCTD12 and glucose-regulated protein 78 (GRP78) are present in a complex with $GABA_B$ receptors (Bartoi et al. 2010). It has been shown that KCTD12 protein T1 domain binds to the C-terminal region of $GABA_{B2}$ and affects the axonal transport and surface stability of $GABA_B$ receptors (Bartoi et al. 2010). Different KCTD proteins display distinct expression profiles in the brain regions (Metz et al. 2011) and it has been shown that the co-assembly of $GABA_B$ receptors with KCTD proteins dramatically alters G-protein signalling by accelerating onset and promoting desensitisation as well as increasing the agonist potency in a KCTD-subtype specific manner (Schwenk et al. 2010).

7.1.4 Atypical Regulation of $GABA_B$ Receptors Surface Expression

$GABA_B$ receptors are regulated via distinct mechanisms from those utilised by many metabotropic receptors such as the β2-adrenergic receptor (Bettler and Tiao 2006). Regulation of $GABA_B$ receptors is covered in detail in Chaps. 4 and 6 of this book but, briefly, agonist exposure results in phosphorylation and endocytosis of most GPCRs from the cell surface and then either down-regulation via degradation or recycling back to the cell surface. In contrast, $GABA_B$ receptor cell surface levels are not significantly changed upon receptor activation in neurons (Bettler and Tiao 2006; Fairfax et al. 2004). There is little internalisation of $GABA_B$ receptors even after prolonged agonist exposure in cultured neurons and they are very stable at the cell membrane. The lack of agonist-induced phosphorylation and β-arrestin recruitment correlates with absence of receptor endocytosis (Couve et al. 2004; Fairfax et al. 2004). Surprisingly, at basal state surface $GABA_B$ receptors are stabilised by increased phosphorylation at serine 892 in $GABA_{B2}$ resulting in less degradation in neurons (Couve et al. 2004; Fairfax et al. 2004). It has been demonstrated by several groups that $GABA_B$ receptors undergo constitutive recycling to maintain specific number of receptors at surface and depending on the stimulation (either agonist or glutamate), there is shift from recycling to degradation in lysosomes (Grampp et al. 2007; Maier et al. 2010; Kantamneni et al. 2014; Zhang et al. 2015).

7.1.5 $GABA_B$ Receptor Regulatory Sites

The main regulatory sites on $GABA_B$ receptors are their intracellular C-terminal tails and extracellular sushi domains. Sushi domains are also known as short consensus repeats that are conserved protein interaction motifs present in other GPCRs and proteins of the complement system (Perrin et al. 2006; Kirkitadze and Barlow 2001). Sushi domains are important for protein interactions, especially membrane targeting (Tiao et al. 2008; Biermann et al. 2010). Depending on the stimulation received by the receptor, the C-terminal domains bind to various proteins including enzymes, scaffold proteins, signalling, and trafficking proteins (De La Rue and Henley 2002; Sheng and Kim 2011). In the majority of cases, intracellular C-terminal domains mediate complex formation and one can imagine them as regulatory sites during receptor activity. This can translate into cross-talk between receptors through protein–protein interactions and modification thus affecting or modulating overall synaptic transmission. For example, multiple studies have demonstrated that $GABA_B$ receptors are enriched at glutamatergic synapses as well as GABAergic synapses (Fritschy et al. 1999; Lujan and Shigemoto 2006), suggesting that $GABA_B$ receptors can modulate glutamatergic transmission. In fact both ionotropic and metabotropic glutamate receptors are regulated directly and sometimes indirectly by $GABA_B$ receptors (Morrisett et al. 1991; Hirono et al. 2001; Sun et al. 2006; Chalifoux and

Carter 2010; Terunuma et al. 2014). $GABA_B$ receptors also regulate release of GABA transmitter, GABA ion channels trafficking and function (Mott and Lewis 1991; Hahner et al. 1991; Otis and Mody 1992; Patenaude et al. 2003; Gerrow and Triller 2014). The sections below in this chapter will follow this theme of regulating other receptors by $GABA_B$ receptors and how this translates to modulation or changes in synaptic transmission.

7.1.6 Importance of Specific $GABA_B$ Subunit Isoforms

In the past 15 years, there is increasing evidence about the diverse roles of individual $GABA_B$ subunits such as $GABA_{B1a}$, $GABA_{B1b}$ and $GABA_{B2}$ helped by mouse models in dissecting the roles of these specific subunits. There is considerable diversity in expression patterns between pre- and postsynaptic $GABA_B$ receptors (Ulrich and Bettler 2007; Pinard et al. 2010). Through heterodimerisation functional $GABA_B$ receptors are assembled into two main $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ receptor forms consisting of molecular subunit isoforms of $GABA_{B1}$ (either 1a or 1b) and the $GABA_{B2}$ subunit. Expression of $GABA_{B1a}$ and $GABA_{B1b}$ subunits is independently modulated in different neurons as well as between different synaptic compartments (Bischoff et al. 1999; Fritschy et al. 1999; Vigot et al. 2006; Margeta-Mitrovic et al. 1999). For example, due to N-terminal 'sushi' domains on the $GABA_{B1a}$ subunit, presynaptic compartments are enriched with $GABA_{B(1a,2)}$ receptors at glutamatergic synapses. Conversely, $GABA_{B(1b,2)}$ receptors are primarily localised at dendrites due to absence of the 'sushi' domains in $GABA_{B1b}$ subunit (Vigot et al. 2006; Tiao et al. 2008; Biermann et al. 2010). In cortical neurons, GABA release is inhibited through activation of presynaptic $GABA_{B(1a,2)}$ autoreceptors (Perez-Garci et al. 2006), whereas in the cornu ammonis 1 (CA1) region of the hippocampus and the thalamus $GABA_{B(1b,2)}$ receptors can inhibit GABA release (Vigot et al. 2006; Ulrich and Bettler 2007).

In dentate gyrus, both $GABA_{B1a}$ and $GABA_{B1b}$ subunits are expressed in granule cells and hilar neurons; however, they show differential localisation (Bischoff et al. 1999; Foster et al. 2013). Functional diversity of the subunits was further dissected using genetic mouse models ($GABA_{B1a}^{-/-}$ and $GABA_{B1b}^{-/-}$) to investigate the individual roles of 1a and 1b subunit-containing $GABA_B$ receptor heterodimers. The granule cell output was measured as population spike (analysed to see if spikes are either inhibited or enhanced) during $GABA_A$ blockade after application of the $GABA_B$ receptor agonist, baclofen. $GABA_{B1a}^{-/-}$ mice revealed differential effects (both inhibition and enhancement), suggesting inhibitory and disinhibitory effects of $GABA_{B(1b,2)}$ receptors. However, in $GABA_{B1b}^{-/-}$ mice population spikes were enhanced by baclofen, suggesting disinhibitory roles of $GABA_{B(1a,2)}$ receptors (Foster et al. 2013). In summary, data from knockout mice studies in dentate gyrus suggest that $GABA_{B(1a,2)}$ receptors gate the signal by mediating disinhibition, therefore playing a crucial role in spatial and pattern learning in hippocampus (Foster et al. 2013). It has also been shown that synaptic plasticity in hippocampus as well as performance of novel objection recognition tasks is impaired in $GABA_{B1a}^{-/-}$ mice, implicating a critical role for $GABA_{B(1a,2)}$ receptors in this process (Vigot et al. 2006; Jacobson et al. 2007).

7.2 $GABA_B$ Receptor Modulation of Glutamate-Mediated Excitatory Neurotransmission

Glutamate is the major excitatory neurotransmitter in the brain and mediates most excitatory neurotransmission, which is crucial for brain development and function. There are two major groups of glutamate receptors, ionotropic and metabotropic, based on the mechanism by which they are activated and downstream properties of the postsynaptic signal. Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels assembled as tetramers and can be divided into three subgroups based on their glutamate binding, structural homology, function, and pharmacology: *N*-methyl D-aspartate receptors (NMDARs) (GluN1, GluN2A–GluN2D, GluN3A, and GluN3B), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (GluA1–GluA4), and kainate receptors (GluK1–GluK5) (Lodge 2009; Traynelis et al. 2010). There are eight receptor subtypes for mGluRs, which are grouped into three families according to their sequence homology, G-protein coupling, and pharmacology. The three groups of mGluRs, group I (mGluR1 and mGluR5), group II (mGluR2–mGluR4), and group III (mGluR7 and mGluR8), belong to class C GPCRs similar to $GABA_B$ receptors (Conn and Pin 1997).

Multiple physiological processes are regulated by glutamate receptors, due to the presence of different subtypes of receptors. Excess glutamate release and subsequent receptor activation have been linked to numerous brain disorders such as epilepsy and neurodegenerative diseases. Hence there is tight control and a critical balance between excitation and inhibition during normal physiological processes. Activation of $GABA_B$ receptors causes inhibition of neurotransmitter release presynaptically, including heterosynaptic inhibition of glutamate release and auto-inhibition of GABA release (Thompson and Gahwiler 1992; Wu and Saggau 1995). $GABA_B$ receptor activation regulates multiple aspects of excitatory neurotransmission in many areas of brain. At excitatory synapses, activation of $GABA_B$ receptors modulates plasticity via post- and presynaptic mechanisms. Presynaptic $GABA_B$ receptors regulate the probability of neurotransmitter release and modulate the synaptic signal mediated via glutamate (Davies and Collingridge 1996; Davies et al. 1991).

7.2.1 $GABA_B$ Receptor Regulation of Glutamate Ion Channels

NMDARs mediate major synaptic Ca^{2+} signals in the brain, which are critical for neurotransmission and activity-dependent plasticity (Bliss and Collingridge 1993; Mainen et al. 1999; Malenka and Bear 2004). At basal state, Mg^{2+} is bound to the NMDA receptor channel pore blocking the entry of Ca^{2+}. When the neuron is sufficiently depolarised, Mg^{2+} ions are unbound allowing the entry of Ca^{2+} ions into the cell. $GABA_B$ receptors inhibit these Ca^{2+} signals via modulation of K^+ channels, resulting in a hyperpolarisation that reduces the Ca^{2+} influx and overall current by enhancing Mg^{2+} blockade of NMDARs (Morrisett et al. 1991; Otmakhova and

Lisman 2004; Deng et al. 2009). Recently, it has been demonstrated that $GABA_B$ receptor activation directly inhibits Ca^{2+} influx through NMDARs (Chalifoux and Carter 2010). This effect on NMDARs is independent of voltage-sensitive Ca^{2+} channel and K^+ channel activation, Gβγ subunits, and internal Ca^{2+} stores. $GABA_B$ receptors, through activation of Gαi/Gαo G-proteins, inhibit adenylate cyclase to reduce PKA activity by decreasing cyclic 3′,5′-adenosine-monophosphate (cAMP) levels. PKA activity normally increases the Ca^{2+} influx through NMDA receptors and reduction of PKA activity by $GABA_B$ receptors inhibits Ca^{2+} signals (Chalifoux and Carter 2010). In the same experiments, synaptic currents mediated by AMPA or NMDA receptors are not affected by $GABA_B$ receptor-mediated modulation via PKA pathway at postsynaptic sites (Chalifoux and Carter 2010).

In a knock-in mouse model in which wild-type $GABA_{B2}$ receptor was replaced with a S783A-mutated version that cannot be phosphorylated, there was an increase in surface expression of AMPA receptors (Terunuma et al. 2014). AMP-dependent protein kinase (AMPK) phosphorylates S783 on the $GABA_{B2}$ subunit, which in turn enhances coupling of receptor to GIRKs (Kuramoto et al. 2007). Transient activation of NMDARs results in increased phosphorylation of $GABA_{B2}$ receptor, whereas prolonged activation results in dephosphorylation of $GABA_B$ receptors by protein phosphatase 2A (PP2A). As result of constitutive phosphorylation of $GABA_{B2}$ receptor, $GABA_B$ receptors are very stable at the cell surface and dephosphorylation of this subunit selectively targets the receptors for lysosomal degradation (Fairfax et al. 2004; Terunuma et al. 2010a). The expression of $GABA_B$ receptor was increased with the S783A-mutated version due to decreased degradation, leading to a reduced level of Arc/Arg3.1 protein, which is necessary for memory consolidation. Consequently, there was an increase in AMPA receptor number at cell surface, PSD95 protein expression, and number of excitatory synapses. These results from knock-in mouse model suggest that $GABA_B$ receptors play a crucial role by regulating excitatory synaptic transmission and modulate neuronal architecture (Terunuma et al. 2014).

7.2.2 *$GABA_B$ Receptor Regulation of Metabotropic Glutamate Receptors*

$GABA_B$ receptors and mGluRs show similar expression profiles (although specific subtypes show restricted expression in certain brain regions) in many brain areas and sometimes closely localised in synaptic sites and modulate transmitter release (Princivalle et al. 2000; Raiteri 2008; Ladera et al. 2007). Specifically, mGluRs are highly expressed in thalamus and $GABA_B$ receptors in thalamus play a crucial role in synchronised oscillations, regulation of Electroencephalogram (EEG) slow waves and also act as a neuromodulator for burst firing excitation (Crunelli and Leresche 1991; von Krosigk et al. 1993; Juhasz et al. 1994). Sensory inputs are modulated in the thalamus, a relay between cerebellar and basal ganglia inputs to the cerebral cortex. Most thalamic nuclei project primarily to the cerebral cortex as well as receiving a reciprocal connection from the cerebral cortex. A population of

inhibitory neurons are present in the reticular thalamic nucleus and project to all thalamic relay subnuclei, where they can modulate the signal transmission through the thalamus. In summary, the major role of thalamus is to gate control and regulate the information received by cortex. To provide a brief overview about mGluRs: Group I mGluRs generate excitatory responses and are predominantly expressed at the periphery of the postsynaptic density and couple to Gq proteins (Ferraguti et al. 2008). Group II and III mGluRs negatively regulate neurotransmitter release, preferentially localised at presynaptic terminals and couple to Gi/o proteins similar to $GABA_B$ receptors (Ferraguti and Shigemoto 2006). $GABA_B$ receptors can modulate the function of mGluRs in thalamus by indirect interaction or cross-talk, similar to examples discussed below. In fact, previously it has been shown that $GABA_B$ receptors and mGluR1 exist in same complex in mouse cerebellum identified by co-immunoprecipitation experiments using specific antibodies (Rives et al. 2009).

$GABA_B$ receptors are concentrated at cerebellar parallel fibre Purkinje cell synapses and have many functions that are both dependent and independent of GABA. mGluR1 and $GABA_B$ receptors are expressed in large amounts in cerebellar Purkinje cells, and show similar subcellular localisations throughout development (Ige et al. 2000; Lujan and Shigemoto 2006; Rives et al. 2009). Activation of the metabotropic glutamate receptor mGluR1 is required for expression of long-term depression (LTD) at cerebellar parallel fibre Purkinje cell synapses which is a form of synaptic plasticity necessary for cerebellar motor learning (Ichise et al. 2000; Ito 2001). Both mGluR1 and $GABA_B$ receptors are co-expressed at extra/peri postsynaptic sites, in parallel fibre Purkinje cells (Lujan et al. 1997; Fritschy et al. 1999; Mateos et al. 2000). Co-activation of $GABA_B$ receptors using baclofen in Purkinje cells enhanced mGluR1 agonist-induced currents and intracellular Ca^{2+} levels. Moreover, this effect was reproduced with endogenous GABA released in cerebral cortex by electrical stimulation (Hirono et al. 2001). It has also been shown that, at Purkinje cell synapses, activation of $GABA_B$ receptors inhibits neurotransmitter release by inhibiting calcium channels as well as affecting release processes (Dittman and Regehr 1996, 1997; Vigot and Batini 1997).

In cerebellar Purkinje cells, extracellular Ca^{2+} interacts with $GABA_B$ receptor producing an increase in glutamate sensitivity of mGluR1. The mediation of glutamate sensitivity of mGluR1 is specific to $GABA_B$ receptors due to its absence in cells derived from $GABA_{B1}^{-/-}$ animals. Furthermore, it has been shown that Gi/o proteins do not translate the extracellular Ca^{2+}-mediated interaction with $GABA_B$ receptors to mGluR1 glutamate sensitivity (Tabata et al. 2004). Activation of $GABA_B$ receptor modulates the mGluR1-mediated induction of LTD and signalling in cerebellar cells. Activation of postsynaptic $GABA_B$ receptor enhanced the mGluR1-mediated LTD of glutamate-evoked current increasing the magnitude of depression at parallel fibre Purkinje cell synapses (Kamikubo et al. 2007). This cross-talk between the receptors is proposed to occur via Gi/o proteins, suggesting that activation of the G-proteins via $GABA_{B2}$ subunit is necessary for this effect (Kamikubo et al. 2007). Similarly, βγ subunits of the Gi/o protein through activation of $GABA_B$ receptor have been shown to enhance mGluR1-mediated calcium

response (Ca^{2+} release) in both human embryonic kidney (HEK) cells and cortical neurons (Kamikubo et al. 2007; Rives et al. 2009). In summary, $GABA_B$ receptors regulate mGluR signalling in specific regions/cell types in the brain and modulate excitatory neurotransmission.

7.3 $GABA_B$ Receptor-Mediated Modulation of Inhibitory Transmission

In the brain, GABA is the most abundant inhibitory neurotransmitter and is involved in many physiological processes by exerting inhibitory control on neuronal networks through $GABA_A$, $GABA_B$, and $GABA_C$ receptors. Pierau and Zimmermann first described inhibition of transmitter release by baclofen in the CNS in 1973 (Pierau and Zimmermann 1973). Baclofen is a specific agonist for $GABA_B$ receptor and has been clinically used primarily for treatment of spasticity (see Chap. 17 of this book). In neuronal networks, $GABA_B$ receptors at excitatory and inhibitory presynaptic synapses induce inhibitory and disinhibitory effects. $GABA_B$ receptor activation is predominantly inhibitory in the hippocampal CA1 and CA3 synaptic circuits due to inhibition of glutamate release via presynaptic receptors (Isaacson et al. 1993; Chen et al. 2006). On the other hand, disinhibition (stop/block inhibition) produced by $GABA_B$ receptors on granule cells in the dentate gyrus is mainly due to reduced GABA release from hilar interneurons (Mott and Lewis 1991; Mott et al. 1993; Burgard and Sarvey 1991). This is very efficient feedback mechanism that can suppress GABAergic inhibition in an activity-dependent manner. Presynaptic $GABA_B$ autoreceptors at GABAergic terminals control the release of GABA. To elucidate the roles of $GABA_B$ autoreceptor-mediated suppression of GABA release, Kobayashi et al. performed multiple whole-cell, patch-clamp recordings from layer V rat insular cortex. Using the $GABA_B$ receptor antagonist, CGP 52432, their data suggested that, in the cerebral cortex, release of GABA is inhibited through activation of $GABA_B$ autoreceptors by a single presynaptic action potential (Kobayashi et al. 2012).

Presynaptic $GABA_B$ receptors inhibit neurotransmitter release at both the inhibitory and excitatory terminals where they are present. Earlier studies show that stimulus-evoked GABA release at inhibitory synapses in dentate gyrus was primarily due to $GABA_B$ receptor-mediated presynaptic inhibition (Otis and Mody 1992). Recently, Gerrow and Triller proposed a novel mechanism for regulating inhibitory transmission and showed that $GABA_B$ receptor activity affects $GABA_A$ receptor enrichment at inhibitory synapses. They showed an association of $GABA_A$ receptor enrichment at synapses via protein kinase C (PKC) signalling with a change in their rate of lateral diffusion (Gerrow and Triller 2014). This effect was dependent on α-subunit composition, where α2- and α5-$GABA_A$ receptors produced opposite changes in diffusion and enrichment at synapses, whereas α1-$GABA_A$ receptors were unaffected. Further experimentation revealed that modulation of α2-$GABA_A$ receptor mobility regulates α5-$GABA_A$ receptor trapping at synapses. $GABA_B$

receptor activity mediates these changes in diffusion via PKC signalling pathway and the intracellular transmembrane TM3-4 loop of α2, whereas the loop of α5 was not involved (Gerrow and Triller 2014). Earlier studies have also suggested that $GABA_B$ receptors can modulate $GABA_A$ receptors function through activation of G-protein, phospholipase C and phosphorylation of a subtype of $GABA_A$ receptor via PKC (Hahner et al. 1991).

At excitatory synapses, activation of $GABA_B$ receptors modulates plasticity via post- and presynaptic mechanisms (Davies and Collingridge 1996; Davies et al. 1991; Chalifoux and Carter 2010). In adult hippocampus, GABAergic synapses contribute to functional synaptic plasticity (long-term potentiation, LTP) via mechanisms dependant on class C GPCRs, intracellular Ca^{2+} levels, and activation of G-proteins in pyramidal cells (Patenaude et al. 2003). Specifically, $GABA_B$ receptor-dependent (as well as group 1 and 2 mGluRs) postsynaptic mechanisms lead to a sustained increase of $GABA_A$ synaptic transmission. This phenomenon was observed when a stimulation paradigm mimicking the endogenous theta rhythm is used (Patenaude et al. 2003). Recently, it has been shown that $GABA_B$ receptors in thalamocortical neurons regulate the magnitude of tonic GABA current via postsynaptic mechanism (Connelly et al. 2013). Specifically, activation of postsynaptic $GABA_B$ receptor regulates extrasynaptic $GABA_A$ receptors via Gi/o proteins, adenylate cyclase, and PKA pathway resulting changes in excitability of thalamocortical neurons (Connelly et al. 2013). The above studies suggest that $GABA_B$ receptors can affect inhibitory synaptic transmission by modulating $GABA_A$ receptor activity.

In cortical networks, GABAergic interneurons fulfil multiple functions, and synapses formed by interneurons are heterogeneous (Miles et al. 1996; Halasy et al. 1996; Xiang et al. 1998). In the specific contexts of network activity, this feature will allow different populations of synapses with distinct postsynaptic effects to be switched on or off. The firing properties of inhibitory cells, dynamics of GABA release, and location of inhibitory synapses determine the net effect of synaptic inhibition. There is evidence that these synapses can be modulated differentially by $GABA_B$ autoreceptors due to different presynaptic Ca^{2+} channels, which are expressed at distinct subgroups of hippocampal inhibitory synapses (Lambert and Wilson 1993; Poncer et al. 1997). Both GABA and glutamate presynaptically modulate these hippocampal inhibitory synapses (Thompson and Gahwiler 1989; Poncer et al. 1995). Poncer et al. examined if there are any differential effects of these two neurotransmitters by using rat hippocampal slice cultures and recorded pairs of visually identified inhibitory and pyramidal cells in the CA3 region (Poncer et al. 2000). They used DCG-IV (group II mGluR agonist) and baclofen, and examined IPSPs. After a series of experiments, they concluded that presynaptic $GABA_B$ receptors are present and functional at both inhibitory synapses, stratum oriens and stratum radiatum, whereas group II mGluRs are present in subpopulation of synapses formed by some interneurons located in the stratum radiatum (Poncer et al. 2000).

Gate control is widely studied mechanism in pain pathways, where non-painful stimulus/input closes the gate to painful input, preventing pain sensation travelling from periphery to CNS. The ability of neural circuits to gate inputs by either facilitating or suppressing specific synaptic activity is called synaptic gating or gate control.

The inhibitory interneurons can gate control, which can diffuse excitatory signal by shunting inhibition that can change the membrane conductance of excitatory axon (O'Donnell and Grace 1995). $GABA_B$ receptors play a pivotal role in disinhibition for the gate control of inputs from the entorhinal cortex to the hippocampus, which are involved in spatial and pattern learning (Gilbert and Franklin 2001; Leutgeb et al. 2007; Moser et al. 2008). The dendrites of granule cells form excitatory synapses from the perforant path, and spikes propagate into the hippocampus that are generated in granule cells. However, a population of local interneurons in the molecular layer and hilus strongly inhibit granule cells (Freund and Buzsaki 1996; Amaral et al. 2007). Both $GABA_A$ and $GABA_B$ receptors on granule cells are activated by release of GABA from these interneurons to induce fast and slow inhibitory currents, respectively (Otis et al. 1993; Otis and Mody 1992). Particularly, interneurons from hilar in the border area are important for the disinhibition role. These hilar interneurons project to proximal dendrites and cell body of granule cells and shunt action potential generation by producing synaptic inhibition (Freund and Buzsaki 1996; Amaral et al. 2007; Cossart et al. 2005). Activation of somatodendritic $GABA_B$ receptors, which are highly expressed in hilar interneurons, decreases firing of hilar border neurons (Bischoff et al. 1999; Kulik et al. 2003; Mott et al. 1999). This decrease in firing results in reduction of GABA release on granule cells and enhances spike discharge (Mott and Lewis 1991; Mott et al. 1993; Burgard and Sarvey 1991). However, GABA release from hilar axonal terminals onto granule cells may also be inhibited by activation of presynaptic $GABA_B$ receptors, which decrease hilar inhibition by reducing excitatory inputs to the hilus (Foster et al. 2013). In summary, $GABA_B$ receptors play a crucial role in modulating inhibitory transmission.

7.4 Mediators of $GABA_B$/$GABA_A$/Glutamate Receptors Cross-Talk

Anchoring proteins, enzymes, and scaffold proteins regulate glutamatergic and GABAergic receptor complexes and can mediate receptor cross-talk. Components of receptor signalling complex are generally localised together via scaffold proteins, which co-assemble regulatory proteins (such as protein kinases and phosphatases) with receptors. Scaffold proteins such as A kinase anchoring proteins (AKAPs) are classic examples of this class and previously shown to localise PKA, PKC, protein phosphatases (PPs), and the calmodulin-activated protein phosphatase calcineurin (CaN) at specific synaptic sites to regulate excitatory synaptic strength (Gomez et al. 2002; Wong and Scott 2004; Smith et al. 2006; Robertson et al. 2009; Jurado et al. 2010). For example, AKAP79/150 through interaction with PSD-95 is linked to NMDARs and other ion channels to regulate activity-dependent signalling processes (Colledge et al. 2000; Tunquist et al. 2008; Sanderson and Dell'acqua 2011). Proteins such as PKA and PP2B that bind to AKAP79/150 also regulate $GABA_B$ receptors (through phosphorylation and dephosphorylation) and it is possible that the scaffolding function of AKAP79/150 may be required for glutamate/$GABA_B$

receptor cross-talk. This may also be true in case of $GABA_A$ receptor enrichment at synapses via PKC signalling, modulated through activation of $GABA_B$ receptors (Gerrow and Triller 2014).

GPCR interacting scaffold protein (GISP) was previously shown to interact with the $GABA_{B1}$ subunit and increases cell surface expression of heteromeric $GABA_{B1/B2}$ complexes (Kantamneni et al. 2007). GISP is an AKAP9 splice variant at the C-terminal but lacks PKA RII regulatory binding domains that are PKA interaction sites (Kantamneni et al. 2007). Yotiao is another AKAP protein derived from alternative splicing of AKAP9 within the N-terminal region that has crucial function in regulation of NMDARs (Lin et al. 1998; Westphal et al. 1999). Yotiao forms a kinase-phosphatase signalling complex, which functions as dual switch with the GluN1A receptor by interacting with both PKA and PP1 simultaneously. In this signalling complex, activation of PP1 exerts an inhibitory effect on NMDAR activity and on the other hand activation of bound PKA enhances NMDAR currents (Westphal et al. 1999; Colledge et al. 2000). Therefore it is possible that AKAP9, which is expressed at the synapses, could interact simultaneously with specific population of $GABA_B$ receptors and NMDARs (Collado-Hilly and Coquil 2009). As well as the receptors, AKAP9 could also bind protein kinases and phosphatases and can function to mediate the observed cross-talk between $GABA_B$ receptors and NMDARs as mentioned above.

An example of a protein that may potentially mediate direct cross-talk between $GABA_B$ receptor signalling and glutamate receptor signalling is Ca^{2+} calmodulin-dependent protein kinase (CaMKII). CaMKII has been shown to interact with both $GABA_B$ and NMDA receptors (Bayer et al. 2001; Guetg et al. 2010). Previously it has been shown that activation of NMDARs reduces the number of surface $GABA_B$ receptors via degradation of $GABA_{B1}$ and $GABA_{B2}$ subunits (Guetg et al. 2010; Terunuma et al. 2010b; Kantamneni et al. 2014). This phenomenon is mediated via phosphorylation of $GABA_{B1}$ subunit (Ser867) by CaMKII, which is triggered by NMDAR activation (Guetg et al. 2010). Thus CaMKII mediates down-regulation of $GABA_B$ receptors as well as regulating NMDAR-mediated plasticity (Guetg et al. 2010; El Gaamouch et al. 2012). Unlike the earlier examples of indirect receptor modulation, CaMKII may potentially function as direct link between $GABA_B$ and glutamate receptors.

7.5 Conclusions

$GABA_B$ receptors are metabotropic class C GPCRs that mediate long-lasting slow inhibitory neurotransmission in the CNS. They regulate both excitatory and inhibitory neurotransmission through modulation of $GABA_A$ and glutamate receptors (both ionotropic and metabotropic). $GABA_B$ receptors also modulate overall transmission and circuitry in the brain by regulating the release of neurotransmitters GABA and glutamate as well as others such as noradrenaline, dopamine (see Chap. 8 of this book), serotonin, and acetylcholine (Bowery et al. 1980; Bowery and

Hudson 1979; Gray and Green 1987; Reimann et al. 1982; Schlicker et al. 1984; Potashner 1979). Specific $GABA_B$ subunits such as $GABA_{B1a}$, $GABA_{B1b}$, and $GABA_{B2}$ show differences in expression between pre- and postsynaptic sites as well as different brain regions to produce diverse physiological functions. There are key regulatory proteins (such as CaMKII, PKA, PKC, and PP) that mediate the cross-talk between $GABA_B$ receptors and other receptors. Future efforts should be directed towards elucidating the physiological roles of regulatory proteins that mediate the cross-talk between $GABA_B$ and other receptors to maintain the balance of excitation and inhibition. This information would be useful to develop future therapeutics to treat neurological diseases such as neurodevelopmental disorders, epilepsy, neurodegenerative, and psychiatric disorders.

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References

Amaral, D. G., Scharfman, H. E., & Lavenex, P. (2007). The dentate gyrus: Fundamental neuroanatomical organization (dentate gyrus for dummies). *Progress in Brain Research, 163*, 3–22.

Bartoi, T., Rigbolt, K. T., Du, D., Kohr, G., Blagoev, B., & Kornau, H. C. (2010). GABAB receptor constituents revealed by tandem affinity purification from transgenic mice. *Journal of Biological Chemistry, 285*, 20625–20633.

Baumann, S. W., Baur, R., & Sigel, E. (2001). Subunit arrangement of gamma-aminobutyric acid type A receptors. *Journal of Biological Chemistry, 276*, 36275–36280.

Bayer, K. U., De Koninck, P., Leonard, A. S., Hell, J. W., & Schulman, H. (2001). Interaction with the NMDA receptor locks CaMKII in an active conformation. *Nature, 411*, 801–805.

Bettler, B., & Tiao, J. Y. (2006). Molecular diversity, trafficking and subcellular localization of GABAB receptors. *Pharmacology & Therapeutics, 110*, 533–543.

Biermann, B., Ivankova-Susankova, K., Bradaia, A., Abdel Aziz, S., Besseyrias, V., Kapfhammer, J. P., et al. (2010). The Sushi domains of GABAB receptors function as axonal targeting signals. *Journal of Neuroscience, 30*, 1385–1394.

Bischoff, S., Leonhard, S., Reymann, N., Schuler, V., Shigemoto, R., Kaupmann, K., et al. (1999). Spatial distribution of GABA(B)R1 receptor mRNA and binding sites in the rat brain. *Journal of Comparative Neurology, 412*, 1–16.

Bliss, T. V., & Collingridge, G. L. (1993). A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature, 361*, 31–39.

Bowery, N. G., Hill, D. R., Hudson, A. L., Doble, A., Middlemiss, D. N., Shaw, J., et al. (1980). (-) Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature, 283*, 92–94.

Bowery, N. G., & Hudson, A. L. (1979). gamma-Aminobutyric acid reduces the evoked release of [3H]-noradrenaline from sympathetic nerve terminals [proceedings]. *British Journal of Pharmacology, 66*, 108p.

Buhl, E. H., Halasy, K., & Somogyi, P. (1994). Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic release sites. *Nature, 368*, 823–828.

Burgard, E. C., & Sarvey, J. M. (1991). Long-lasting potentiation and epileptiform activity produced by GABAB receptor activation in the dentate gyrus of rat hippocampal slice. *Journal of Neuroscience, 11*, 1198–1209.

Chalifoux, J. R., & Carter, A. G. (2010). GABAB receptors modulate NMDA receptor calcium signals in dendritic spines. *Neuron, 66*, 101–113.

Chen, Y., Menendez-Roche, N., & Sher, E. (2006). Differential modulation by the GABAB receptor allosteric potentiator 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethylpropyl)-phenol (CGP7930) of synaptic transmission in the rat hippocampal CA1 area. *Journal of Pharmacology and Experimental Therapeutics, 317*, 1170–1177.

Cherubini, E., Gaiarsa, J. L., & Ben-Ari, Y. (1991). GABA: An excitatory transmitter in early postnatal life. *Trends in Neurosciences, 14*, 515–519.

Cobb, S. R., Manuel, N. A., Morton, R. A., Gill, C. H., Collingridge, G. L., & Davies, C. H. (1999). Regulation of depolarizing GABA(A) receptor-mediated synaptic potentials by synaptic activation of GABA(B) autoreceptors in the rat hippocampus. *Neuropharmacology, 38*, 1723–1732.

Collado-Hilly, M., & Coquil, J. F. (2009). Ins(1,4,5)P3 receptor type 1 associates with AKAP9 (AKAP450 variant) and protein kinase A type IIbeta in the Golgi apparatus in cerebellar granule cells. *Biology of the Cell, 101*, 469–480.

Colledge, M., Dean, R. A., Scott, G. K., Langeberg, L. K., Huganir, R. L., & Scott, J. D. (2000). Targeting of PKA to glutamate receptors through a MAGUK-AKAP complex. *Neuron, 27*, 107–119.

Conn, P. J., & Pin, J. P. (1997). Pharmacology and functions of metabotropic glutamate receptors. *Annual Review of Pharmacology and Toxicology, 37*, 205–237.

Connelly, W. M., Fyson, S. J., Errington, A. C., McCafferty, C. P., Cope, D. W., Di Giovanni, G., et al. (2013). GABAB receptors regulate extrasynaptic GABAA receptors. *Journal of Neuroscience, 33*, 3780–3785.

Cossart, R., Bernard, C., & Ben-Ari, Y. (2005). Multiple facets of GABAergic neurons and synapses: Multiple fates of GABA signalling in epilepsies. *Trends in Neurosciences, 28*, 108–115.

Couve, A., Calver, A. R., Fairfax, B., Moss, S. J., & Pangalos, M. N. (2004). Unravelling the unusual signalling properties of the GABA(B) receptor. *Biochemical Pharmacology, 68*, 1527–1536.

Crunelli, V., & Leresche, N. (1991). A role for GABAB receptors in excitation and inhibition of thalamocortical cells. *Trends in Neurosciences, 14*, 16–21.

Cruz, H. G., Ivanova, T., Lunn, M. L., Stoffel, M., Slesinger, P. A., & Luscher, C. (2004). Bi-directional effects of GABA(B) receptor agonists on the mesolimbic dopamine system. *Nature Neuroscience, 7*, 153–159.

Davies, C. H., & Collingridge, G. L. (1996). Regulation of EPSPs by the synaptic activation of GABAB autoreceptors in rat hippocampus. *Journal of Physiology, 496*(Pt 2), 451–470.

Davies, C. H., Starkey, S. J., Pozza, M. F., & Collingridge, G. L. (1991). GABA autoreceptors regulate the induction of LTP. *Nature, 349*, 609–611.

De La Rue, S. A., & Henley, J. M. (2002). Proteins involved in the trafficking and functional synaptic expression of AMPA and KA receptors. *ScientificWorldJournal, 2*, 461–482.

Deisz, R. A., Billard, J. M., & Zieglgansberger, W. (1997). Presynaptic and postsynaptic GABAB receptors of neocortical neurons of the rat in vitro: Differences in pharmacology and ionic mechanisms. *Synapse, 25*, 62–72.

Deng, P. Y., Xiao, Z., Yang, C., Rojanathammanee, L., Grisanti, L., Watt, J., et al. (2009). GABA(B) receptor activation inhibits neuronal excitability and spatial learning in the entorhinal cortex by activating TREK-2 K+ channels. *Neuron, 63*, 230–243.

Dingledine, R., Borges, K., Bowie, D., & Traynelis, S. F. (1999). The glutamate receptor ion channels. *Pharmacological Reviews, 51*, 7–61.

Dittman, J. S., & Regehr, W. G. (1996). Contributions of calcium-dependent and calcium-independent mechanisms to presynaptic inhibition at a cerebellar synapse. *Journal of Neuroscience, 16*, 1623–1633.

Dittman, J. S., & Regehr, W. G. (1997). Mechanism and kinetics of heterosynaptic depression at a cerebellar synapse. *Journal of Neuroscience, 17*, 9048–9059.

Dutar, P., & Nicoll, R. A. (1988). A physiological role for GABAB receptors in the central nervous system. *Nature, 332*, 156–158.

El Gaamouch, F., Buisson, A., Moustie, O., Lemieux, M., Labrecque, S., Bontempi, B., et al. (2012). Interaction between alphaCaMKII and GluN2B controls ERK-dependent plasticity. *Journal of Neuroscience, 32*, 10767–10779.

Fairfax, B. P., Pitcher, J. A., Scott, M. G., Calver, A. R., Pangalos, M. N., Moss, S. J., et al. (2004). Phosphorylation and chronic agonist treatment atypically modulate GABAB receptor cell surface stability. *Journal of Biological Chemistry, 279*, 12565–12573.

Fernandez, F., & Garner, C. C. (2007). Over-inhibition: A model for developmental intellectual disability. *Trends in Neurosciences, 30*, 497–503.

Ferraguti, F., Crepaldi, L., & Nicoletti, F. (2008). Metabotropic glutamate 1 receptor: Current concepts and perspectives. *Pharmacological Reviews, 60*, 536–581.

Ferraguti, F., & Shigemoto, R. (2006). Metabotropic glutamate receptors. *Cell and Tissue Research, 326*, 483–504.

Foster, J. D., Kitchen, I., Bettler, B., & Chen, Y. (2013). GABAB receptor subtypes differentially modulate synaptic inhibition in the dentate gyrus to enhance granule cell output. *British Journal of Pharmacology, 168*, 1808–1819.

Freund, T. F., & Buzsaki, G. (1996). Interneurons of the hippocampus. *Hippocampus, 6*, 347–470.

Fritschy, J. M., Meskenaite, V., Weinmann, O., Honer, M., Benke, D., & Mohler, H. (1999). GABAB-receptor splice variants GB1a and GB1b in rat brain: Developmental regulation, cellular distribution and extrasynaptic localization. *European Journal of Neuroscience, 11*, 761–768.

Gahwiler, B. H., & Brown, D. A. (1985). GABAB-receptor-activated K+ current in voltage-clamped CA3 pyramidal cells in hippocampal cultures. *Proceedings of the National Academy of Sciences of the United States of America, 82*, 1558–1562.

Gerrow, K., & Triller, A. (2014). GABAA receptor subunit composition and competition at synapses are tuned by GABAB receptor activity. *Molecular and Cellular Neuroscience, 60*, 97–107.

Gilbert, A. K., & Franklin, K. B. (2001). GABAergic modulation of descending inhibitory systems from the rostral ventromedial medulla (RVM). Dose-response analysis of nociception and neurological deficits. *Pain, 90*, 25–36.

Gomez, L. L., Alam, S., Smith, K. E., Horne, E., & Dell'acqua, M. L. (2002). Regulation of A-kinase anchoring protein 79/150-cAMP-dependent protein kinase postsynaptic targeting by NMDA receptor activation of calcineurin and remodeling of dendritic actin. *Journal of Neuroscience, 22*, 7027–7044.

Grampp, T., Sauter, K., Markovic, B., & Benke, D. (2007). Gamma-aminobutyric acid type B receptors are constitutively internalized via the clathrin-dependent pathway and targeted to lysosomes for degradation. *Journal of Biological Chemistry, 282*, 24157–24165.

Gray, J. A., & Green, A. R. (1987). GABAB-receptor mediated inhibition of potassium-evoked release of endogenous 5-hydroxytryptamine from mouse frontal cortex. *British Journal of Pharmacology, 91*, 517–522.

Guetg, N., Abdel Aziz, S., Holbro, N., Turecek, R., Rose, T., Seddik, R., et al. (2010). NMDA receptor-dependent GABAB receptor internalization via CaMKII phosphorylation of serine 867 in GABAB1. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 13924–13929.

Hahner, L., Mcquilkin, S., & Harris, R. A. (1991). Cerebellar GABAB receptors modulate function of GABAA receptors. *FASEB Journal, 5*, 2466–2472.

Halasy, K., Buhl, E. H., Lorinczi, Z., Tamas, G., & Somogyi, P. (1996). Synaptic target selectivity and input of GABAergic basket and bistratified interneurons in the CA1 area of the rat hippocampus. *Hippocampus, 6*, 306–329.

Hirono, M., Yoshioka, T., & Konishi, S. (2001). GABA(B) receptor activation enhances mGluR-mediated responses at cerebellar excitatory synapses. *Nature Neuroscience, 4*, 1207–1216.

Hollmann, M., & Heinemann, S. (1994). Cloned glutamate receptors. *Annual Review of Neuroscience, 17*, 31–108.

Ichise, T., Kano, M., Hashimoto, K., Yanagihara, D., Nakao, K., Shigemoto, R., et al. (2000). mGluR1 in cerebellar Purkinje cells essential for long-term depression, synapse elimination, and motor coordination. *Science, 288*, 1832–1835.

Ige, A. O., Bolam, J. P., Billinton, A., White, J. H., Marshall, F. H., & Emson, P. C. (2000). Cellular and sub-cellular localisation of GABA(B1) and GABA(B2) receptor proteins in the rat cerebellum. *Brain Research. Molecular Brain Research, 83*, 72–80.

Isaacson, J. S., Solis, J. M., & Nicoll, R. A. (1993). Local and diffuse synaptic actions of GABA in the hippocampus. *Neuron, 10*, 165–175.

Ito, M. (2001). Cerebellar long-term depression: Characterization, signal transduction, and functional roles. *Physiological Reviews, 81*, 1143–1195.

Jacobson, L. H., Kelly, P. H., Bettler, B., Kaupmann, K., & Cryan, J. F. (2007). Specific roles of GABA(B(1)) receptor isoforms in cognition. *Behavioural Brain Research, 181*, 158–162.

Juhasz, G., Emri, Z., Kekesi, K. A., Salfay, O., & Crunelli, V. (1994). Blockade of thalamic GABAB receptors decreases EEG synchronization. *Neuroscience Letters, 172*, 155–158.

Jurado, S., Biou, V., & Malenka, R. C. (2010). A calcineurin/AKAP complex is required for NMDA receptor-dependent long-term depression. *Nature Neuroscience, 13*, 1053–1055.

Kamikubo, Y., Tabata, T., Kakizawa, S., Kawakami, D., Watanabe, M., Ogura, A., et al. (2007). Postsynaptic GABAB receptor signalling enhances LTD in mouse cerebellar Purkinje cells. *Journal of Physiology, 585*, 549–563.

Kantamneni, S., Correa, S. A., Hodgkinson, G. K., Meyer, G., Vinh, N. N., Henley, J. M., et al. (2007). GISP: A novel brain-specific protein that promotes surface expression and function of GABA(B) receptors. *Journal of Neurochemistry, 100*, 1003–1017.

Kantamneni, S., Gonzalez-Gonzalez, I. M., Luo, J., Cimarosti, H., Jacobs, S. C., Jaafari, N., et al. (2014). Differential regulation of GABAB receptor trafficking by different modes of N-methyl-D-aspartate (NMDA) receptor signaling. *Journal of Biological Chemistry, 289*, 6681–6694.

Kirkitadze, M. D., & Barlow, P. N. (2001). Structure and flexibility of the multiple domain proteins that regulate complement activation. *Immunology Reviews, 180*, 146–161.

Kobayashi, M., Takei, H., Yamamoto, K., Hatanaka, H., & Koshikawa, N. (2012). Kinetics of GABAB autoreceptor-mediated suppression of GABA release in rat insular cortex. *Journal of Neurophysiology, 107*, 1431–1442.

Kulik, A., Vida, I., Lujan, R., Haas, C. A., Lopez-Bendito, G., Shigemoto, R., et al. (2003). Subcellular localization of metabotropic GABA(B) receptor subunits GABA(B1a/b) and GABA(B2) in the rat hippocampus. *Journal of Neuroscience, 23*, 11026–11035.

Kuramoto, N., Wilkins, M. E., Fairfax, B. P., Revilla-Sanchez, R., Terunuma, M., Tamaki, K., et al. (2007). Phospho-dependent functional modulation of GABA(B) receptors by the metabolic sensor AMP-dependent protein kinase. *Neuron, 53*, 233–247.

Ladera, C., Godino Mdel, C., Martin, R., Lujan, R., Shigemoto, R., Ciruela, F., et al. (2007). The coexistence of multiple receptors in a single nerve terminal provides evidence for pre-synaptic integration. *Journal of Neurochemistry, 103*, 2314–2326.

Lambert, N. A., & Wilson, W. A. (1993). Heterogeneity in presynaptic regulation of GABA release from hippocampal inhibitory neurons. *Neuron, 11*, 1057–1067.

Leutgeb, J. K., Leutgeb, S., Moser, M. B., & Moser, E. I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science, 315*, 961–966.

Lin, J. W., Wyszynski, M., Madhavan, R., Sealock, R., Kim, J. U., & Sheng, M. (1998). Yotiao, a novel protein of neuromuscular junction and brain that interacts with specific splice variants of NMDA receptor subunit NR1. *Journal of Neuroscience, 18*, 2017–2027.

Lodge, D. (2009). The history of the pharmacology and cloning of ionotropic glutamate receptors and the development of idiosyncratic nomenclature. *Neuropharmacology, 56*, 6–21.

Lujan, R., Roberts, J. D., Shigemoto, R., Ohishi, H., & Somogyi, P. (1997). Differential plasma membrane distribution of metabotropic glutamate receptors mGluR1 alpha, mGluR2 and mGluR5, relative to neurotransmitter release sites. *Journal of Chemical Neuroanatomy, 13*, 219–241.

Lujan, R., & Shigemoto, R. (2006). Localization of metabotropic GABA receptor subunits GABAB1 and GABAB2 relative to synaptic sites in the rat developing cerebellum. *European Journal of Neuroscience, 23*, 1479–1490.

Luscher, C., Jan, L. Y., Stoffel, M., Malenka, R. C., & Nicoll, R. A. (1997). G protein-coupled inwardly rectifying K+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron, 19*, 687–695.

Maier, P. J., Marin, I., Grampp, T., Sommer, A., & Benke, D. (2010). Sustained glutamate receptor activation down-regulates GABAB receptors by shifting the balance from recycling to lysosomal degradation. *Journal of Biological Chemistry, 285*, 35606–35614.

Mainen, Z. F., Malinow, R., & Svoboda, K. (1999). Synaptic calcium transients in single spines indicate that NMDA receptors are not saturated. *Nature, 399*, 151–155.

Malenka, R. C., & Bear, M. F. (2004). LTP and LTD: An embarrassment of riches. *Neuron, 44*, 5–21.

Malitschek, B., Schweizer, C., Keir, M., Heid, J., Froestl, W., Mosbacher, J., et al. (1999). The N-terminal domain of gamma-aminobutyric Acid(B) receptors is sufficient to specify agonist and antagonist binding. *Molecular Pharmacology, 56*, 448–454.

Margeta-Mitrovic, M., Mitrovic, I., Riley, R. C., Jan, L. Y., & Basbaum, A. I. (1999). Immunohistochemical localization of GABA(B) receptors in the rat central nervous system. *Journal of Comparative Neurology, 405*, 299–321.

Marshall, F. H., Jones, K. A., Kaupmann, K., & Bettler, B. (1999). GABAB receptors—The first 7TM heterodimers. *Trends in Pharmacological Sciences, 20*, 396–399.

Mateos, J. M., Benitez, R., Elezgarai, I., Azkue, J. J., Lazaro, E., Osorio, A., et al. (2000). Immunolocalization of the mGluR1b splice variant of the metabotropic glutamate receptor 1 at parallel fiber-Purkinje cell synapses in the rat cerebellar cortex. *Journal of Neurochemistry, 74*, 1301–1309.

Metz, M., Gassmann, M., Fakler, B., Schaeren-Wiemers, N., & Bettler, B. (2011). Distribution of the auxiliary GABAB receptor subunits KCTD8, 12, 12b, and 16 in the mouse brain. *Journal of Comparative Neurology, 519*, 1435–1454.

Miles, R., Toth, K., Gulyas, A. I., Hajos, N., & Freund, T. F. (1996). Differences between somatic and dendritic inhibition in the hippocampus. *Neuron, 16*, 815–823.

Morrisett, R. A., Mott, D. D., Lewis, D. V., Swartzwelder, H. S., & Wilson, W. A. (1991). GABAB-receptor-mediated inhibition of the N-methyl-D-aspartate component of synaptic transmission in the rat hippocampus. *Journal of Neuroscience, 11*, 203–209.

Moser, E. I., Kropff, E., & Moser, M. B. (2008). Place cells, grid cells, and the brain's spatial representation system. *Annual Review of Neuroscience, 31*, 69–89.

Mott, D. D., & Lewis, D. V. (1991). Facilitation of the induction of long-term potentiation by GABAB receptors. *Science, 252*, 1718–1720.

Mott, D. D., Li, Q., Okazaki, M. M., Turner, D. A., & Lewis, D. V. (1999). GABAB-receptor-mediated currents in interneurons of the dentate-hilus border. *Journal of Neurophysiology, 82*, 1438–1450.

Mott, D. D., Xie, C. W., Wilson, W. A., Swartzwelder, H. S., & Lewis, D. V. (1993). GABAB autoreceptors mediate activity-dependent disinhibition and enhance signal transmission in the dentate gyrus. *Journal of Neurophysiology, 69*, 674–691.

Nicoll, R. A., Malenka, R. C., & Kauer, J. A. (1990). Functional comparison of neurotransmitter receptor subtypes in mammalian central nervous system. *Physiological Reviews, 70*, 513–565.

O'Donnell, P., & Grace, A. A. (1995). Synaptic interactions among excitatory afferents to nucleus accumbens neurons: Hippocampal gating of prefrontal cortical input. *Journal of Neuroscience, 15*, 3622–3639.

Otis, T. S., De Koninck, Y., & Mody, I. (1993). Characterization of synaptically elicited GABAB responses using patch-clamp recordings in rat hippocampal slices. *Journal of Physiology, 463*, 391–407.

Otis, T. S., & Mody, I. (1992). Differential activation of GABAA and GABAB receptors by spontaneously released transmitter. *Journal of Neurophysiology, 67*, 227–235.

Otmakhova, N. A., & Lisman, J. E. (2004). Contribution of Ih and GABAB to synaptically induced afterhyperpolarizations in CA1: A brake on the NMDA response. *Journal of Neurophysiology, 92*, 2027–2039.

Patenaude, C., Chapman, C. A., Bertrand, S., Congar, P., & Lacaille, J. C. (2003). GABAB receptor- and metabotropic glutamate receptor-dependent cooperative long-term potentiation of rat hippocampal GABAA synaptic transmission. *Journal of Physiology, 553*, 155–167.

Perez-Garci, E., Gassmann, M., Bettler, B., & Larkum, M. E. (2006). The GABAB1b isoform mediates long-lasting inhibition of dendritic Ca^{2+} spikes in layer 5 somatosensory pyramidal neurons. *Neuron, 50*, 603–616.

Perrin, M. H., Grace, C. R., Riek, R., & Vale, W. W. (2006). The three-dimensional structure of the N-terminal domain of corticotropin-releasing factor receptors: Sushi domains and the B1 family of G protein-coupled receptors. *Annals of the New York Academy of Sciences, 1070*, 105–119.

Pierau, F. K., & Zimmermann, P. (1973). Action of a GABA-derivative on postsynaptic potentials and membrane properties of cats' spinal motoneurones. *Brain Research, 54*, 376–380.

Pinard, A., Seddik, R., & Bettler, B. (2010). GABAB receptors: Physiological functions and mechanisms of diversity. *Advances in Pharmacology, 58*, 231–255.

Poncer, J. C., Mckinney, R. A., Gahwiler, B. H., & Thompson, S. M. (1997). Either N- or P-type calcium channels mediate GABA release at distinct hippocampal inhibitory synapses. *Neuron, 18*, 463–472.

Poncer, J. C., Mckinney, R. A., Gahwiler, B. H., & Thompson, S. M. (2000). Differential control of GABA release at synapses from distinct interneurons in rat hippocampus. *Journal of Physiology, 528*(Pt 1), 123–130.

Poncer, J. C., Shinozaki, H., & Miles, R. (1995). Dual modulation of synaptic inhibition by distinct metabotropic glutamate receptors in the rat hippocampus. *Journal of Physiology, 485*(Pt 1), 121–134.

Potashner, S. J. (1979). Baclofen: Effects on amino acid release and metabolism in slices of guinea pig cerebral cortex. *Journal of Neurochemistry, 32*, 103–109.

Princivalle, A., Regondi, M. C., Frassoni, C., Bowery, N. G., & Spreafico, R. (2000). Distribution of GABA(B) receptor protein in somatosensory cortex and thalamus of adult rats and during postnatal development. *Brain Research Bulletin, 52*, 397–405.

Raiteri, M. (2008). Presynaptic metabotropic glutamate and GABAB receptors. *Handbook of Experimental Pharmacology***,** (184), 373–407.

Reimann, W., Zumstein, A., & Starke, K. (1982). Gamma-aminobutyric acid can both inhibit and facilitate dopamine release in the caudate nucleus of the rabbit. *Journal of Neurochemistry, 39*, 961–969.

Rives, M. L., Vol, C., Fukazawa, Y., Tinel, N., Trinquet, E., Ayoub, M. A., et al. (2009). Crosstalk between GABAB and mGlu1a receptors reveals new insight into GPCR signal integration. *EMBO Journal, 28*, 2195–2208.

Robbins, M. J., Calver, A. R., Filippov, A. K., Hirst, W. D., Russell, R. B., Wood, M. D., et al. (2001). GABA(B2) is essential for g-protein coupling of the GABA(B) receptor heterodimer. *Journal of Neuroscience, 21*, 8043–8052.

Robertson, H. R., Gibson, E. S., Benke, T. A., & Dell'acqua, M. L. (2009). Regulation of postsynaptic structure and function by an A-kinase anchoring protein-membrane-associated guanylate kinase scaffolding complex. *Journal of Neuroscience, 29*, 7929–7943.

Rubenstein, J. L., & Merzenich, M. M. (2003). Model of autism: Increased ratio of excitation/inhibition in key neural systems. *Genes, Brain, and Behavior, 2*, 255–267.

Sanderson, J. L., & Dell'acqua, M. L. (2011). AKAP signaling complexes in regulation of excitatory synaptic plasticity. *The Neuroscientist, 17*, 321–336.

Scanziani, M. (2000). GABA spillover activates postsynaptic GABA(B) receptors to control rhythmic hippocampal activity. *Neuron, 25*, 673–681.

Scanziani, M. (2002). Competing on the edge. *Trends in Neurosciences, 25*, 282–283.

Schlicker, E., Classen, K., & Gothert, M. (1984). GABAB receptor-mediated inhibition of serotonin release in the rat brain. *Naunyn-Schmiedeberg's Archives of Pharmacology, 326*, 99–105.

Schwenk, J., Metz, M., Zolles, G., Turecek, R., Fritzius, T., Bildl, W., et al. (2010). Native GABA(B) receptors are heteromultimers with a family of auxiliary subunits. *Nature, 465*, 231–235.

Sheng, M., & Kim, E. (2011). The postsynaptic organization of synapses. *Cold Spring Harbor Perspectives in Biology, 3*.

Smith, K. E., Gibson, E. S., & Dell'acqua, M. L. (2006). cAMP-dependent protein kinase postsynaptic localization regulated by NMDA receptor activation through translocation of an A-kinase anchoring protein scaffold protein. *Journal of Neuroscience, 26*, 2391–2402.

Sun, H., Ma, C. L., Kelly, J. B., & Wu, S. H. (2006). GABAB receptor-mediated presynaptic inhibition of glutamatergic transmission in the inferior colliculus. *Neuroscience Letters, 399*, 151–156.

Tabata, T., Araishi, K., Hashimoto, K., Hashimotodani, Y., van der Putten, H., Bettler, B., et al. (2004). Ca^{2+} activity at GABAB receptors constitutively promotes metabotropic glutamate signaling in the absence of GABA. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 16952–16957.

Tabuchi, K., Blundell, J., Etherton, M. R., Hammer, R. E., Liu, X., Powell, C. M., et al. (2007). A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. *Science, 318*, 71–76.

Takahashi, T., Kajikawa, Y., & Tsujimoto, T. (1998). G-Protein-coupled modulation of presynaptic calcium currents and transmitter release by a GABAB receptor. *Journal of Neuroscience, 18*, 3138–3146.

Terunuma, M., Pangalos, M. N., & Moss, S. J. (2010a). Functional modulation of GABAB receptors by protein kinases and receptor trafficking. *Advances in Pharmacology, 58*, 113–122.

Terunuma, M., Revilla-Sanchez, R., Quadros, I. M., Deng, Q., Deeb, T. Z., Lumb, M., et al. (2014). Postsynaptic GABAB receptor activity regulates excitatory neuronal architecture and spatial memory. *Journal of Neuroscience, 34*, 804–816.

Terunuma, M., Vargas, K. J., Wilkins, M. E., Ramirez, O. A., Jaureguiberry-Bravo, M., Pangalos, M. N., et al. (2010b). Prolonged activation of NMDA receptors promotes dephosphorylation and alters postendocytic sorting of GABAB receptors. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 13918–13923.

Thompson, S. M., & Gahwiler, B. H. (1989). Activity-dependent disinhibition. III. Desensitization and GABAB receptor-mediated presynaptic inhibition in the hippocampus in vitro. *Journal of Neurophysiology, 61*, 524–533.

Thompson, S. M., & Gahwiler, B. H. (1992). Comparison of the actions of baclofen at pre- and postsynaptic receptors in the rat hippocampus in vitro. *Journal of Physiology, 451*, 329–345.

Tiao, J. Y., Bradaia, A., Biermann, B., Kaupmann, K., Metz, M., Haller, C., et al. (2008). The sushi domains of secreted GABA(B1) isoforms selectively impair GABA(B) heteroreceptor function. *Journal of Biological Chemistry, 283*, 31005–31011.

Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., et al. (2010). Glutamate receptor ion channels: Structure, regulation, and function. *Pharmacological Reviews, 62*, 405–496.

Tunquist, B. J., Hoshi, N., Guire, E. S., Zhang, F., Mullendorff, K., Langeberg, L. K., et al. (2008). Loss of AKAP150 perturbs distinct neuronal processes in mice. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 12557–12562.

Ulrich, D., & Bettler, B. (2007). GABA(B) receptors: Synaptic functions and mechanisms of diversity. *Current Opinion in Neurobiology, 17*, 298–303.

Ventura, R., & Harris, K. M. (1999). Three-dimensional relationships between hippocampal synapses and astrocytes. *Journal of Neuroscience, 19*, 6897–6906.

Vigot, R., Barbieri, S., Brauner-Osborne, H., Turecek, R., Shigemoto, R., Zhang, Y. P., et al. (2006). Differential compartmentalization and distinct functions of GABAB receptor variants. *Neuron, 50*, 589–601.

Vigot, R., & Batini, C. (1997). GABA(B) receptor activation of Purkinje cells in cerebellar slices. *Neuroscience Research, 29*, 151–160.

von Krosigk, M., Bal, T., & McCormick, D. A. (1993). Cellular mechanisms of a synchronized oscillation in the thalamus. *Science, 261*, 361–364.

Watanabe, M., Maemura, K., Kanbara, K., Tamayama, T., & Hayasaki, H. (2002). GABA and GABA receptors in the central nervous system and other organs. *International Review of Cytology, 213*, 1–47.

Westphal, R. S., Tavalin, S. J., Lin, J. W., Alto, N. M., Fraser, I. D., Langeberg, L. K., et al. (1999). Regulation of NMDA receptors by an associated phosphatase-kinase signaling complex. *Science, 285*, 93–96.

Wong, W., & Scott, J. D. (2004). AKAP signalling complexes: Focal points in space and time. *Nature Reviews Molecular Cell Biology, 5*, 959–970.

Wu, L. G., & Saggau, P. (1995). GABAB receptor-mediated presynaptic inhibition in guinea-pig hippocampus is caused by reduction of presynaptic Ca^{2+} influx. *Journal of Physiology, 485*(Pt 3), 649–657.

Xiang, Z., Huguenard, J. R., & Prince, D. A. (1998). Cholinergic switching within neocortical inhibitory networks. *Science, 281*, 985–988.

Xu, J., & Wojcik, W. J. (1986). Gamma aminobutyric acid B receptor-mediated inhibition of adenylate cyclase in cultured cerebellar granule cells: Blockade by islet-activating protein. *Journal of Pharmacology and Experimental Therapeutics, 239*, 568–573.

Zhang, Z., Zhang, W., Huang, S., Sun, Q., Wang, Y., Hu, Y., et al. (2015). GABAB receptor promotes its own surface expression by recruiting a Rap1-dependent signaling cascade. *Journal of Cell Science, 128*, 2302–2313.

Zoghbi, H. Y. (2003). Postnatal neurodevelopmental disorders: Meeting at the synapse? *Science, 302*, 826–830.

Chapter 8
$GABA_B$ Receptor Functions in the Mesolimbic Dopamine System

Arnaud L. Lalive and Christian Lüscher

Abstract $GABA_B$ receptors are expressed in neurons of the dopamine system where they bidirectionally modulate activity and release of glutamate, GABA, and dopamine itself. Dopamine has many functions including signaling of salient external stimuli and prediction errors that optimize decision-making, as well as motivation and initiation of movement. $GABA_B$ receptors thus exert a second-order modulation, with effects on locomotion, motivation, and reward learning. Moreover, recent findings indicate that neuronal activity may induce a plasticity of $GABA_B$ receptor signaling. In this chapter, we review the structural and functional features of $GABA_B$ receptor signaling in the dopaminergic system, from subcellular specialization to plasticity and fine-tuning of mesolimbic circuits. Beyond a physiological role $GABA_B$ receptors may also affect disease, such as addiction. $GABA_B$ receptors may therefore constitute an interesting target for pharmacological interventions to treat this condition.

Keywords $GABA_B$ receptor • Synaptic plasticity • Dopamine • Ventral tegmental area • Addiction • Mesolimbic system

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8.1 The Midbrain DA System

8.1.1 Projection- and Input-Specific DA Populations

The midbrain dopamine (DA) system has its origin in two major nuclei: the Ventral Tegmental Area (VTA) and the Substantia Nigra compacta (SNc), which are anatomically and functionally distinct. The VTA DA neurons, close to the midline at the caudal end of the midbrain, project most notably to the Nucleus Accumbens (NAc) and Prefrontal Cortex (PFC), forming the mesocorticolimbic system. Increased DA activity is typically associated with positive outcome, whereas DA inhibition is aversive (Schultz 1998; Tan et al. 2012; van Zessen et al. 2012). DA neurons of the SNc are located lateral to the VTA, project to the dorsal striatum (the mesostriatal system) where they modulate locomotion. DA neurons also receive reciprocal inputs from their striatal target; the dorsal striatum mostly projects to the SNc, the NAc preferentially targets the VTA. Additionally, nuclei associated with motivational states like the dorsal raphe and the lateral hypothalamus selectively target the VTA over the SNc, further differentiating these two populations. In comparison, both DA nuclei receive equal projections from the cortex (Watabe-Uchida et al. 2012).

8.1.2 VTA Microcircuit

The VTA contains three cell types: DA, γ-aminobutyric acid (GABA)-ergic, and glutamatergic. The DA neurons form 80 % of the total population. The GABAergic neurons are fewer and provide a major inhibitory input to DA neurons (Johnson and North 1992a). The functional relevance of this microcircuit was initially demonstrated by showing that μ-opioid receptors specifically inhibit GABA neurons, leading to the disinhibition of DA neurons. Lastly, a small population of glutamatergic neurons forms local synapses with DA and GABA neurons (Dobi et al. 2010).

The recent development of transgenic mouse lines and optogenetics has allowed investigators to manipulate neuronal populations of the VTA in vivo and measure their impact on behavior, further validating the functional organization of the VTA microcircuit. For example, direct activation of DA neurons is sufficient to drive reward-related behaviors (Tsai et al. 2009; Adamantidis et al. 2011). In contrast, activation of GABA neurons inhibits DA firing and is sufficient to drive aversive behavioral responses (Tan et al. 2012; van Zessen et al. 2012). Finally, activation of the local glutamatergic population excites DA neurons and is rewarding (Wang et al. 2015).

8.1.3 Inputs to VTA Neurons

In the VTA, both DA and GABAergic populations receive quantitatively similar proportions of excitatory and inhibitory inputs from outside the VTA (Beier et al. 2015). Major excitatory inputs include the frontal cortex, central amygdala,

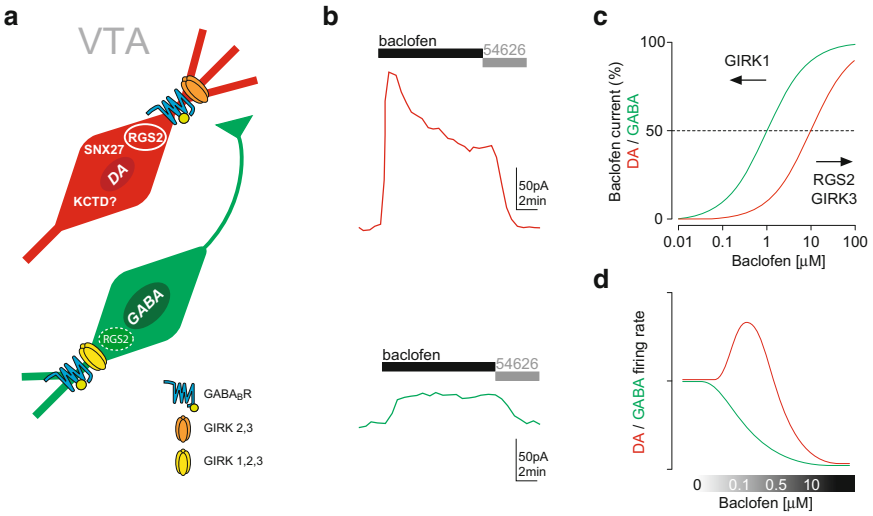

Fig. 8.1 Cell-type-specific $GABA_B$ receptor signaling in the VTA. (**a**) VTA microcircuit depicting GABA neuron (*green*) inhibition onto DA neuron (*red*), and cell-type-specific expression of proteins involved in $GABA_B$ receptor signaling. (**b**) Example voltage clamp recordings of maximal baclofen-evoked currents (300 µM) in DA (*red*, *upper panel*) and GABA (*green*, *lower panel*) neurons. Currents are blocked by $GABA_B$ receptor antagonist CGP54626 (2 µM). (**c**) Dose–response curve for baclofen in DA (*red*) and GABA (*green*) neurons, showing the difference in EC50. Insets indicate relevant proteins and their effect on coupling efficiency. (**d**) Schematic representation of DA (*red*) and GABA (*green*) neuron firing rate to increasing concentrations of baclofen [Traces in (**b**) reproduced from Labouèbe et al. 2007]

hippocampal septum, lateral habenula, and dorsal raphe, while major inhibitory inputs comprise the Nac, ventral pallidum, and globus pallidus. However, specific inputs from a given region may be qualitatively biased toward one cell type. For example, the GABAergic projection from the NAc preferentially inhibits VTA GABA neurons, and eventually disinhibits DA neurons (Xia et al. 2011; Bocklisch et al. 2013). Therefore, the ultimate DA output is controlled by balanced modulation of both DA and GABA neurons (Fig. 8.1).

8.1.4 $GABA_B$ Receptors in the Midbrain DA System

$GABA_B$ receptors are enriched across DA neurons of the midbrain, and also found in VTA GABA neurons, whereas their presence in VTA glutamatergic neurons remains to be established. It has been proposed that local and long-range inhibitory inputs to DA neurons have a specialization with synapses that contain only $GABA_A$ or $GABA_B$ receptors, respectively (Sugita et al. 1992; Johnson and North 1992b; Cameron and Williams 1993). However, recent investigations have suggested the presence of $GABA_A$ receptors at all inhibitory synapses in DA neurons. For example, optogenetic activation of long-range projections from the NAc evokes $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) in VTA DA neurons (Bocklisch et al. 2013).

Additionally, in vivo electrical stimulation of inhibitory nuclei targeting the SNc evokes a combined $GABA_A$, $GABA_B$ receptor-mediated inhibitory postsynaptic potential (IPSP) (Brazhnik et al. 2008). Whether VTA GABA neurons can activate $GABA_B$ receptors in DA neurons remains to be investigated. Dendritic $GABA_B$ receptors are typically extrasynaptically located (Boyes and Bolam 2003; Koyrakh et al. 2005), therefore a single pulse of stimulation evokes mostly $GABA_A$ receptor-mediated currents in the VTA. Higher intensity and frequency stimulation is usually required to drive GABA spillover outside the synaptic cleft and reach extrasynaptic $GABA_B$ receptors, suggesting that only sustained GABA release engages $GABA_B$ receptor signaling, independently of the synaptic input.

8.2 $GABA_B$ Receptor Signaling in the Midbrain

8.2.1 Common Features of $GABA_B$ Receptor Signaling

8.2.1.1 $GABA_B$ Receptor Structure and Main Effectors

Functional $GABA_B$ receptors are heterodimers that require the assembly of one $GABA_{B1}$ isoform (1a or 1b) with $GABA_{B2}$ (Jones et al. 1998; White et al. 1998; Kaupmann et al. 1998; see also Chap. 4 of this book). The $GABA_{B1a}$ subunit differs from $GABA_{B1b}$ by the expression of two sushi domains, which guide trafficking to axon terminals. Accordingly, $GABA_{B1a/2}$ receptors are mainly found presynaptically, whereas postsynaptic compartments mostly express $GABA_{B1b/2}$ dimers (Vigot et al. 2006). Because $GABA_B$ receptor subunit composition is similar across most neurons, any variability in $GABA_B$ receptor signaling is likely to be accounted for by associated proteins.

Both $GABA_{B1a/2}$ and $GABA_{B1b/2}$ similarly couple to $G_{i/o}$ protein. Gα inhibits the adenylate cyclase pathway while Gβγ gates ion channels. In presynaptic boutons, $GABA_B$ receptors decrease neurotransmitter release via Gβγ-mediated closing of Voltage-Gated Ca^{2+} (CaV) channels and direct interaction with the release machinery. Postsynaptically, the major effect of Gβγ is the opening of G protein-activated inwardly rectifying K^+ channels (GIRK, also known as Kir3), which hyperpolarize the membrane and decrease neuronal excitability (Bettler et al. 2004; Lüscher et al. 1997). (For a more detailed description of $GABA_B$ receptor signaling, see Chap. 6 of this book).

8.2.1.2 Macromolecular Signaling Complex and Associated Proteins

Accumulating evidence suggests that $GABA_B$ receptors and associated proteins form macromolecular complexes to promote the specificity of spatial and temporal control of signaling (Doupnik et al. 2004). Electron microscopy, co-immunoprecipitation, as well as bioluminescence and fluorescence resonance energy transfer experiments initially revealed close interactions between $GABA_B$ and GIRK subunits, suggesting the existence of those complexes (David et al. 2006; Fowler et al. 2007; Ciruela et al. 2010).

These approaches also identified the interaction of $GABA_B$ receptors and GIRK channels with Regulator of G protein signaling (RGS) proteins (Fowler et al. 2007). RGS provide powerful modulation of the coupling efficiency between GPCR and effectors by accelerating the activation and deactivation kinetics of G proteins, and have also been proposed to influence signaling desensitization, a process of signal attenuation upon receptor activation (Mutneja et al. 2005). For most G protein-coupled receptors (GPCR), desensitization is expressed by rapid agonist-induced internalization of the receptor. However $GABA_B$ receptors behave differently, and desensitization mechanisms remain elusive (Couve et al. 2002). More recently, affinity purification assays combined with quantitative mass spectrometry from native membrane preparations allowed the identification of four KCTD (K^+ Channel Tetramerization Domain-containing) proteins as auxiliary subunits of the $GABA_B$ receptors (Schwenk et al. 2010). KCTDs bind to the $GABA_{B2}$ subunit and modulate coupling, onset kinetics and desensitization of receptor signaling (Turecek et al. 2014). Further functional proteomics analyses revealed co-assembly with unsuspected partners like HCN (hyperpolarization-activated cyclic nucleotide-gated) channels. Surprisingly, no physical interaction was found between $GABA_B$ receptors or KCTD proteins and GIRK channels despite being major effector of $GABA_B$ receptors (Schwenk et al. 2016).

Altogether, there are many proteins that actively shape $GABA_B$ receptor signaling and even more possible combinations to build macromolecular complexes. Such abundance allows region and cell-type-specific variability of $GABA_B$ receptor functional expression, which is highly relevant in the midbrain DA system.

8.2.2 Cell-Type-Specific $GABA_B$ Receptor Signaling in the Midbrain

8.2.2.1 Differences in $GABA_B$ Receptor Signaling in VTA DA and GABA Neurons

In the VTA both DA and GABA neurons express $GABA_B$ receptors, however there are a number of functional differences that can be assessed by recording from VTA neurons in acute brain slices and applying baclofen, a $GABA_B$ receptor agonist, to evoke GIRK currents. First, DA neurons have a much lower sensitivity to baclofen, measured by an EC50 value (the concentration required to reach 50 % of the maximal response) ten-fold higher than in GABA neurons. Yet, the peak current evoked by a saturating concentration of baclofen is around three-fold larger in DA neurons. Lastly, in DA neurons, baclofen-evoked currents desensitize by around 40 % over minutes, whereas GABA neurons show a steady current upon continuous baclofen application (Cruz et al. 2004). These three differences in $GABA_B$ receptor signaling have been partially explained by differential composition and expression levels of proteins involved in $GABA_B$ receptor macromolecular signaling complex, which we review here (Table 8.1). They also provide the mesocorticolimbic system with a bidirectional handle on DA output, which will be discussed later.

Table 8.1 Cell-type-specific expression of proteins modulating $GABA_B$ receptor signaling in the midbrain DA system

	DA	GABA	References
GIRK	2a (SNc only)	1	Inanobe et al. (1999)
	2c	2c	Cruz et al. (2004)
	3	3	Labouèbe et al. (2007)
RGS	2		Labouèbe et al. (2007)
	4	4	
KCTD	12?	12?	Metz et al. (2011)
	16?	16?	
SNX	27	27?	Munoz and Slesinger (2014)
HCN	high	low	Lacey et al. (1989)

8.2.2.2 Cell-Type-Specific GIRK Channel Expression

VTA DA and GABA neurons express a specific set of GIRK channel subunits. Of the four known GIRK subunits, GIRK1, 2, and 3 are found in the brain, whereas GIRK4 expression is low and probably functionally irrelevant (Wickman et al. 2000). GIRK channels assemble as heterotetramers formed by two pairs of two subunits (1/2, 1/3, 2/3), or GIRK2 homotetramers, with GIRK2 available in three isorforms, GIRKa-c (Lüscher and Slesinger 2010). GIRK2-containing channels seem to be most commonly expressed throughout the brain, as knocking-out GIRK2 ablates almost entirely GIRK-mediated currents in many different cell types, including midbrain DA and GABA neurons (Lüscher et al. 1997; Arora et al. 2010; Cruz et al. 2004; Hearing et al. 2013).

VTA GABA neurons express GIRK1, 2c, and 3 subunits, whereas VTA and SNc DA neurons do not express GIRK1, as observed by single cell RT-PCR and electron microscopy (Inanobe et al. 1999; Cruz et al. 2004; Labouèbe et al. 2007). The presence of GIRK1 partially explains why $GABA_B$ receptors have a higher coupling efficiency in GABA neurons. Indeed, ectopic expression of GIRK1 in VTA DA neurons reduces the EC50 value for baclofen-evoked GIRK currents. Conversely, the same result is obtained by knocking out GIRK3 from DA neurons, suggesting that GIRK1 increases, whereas GIRK3 decreases the coupling efficiency of $GABA_B$ receptors (Labouèbe et al. 2007).

Cell-type-specific GIRK channel composition is also found among the midbrain DA population. VTA DA neurons express mostly GIRK2c/3 heteromers, whereas SNc DA neurons express both GIRK2a and 2c isoforms and form GIRK2a/2c homomers (Inanobe et al. 1999). Although not directly compared, maximal currents and desensitization rate seem similar overall in both populations (VTA: Labouèbe et al. 2007; SNc: Arora et al. 2010). Accordingly, GIRK3 knockout does not affect amplitude or desensitization in SNc DA neurons, although the latter was not systematically quantified (Arora et al. 2010). Thus, it appears that GIRK channel composition broadly influences coupling efficiency, whereas a potential role for GIRK1 in desensitization remains to be explored.

8.2.2.3 Cell-Type-Specific RGS Expression

Another major difference observed so far is the selective expression of RGS2 in VTA DA neurons versus GABA neurons. Whereas RGS4 is present in both, RGS2 associates with the GIRK3 subunit and acts as a brake on coupling efficiency of $GABA_B$ receptor signaling in DA neurons. Either inhibiting RGS proteins or knocking out RGS2 results in lower EC50 values for baclofen-evoked GIRK currents in DA neurons, similar to GIRK3 knockout. RGS2 and GIRK3 double knockout do not further decrease the EC50 value, suggesting that RGS2 decreases the coupling efficiency through its interaction with GIRK3 (Labouèbe et al. 2007).

8.2.2.4 KCTD

Notably, neither GIRK3 knockout, RGS2 knockout nor their combination in DA neurons fully recapitulates the high coupling efficiency observed in GABA neurons. Therefore, other components of the signaling complex must account for this cell-type-specific aspect $GABA_B$ receptor signaling. The missing piece of the puzzle could very well be found in a newly identified function of KCTD proteins, as auxiliary subunits for $GABA_B$ receptors (Schwenk et al. 2010). Affinity purification assays have identified four members of the KCTD family that interact with $GABA_{B2}$: KCTD8, 12, 12b, and 16. In cultured hippocampal neurons, all four KCTD sharpen the rise-time of GIRK currents. KCTD12 and 12b also shorten current onset, and most interestingly, strongly increase the desensitization rate. The acceleration of signal transduction is mediated by the binding of KCTDs to G proteins, keeping them close to and stabilizing $GABA_B$ receptors. The pronounced desensitization is due to the ability of KCTD12 and 12b, but not 8 or 16, to bind activated Gβγ proteins and uncouple them from GIRK channels (Turecek et al. 2014). KCTD12 and 16 are enriched in the VTA (Metz et al. 2011), however cell-type-specific expression patterns remain so far unexplored in this same region. Selective expression of KCTD12 in DA neurons is a likely molecular candidate to explain the signature desensitizing GIRK currents in these cells, and differential KCTD expression may also explain discrepancies in coupling efficiency in GABA versus DA neurons.

8.2.2.5 Sorting Nexin 27

The expression of GIRK2c/3 heteromers in VTA DA neurons allows their targeting by Sorting Nexin 27 (SNX27), an endosomal protein involved in the trafficking of several G protein-coupled receptors (GPCR) (Joubert et al. 2004; Lauffer et al. 2010). SNX27 contains a PDZ domain that selectively associates with the C-terminal PDZ-binding motif on GIRK2c and GIRK3 subunits (Balana et al. 2013; Lunn et al. 2007). In mice lacking SNX27 only in DA neurons, maximal baclofen currents are

blunted, presumably due to a decreased surface expression of GIRK channels (Munoz and Slesinger 2014). SNX27 is also expressed in non-DA neurons of the VTA, and might therefore fulfill a similar function in GABA neurons.

8.2.2.6 HCN Channels

Midbrain DA neurons, but not GABA neurons express HCN channels, which enable the pacemaker-like tonic firing of SNc DA neurons (Neuhoff et al. 2002; Khaliq and Bean 2010). HCN channels co-assemble with $GABA_{B2}$ via KCTD16. It has been suggested that $GABA_B$ receptor activation leads to HCN channel opening over several hundreds of milliseconds, closely following the timescale of G protein signaling. HCN opening potentially decrease the amplitude and accelerates the decay of GIRK-mediated postsynaptic potentials (Schwenk et al. 2016). The mechanistic underpinnings of this phenomenon remain to be explored. For example, HCN channels are typically activated by hyperpolarization, which is driven by GIRK channels. Therefore it is possible that HCN channel opening is unrelated to $GABA_B$ receptor signaling and simply responds to GIRK activation, providing a depolarizing drive. However HCN channels are also gated by cyclic Adenosine Monophosphate (cAMP), which is decreased by Gαi/o-mediated inhibition of adenylate cyclase, so in theory $GABA_B$ receptor activation could shift the threshold for HCN opening toward more hyperpolarized potentials. Another possibility is that $GABA_B$ receptor signaling directly interacts with HCN channels, as with main effectors like GIRK and CaV channels. This distinction is crucial to understanding the functional relevance of the interplay between $GABA_B$ receptors and HCN channels. Regardless of mechanism, this DA-specific effect may also shape the unique $GABA_B$ receptor-GIRK signaling characteristics observed in these cells.

8.3 $GABA_B$ Receptor Modulation of DA Neuron Activity

8.3.1 DA Neuron Firing Patterns

DA neurons typically display two firing patterns: tonic firing, a regular pacemaker-like rhythm (1–8 Hz), and burst or phasic firing, characterized by several action potentials at higher frequencies (>15 Hz), also known as phasic firing (Grace and Bunney 1984a, b). Tonic firing relies on the intrinsic expression of a variety of CaV and voltage-dependent Na^+ channels in DA neurons as well as HCN channels in the SNc (Neuhoff et al. 2002; Khaliq and Bean 2010). By contrast the transition to bursting requires glutamate release from afferent excitatory inputs and *N*-methyl-D-aspartate (NMDA) receptor activation (Grace et al. 2007; Zweifel et al. 2009). Tonic firing provides background concentrations of extracellular DA, whereas phasic activity drives significantly larger release of DA, necessary to drive motivated behaviors. For example, phasic, but not tonic, DA neurons stimulation is sufficient

to drive conditioned place preference and operant self-stimulation (Tsai et al. 2009; Adamantidis et al. 2011). However, DA neuron inhibition below tonic firing frequencies is also necessary to signal reward prediction error and can be aversive (Schultz 1998; Tan et al. 2012). Therefore, the fine-tuning of DA firing modes allows optimal encoding of external stimuli and adequate behavioral adaptation. Here we review the molecular pathways by which $GABA_B$ receptors in DA neurons modulate overall firing rate, occurrence of bursts and DA release. In other words, we define how $GABA_B$ receptors shape the appropriate responsiveness of DA neurons.

8.3.2 $GABA_B$ Receptor-Mediated Modulation of Firing

8.3.2.1 GIRK Channels

The main effect of somatodendritic $GABA_B$ receptors results from the opening of GIRK channels. The efflux of K^+ hyperpolarizes the membrane and decreases input resistance, all of which elevate the threshold for action potential generation. In slice recordings of SNc, bath-application of baclofen readily hyperpolarizes the membrane potential and decreases firing frequency of both DA and GABA neurons (Lacey et al. 1989). In anesthetized rats, systemic injections of baclofen decrease the firing rate and reduce the occurrence of bursts in VTA and SNc DA neurons, in a dose-dependent manner (Olpe et al. 1977). Local microiontophoresis of baclofen in the VTA yields similar results (Engberg et al. 1993; Erhardt et al. 2002). At low concentrations, this effect is probably mediated in part by activation of presynaptic $GABA_B$ receptors at excitatory terminals onto VTA DA neurons, which display higher coupling efficiency than dendritic receptors in DA neurons. However, the effects of higher concentrations of baclofen are likely to reflect a direct hyperpolarization by DA neuron $GABA_B$ receptors. Supporting this hypothesis, baclofen infusion in the VTA and SNc efficiently decrease DA concentrations in the striatum (Westerink et al. 1992). Similarly, baclofen and saclofen (a $GABA_B$ receptor antagonist) decrease and increase, respectively, DA signals in the NAc, recorded in vivo in awake rats (Xi and Stein 1998). It is therefore likely that somatodendritic $GABA_B$ receptors, by decreasing firing at the cell body, indirectly modulate DA release at the terminals.

8.3.2.2 CaV

Unlike many other neuron types, tonic firing in DA cells heavily relies on external Ca^{2+} and CaV channel opening (Harris et al. 1989). In midbrain DA neurons, $GABA_B$ receptor activation decreases Ca^{2+} currents mediated by high voltage-activated CaV channels such as L-, N-, and P/Q-type (Cardozo and Bean 1995), which were shown to help generate tonic firing (Nedergaard et al. 1993; Puopolo et al. 2007). Therefore it is likely that $GABA_B$ receptor activation also inhibits Ca^{2+} channels, potentially decreasing firing in synergy with GIRK channel opening.

8.3.2.3 HCN

HCN channels, recently identified as members of the extended $GABA_B$ receptor signaling complex, are opened following $GABA_B$ receptor activation and shorten the GIRK channel-mediated hyperpolarization. This repolarization goes physiologically against the grain of all other $GABA_B$ receptor modulations, but may rather provide a passive feedback signal to $GABA_B$ receptors. Additionally, HCN channels sustain tonic firing in SNc DA neurons, where their activation by $GABA_B$ receptors could paradoxically maintain, rather than inhibit, firing, however this question remains to be investigated (Schwenk et al. 2016).

8.3.2.4 NMDA Receptors

Finally, $GABA_B$ receptors can decrease Ca^{2+} current through NMDA receptors in cortical neurons, without affecting the amplitude of NMDA or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor EPSC (Chalifoux and Carter 2010). This effect requires the Gα-mediated inhibition of adenylate cyclase and PKA activity, and is independent of GIRK and CaV channels. The existence of such a mechanism has not yet been investigated in DA neurons, but since bursting requires NMDA receptor activation, this signaling pathway might reduce tonic to phasic firing transitions. Additionally, membrane hyperpolarization through GIRK channels may increase the blockade of NMDAR by Mg^{2+} as suggested in the hippocampus, further decreasing NMDA receptor-mediated transmission (Morrisett et al. 1991; Otmakhova and Lisman 2004). However, it is not clear to which extent Ca^{2+} flowing through NMDA receptors contributes to action potential generation.

8.3.3 $GABA_B$ Receptor-Mediated Modulation of DA Release

DA neurons release DA not only from axons in target regions, but also in the vicinity of their cell body, through somatodendritic vesicular release (Björklund and Lindvall 1975; Geffen et al. 1976; Jaffe et al. 1998). Numerous studies have investigated the mechanisms for these two types of DA release, mostly with fast-scan cycling voltammetry and amperometry measurements of external DA concentrations in slice preparations. These methods take advantage of the unique oxidation/reduction potential of DA to measure rapid variations of external concentration or exocytosis, respectively, with the use of carbon fiber microelectrodes. Overall, in both cases action potential-triggered Ca^{2+} influx through CaV channels seems necessary, although there may be regional or species differences in the source of Ca^{2+} and the exact channels involved (Rice et al. 1997; Ford et al. 2010; Hoffman and Gerhardt 1999; Chen et al. 2006).

8.3.3.1 Dendritic Release

Only a few studies have investigated the effect of $GABA_B$ receptor on somatodendritic DA release. As previously mentioned, postsynaptic $GABA_B$ receptors in DA neurons inhibit CaV channels to modulate firing (Cardozo and Bean 1995). Among these, N-, L-, and T-type channels drive dendritic DA release in the SNc (Beckstead et al. 2004; Kim et al. 2008). Thus, although not directly demonstrated, dendritic $GABA_B$ receptors could inhibit CaV channels and decrease vesicular fusion. Indeed, in vivo local infusion of baclofen nearly abolishes external DA concentrations in the SNc and VTA (Westerink et al. 1992; Santiago et al. 1993b; Klitenick et al. 1992). Conversely, inhibiting $GABA_B$ receptors in SNc slices increases voltammetry DA signals (but not in the VTA, Chen and Rice 2002).

8.3.3.2 Axonal Release

DA axonal release in the dorsal and ventral striatum, emanating from the SNc or VTA DA projections, relies mainly on N- and P/Q-type channels activation, with a potential minor participation of R-type channels (Phillips and Stamford 2000; Chen et al. 2006). All of these channels can be inhibited by $GABA_B$ receptors in different brain regions and cell-types, although this has not been directly demonstrated in DA neurons. Actually, the expression and function of $GABA_B$ receptors in DA neuron terminals remains poorly understood. $GABA_B$ receptor messenger ribonucleic acid (mRNA) autoradiography reveals low expression in the striatum (Kaupmann et al. 1998). Electron microscopy in rats revealed the expression of $GABA_B$ receptors at VGlut2-positive terminals in the striatum, which could belong to either thalamic excitatory neurons or VTA DA neurons (Lacey et al. 2005). In primates, rare presynaptic boutons, displaying the structural features of SNc DA terminals, also express the $GABA_{B1}$ subunit (Charara et al. 2000). At the functional level, microdialysis experiments have shown a lack of, or minimal effect of baclofen infusion in the NAc on DA contents (Santiago et al. 1993a; Westerink et al. 1994; Xi et al. 2003), and a modest decrease or increase of DA in the PFC upon $GABA_B$ receptor activation or antagonization, respectively (Santiago et al. 1993a). A couple of more recent studies, recording electrically evoked DA voltammetry signals in the NAc (Pitman et al. 2014) and dorsal striatum (Schmitz et al. 2002) showed that at saturating concentration, baclofen only blocks 20–40 % of the total signal evoked by single pulses, whereas this number further dropped with increased stimulation frequency stimulation. This is highly divergent from the action of presynaptic $GABA_B$ receptors in most other brain regions and cell types, which usually show high coupling efficiency and almost complete blockade of neurotransmitter release (Lüscher et al. 1997; Padgett et al. 2012). Altogether, these results suggest that $GABA_B$ receptors are present in DA neurons terminals, but assume much less of their traditional function as gatekeepers of release.

The assessment of DA axonal $GABA_B$ receptors modulation of neurotransmitter release has been so far difficult because of the absence of fast, DA-mediated postsynaptic current. However, it was recently discovered that DA neuron subpopulations corelease glutamate (Hnasko et al. 2010) or GABA (Tritsch et al. 2012), as measured by postsynaptic excitatory and inhibitory currents in striatal neurons, respectively. Future studies using these ionotropic responses as readouts for presynaptic regulation of release will hopefully yield more specific insight into the function of presynaptic $GABA_B$ receptors in DA terminals.

8.4 Bidirectional Control of DA Neurons by $GABA_B$ Receptor Agonists

In the VTA, GABA neurons provide a major inhibitory input to DA neurons (Johnson and North 1992a). Inhibition of GABA neurons disinhibits DA neurons, whereas GABA neuron activation shuts DA neurons down, and is sufficient to drive aversive behavioral responses (Tan et al. 2012; van Zessen et al. 2012). Therefore, modulating excitability and release from VTA GABA neurons allows for an indirect, yet powerful handle over DA neuron activity. Here, we review how adequate dosage of $GABA_B$ receptor agonists takes advantage of the VTA microcircuit to bidirectionally modulate DA neuron activity, by selectively inhibiting GABA neurons only or both GABA and DA neurons.

8.4.1 Tailored Agonist Dosage for DA Neuron Disinhibition

As mentioned earlier, VTA GABA neurons express $GABA_B$ receptors and GIRK channels, although with a much higher coupling efficiency than DA neurons, reflected by the ten-fold lower EC50 value for baclofen-mediated GIRK currents in GABA versus DA neurons (Fig. 8.1). In addition, and contrary to DA neurons, axonal $GABA_B$ receptors in GABA neurons strongly modulate synaptic release in the VTA, with an EC50 value for baclofen similar to dendritic receptors (Cruz et al. 2004; Padgett et al. 2012). In other words, there is a window of minimal baclofen concentration (about 0.1 μM) at which dendritic and axonal $GABA_B$ receptors are activated in GABA neurons, but not in DA neurons (Fig. 8.1). Thus, this minimal baclofen concentration inhibits GABA neurons, removes inhibition from GABA to DA neurons and therefore disinhibits DA neurons in slices. Higher concentrations then activate $GABA_B$ receptors in DA neurons, initially normalizing and eventually decreasing DA firing (Cruz et al. 2004).

Interestingly, similar observations were reported with the club drug γ-hydroxybutirate (GHB), a low-affinity $GABA_B$ receptor agonist. As with baclofen, the EC50 value for GIRK currents is one order of magnitude lower in GABA neurons than in DA neurons. Furthermore, saturating concentrations of GHB and baclofen evoke GIRK currents of comparable amplitude in GABA neurons, whereas

GHB yields only 30 % of the maximal baclofen-evoked current in DA neurons. Therefore, a low concentration of GHB, sufficient to activate $GABA_B$ receptors in GABA neurons but without effect in DA neurons, leads to the disinhibition of DA neurons (Labouèbe et al. 2007). For both baclofen and GHB, bidirectional control over DA neuron activity takes advantage of two key features of the VTA: First, the microcircuit formed by GABA neurons' inhibitory innervation of DA neurons, and second, the cell-type-specific coupling efficiency of $GABA_B$ receptor signaling.

8.4.2 Physiological Relevance

These experiments provide a cellular mechanism by which increasing concentrations of $GABA_B$ receptor agonists bidirectionally modulate DA neuron activity. More specifically, at low doses, GABA neuron inhibition leads to the disinhibition of DA neurons and increases DA release throughout the brain, which is the hallmark of addictive drugs (Di Chiara and Imperato 1988). Indeed, GHB is self-administered in rodents (Martellotta et al. 1998) and has abuse potential in humans (Nicholson and Balster 2001) at concentrations leading to DA neuron disinhibition in slices. Similarly, human volunteers engaged in a gambling task show increased reinforcement learning with a low dose of baclofen (compared to a larger dose), presumably through the disinhibition of DA neurons (Terrier et al. 2011).

In contrast, higher concentrations of baclofen or GHB decrease DA neuron activity and DA release, by hyperpolarizing the membrane and increasing the threshold for burst firing. Moreover, agonist concentrations sufficient to activate $GABA_B$ receptors in DA neurons are also likely to activate high coupling efficiency presynaptic receptors at excitatory inputs, decreasing glutamate release (Padgett et al. 2012). Accordingly, baclofen infusion in the VTA of rodents reduces self-administration of various addictive drugs (Shoaib et al. 1998; Xi and Stein 2000; Corrigall et al. 2000; see also Chaps. 14 and 15 of this book). Baclofen is also used in humans as an anticraving agent for addictive drugs like cocaine and alcohol (Ling et al. 1998; Ameisen 2005; Addolorato et al. 2009; see also Chaps. 14 and 15 of this book), an effect occasionally observed with GHB itself (Gallimberti et al. 1989).

8.5 Plasticity of $GABA_B$ Receptor Signaling

While most studies on synaptic plasticity examine excitatory transmission, various forms of plasticity of $GABA_A$ receptor-mediated transmission have been identified (Castillo et al. 2011; Kullmann et al. 2012). Only a handful of studies have characterized activity-dependent and drug-evoked plasticity of $GABA_B$ receptor signaling. Here we review these observations with an emphasis on those occurring in the mesolimbic circuit, and discuss their induction and expression mechanisms as well as their functional implications.

8.5.1 Activity-Dependent Plasticity

8.5.1.1 Induction

The induction of all forms of activity-dependent $GABA_B$ receptor signaling plasticity described so far requires NMDA receptor activation. Various induction protocols, like electrical stimulation of glutamate release and postsynaptic membrane depolarization in slices, or bath application of NMDA receptor agonists in cell cultures, lead to potentiation or depression of $GABA_B$ receptor signaling. The direction of the plasticity is then specified by distinct expression mechanisms.

8.5.1.2 Potentiation

The first electrophysiological study to report activity-dependent synaptic plasticity of $GABA_B$ receptors in cell cultures and acute hippocampal slices showed that a classic AMPA receptor long-term potentiation protocol potentiates $GABA_B$ receptor-mediated IPSCs. The induction requires electrical stimulation of synaptic glutamate release paired with postsynaptic membrane depolarization, which activates NMDA receptors, increases intracellular Ca^{2+}, and recruits Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII; Huang et al. 2005). Similar induction mechanism was recently observed in VTA DA neurons, in which sustained depolarization or burst firing leads to the potentiation of $GABA_B$ receptor-GIRK currents (Lalive et al. 2014). Blocking the trafficking of GIRK channels by interfering specifically with PDZ-binding domains on GIRK2c or GIRK3 prevents the potentiation of $GABA_B$ receptor-GIRK currents in DA neurons, suggesting that $GABA_B$-GIRK plasticity is expressed through surface delivery of heteromeric GIRK2c/3 channels in DA neurons (Fig. 8.2). Expression mechanisms in the hippocampus could additionally involve protein phosphatase (PP) 1-dependent phosphorylation of Serine (S) 9 residue on GIRK2 to modulate channel trafficking (Chung et al. 2009). Lastly, NMDA application transiently increases $GABA_B$ receptor surface expression by recruiting 5′-adenosine monophosphate-activated kinase (AMPK)-mediated phosphorylation at S783 on the $GABA_{B2}$ subunit in cortical neuron cultures (Terunuma et al. 2010). A similar increase was also reported following glycine-mediated synaptic NMDA receptor activation, which was prevented by inhibiting protein recycling (Kantamneni et al. 2014).

8.5.1.3 Depression

In cultured hippocampal neurons, prolonged (30 min) NMDA receptor activation induces a Ca^{2+}- and CaMKII-dependent depression of $GABA_B$ receptor signaling. CaMKII specifically phosphorylates S892 on $GABA_{B1b}$, which drives the internalization of $GABA_B$ receptors (Guetg et al. 2010). In cultured cortical neurons, a similar induction protocol depresses baclofen-evoked currents. The exact induction mechanism is not identified, however expression requires increased PP2 activity

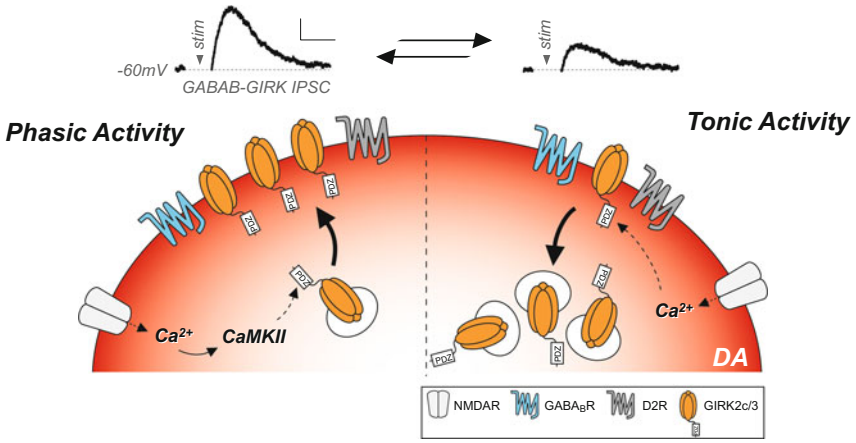

Fig. 8.2 Dopamine neuron firing drives bidirectional GIRK channel plasticity. (*Left*) NMDA receptor and phasic activity drive Ca^{2+} entry and CaMKII activation, engaging a molecular pathway interacting with GIRK2c/3 PDZ domain and trafficking channels at the membrane. This results in the potentiation of GABA$_B$ and D2 receptor-GIRK currents, illustrated by the example recording of a large GABA$_B$ receptor-GIRK IPSC, evoked by electrical stimulation at −60 mV (*top inset*). (*Right*) Tonic activity or tonic NMDA receptor activation drives Ca^{2+} entry, triggering an undetermined molecular cascade eventually driving the internalization of GIRK2c/3 channels through a PDZ domain interaction, resulting in the depression of GABA$_B$ and potentially D2 receptor-GIRK currents, as shown by the reduced amplitude of electrically evoked GABA$_B$ receptor-GIRK IPSC (*top inset*). Scale: 20pA, 200 ms [*Inset* traces reproduced from Lalive et al. 2014]

and dephosphorylation of GABA$_{B2}$ S783, leading to decreased surface expression of GABA$_B$ receptors (Terunuma et al. 2010). It appears that dephosphorylation of GABA$_{B2}$ does not directly trigger the internalization of the receptor, but rather modifies basal recycling at the level of postendocytic sorting. This mechanism has since been implicated in GABA$_B$ receptor plasticity in VTA GABA neurons and PFC (Padgett et al. 2012; Hearing et al. 2013) (further described in the drug-evoked plasticity section below), and in the lateral habenula following exposure to aversive stimuli (Lecca et al. 2016), suggesting a crucial and widely distributed plasticity mechanism. In VTA DA neurons, low frequency tonic firing or synaptic activation of NMDA receptors for 5 min decreases the amplitude of GABA$_B$ receptor-GIRK IPSC (Fig. 8.2). Similarly to the burst-induced potentiation, this plasticity is expressed by trafficking of GIRK3-containing channels (Lalive et al. 2014).

8.5.1.4 Functional Significance

Overall, GABA$_B$ receptor plasticity seems tightly tuned to neuronal excitation. It is striking that all of the above-described mechanisms rely on excitatory input stimulation for their induction. This may then lead to NMDA receptor activation

or action potential firing, suggesting that plasticity of $GABA_B$ receptor signaling is an adaptive response to changes in neuronal activity (Fig. 8.2). A unifying interpretation would suggest that this inhibitory plasticity is called in as a compensatory, homeostatic-like adaption, where $GABA_B$ receptor function is potentiated following sustained activity, but reduced after periods of little activity. This hypothesis is especially supported in VTA DA neurons, where GIRK channel upregulation is functionally not restricted to $GABA_B$ receptors, but also potentiates Dopamine 2 receptor-GIRK currents, thereby strengthening another inhibitory pathway (Lalive et al. 2014). Overall, $GABA_B$ receptor plasticity further emphasizes the second-order nature of $GABA_B$ receptor function in tempering neuronal excitability and responsiveness to relevant synaptic signals.

8.5.2 Drug-Evoked Plasticity of $GABA_B$ Receptor Signaling

A hallmark of addictive drugs is the induction of excitatory synaptic plasticity in VTA DA neurons after a single exposure, which eventually spreads to other nuclei following repeated intake or withdrawal periods and underlies the development of addictive behaviors (Lüscher and Malenka 2011). Similarly, drugs of abuse evoke various forms of $GABA_B$ receptor signaling plasticity (Table 8.2), however their functional significance is debated.

8.5.2.1 Early Observations

Early work revealed a decrease in $G\alpha_{i/o}$ protein in the VTA and NAc following chronic but not acute cocaine treatment in rats, hinting at metabotropic signaling alterations (Nestler et al. 1990). Similar results were later obtained after withdrawal from chronic psychostimulants (Zhang et al. 2000; Xi et al. 2003; Giorgetti et al. 2002). Additionally, most GIRK knockout mice show altered drug-related behavior (Luján et al. 2014). Altogether these results pointed early on toward a potential modulation of $GABA_B$ receptor signaling following exposure to drugs of abuse. Unfortunately the techniques employed in these studies do not allow the distinction between neuron types and axonal from dendritic $GABA_B$ receptors, blurring functional interpretation. Patch-clamp studies in identified cell types have then helped overcome these confounds and shed light on the molecular mechanisms underlying drug-evoked plasticity of $GABA_B$ receptor signaling.

8.5.2.2 Plasticity in VTA Neurons

A single exposure to psychostimulants like cocaine and methamphetamine is sufficient to evoke a decrease in $GABA_B$ receptor-GIRK currents in VTA DA and GABA neurons (Padgett et al. 2012; Arora et al. 2011). This adaptation is also observed in DA neurons

Table 8.2 Summary of $GABA_B$ receptor signaling plasticity in the VTA

	Cell-type	Plasticity	Protocol	Induction	Expression
Activity-dependent	DA	Potentiation (IPSC and baclofen)	Depolarization, burst firing	NMDAR	GIRK2c/3 trafficking
				Ca^{2+}	Lalive et al. (2014)
				CaMKII	
	DA	Depression (IPSC)	Tonic firing	NMDAR	GIRK2c/3 trafficking
					Lalive et al. (2014)
Drug-evoked	DA	Depression (baclofen, but IPSC unchanged)	Cocaine, meth (1–5 days)	D1R, D2R	GIRK channel internalization
		Self-administration (14 days)			Arora et al. (2011), Munoz et al. (2016), and Sharpe et al. (2015)
	DA	Increased coupling efficiency	Morphine, GHB (5 days)	?	RGS2 downregulation
					Labouèbe et al. (2007)
	GABA	Depression (IPSC, baclofen)	Cocaine, meth (1 day)	D1R	PP2A dephosphorylation of GABAB2 S783; GABABR-GIRK internalization
					Padgett et al. (2012)
	VTA-projecting PFC	Depression (baclofen, somatodendritic)	Cocaine (5 days)	D1R	PP2A dephosphorylation of GABAB2 S783; GABABR-GIRK internalization
					Hearing et al. (2013)
	Excitatory inputs to DA	Increased coupling efficiency (axonal)	Morphine (6 days)	?	?
					Manzoni and Williams (1999)

after repeated passive injections (Munoz et al. 2016) or active self-administration of psychostimulants (Sharpe et al. 2015). In both cell types, plasticity induction requires DA type 1 (D1) and DA type 2 (D2) receptor activation, however the molecular pathways underlying plasticity expression are highly different.

In DA neurons, the reduction in $GABA_B$ receptor signaling is mediated by a decrease in GIRK channel density (but not $GABA_B$ receptors) at the membrane (Arora et al. 2011). Interestingly, in DA neurons lacking the GIRK3 subunit, psychostimulants fail to depress baclofen-evoked currents, suggesting that trafficking of GIRK2c/3 heteromers is required (Munoz et al. 2016), similar to activity-dependent GIRK plasticity described in the same cells (Lalive et al. 2014). Additionally, DA neurons lacking SNX27 display blunted baclofen-evoked currents (Munoz and Slesinger 2014). SNX27 is involved in trafficking of GIRK2c/3 channels and is sensitive to drugs of abuse (Kajii et al. 2003), and may therefore participate to $GABA_B$ receptor signaling plasticity in DA neurons.

In GABA neurons, the decrease in $GABA_B$ receptor signaling is also observed in axon terminals, where the coupling efficiency is reduced (Padgett et al. 2012). The reduction in $GABA_B$ receptor-GIRK currents is due to enhanced PP2A-mediated dephosphorylation of $GABA_{B2}$ S783, a pathway previously identified for activity-dependent depression of $GABA_B$ receptor signaling (Terunuma et al. 2010). As a result, $GABA_B$ receptors and GIRK channels are internalized but not degraded, and acute PP2A inhibition rescues the full amplitude of baclofen-evoked currents. Furthermore, this plasticity is blocked in a mouse expressing a $GABA_{B2}$ subunit insensitive to PP2A dephosphorylation (Munoz et al. 2016). Interestingly, GIRK channels do not interact with PP2A but are also removed from the membrane, possibly because of their proximity to $GABA_B$ receptors (Padgett et al. 2012).

Drugs other than psychostimulants also evoke $GABA_B$ receptor signaling plasticity. Chronic GHB or morphine treatment increases the coupling efficiency of $GABA_B$ receptors to GIRK channels in VTA DA neurons. This specific effect is mediated by the downregulation of RGS2, a protein acting as a brake on G protein signaling. Consistent with this, the coupling efficiency is increased in RGS2 knockout mice, and drug treatment has no further effect (Labouèbe et al. 2007).

8.5.2.3 Plasticity of VTA Afferents

In VTA-projecting PFC neurons, repeated cocaine injection decreases baclofen-evoked currents in a D1 receptor-dependent fashion (Hearing et al. 2013). $GABA_B$ receptors are internalized following PP2A-dependent S783 GABAB2 dephosphorylation, similar to what was described in VTA GABA neurons (Padgett et al. 2012). It is not known whether $GABA_B$ receptor signaling is also altered in axons projecting to the VTA. Interestingly, chronic morphine increases the coupling efficiency of presynaptic $GABA_B$ receptors at excitatory terminals onto DA neurons (Manzoni and Williams 1999). However, this was observed with nonspecific electrical stimulation and may not reflect $GABA_B$ receptor function at all axon terminals.

8.5.2.4 Functional Significance

In DA neurons, the reduction in maximal baclofen-evoked current after psychostimulant exposure is paralleled with a mild decrease in the ability of $GABA_B$ receptors to block neuronal activity (Munoz et al. 2016). In other words, DA neurons are partially relieved from the rule of $GABA_B$ receptors. However, in the same conditions synaptically evoked $GABA_B$ receptor GIRK IPSCs, which reflect a more physiological mode of $GABA_B$ receptor activation, are unaffected (Padgett et al. 2012). This suggests that despite GIRK channel internalization, $GABA_B$ receptor signaling is still fully functional under physiological levels of activation. In stark contrast, synaptically evoked $GABA_B$ receptor-GIRK IPSC in VTA GABA neurons are absent following drug exposure, arguing for a physiologically relevant functional deficiency. Accordingly, $GABA_B$ receptor activation, even with saturating concentrations of baclofen, is unable to decrease firing (Padgett et al. 2012). Therefore, the VTA microcircuit is altered: GABA neuron activity is less likely to be inhibited, and its inhibitory control over DA neurons may be strengthened. Similarly, the increase in coupling efficiency in DA neurons after morphine treatment empowers $GABA_B$ receptors to modulate DA neuron excitability. Indeed, DA neurons are now inhibited by a minimal concentration of baclofen (0.1 μM) that would only affect GABA neurons in control conditions (Labouèbe et al. 2007). To fully predict the net functional effect of these forms of plasticity, two key questions need to be answered. First of all, do these plasticities represent a uniform and simultaneous adaptation following exposure to any drug of abuse, like it has been suggested for excitatory plasticity (Lüscher and Ungless 2006), or are they tailor-cut to specific drugs? For example, a decrease in $GABA_B$ receptor GIRK current amplitude in DA neurons, as seen after psychostimulant exposure (Arora et al. 2011), might be compensated for by an increase in coupling efficiency, as observed after morphine treatment (Labouèbe et al. 2007), resulting in no functional difference. Second, what is the actual concentration of endogenous agonist to which $GABA_B$ receptors are exposed in vivo? In other words, thus far it has been difficult to infer the physiological function of $GABA_B$ receptors from maximal baclofen-evoked current amplitude.

8.5.2.5 Drug-Evoked Plasticity: Compensatory or Contributory?

Drug-evoked excitatory synaptic plasticity is believed to progressively modify circuit function and eventually lead to the development of addictive behaviors (Lüscher and Malenka 2011). Repeated drug exposure increases DA neuron activity and excitability (White 1996; Henry et al. 1989). However, it is not clear whether $GABA_B$ receptor signaling plasticity subserves or counteracts these circuit changes. The loss of $GABA_B$ receptor function in VTA GABA neurons argues for a compensatory mechanism, promoting GABA release onto DA neurons to limit their activity (Padgett et al. 2012). However, mimicking the drug-evoked plasticity by downregulating GIRK channels in VTA DA neurons or PFC increases the acute locomotor response to psychostimulant, suggesting an active participation to the behavioral effects of addictive drugs (Munoz and Slesinger 2014; Hearing et al. 2013).

Over recent years $GABA_B$ receptor plasticity has emerged as a cellular mechanism relevant to neuronal activity and circuit function. Activity-dependent and drug-evoked adaptations occur in different cell types and regions, concurrently with excitatory plasticity. Whereas excitatory synaptic plasticity is usually perceived as the primary neural correlate of experience, $GABA_B$ receptor plasticity rather appears as a secondary adaptation that may either stabilize or derail neuronal excitability, especially in DA neurons. Future studies will have to causally assess the contribution of $GABA_B$ receptor signaling plasticity to neuronal activity and behavioral alterations in the context of addiction disease.

8.6 Conclusions

$GABA_B$ receptors engage diverse signaling pathways to hyperpolarize somatodendritic compartments and reduce probability of neurotransmitter release, thus exerting a pre- and postsynaptic modulation of neural activity. In the VTA, the cell-type-specific composition of $GABA_B$ receptor complexes determines the signaling efficacy and allows for bidirectional control of DA output through inhibition of local interneurons. $GABA_B$ receptor agonists may thus lead to disinhibition at low concentration, while inhibiting DA neurons at higher doses. This may explain the reinforcing effects of baclofen and GHB and associated risk for addiction. This model also accounts for the anti-craving effect of high doses of baclofen.

In DA neurons, where dynamic switching between pauses, tonic, and phasic firing occurs with experience, $GABA_B$ receptors control the excitability and adjust responsiveness to relevant synaptic inputs. Furthermore, $GABA_B$ receptor signaling can undergo plasticity and adapts to changes in neuronal activity, which may constitute a compensatory mechanism to maintain physiological neuronal activity. Addictive drugs also alter $GABA_B$ receptor signaling throughout the mesolimbic system, however the relevance of this plasticity for the development of addictive behavior remains elusive.

Altogether, $GABA_B$ receptors are effective and dynamic modulators of the DA system, and form a target with potential for pharmacological interventions in humans.

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References

Adamantidis, A. R., Tsai, H.-C., Boutrel, B., Zhang, F., Stuber, G. D., Budygin, E. A., et al. (2011). Optogenetic interrogation of dopaminergic modulation of the multiple phases of reward-seeking behavior. *The Journal of Neuroscience, 31*, 10829–10835.

Addolorato, G., Leggio, L., Cardone, S., Ferrulli, A., & Gasbarrini, G. (2009). Role of the GABA(B) receptor system in alcoholism and stress: Focus on clinical studies and treatment perspectives. *Alcohol (Fayetteville, NY), 43*, 559–563.

Ameisen, O. (2005). Complete and prolonged suppression of symptoms and consequences of alcohol-dependence using high-dose baclofen: A self-case report of a physician. *Alcohol and Alcoholism (Oxford, Oxfordshire), 40*, 147–150.

Arora, D., Haluk, D. M., Kourrich, S., Pravetoni, M., Fernández-Alacid, L., Nicolau, J. C., et al. (2010). Altered neurotransmission in the mesolimbic reward system of Girk mice. *Journal of Neurochemistry, 114*, 1487–1497.

Arora, D., Hearing, M., Haluk, D. M., Mirkovic, K., Fajardo-Serrano, A., Wessendorf, M. W., et al. (2011). Acute cocaine exposure weakens GABAB receptor-dependent G-protein-gated inwardly rectifying K+ signaling in dopamine neurons of the ventral tegmental area. *The Journal of Neuroscience, 31*, 12251–12257.

Balana, B., Bahima, L., Bodhinathan, K., Taura, J. J., Taylor, N. M., Nettleton, M. Y., et al. (2013). Ras-association domain of sorting Nexin 27 is critical for regulating expression of GIRK potassium channels. *PLoS One, 8*, e59800.

Beckstead, M. J., Grandy, D. K., Wickman, K., & Williams, J. T. (2004). Vesicular dopamine release elicits an inhibitory postsynaptic current in midbrain dopamine neurons. *Neuron, 42*, 939–946.

Beier, K. T., Steinberg, E. E., DeLoach, K. E., Xie, S., Miyamichi, K., Schwarz, L., et al. (2015). Circuit architecture of VTA dopamine neurons revealed by systematic input-output mapping. *Cell, 162*, 622–634.

Bettler, B., Kaupmann, K., Mosbacher, J., & Gassmann, M. (2004). Molecular structure and physiological functions of GABA(B) receptors. *Physiological Reviews, 84*, 835–867.

Björklund, A., & Lindvall, O. (1975). Dopamine in dendrites of substantia nigra neurons: Suggestions for a role in dendritic terminals. *Brain Research, 83*, 531–537.

Bocklisch, C., Pascoli, V., Wong, J. C. Y., House, D. R. C., Yvon, C., De Roo, M., et al. (2013). Cocaine disinhibits dopamine neurons by potentiation of GABA transmission in the ventral tegmental area. *Science, 341*, 1521–1525.

Boyes, J., & Bolam, J. P. (2003). The subcellular localization of GABA(B) receptor subunits in the rat substantia nigra. *The European Journal of Neuroscience, 18*, 3279–3293.

Brazhnik, E., Shah, F., & Tepper, J. M. (2008). GABAergic afferents activate both GABAA and GABAB receptors in mouse substantia nigra dopaminergic neurons in vivo. *The Journal of Neuroscience, 28*, 10386–10398.

Cameron, D. L., & Williams, J. T. (1993). Dopamine D1 receptors facilitate transmitter release. *Nature, 366*, 344–347.

Cardozo, D. L., & Bean, B. P. (1995). Voltage-dependent calcium channels in rat midbrain dopamine neurons: Modulation by dopamine and GABAB receptors. *Journal of Neurophysiology, 74*, 1137–1148.

Castillo, P. E., Chiu, C. Q., & Carroll, R. C. (2011). Long-term plasticity at inhibitory synapses. *Current Opinion in Neurobiology, 21*, 328–338.

Chalifoux, J. R., & Carter, A. G. (2010). GABAB receptors modulate NMDA receptor calcium signals in dendritic spines. *Neuron, 66*, 101–113.

Charara, A., Heilman, T. C., Levey, A. I., & Smith, Y. (2000). Pre- and postsynaptic localization of GABA(B) receptors in the basal ganglia in monkeys. *Neuroscience, 95*, 127–140.

Chen, B. T., Moran, K. A., Avshalumov, M. V., & Rice, M. E. (2006). Limited regulation of somatodendritic dopamine release by voltage-sensitive Ca channels contrasted with strong regulation of axonal dopamine release. *Journal of Neurochemistry, 96*, 645–655.

Chen, B. T., & Rice, M. E. (2002). Synaptic regulation of somatodendritic dopamine release by glutamate and GABA differs between substantia nigra and ventral tegmental area. *Journal of Neurochemistry, 81*, 158–169.

Chung, H. J., Qian, X., Ehlers, M., Jan, Y. N., & Jan, L. Y. (2009). Neuronal activity regulates phosphorylation-dependent surface delivery of G protein-activated inwardly rectifying potassium channels. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 629–634.

Ciruela, F., Fernández-Dueñas, V., Sahlholm, K., Fernández-Alacid, L., Nicolau, J. C., Watanabe, M., et al. (2010). Evidence for oligomerization between GABA(B) receptors and GIRK channels containing the GIRK1 and GIRK3 subunits. *The European Journal of Neuroscience, 32*(8), 1265–1277.

Corrigall, W. A., Coen, K. M., Adamson, K. L., Chow, B. L., & Zhang, J. (2000). Response of nicotine self-administration in the rat to manipulations of mu-opioid and gamma-aminobutyric acid receptors in the ventral tegmental area. *Psychopharmacology, 149*, 107–114.

Couve, A., Thomas, P., Calver, A. R., Hirst, W. D., Pangalos, M. N., Walsh, F. S., et al. (2002). Cyclic AMP-dependent protein kinase phosphorylation facilitates GABA(B) receptor-effector coupling. *Nature Neuroscience, 5*, 415–424.

Cruz, H. G., Ivanova, T., Lunn, M.-L., Stoffel, M., Slesinger, P. A., & Lüscher, C. (2004). Bi-directional effects of GABA(B) receptor agonists on the mesolimbic dopamine system. *Nature Neuroscience, 7*, 153–159.

David, M., Richer, M., Mamarbachi, A. M., Villeneuve, L. R., Dupré, D. J., & Hebert, T. E. (2006). Interactions between GABA-B1 receptors and Kir 3 inwardly rectifying potassium channels. *Cellular Signalling, 18*, 2172–2181.

Di Chiara, G., & Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proceedings of the National Academy of Sciences of the United States of America, 85*, 5274–5278.

Dobi, A., Margolis, E. B., Wang, H.-L., Harvey, B. K., & Morales, M. (2010). Glutamatergic and nonglutamatergic neurons of the ventral tegmental area establish local synaptic contacts with dopaminergic and nondopaminergic neurons. *The Journal of Neuroscience, 30*, 218–229.

Doupnik, C. A., Jaén, C., & Zhang, Q. (2004) *Measuring the modulatory effects of RGS proteins on GIRK channels* (pp 131–154). Department of Physiology and Biophysics, University of South Florida College of Medicine. Tampa: Elsevier.

Engberg, G., Kling-Petersen, T., & Nissbrandt, H. (1993). GABAB-receptor activation alters the firing pattern of dopamine neurons in the rat substantia nigra. *Synapse (New York, NY), 15*, 229–238.

Erhardt, S., Mathé, J. M., Chergui, K., Engberg, G., & Svensson, T. H. (2002). GABA(B) receptor-mediated modulation of the firing pattern of ventral tegmental area dopamine neurons in vivo. *Naunyn-Schmiedeberg's Archives of Pharmacology, 365*, 173–180.

Ford, C. P., Gantz, S. C., Phillips, P. E. M., & Williams, J. T. (2010). Control of extracellular dopamine at dendrite and axon terminals. *The Journal of Neuroscience, 30*, 6975–6983.

Fowler, C. E., Aryal, P., Suen, K.-F., & Slesinger, P. A. (2007). Evidence for association of GABAB receptors with Kir3 channels and regulators of G protein signalling (RGS4) proteins. *The Journal of Physiology, 580*, 51–65.

Gallimberti, L., Canton, G., Gentile, N., Ferri, M., Cibin, M., Ferrara, S. D., et al. (1989). Gamma-hydroxybutyric acid for treatment of alcohol withdrawal syndrome. *Lancet (London, England), 2*, 787–789.

Geffen, L. B., Jessell, T. M., Cuello, A. C., & Iversen, L. L. (1976). Release of dopamine from dendrites in rat substantia nigra. *Nature, 260*, 258–260.

Giorgetti, M., Hotsenpiller, G., Froestl, W., & Wolf, M. E. (2002). In vivo modulation of ventral tegmental area dopamine and glutamate efflux by local GABA(B) receptors is altered after repeated amphetamine treatment. *Neuroscience, 109*, 585–595.

Grace, A. A., & Bunney, B. S. (1984a). The control of firing pattern in nigral dopamine neurons: Burst firing. *The Journal of Neuroscience, 4*, 2877–2890.

Grace, A. A., & Bunney, B. S. (1984b). The control of firing pattern in nigral dopamine neurons: Single spike firing. *The Journal of Neuroscience, 4*, 2866–2876.

Grace, A. A., Floresco, S. B., Goto, Y., & Lodge, D. J. (2007). Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. *Trends in Neurosciences, 30*, 220–227.

Guetg, N., Abdel Aziz, S., Holbro, N., Turecek, R., Rose, T., Seddik, R., et al. (2010). NMDA receptor-dependent GABAB receptor internalization via CaMKII phosphorylation of serine 867 in GABAB1. *Proceedings of the National Academy of Sciences of the United States of America, 107*(31), 13924–13929.

Harris, N. C., Webb, C., & Greenfield, S. A. (1989). A possible pacemaker mechanism in pars compacta neurons of the guinea-pig substantia nigra revealed by various ion channel blocking agents. *Neuroscience, 31*, 355–362.

Hearing, M., Kotecki, L., Marron Fernandez de Velasco, E., Fajardo-Serrano, A., Chung, H. J., Luján, R., et al. (2013). Repeated cocaine weakens GABAB-Girk signaling in layer 5/6 pyramidal neurons in the prelimbic cortex. *Neuron, 80*, 159–170.

Henry, D. J., Greene, M. A., & White, F. J. (1989). Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: Repeated administration. *The Journal of Pharmacology and Experimental Therapeutics, 251*, 833–839.

Hnasko, T. S., Chuhma, N., Zhang, H., Goh, G. Y., Sulzer, D., Palmiter, R. D., et al. (2010). Vesicular glutamate transport promotes dopamine storage and glutamate corelease in vivo. *Neuron, 65*, 643–656.

Hoffman, A. F., & Gerhardt, G. A. (1999). Differences in pharmacological properties of dopamine release between the substantia nigra and striatum: An in vivo electrochemical study. *The Journal of Pharmacology and Experimental Therapeutics, 289*, 455–463.

Huang, C. S., Shi, S.-H., Ule, J., Ruggiu, M., Barker, L. A., Darnell, R. B., et al. (2005). Common molecular pathways mediate long-term potentiation of synaptic excitation and slow synaptic inhibition. *Cell, 123*, 105–118.

Inanobe, A., Yoshimoto, Y., Horio, Y., Morishige, K. I., Hibino, H., Matsumoto, S., et al. (1999). Characterization of G-protein-gated K+ channels composed of Kir3.2 subunits in dopaminergic neurons of the substantia nigra. *The Journal of Neuroscience, 19*, 1006–1017.

Jaffe, E. H., Marty, A., Schulte, A., & Chow, R. H. (1998). Extrasynaptic vesicular transmitter release from the somata of substantia nigra neurons in rat midbrain slices. *The Journal of Neuroscience, 18*, 3548–3553.

Johnson, S. W., & North, R. A. (1992a). Opioids excite dopamine neurons by hyperpolarization of local interneurons. *The Journal of Neuroscience, 12*, 483–488.

Johnson, S. W., & North, R. A. (1992b). Two types of neurone in the rat ventral tegmental area and their synaptic inputs. *The Journal of Physiology, 450*, 455–468.

Jones, K. A., Borowsky, B., Tamm, J. A., Craig, D. A., Durkin, M. M., Dai, M., et al. (1998). GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2. *Nature, 396*, 674–679.

Joubert, L., Hanson, B., Barthet, G., Sebben, M., Claeysen, S., Hong, W., et al. (2004). New sorting nexin (SNX27) and NHERF specifically interact with the 5-HT4a receptor splice variant: Roles in receptor targeting. *Journal of Cell Science, 117*, 5367–5379.

Kajii, Y., Muraoka, S., Hiraoka, S., Fujiyama, K., Umino, A., & Nishikawa, T. (2003). A developmentally regulated and psychostimulant-inducible novel rat gene mrt1 encoding PDZ-PX proteins isolated in the neocortex. *Molecular Psychiatry, 8*, 434–444.

Kantamneni, S., Gonzàlez-Gonzàlez, I. M., Luo, J., Cimarosti, H., Jacobs, S. C., Jaafari, N., et al. (2014). Differential regulation of GABAB receptor trafficking by different modes of N-methyl-D-aspartate (NMDA) receptor signaling. *The Journal of Biological Chemistry, 289*, 6681–6694.

Kaupmann, K., Malitschek, B., Schuler, V., Heid, J., Froestl, W., Beck, P., et al. (1998). GABA(B)-receptor subtypes assemble into functional heteromeric complexes. *Nature, 396*, 683–687.

Khaliq, Z. M., & Bean, B. P. (2010). Pacemaking in dopaminergic ventral tegmental area neurons: Depolarizing drive from background and voltage-dependent sodium conductances. *The Journal of Neuroscience, 30*, 7401–7413.

Kim, Y., Park, M. K., & Chung, S. (2008). Voltage-operated Ca^{2+} channels regulate dopamine release from somata of dopamine neurons in the substantia nigra pars compacta. *Biochemical and Biophysical Research Communications, 373*, 665–669.

Klitenick, M. A., DeWitte, P., & Kalivas, P. W. (1992). Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA: An in vivo microdialysis study. *The Journal of Neuroscience, 12*, 2623–2632.

Koyrakh, L., Luján, R., Colón, J., Karschin, C., Kurachi, Y., Karschin, A., et al. (2005). Molecular and cellular diversity of neuronal G-protein-gated potassium channels. *The Journal of Neuroscience, 25*, 11468–11478.

Kullmann, D. M., Moreau, A. W., Bakiri, Y., & Nicholson, E. (2012). Plasticity of inhibition. *Neuron, 75*, 951–962.

Labouèbe, G., Lomazzi, M., Cruz, H. G., Creton, C., Luján, R., Li, M., et al. (2007). RGS2 modulates coupling between GABAB receptors and GIRK channels in dopamine neurons of the ventral tegmental area. *Nature Neuroscience, 10*, 1559–1568.

Lacey, C. J., Boyes, J., Gerlach, O., Chen, L., Magill, P. J., & Bolam, J. P. (2005). GABA(B) receptors at glutamatergic synapses in the rat striatum. *Neuroscience, 136*, 1083–1095.

Lacey, M. G., Mercuri, N. B., & North, R. A. (1989). Two cell types in rat substantia nigra zona compacta distinguished by membrane properties and the actions of dopamine and opioids. *The Journal of Neuroscience, 9*, 1233–1241.

Lalive, A. L., Munoz, M. B., Bellone, C., Slesinger, P. A., Lüscher, C., & Tan, K. R. (2014). Firing modes of dopamine neurons drive bidirectional GIRK channel plasticity. *The Journal of Neuroscience, 34*, 5107–5114.

Lauffer, B. E. L., Melero, C., Temkin, P., Lei, C., Hong, W., Kortemme, T., et al. (2010). SNX27 mediates PDZ-directed sorting from endosomes to the plasma membrane. *The Journal of Cell Biology, 190*, 565–574.

Lecca, S., Pelosi, A., Tchenio, A., Moutkine, I., Luján, R., Hervé, D., et al. (2016). Rescue of GABAB and GIRK function in the lateral habenula by protein phosphatase 2A inhibition ameliorates depression-like phenotypes in mice. *Nature Medicine, 22*, 254–261.

Ling, W., Shoptaw, S., & Majewska, D. (1998). Baclofen as a cocaine anti-craving medication: A preliminary clinical study. *Neuropsychopharmacology, 18*, 403–404.

Luján, R., Marron Fernandez de Velasco, E., Aguado, C., & Wickman, K. (2014). New insights into the therapeutic potential of Girk channels. *Trends in Neurosciences, 37*, 20–29.

Lunn, M.-L., Nassirpour, R., Arrabit, C., Tan, J., McLeod, I., Arias, C. M., et al. (2007). A unique sorting nexin regulates trafficking of potassium channels via a PDZ domain interaction. *Nature Neuroscience, 10*, 1249–1259.

Lüscher, C., Jan, L. Y., Stoffel, M., Malenka, R. C., & Nicoll, R. A. (1997). G protein-coupled inwardly rectifying K+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron, 19*, 687–695.

Lüscher, C., & Malenka, R. C. (2011). Drug-evoked synaptic plasticity in addiction: From molecular changes to circuit remodeling. *Neuron, 69*, 650–663.

Lüscher, C., & Slesinger, P. A. (2010). Emerging roles for G protein-gated inwardly rectifying potassium (GIRK) channels in health and disease. *Nature Reviews Neuroscience, 11*(5), 301–315.

Lüscher, C., & Ungless, M. A. (2006). The mechanistic classification of addictive drugs. *PLoS Medicine, 3*, e437.

Manzoni, O. J., & Williams, J. T. (1999). Presynaptic regulation of glutamate release in the ventral tegmental area during morphine withdrawal. *The Journal of Neuroscience, 19*, 6629–6636.

Martellotta, M. C., Cossu, G., Fattore, L., Gessa, G. L., & Fratta, W. (1998). Intravenous self-administration of gamma-hydroxybutyric acid in drug-naive mice. *European Neuropsychophar macology, 8*, 293–296.

Metz, M., Gassmann, M., Fakler, B., Schaeren-Wiemers, N., & Bettler, B. (2011). Distribution of the auxiliary GABAB receptor subunits KCTD8, 12, 12b, and 16 in the mouse brain. *The Journal of Comparative Neurology, 519*, 1435–1454.

Morrisett, R. A., Mott, D. D., Lewis, D. V., Swartzwelder, H. S., & Wilson, W. A. (1991). GABAB-receptor-mediated inhibition of the N-methyl-D-aspartate component of synaptic transmission in the rat hippocampus. *The Journal of Neuroscience, 11*, 203–209.

Munoz, M. B., Padgett, C. L., Rifkin, R., Terunuma, M., Wickman, K., Contet, C., et al. (2016). A role for the GIRK3 subunit in methamphetamine-induced attenuation of GABAB receptor-activated GIRK currents in VTA dopamine neurons. *The Journal of Neuroscience, 36*, 3106–3114.

Munoz, M. B., & Slesinger, P. A. (2014). Sorting nexin 27 regulation of G protein-gated inwardly rectifying K^+ channels attenuates in vivo cocaine response. *Neuron, 82*, 659–669.

Mutneja, M., Berton, F., Suen, K.-F., Lüscher, C., & Slesinger, P. A. (2005). Endogenous RGS proteins enhance acute desensitization of GABA(B) receptor-activated GIRK currents in HEK-293T cells. *Pflügers Archiv / European Journal of Physiology, 450*, 61–73.

Nedergaard, S., Flatman, J. A., & Engberg, I. (1993). Nifedipine- and omega-conotoxin-sensitive Ca^{2+} conductances in guinea-pig substantia nigra pars compacta neurones. *The Journal of Physiology, 466*, 727–747.

Nestler, E. J., Terwilliger, R. Z., Walker, J. R., Sevarino, K. A., & Duman, R. S. (1990). Chronic cocaine treatment decreases levels of the G protein subunits Gi alpha and Go alpha in discrete regions of rat brain. *Journal of Neurochemistry, 55*, 1079–1082.

Neuhoff, H., Neu, A., Liss, B., & Roeper, J. (2002). I(h) channels contribute to the different functional properties of identified dopaminergic subpopulations in the midbrain. *The Journal of Neuroscience, 22*, 1290–1302.

Nicholson, K. L., & Balster, R. L. (2001). GHB: A new and novel drug of abuse. *Drug and Alcohol Dependence, 63*, 1–22.

Olpe, H. R., Koella, W. P., Wolf, P., & Haas, H. L. (1977). The action of baclofen on neurons of the substantia nigra and of the ventral tegmental area. *Brain Research, 134*, 577–580.

Otmakhova, N. A., & Lisman, J. E. (2004). Contribution of Ih and GABAB to synaptically induced afterhyperpolarizations in CA1: A brake on the NMDA response. *Journal of Neurophysiology, 92*, 2027–2039.

Padgett, C. L., Lalive, A. L., Tan, K. R., Terunuma, M., Munoz, M. B., Pangalos, M. N., et al. (2012). Methamphetamine-evoked depression of GABA(B) receptor signaling in GABA neurons of the VTA. *Neuron, 73*, 978–989.

Phillips, P. E., & Stamford, J. A. (2000). Differential recruitment of N-, P- and Q-type voltage-operated calcium channels in striatal dopamine release evoked by 'regular' and 'burst' firing. *Brain Research, 884*, 139–146.

Pitman, K. A., Puil, E., & Borgland, S. L. (2014). GABA(B) modulation of dopamine release in the nucleus accumbens core. *The European Journal of Neuroscience, 40*, 3472–3480.

Puopolo, M., Raviola, E., & Bean, B. P. (2007). Roles of subthreshold calcium current and sodium current in spontaneous firing of mouse midbrain dopamine neurons. *The Journal of Neuroscience, 27*, 645–656.

Rice, M. E., Cragg, S. J., & Greenfield, S. A. (1997). Characteristics of electrically evoked somatodendritic dopamine release in substantia nigra and ventral tegmental area in vitro. *Journal of Neurophysiology, 77*, 853–862.

Santiago, M., Machado, A., & Cano, J. (1993a). Regulation of the prefrontal cortical dopamine release by GABAA and GABAB receptor agonists and antagonists. *Brain Research, 630*, 28–31.

Santiago, M., Machado, A., & Cano, J. (1993b). In vivo release of dopamine from rat striatum, substantia nigra and prefrontal cortex: Differential modulation by baclofen. *British Journal of Pharmacology, 109*, 814–818.

Schmitz, Y., Schmauss, C., & Sulzer, D. (2002). Altered dopamine release and uptake kinetics in mice lacking D2 receptors. *The Journal of Neuroscience, 22*, 8002–8009.

Schultz, W. (1998). Predictive reward signal of dopamine neurons. *Journal of Neurophysiology, 80*, 1–27.

Schwenk, J., Metz, M., Zolles, G., Turecek, R., Fritzius, T., Bildl, W., et al. (2010). Native GABA(B) receptors are heteromultimers with a family of auxiliary subunits. *Nature, 465*, 231–235.

Schwenk, J., Pérez-Garci, E., Schneider, A., Kollewe, A., Gauthier-Kemper, A., Fritzius, T., et al. (2016). Modular composition and dynamics of native GABAB receptors identified by high-resolution proteomics. *Nature Neuroscience, 19*, 233–242.

Sharpe, A. L., Varela, E., Bettinger, L., & Beckstead, M. J. (2015). Methamphetamine self-administration in mice decreases GIRK channel-mediated currents in midbrain dopamine neurons. *The International Journal of Neuropsychopharmacology, 18*.

Shoaib, M., Swanner, L. S., Beyer, C. E., Goldberg, S. R., & Schindler, C. W. (1998). The GABAB agonist baclofen modifies cocaine self-administration in rats. *Behavioural Pharmacology, 9*, 195–206.

Sugita, S., Johnson, S. W., & North, R. A. (1992). Synaptic inputs to GABAA and GABAB receptors originate from discrete afferent neurons. *Neuroscience Letters, 134*, 207–211.

Tan, K. R., Yvon, C., Turiault, M., Mirzabekov, J. J., Doehner, J., Labouèbe, G., et al. (2012). GABA neurons of the VTA drive conditioned place aversion. *Neuron, 73*, 1173–1183.

Terrier, J., Ort, A., Yvon, C., Saj, A., Vuilleumier, P., & Lüscher, C. (2011). Bi-directional effect of increasing doses of baclofen on reinforcement learning. *Frontiers in Behavioral Neuroscience, 5*, 40.

Terunuma, M., Vargas, K. J., Wilkins, M. E., Ramírez, O. A., Jaureguiberry-Bravo, M., Pangalos, M. N., et al. (2010). Prolonged activation of NMDA receptors promotes dephosphorylation and

alters postendocytic sorting of GABAB receptors. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 13918–13923.

Tritsch, N. X., Ding, J. B., & Sabatini, B. L. (2012). Dopaminergic neurons inhibit striatal output through non-canonical release of GABA. *Nature, 490*, 262–266.

Tsai, H.-C., Zhang, F., Adamantidis, A., Stuber, G. D., Bonci, A., De Lecea, L., et al. (2009). Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. *Science (New York, NY), 324*, 1080–1084.

Turecek, R., Schwenk, J., Fritzius, T., Ivankova, K., Zolles, G., Adelfinger, L., et al. (2014). Auxiliary GABAB receptor subunits uncouple G protein βγ subunits from effector channels to induce desensitization. *Neuron, 82*, 1032–1044.

van Zessen, R., Phillips, J. L., Budygin, E. A., & Stuber, G. D. (2012). Activation of VTA GABA neurons disrupts reward consumption. *Neuron, 73*, 1184–1194.

Vigot, R., Barbieri, S., Bräuner-Osborne, H., Turecek, R., Shigemoto, R., Zhang, Y. P., et al. (2006). Differential compartmentalization and distinct functions of GABAB receptor variants. *Neuron, 50*, 589–601.

Wang, H.-L., Qi, J., Zhang, S., Wang, H., & Morales, M. (2015). Rewarding effects of optical stimulation of ventral tegmental area glutamatergic neurons. *The Journal of Neuroscience, 35*, 15948–15954.

Watabe-Uchida, M., Zhu, L., Ogawa, S. K., Vamanrao, A., & Uchida, N. (2012). Whole-brain mapping of direct inputs to midbrain dopamine neurons. *Neuron, 74*, 858–873.

Westerink, B. H., de Boer, P., Santiago, M., & De Vries, J. B. (1994). Do nerve terminals and cell bodies of nigrostriatal dopaminergic neurons of the rat contain similar receptors? *Neuroscience Letters, 167*, 109–112.

Westerink, B. H., Santiago, M., & De Vries, J. B. (1992). The release of dopamine from nerve terminals and dendrites of nigrostriatal neurons induced by excitatory amino acids in the conscious rat. *Naunyn-Schmiedeberg's Archives of Pharmacology, 345*, 523–529.

White, F. J. (1996). Synaptic regulation of mesocorticolimbic dopamine neurons. *Annual Review of Neuroscience, 19*, 405–436.

White, J. H., Wise, A., Main, M. J., Green, A., Fraser, N. J., Disney, G. H., et al. (1998). Heterodimerization is required for the formation of a functional GABA(B) receptor. *Nature, 396*, 679–682.

Wickman, K., Karschin, C., Karschin, A., Picciotto, M. R., & Clapham, D. E. (2000). Brain localization and behavioral impact of the G-protein-gated K+ channel subunit GIRK4. *The Journal of Neuroscience, 20*, 5608–5615.

Xi, Z.-X., Ramamoorthy, S., Shen, H., Lake, R., Samuvel, D. J., & Kalivas, P. W. (2003). GABA transmission in the nucleus accumbens is altered after withdrawal from repeated cocaine. *The Journal of Neuroscience, 23*, 3498–3505.

Xi, Z. X., & Stein, E. A. (1998). Nucleus accumbens dopamine release modulation by mesolimbic GABAA receptors-an in vivo electrochemical study. *Brain Research, 798*, 156–165.

Xi, Z. X., & Stein, E. A. (2000). Increased mesolimbic GABA concentration blocks heroin self-administration in the rat. *The Journal of Pharmacology and Experimental Therapeutics, 294*, 613–619.

Xia, Y., Driscoll, J. R., Wilbrecht, L., Margolis, E. B., Fields, H. L., & Hjelmstad, G. O. (2011). Nucleus accumbens medium spiny neurons target non-dopaminergic neurons in the ventral tegmental area. *The Journal of Neuroscience, 31*, 7811–7816.

Zhang, K., Tarazi, F. I., Campbell, A., & Baldessarini, R. J. (2000). GABA(B) receptors: Altered coupling to G-proteins in rats sensitized to amphetamine. *Neuroscience, 101*, 5–10.

Zweifel, L. S., Parker, J. G., Lobb, C. J., Rainwater, A., Wall, V. Z., Fadok, J. P., et al. (2009). Disruption of NMDAR-dependent burst firing by dopamine neurons provides selective assessment of phasic dopamine-dependent behavior. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 7281–7288.

Part III
Pharmacology

Chapter 9
Drug Discrimination Studies for Investigations on the Mechanisms of Action of $GABA_B$ Receptor Ligands

Michelle G. Baladi and Lawrence P. Carter

Abstract Drug discrimination is a reliable, sensitive, and pharmacologically specific behavioral procedure for studying the mechanism of action of drugs. These qualities have enabled investigators to classify and differentiate closely related compounds and gather important information concerning their mechanism(s) of action in vivo. Although drugs from various pharmacological classes have been used to establish discriminative stimulus control, the focus of this chapter is on how drug discrimination procedures have been used to study the in vivo pharmacology of $GABA_B$ receptor ligands. More specifically, we will review several different drug discrimination procedures and discuss how they have been used to qualitatively and quantitatively study different components of a drug with a relatively simple mechanism of action (baclofen) and a drug with a more complex mechanism of action [gamma-hydroxybutyrate (GHB)]. A number of studies have provided evidence that the behavioral effects of baclofen and GHB are mediated predominantly by $GABA_B$ receptors; however, there is growing evidence that the mechanisms mediating the effects of these two drugs are not identical. The differences between baclofen and GHB are relevant not only for understanding their distinct therapeutic and abuse-related effects, but also for understanding their susceptibility to positive modulatory effects, which could allow for a more selective modulation of the $GABA_B$ receptor system, thereby leading to safer and more effective therapeutics.

Keywords Drug discrimination • Generalization • Baclofen • gamma-Hydroxybutyrate (GHB) • Positive allosteric modulator

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9.1 Introduction

Drug discrimination is a behavioral procedure that has been widely used in a number of species, including humans, and under a variety of conditions to investigate the effects of drugs from many different pharmacological classes. Drug discrimination can be used to investigate a wide variety of in vivo pharmacological aspects related to the stimulus properties of a drug. These aspects include time of onset, duration of action, mechanism of action, activity of drug metabolites, and identification and characterization of related ligands such as agonists, antagonists, and allosteric receptor modulators. In addition, drug discrimination has been used extensively in the field of drug abuse research to investigate the processes of tolerance and withdrawal [for example, see (France and Woods 1989; Young 1991; Walker et al. 1997)] and to evaluate potential candidate medications for the treatment of drug addiction (Bigelow and Preston 1992; Brandt et al. 1997; Sell et al. 2003; Stoops 2006; Banks et al. 2015).

The most commonly employed apparatus for conducting drug discrimination studies is an operant chamber with two or more species-appropriate response options (e.g., nose poke holes for mice, illuminated keys for pigeons, levers for rats and monkeys) and equipment to deliver a positive or negative reinforcer (e.g., food or electric shock) to maintain behavior (France and Woods 1985; Callahan and Appel 1990; Sannerud and Ator 1995; Carter et al. 2004; Li et al. 2009). However, drug discrimination studies have also employed a variety of different procedures such as food-reinforced T-mazes for rats (Colombo et al. 1998) and money-reinforced button pressing for humans (Rush et al. 2002). In a typical two-lever drug discrimination procedure, subjects are trained to discriminate between a particular dose of a training drug (i.e., training dose) and a nondrug state by reinforcing one response option after administration of the training dose and by reinforcing the other response option after administration of vehicle (e.g., saline or placebo) under a schedule of reinforcement. The two primary endpoints are: (1) the relative allocation of responses between the response options (e.g., the percentage of drug-appropriate responding or %DR) or the first response option that is selected and (2) the rate of responding as a nonselective indicator of pharmacological effect (e.g., see Fig. 9.1).

During training sessions, only one response option is reinforced within a session because there is a "correct" response that is based on what was administered prior to the session (i.e., training drug or vehicle). For example, during drug training sessions, only responses on the drug-associated lever are reinforced while during vehicle training sessions, only responses on the vehicle-associated lever are reinforced. However, test sessions are conducted under conditions in which both response options are reinforced (i.e., there are no "incorrect answers"). Training and test sessions may be designed to comprise a single administration and response opportunity (single trial session) or multiple administration and response opportunities (multiple trial session); the advantage of the latter approach is that cumulative dose effect curves may be generated within a test session (Schechter 1997).

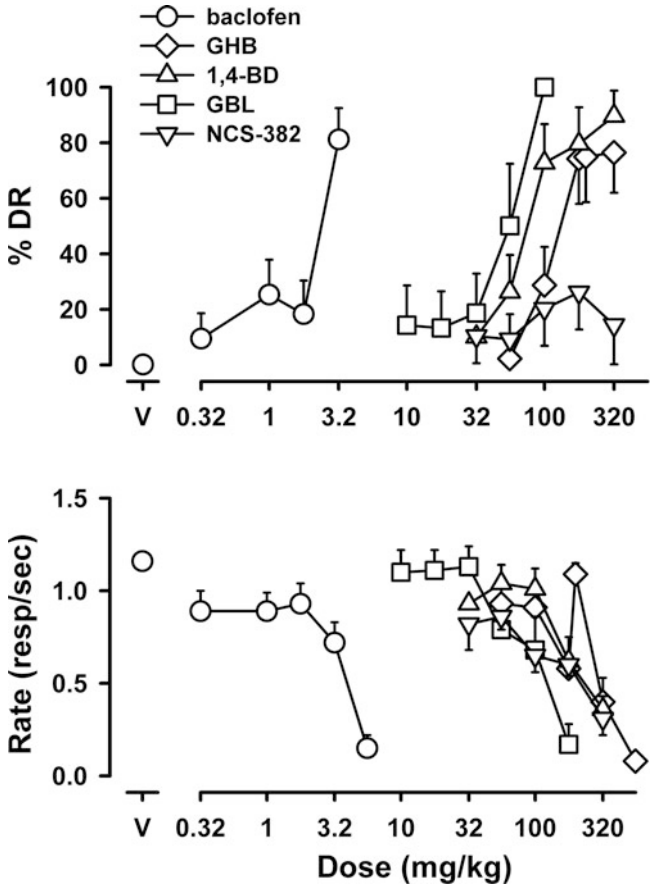

Fig. 9.1 Effects of baclofen, GHB, 1,4-BD, GBL, and NCS-382 in rats trained to discriminate 3.2 mg/kg baclofen from saline. Ordinates: *top panel*, the percentage of responses on the drug-appropriate lever (%DR); *bottom panel*, rate of responding in responses per second. Abscissa: dose in mg/kg body weight; data above "V" show the effect of saline vehicle. Averaged data for 11 rats ±1 S.E.M. are shown, except for discrimination data under the following conditions: $n=8$ for 178 mg/kg GHB; $n=7$ for 320 mg/kg GHB; $n=10$ for 32, 178, and 320 mg/kg 1,4-BD; $n=10$ for 32 and 100 mg/kg NCS-382; and $n=7$ for 320 mg/kg NCS-382. Reproduced from Carter et al. (2004) with permission from ASPET

Tests sessions essentially serve three main purposes: (1) test sessions in which the dose of the training drug is administered confirm that discriminative stimulus control has been established and is maintained; (2) test sessions in which doses of other drugs are administered permit inferences to be made regarding the similarity of the mechanism of action of the test drug and the training drug; and (3) test sessions in which multiple drugs (e.g., antagonist and agonist) are administered are used to confirm mechanism of action and quantify the nature of the pharmacological interaction by examining shifts in dose effect curves.

After repeated presentations (typically during daily training sessions), discriminative stimulus control is established when the subjects reach predetermined performance criteria and reliably respond on the drug-associated response option following drug administration and respond on the nondrug or vehicle-associated response option following vehicle administration. This predetermined performance criteria could be as follows: the first response requirement and at least 80 % of the total responses occur on the correct response option for five consecutive or six of seven training sessions [for example, see (Li et al. 2009)].

The speed of the acquisition of drug discrimination can vary as a function of several factors including the type of drug and the training dose [for example, see (Koek et al. 2006)]. When the criteria for discriminative stimulus control have been met, additional doses of the training drug may be evaluated under test conditions. Subjects who have acquired the discrimination may be tested multiple times per week so long as training or test sessions with the training dose indicate that discriminative stimulus control is maintained. For this purpose, training sessions (to ensure that subjects are under stimulus control) are often scheduled between the test sessions. The training dose is expected to occasion greater than 80 % DR with a minimal, if any, effect on response rate (which may be due to tolerance) compared to a vehicle control. Doses of the training drug lower than the training dose may occasion less %DR than the training dose, or no %DR at all. Doses of the training drug higher than the training dose are likely to occasion an equal or greater %DR than the training dose with a potential effect on the response rate. In the absence of an increase in %DR, changes in the rate of responding are used to confirm that behaviorally active doses of a test drug have been studied (e.g., see the dose of 320 mg/kg NCS-382 in Fig. 9.1).

One reason that the drug discrimination procedure has proved so useful for studying the pharmacological mechanism of action of drugs is that it is not simply a “drug vs. no drug” discrimination that is learned, but rather, the discriminative stimulus that is trained has a high degree of pharmacological specificity (Colpaert 1978; Mansbach and Balster 1991). For example, drugs that share a mechanism of action typically generalize or substitute for the discriminative stimulus effects of each other, whereas pharmacologically unrelated drugs do not generalize to each other. The pharmacological mechanism of action of a drug can be further characterized by investigating the ability of antagonists to attenuate or block the discriminative stimulus effects of drugs that occasion %DR. For example, in animals trained to discriminate the μ opioid receptor agonist morphine from saline: (1) drugs with agonist activity at the μ opioid receptor, such as fentanyl, produce dose-related similar (i.e., morphine-like %DR) discriminative effects; (2) drugs that lack activity at μ opioid receptors, such as D-amphetamine, fail to produce morphine-like discriminative effects (Shannon and Holtzman 1976a, 1977); and (3) μ opioid receptor antagonists such as naltrexone block the discriminative effects of morphine and shift the dose effect curve rightward (Shannon and Holtzman 1976b). In addition, cross-generalization occurs regardless of which μ opioid receptor agonist is used as the training drug; for example, in rats trained to discriminate fentanyl from saline, morphine produces fentanyl-like discriminative effects (Colpaert et al. 1976). These

sorts of symmetrical cross-generalization data provide strong evidence in vivo that the drugs share a pharmacological mechanism of action at the receptor level; however, the precise mechanism of action must be confirmed through antagonism studies. For example, diazepam and pentobarbital are both $GABA_A$ receptor-positive modulators, but they act through different binding sites on $GABA_A$ receptors (benzodiazepine and barbiturate binding sites, respectively). Cross-generalization studies indicate very clearly that diazepam and pentobarbital exert similar discriminative stimulus effects regardless of the training drug [i.e., diazepam-like or pentobarbital-like cross-generalization (Jarbe 1976; Winger and Herling 1982; Ator and Griffiths 1983; Nader et al. 1991)], suggesting a shared mechanism of action (i.e., positive modulation of the $GABA_A$ receptor). However, the discriminative stimulus effects of diazepam and pentobarbital can be differentiated by flumazenil, a benzodiazepine site antagonist that shifts the diazepam dose effect curve rightward, but not the pentobarbital dose effect curve (Ator and Griffiths 1983; Woolverton and Nader 1995). Taken together, these results emphasize that although stimulus generalization studies provide evidence that a training drug and a test dug can produce a similar *qualitative* discriminative stimulus effect, antagonism studies can more thoroughly conclude whether they do so through an identical mechanism of action at the receptor level.

Schild analysis is a *quantitative* pharmacological tool for estimating the affinity of antagonists as well as understanding interactions between drugs and receptors. Despite the challenges inherent with this analysis [see (Kenakin 1982)], particularly in vivo when assumptions (e.g., equilibrium) cannot be confirmed, it is noteworthy that orderly data can be obtained with this approach using the drug discrimination procedure (Dykstra et al. 1988; Paronis and Bergman 1999; Gerak and France 2007). For example, Schild analysis has been used to evaluate the discriminative stimulus effects of drugs at opioid receptors (France et al. 1990), $GABA_A$ receptors (Paronis and Bergman 1999; Gerak and France 2012), and serotonin receptors (Li et al. 2009). This method compares the pattern of antagonism, by comparing families of dose-response curves for combinations of agonists and antagonists that vary in selectivity for different receptors, to that predicted by the simple, competitive, and reversible model (i.e., the agonist and antagonist compete for the same recognition sites on the receptor). Schild analyses provide *quantitative* estimates of the potency of an antagonist (i.e., pA_2). If only one receptor type mediates the effects of all drugs under a particular set of conditions, then under those conditions, the potency of an antagonist should be the same in blocking the actions of all agonists that have activity at that receptor. For example, the potency of naltrexone to antagonize the discriminative stimulus effects of the opioid receptor agonists etorphine, buprenorphine, morphine, and fentanyl is the same, which provides strong evidence that the discriminative stimulus effects of these drugs are mediated by the μ opioid receptor in vivo (Walker et al. 1994).

In addition to the typical drug vs. saline discrimination procedure, many subjects are able to learn more complex drug discriminations such as those with three response options (White and Holtzman 1981), drug versus drug training (Boja and Schechter 1990), and discriminations between a drug mixture and the components of the

mixture alone (AND-OR procedure) (Stolerman and Mariathasan 1990). The utility of these more complex procedures is that they allow for further dissection of complex or multifactorial mechanisms of action. For example, we previously mentioned how antagonism studies with flumazenil could distinguish between the similar discriminative stimulus effects of the positive $GABA_A$ receptor modulators diazepam and pentobarbital. Using a three-lever drug discrimination procedure (drug vs. drug vs. vehicle), it was shown that the rats themselves could discriminate *between* two positive $GABA_A$ receptor modulators (chlordiazepoxide and pentobarbital) based upon an apparent qualitative difference in stimuli (Krimmer and Barry 1979).

Furthermore, there are several other factors (e.g., schedule of reinforcement, training dose, type of reinforcer) that can potentially impact the result of drug discrimination studies (Snodgrass and McMillan 1991; Baker et al. 2004). For example, a number of investigators have shown that changes in training dose can affect the slopes of generalization gradients, profiles of substitution, and antagonism (Shannon and Holtzman 1979; Colpaert et al. 1980a, b; Koek and Slangen 1982). The effects that occur as training dose is manipulated could be a result of a variety of factors including changes in levels of discriminative stimulus control, changes in the pharmacological selectivity of a training drug, or differences in efficacy among drugs and efficacy requirements across experimental conditions. A comprehensive discussion of training dose and other factors that impact the drug discrimination paradigm is beyond the scope of this chapter, but can be found elsewhere [for review, see (Colpaert and Janssen 1982; Comer et al. 1991; McMillan et al. 2002)].

Although drugs from many pharmacological classes have been used to establish discriminative stimulus control, this chapter will focus on how drug discrimination procedures have been used to study the mechanism of action of $GABA_B$ receptor ligands. There are a variety of pharmacological tools available to study $GABA_B$ receptors (see Chaps. 2, 3, and 18 of this book), including agonists such as baclofen and 3-Aminopropyl(methyl)phosphinic acid (SKF97541), antagonists such as 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP35348) and 3-[[[((3,4 dichlorophenyl)methyl]amino]propyl]diethoxymethyl)phosphinic acid (CGP52432), and most recently, positive allosteric modulators such as 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethylpropyl) phenol (CGP7930) and 5,7-bis(1,1-dimethylethyl)-3-hydroxy-3-(trifluoromethyl)-2(3H)-benzofuranone (rac-BHFF). This chapter will first summarize the discriminative stimulus effects of the prototypical $GABA_B$ receptor agonist baclofen and then highlight the ways drug discrimination has been utilized to characterize the pharmacology of novel ligands at $GABA_B$ receptors such as gamma-hydroxybutyrate (GHB) and $GABA_B$ receptor-positive modulators. Drug discrimination procedures have been useful for showing that different $GABA_B$ receptor populations might be responsible for the different pharmacological effects of baclofen and GHB. Similarly, more recent drug discrimination studies have provided further evidence of pharmacologically distinct $GABA_B$ receptor subtypes in vivo based on the differential effects of $GABA_B$ receptor-positive modulators on the actions of baclofen and GHB. Lastly, the chapter will conclude with a commentary on the value of drug discrimination procedures in facilitating new insights into

the heterogeneity of $GABA_B$ receptors, elucidating how the mechanisms of action of drugs with action at $GABA_B$ receptors relate to therapeutic effects, and understanding how recent positive allosteric modulators might offer therapeutic advantages over direct-acting $GABA_B$ receptor agonists.

9.2 Characterization of the Discriminative Stimulus Effects of Baclofen

Baclofen, the prototypical $GABA_B$ receptor agonist (see Chap. 17 of this book), remains one of the most potent and selective agents for stimulation of $GABA_B$ receptors (Bowery et al. 1980; Bowery and Enna 2000). Baclofen is classified as a $GABA_B$ receptor agonist based on pharmacological effects that are independent of the $GABA_A$ receptor, the ability of selective antagonists to block these effects, and the ability of baclofen to induce tolerance and selectively exhibit cross-tolerance to agonists with activity at $GABA_B$ receptors (Nielsen et al. 2002; Eckermann et al. 2004; Smith et al. 2006; Beveridge et al. 2013). For instance, in mice, the $GABA_B$ receptor-selective antagonist CGP35348 blocks baclofen-induced decreases in locomotor activity and increases in forebrain dopamine concentration, suggesting that these effects are mediated through $GABA_B$ receptors (Nissbrandt and Engberg 1996).

9.2.1 Baclofen as a Discriminative Stimulus and Tests of Stimulus Generalization to Baclofen

In the first published report in which baclofen was trained as a discriminative stimulus, rats were trained to discriminate 3.2 mg/kg baclofen from saline using a two-lever, food-reinforced operant procedure. All animals acquired the discrimination in a median of 69 days [range: 67–82; (Carter et al. 2004)]. Under test conditions, baclofen dose-dependently occasioned responding on the baclofen-associated lever up to 81.2 % at the training dose and the discriminative stimulus effects of 3.2 mg/kg baclofen were evident (>80 %) at 10 min and for up to 90 min after intraperitoneal (i.p.) administration (Carter et al. 2004). A subsequent study in pigeons attempted to train an intramuscular (i.m.) dose of 5.6 mg/kg baclofen from saline using a two-key, food-reinforced operant procedure. None of the animals acquired the discrimination within 50 sessions, so the training dose was increased to 7.5 mg/kg baclofen (i.m.). All animals acquired the discrimination of 7.5 mg/kg baclofen from saline in a median of 37 days [range: 11–45; (Koek et al. 2012)]. Under test conditions, baclofen dose-dependently increased responding on the baclofen-associated key up to 93 % at the training dose (Koek et al. 2012).

Results of stimulus generalization studies in rats and pigeons trained to discriminate baclofen from saline have been remarkably consistent, despite differences in species, training dose, and route of administration. In both studies, the test drug found to occasion the greatest baclofen-appropriate responding was GHB (Fig. 9.1; Carter et al. 2004; Koek et al. 2012). For example, the largest dose of GHB that did not markedly decrease response rate (typically if an animal responds at a rate less than 20 % of the saline control rate, discrimination data are not included, see Fig. 9.1, diamond symbol at 560 mg/kg GHB) occasioned a maximum of 76.5 % and >90 % baclofen-appropriate responding in rats trained to discriminate 3.2 mg/kg baclofen from saline (Carter et al. 2004) and in pigeons trained to discriminate 7.5 mg/kg baclofen from saline (Koek et al. 2012), respectively. In rats, the GHB metabolic precursors 1,4-butanediol (1,4-BD) and gamma-butyrolactone (GBL) also occasioned high levels of baclofen-appropriate responding at 89.7 % and 89.4 %, respectively (Fig. 9.1; Carter et al. 2004). These findings are consistent with GHB binding to $GABA_B$ receptors and previous studies in which baclofen fully substituted for the GHB discriminative stimulus in these species (Winter 1981; Colombo et al. 1998, 2000; Carter et al. 2003; Koek et al. 2004). Further, and as expected, the baclofen drug discrimination assays in rats and pigeons exhibited pharmacological specificity. Diazepam, a benzodiazepine that lacks activity at $GABA_B$ receptors, occasioned only 50.9 % and 58 % baclofen-appropriate responding in rats and pigeons, respectively (Carter et al. 2004; Koek et al. 2012). Morphine, a μ opioid receptor agonist that also lacks activity at $GABA_B$ receptors, occasioned only 49.6 % and 43 % baclofen-appropriate responding, respectively (Carter et al. 2004; Koek et al. 2012).

9.2.2 Antagonism of the Baclofen Discriminative Stimulus

If the discriminative stimulus effects of baclofen and other drugs that occasion baclofen-appropriate responding are mediated by $GABA_B$ receptors, then one should be able to attenuate those effects with a $GABA_B$ receptor antagonist. In rats trained to discriminate baclofen, increasing doses of the selective $GABA_B$ receptor antagonist CGP35348 were studied together with the training dose (3.2 mg/kg) of baclofen. Doses of 32–100 mg/kg CGP35348 dose-dependently decreased baclofen-appropriate responding to approximately 50 %; however, larger doses from 178 to 560 mg/kg failed to completely attenuate the discriminative stimulus effects of baclofen (Carter et al. 2004). In contrast, in pigeons trained to discriminate baclofen, 100 mg/kg CGP35348 decreased baclofen-appropriate responding to less than 25 % and significantly shifted the dose-response curve for baclofen 5.3-fold to the right in a parallel manner (Koek et al. 2012). It is possible that the apparent limited antagonism of the baclofen discriminative stimulus in rats is due to the involvement of more than one receptor type in the discriminative stimulus effects of baclofen. There are several lines of evidence that there are multiple subtypes of $GABA_B$ receptors in brain and spinal cord [for review, see (Bonanno and Raiteri 1993; Yamada et al.

1999; Bowery et al. 2002)]. For example, electrophysiological studies have shown that pre- and postsynaptic $GABA_B$ receptors are differentially sensitive to $GABA_B$ receptor ligands (Seabrook et al. 1990; Lambert and Wilson 1993; Yamada et al. 1999). Thus, Carter and colleagues (2004) speculated that the partial antagonism of baclofen by CGP35348 might have been due to the actions of baclofen at CGP35348-insensitive $GABA_B$ receptors or at non-GABAergic receptors. However, the totality of the evidence showed that in rats and pigeons, baclofen could be readily trained as a discriminative stimulus, other $GABA_B$ receptor agonists substituted for the baclofen discriminative stimulus, and selective $GABA_B$ receptor antagonists partially (rats) or fully (pigeons) attenuated the discriminative stimulus effects of baclofen. Taken together, these data indicate that the discriminative stimulus effects of baclofen are mediated by $GABA_B$ receptors and that the baclofen drug discrimination procedure may serve as an in vivo assay for identifying functional $GABA_B$ receptor ligands.

9.3 Example 1: Drug Discrimination and the Role of $GABA_B$ Receptors in the Discriminative Stimulus Effects of GHB

$GABA_B$ receptors can be activated by baclofen, but also by other drugs, such as GHB (Mathivet et al. 1997). However, the $GABA_B$ receptor mechanisms underlying the effects of baclofen and GHB do not seem to be identical [for review, see (Carter et al. 2009)]. The previous section discussed how a drug (i.e., baclofen) with a relatively simple or known mechanism of action can be trained as a discriminative stimulus such that test drugs with a more complex or somewhat unknown mechanism of action (e.g., GHB) can be evaluated for having discriminative stimulus effects that are similar to the known drug. The same approach can be used to study drugs for which the mechanism of action is complex or not known, whereby the complex drug is trained as a discriminative stimulus and compounds with a known mechanism of action are evaluated for stimulus generalization.

9.3.1 Generalization of Baclofen to the GHB Discriminative Stimulus

Rats can discriminate GHB from saline (Winter 1981; Colombo et al. 1995a, b, 1998; Metcalf et al. 2001; Carter et al. 2003; Baker et al. 2004, 2005, 2008) and the discriminative stimulus effects are pharmacologically specific because pharmacologically unrelated drugs (e.g., phencyclidine, ketamine) do not substitute for GHB (Winter 1981; Carter et al. 2003; Baker et al. 2004). Consistent with studies in which the discriminative stimulus effects of GHB were examined in animals discriminating baclofen as the training drug (Carter et al. 2004; Koek et al. 2012), in rats trained to discriminate 200 mg/kg GHB (i.p.) from saline, baclofen occasioned

the greatest amount of GHB-appropriate responding, whereas a $GABA_A$ receptor agonist, several different $GABA_A$ receptor-positive modulators, and GBL occasioned less GHB-appropriate responding (Winter 1981; Carter et al. 2003). For example, in the first study in which GHB was trained as a discriminative stimulus, baclofen occasioned the greatest GHB-appropriate responding when more than half of the rats responded (70 % DR at a dose of 3 mg/kg) out of the 13 drugs that were tested for generalization to GHB (Winter 1981). Similar results were reported in subsequent studies in which rats were trained to discriminate 300 mg/kg GHB; baclofen and to a lesser extent $GABA_A$ receptor-positive modulators (e.g., diazepam) occasioned GHB-appropriate responding. In studies in which rats were trained to discriminate a larger dose of GHB (700 mg/kg), baclofen, but not diazepam, occasioned GHB-appropriate responding (Colombo et al. 1998; Lobina et al. 1999; Baker et al. 2004, 2005, 2008). These data are consistent with the concept that the specificity of a drug's discriminative stimulus generally increases with training dose [also see (Koek et al. 2006)], and taken together, suggest that the discriminative stimulus effects of GHB involve multiple mechanisms, with a predominant role for $GABA_B$ receptors, particularly at large doses of GHB.

A second line of evidence that multiple $GABA_B$ receptor mechanisms might underlie the effects of GHB and baclofen comes from studies that have examined the effects of baclofen on the dose effects curves of GHB and the $GABA_B$ receptor agonist SKF97541 in rats discriminating GHB (Carter et al. 2006). If these drugs have an identical mechanism of action at the same population of $GABA_B$ receptors, then when studied in combination, baclofen should shift the GHB and SKF97541 dose effect curves leftward in a parallel manner. Indeed, baclofen shifted the discriminative stimulus and rate-decreasing effects of SKF97541 leftward in a parallel manner, as would be expected for drugs with a shared mechanism of action; however, baclofen did not shift the discriminative stimulus and rate-decreasing effects of GHB leftward in a similar manner (Carter et al. 2006). Taken together, these data suggest that the mechanism of action of baclofen and GHB are not identical.

9.3.2 Attenuation of the GHB Discriminative Stimulus by $GABA_B$ Receptor Antagonists

As mentioned previously, symmetrical cross-generalization (baclofen and GHB occasion high levels of %DR regardless of the training drug) suggests that there is a shared mechanism of action between two drugs; however, evaluation of the precise mechanism of action requires antagonism studies. If, as the generalization data suggest, the discriminative stimulus effects of GHB are largely mediated by $GABA_B$ receptors, then these effects should be attenuated by selective $GABA_B$ receptor antagonists. Indeed, in several studies, $GABA_B$ antagonists block the discriminative stimulus effects of GHB. For instance, in rats discriminating GHB, CGP35348 antagonized the discriminative stimulus effects of GHB across different training doses [i.e., 300 or 700 mg/kg (Colombo et al. 1998; Baker et al. 2005, 2008)]. Further, in rats

discriminating 200 mg/kg GHB, CGP35348 attenuated the discriminative stimulus effects of GHB and also the $GABA_B$ receptor agonists baclofen and SKF97541 (Carter et al. 2003). Finally, in pigeons discriminating GHB, CGP35348 also antagonized the discriminative stimulus effects of GHB across several training doses (Koek et al. 2004, 2006). Thus, across studies, species, and training doses, the discriminative stimulus effects of GHB are attenuated by the $GABA_B$ receptor antagonist, CGP35348. In contrast, several studies have reported that flumazenil (an antagonist at the benzodiazepine site on the $GABA_A$ receptor) did not attenuate the discriminative stimulus effects of GHB in animals trained to discriminate GHB (Carter et al. 2003; Koek et al. 2006). The purported GHB receptor antagonist NCS-382 also does not appear to reliably attenuate the discriminative stimulus effects of GHB. One study reported complete antagonism of GHB discriminative stimulus effects with NCS-382 (Colombo et al. 1995a) while others reported partial or no antagonism at all (Carter et al. 2003; Baker et al. 2005; Koek et al. 2006). These discrepant findings might be due to an incomplete understanding of the mechanism of action of NCS-382 as there is some evidence that NCS-382 might have partial agonist effects at $GABA_B$ receptors (Koek et al. 2004). Although Schild analysis has not been used to date to examine the discriminative stimulus effects of drugs acting at $GABA_B$ receptors, it has been used to further explore possible differences in underlying $GABA_B$ receptor mechanisms by comparing the effects of GHB and baclofen on rate of responding and comparing the antagonism of their dose-response curves by CGP35348 (Koek et al. 2009). Briefly, CGP35348 was significantly more potent to antagonize the behavioral effects of baclofen than those of GHB. These findings are consistent with previous observations of differential antagonism by CGP35348 of the discriminative stimulus effects of baclofen and GHB in rats (Carter et al. 2006) as well as of the cataleptic effects of baclofen and GHB in mice (Koek et al. 2007). Together, these data provide further evidence that $GABA_B$ receptors mediate many behavioral effects of GHB and that the underlying $GABA_B$ receptor mechanisms that mediate the effects of GHB differ from those that mediate the effects of the prototypical $GABA_B$ receptor agonist baclofen.

9.3.3 Baclofen vs. GHB Discrimination

Although the discriminative stimulus effects of GHB and baclofen appeared to be *qualitatively* similar and were attenuated by selective $GABA_B$ receptor antagonists such as CGP35348, there appeared to be some differences between GHB and baclofen and the antagonism of their behavioral effects. If there are differences between the discriminative stimulus effects of baclofen and GHB, then it might be possible to train animals to discriminate *between* baclofen and GHB. In 2005, Koek and colleagues demonstrated that rats could be trained to discriminate GHB from baclofen (and saline) in a two-lever response procedure, thereby demonstrating that the discriminative stimulus effects of baclofen and GHB are not identical (Koek et al. 2005). The baclofen vs. GHB discrimination effectively removes the baclofen-like component from the GHB discriminative stimulus, thereby permitting one to

study the remaining (non-baclofen) component of the complex GHB stimulus (Koek et al. 2005). Perhaps not surprisingly, when one removes the baclofen-like component from the GHB discriminative stimulus, $GABA_B$ receptor agonists such as baclofen and SKF97541 no longer occasion GHB-like responding (but they occasion baclofen-appropriate responding; Koek et al. 2005). A more unexpected finding, however, was that the discriminative stimulus effects of GHB were differentially attenuated by the $GABA_B$ receptor antagonist CGP52432 in the GHB vs. baclofen (or saline) discrimination as compared to the GHB vs. saline discrimination. Specifically, the $GABA_B$ receptor antagonist CGP52432 was less potent in attenuating the discriminative stimulus effects of GHB in the GHB vs. baclofen (or saline) discrimination as compared to the GHB vs. saline discrimination (Koek et al. 2005). This was in contrast to the effects of another antagonist CGP35348, which was equipotent in the two different discrimination procedures. One difference between CGP52432 and CGP35348 is that CGP52432 has greater affinity at $GABA_B$ autoreceptors ($GABA_B$ receptors on GABAergic neurons) as compared to $GABA_B$ heteroreceptors ($GABA_B$ receptors on glutamatergic neurons; Lanza et al. 1993). The reduced potency of CGP52432 in the GHB vs. baclofen (or saline) discrimination suggests that the remaining component of the GHB discriminative stimulus might be mediated by $GABA_B$ heteroreceptors on glutamatergic neurons, whereas the baclofen discriminative stimulus might be predominantly mediated by $GABA_B$ autoreceptors on GABAergic neurons (Koek et al. 2005; Carter et al. 2009). Together, these findings highlight the immense value that drug discrimination procedures offer for the evaluation and understanding of the qualitative differences between drugs in vivo.

9.4 Example 2: Discriminative Stimulus Effects of $GABA_B$ Receptor-Positive Modulators

In 2012, Koek and colleagues used separate groups of pigeons that they had trained to discriminate baclofen or GHB from saline to evaluate the effects of two $GABA_B$ receptor-positive modulators CGP7930 and rac-BHFF (Koek et al. 2012). There were several interesting findings from this work that further supported previous studies that the discriminative stimulus effects, and by extension pharmacological mechanisms and effects at the receptor level, of baclofen and GHB are not identical and might involve different populations of $GABA_B$ receptors. First, both of the $GABA_B$ receptor-positive modulators studied occasioned greater %DR in pigeons trained to discriminate baclofen (41 % and 74 % baclofen-appropriate responding for CGP7930 and rac-BHFF, respectively) than in pigeons trained to discriminate GHB (1 % and 49 % GHB-appropriate responding for CGP7930 and rac-BHFF, respectively; Koek et al. 2012). Second, in both drug discrimination procedures, CGP7930 and rac-BHFF enhanced the discriminative stimulus effects of baclofen, but not GHB (Koek et al. 2012). As the investigators noted in their discussion, these findings are consistent with in vitro evidence that CGP7930 preferentially potentiates activity at $GABA_B$ autoreceptors and not at $GABA_B$ heteroreceptors (Chen

et al. 2006; Parker et al. 2008). Thus, the findings that CGP7930 occasioned baclofen-appropriate, but not GHB-appropriate responding and that CGP7930 potentiated the discriminative stimulus effects of baclofen, but not GHB, are consistent with previous findings in which the autoreceptor-preferring antagonist CGP52432 was less potent in attenuating the discriminative stimulus effects of GHB in the GHB vs. baclofen (or saline) discrimination as compared to the GHB vs. saline discrimination (Koek et al. 2005). Each of these findings support the interpretation that the discriminative stimulus effects of baclofen are mediated predominantly by $GABA_B$ autoreceptors, whereas the discriminative stimulus effects of GHB are mediated predominantly by $GABA_B$ heteroreceptors. Assuming that the discriminative stimulus effects of these drugs are related to their therapeutic effects, these findings have useful implications for future drug development endeavors.

In 2013, Koek and colleagues followed up on this work by training pigeons to discriminate a dose of 178 mg/kg per os (p.o.) of the $GABA_B$ receptor-positive allosteric modulator rac-BHFF from saline in two-key drug discrimination procedure (178 mg/kg was the dose of rac-BHFF that had occasioned the highest %DR in pigeons trained to discriminate baclofen from saline in the previous study; Koek et al. 2013). In pigeons trained to discriminate rac-BHFF from saline, neither baclofen nor GHB fully substituted for the discriminative stimulus effects of rac-BHFF (Koek et al. 2013). It is not uncommon for an allosteric positive modulator to have different discriminative stimulus effects than an orthosteric (direct-acting) agonist. For example, in the 2004 study by Carter and colleagues, the $GABA_A$ receptor-positive modulators pregnanolone and pentobarbital fully generalized to the discriminative stimulus effects of the $GABA_A$ receptor-positive modulator diazepam occasioning 96 and 88 %DR, respectively; however, the direct-acting $GABA_A$ receptor agonist muscimol did not occasion more than 1 % diazepam-appropriate responding (Carter et al. 2004). In pigeons discriminating rac-BHFF, however, baclofen and GHB occasioned partial generalization and consistent with the prior studies of rac-BHFF in baclofen- and GHB-discriminating animals, rac-BHFF potentiated the discriminative stimulus effects of baclofen, but not GHB (Koek et al. 2013). Taken together, these drug discrimination studies provide evidence that CGP7930 and rac-BHFF act in vivo as positive $GABA_B$ receptor modulators at some, but not all, $GABA_B$ receptors. If different $GABA_B$ receptor populations differ in their susceptibility to positive modulatory effects, this could allow for more selective therapeutic modulation of the $GABA_B$ receptor system (see Chap. 18 of this book).

9.5 Conclusions

In this chapter we have attempted to highlight the incredible value that drug discrimination procedures can provide with regard to the functional evaluation of the pharmacological mechanism of action of drugs in intact behaving organisms. Although drug discrimination can and has been used to study drugs from a wide

variety of pharmacological classes, for the purpose of this book, we have focused on how drug discrimination procedures have been used to evaluate and characterize novel $GABA_B$ receptor ligands and how the characterization of these compounds has led to a better understanding of functional $GABA_B$ receptor heterogeneity. These insights could have implications for the development of more targeted therapeutics that act at specific $GABA_B$ receptor subtypes for the treatment of several conditions (see Chaps. 10–16 of this book)and for the modulation of the effects of direct-acting $GABA_B$ receptor agonists such as baclofen and GHB.

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References

Ator, N. A., & Griffiths, R. R. (1983). Lorazepam and pentobarbital drug discrimination in baboons: Cross-drug generalization and interaction with Ro 15-1788. *Journal of Pharmacology and Experimental Therapeutics, 226*, 776–782.

Baker, L. E., Pynnonen, D., & Poling, A. (2004). Influence of reinforcer type and route of administration on gamma-hydroxybutyrate discrimination in rats. *Psychopharmacology, 174*, 220–227.

Baker, L. E., Searcy, G. D., Pynnonen, D. M., & Poling, A. (2008). Differentiating the discriminative stimulus effects of gamma-hydroxybutyrate and ethanol in a three-choice drug discrimination procedure in rats. *Pharmacology, Biochemistry and Behavior, 89*, 598–607.

Baker, L. E., Van Tilburg, T. J., Brandt, A. E., & Poling, A. (2005). Discriminative stimulus effects of gamma-hydroxybutyrate (GHB) and its metabolic precursor, gamma-butyrolactone (GBL) in rats. *Psychopharmacology, 181*, 458–466.

Banks, M. L., Hutsell, B. A., Blough, B. E., Poklis, J. L., & Negus, S. S. (2015). Preclinical assessment of lisdexamfetamine as an agonist medication candidate for cocaine addiction: Effects in rhesus monkeys trained to discriminate cocaine or to self-administer cocaine in a cocaine versus food choice procedure. *International Journal of Neuropsychopharmacology, 18*, 1–10.

Beveridge, T. J., Smith, H. R., & Porrino, L. J. (2013). Differential development of tolerance to the functional and behavioral effects of repeated baclofen treatment in rats. *Pharmacology, Biochemistry and Behavior, 106*, 27–32.

Bigelow, G. E., & Preston, K. L. (1992). Assessment of buprenorphine in a drug discrimination procedure in humans. *NIDA Research Monograph, 121*, 28–37.

Boja, J. W., & Schechter, M. D. (1990). Increased drug sensitivity in the drug discrimination procedure afforded by drug versus drug training. *Psychopharmacology, 102*, 221–226.

Bonanno, G., & Raiteri, M. (1993). gamma-Aminobutyric acid (GABA) autoreceptors in rat cerebral cortex and spinal cord represent pharmacologically distinct subtypes of the GABAB receptor. *Journal of Pharmacology and Experimental Therapeutics, 265*, 765–770.

Bowery, N. G., Bettler, B., Froestl, W., Gallagher, J. P., Marshall, F., Raiteri, M., et al. (2002). International Union of Pharmacology. XXXIII. Mammalian gamma-aminobutyric acid(B) receptors: Structure and function. *Pharmacological Reviews, 54*, 247–264.

Bowery, N. G., & Enna, S. J. (2000). gamma-Aminobutyric acid(B) receptors: First of the functional metabotropic heterodimers. *Journal of Pharmacology and Experimental Therapeutics, 292*, 2–7.

Bowery, N. G., Hill, D. R., Hudson, A. L., Doble, A., Middlemiss, D. N., Shaw, J., et al. (1980). (-) Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature, 283*, 92–94.

Brandt, M. R., Cabansag, S. R., & France, C. P. (1997). Discriminative stimulus effects of l-alpha-acetylmethadol (LAAM), buprenorphine and methadone in morphine-treated rhesus monkeys. *Journal of Pharmacology and Experimental Therapeutics, 282*, 574–584.

Callahan, P. M., & Appel, J. B. (1990). Differentiation between the stimulus effects of (+)-lysergic acid diethylamide and lisuride using a three-choice, drug discrimination procedure. *Psychopharmacology, 100*, 13–18.

Carter, L. P., Chen, W., Coop, A., Koek, W., & France, C. P. (2006). Discriminative stimulus effects of GHB and GABA(B) agonists are differentially attenuated by CGP35348. *European Journal of Pharmacology, 538*, 85–93.

Carter, L. P., Flores, L. R., Wu, H., Chen, W., Unzeitig, A. W., Coop, A., et al. (2003). The role of GABAB receptors in the discriminative stimulus effects of gamma-hydroxybutyrate in rats: Time course and antagonism studies. *Journal of Pharmacology and Experimental Therapeutics, 305*, 668–674.

Carter, L. P., Koek, W., & France, C. P. (2009). Behavioral analyses of GHB: Receptor mechanisms. *Pharmacology & Therapeutics, 121*, 100–114.

Carter, L. P., Unzeitig, A. W., Wu, H., Chen, W., Coop, A., Koek, W., et al. (2004). The discriminative stimulus effects of gamma-hydroxybutyrate and related compounds in rats discriminating baclofen or diazepam: The role of GABA(B) and GABA(A) receptors. *Journal of Pharmacology and Experimental Therapeutics, 309*, 540–547.

Chen, Y., Menendez-Roche, N., & Sher, E. (2006). Differential modulation by the GABAB receptor allosteric potentiator 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethylpropyl)-phenol (CGP7930) of synaptic transmission in the rat hippocampal CA1 area. *Journal of Pharmacology and Experimental Therapeutics, 317*, 1170–1177.

Colombo, G., Agabio, R., Bourguignon, J., Fadda, F., Lobina, C., Maitre, M., et al. (1995a). Blockade of the discriminative stimulus effects of gamma-hydroxybutyric acid (GHB) by the GHB receptor antagonist NCS-382. *Physiology & Behavior, 58*, 587–590.

Colombo, G., Agabio, R., Carai, M. A., Lobina, C., Pani, M., Reali, R., et al. (2000). Characterization of the discriminative stimulus effects of gamma-hydroxybutyric acid as a means for unraveling the neurochemical basis of gamma-hydroxybutyric acid actions and its similarities to those of ethanol. *Alcohol, 20*, 237–245.

Colombo, G., Agabio, R., Lobina, C., Reali, R., Fadda, F., & Gessa, G. L. (1995b). Symmetrical generalization between the discriminative stimulus effects of gamma-hydroxybutyric acid and ethanol: Occurrence within narrow dose ranges. *Physiology & Behavior, 57*, 105–111.

Colombo, G., Agabio, R., Lobina, C., Reali, R., & Gessa, G. L. (1998). Involvement of GABA(A) and GABA(B) receptors in the mediation of discriminative stimulus effects of gamma-hydroxybutyric acid. *Physiology & Behavior, 64*, 293–302.

Colpaert, F. C. (1978). Discriminative stimulus properties of narcotic analgesic drugs. *Pharmacology, Biochemistry and Behavior, 9*, 863–887.

Colpaert, F. C., & Janssen, P. A. (1982). Factors regulating drug cue sensitivity: Limits of discriminability and the role of a progressively decreasing training dose in cocaine-saline discrimination. *Neuropharmacology, 21*, 1187–1194.

Colpaert, F. C., Kuyps, J. J., Niemegeers, C. J., & Janssen, P. A. (1976). Discriminative stimulus properties of fentanyl and morphine: Tolerance and dependence. *Pharmacology, Biochemistry and Behavior, 5*, 401–408.

Colpaert, F. C., Niemegeers, C. J., & Janssen, P. A. (1980a). Factors regulating drug cue sensitivity: Limits of discriminability and the role of a progressively decreasing training dose in fentanyl-saline discrimination. *Journal of Pharmacology and Experimental Therapeutics, 212*, 474–480.

Colpaert, F. C., Niemegeers, C. J., & Janssen, P. A. (1980b). Factors regulating drug cue sensitivity: The effect of training dose in fentanyl-saline discrimination. *Neuropharmacology, 19*, 705–713.

Comer, S. D., France, C. P., & Woods, J. H. (1991). Training dose: Influences in opioid drug discrimination. *NIDA Research Monograph, 116*, 145–161.

Dykstra, L. A., Bertalmio, A. J., & Woods, J. H. (1988). Discriminative and analgesic effects of mu and kappa opioids: In vivo pA2 analysis. *Psychopharmacology Series, 4*, 107–121.

Eckermann, K. A., Koek, W., & France, C. P. (2004). Chronic 1,4-butanediol treatment in rats: Cross-tolerance to gamma-hydroxybutyrate and (+/-)-baclofen. *European Journal of Pharmacology, 484*, 259–262.

France, C. P., de Costa, B. R., Jacobson, A. E., Rice, K. C., & Woods, J. H. (1990). Apparent affinity of opioid antagonists in morphine-treated rhesus monkeys discriminating between saline and naltrexone. *Journal of Pharmacology and Experimental Therapeutics, 252*, 600–604.

France, C. P., & Woods, J. H. (1985). Opiate agonist-antagonist interactions: Application of a three-key drug discrimination procedure. *Journal of Pharmacology and Experimental Therapeutics, 234*, 81–89.

France, C. P., & Woods, J. H. (1989). Discriminative stimulus effects of naltrexone in morphine-treated rhesus monkeys. *Journal of Pharmacology and Experimental Therapeutics, 250*, 937–943.

Gerak, L. R., & France, C. P. (2007). Time-dependent decreases in apparent pA2 values for naltrexone studied in combination with morphine in rhesus monkeys. *Psychopharmacology, 193*, 315–321.

Gerak, L. R., & France, C. P. (2012). Quantitative analyses of antagonism: Combinations of midazolam and either flunitrazepam or pregnanolone in rhesus monkeys discriminating midazolam. *Journal of Pharmacology and Experimental Therapeutics, 340*, 742–749.

Jarbe, T. U. (1976). Characteristics of pentobarbital discrimination in the gerbil: Transfer and antagonism. *Psychopharmacology, 49*, 33–40.

Kenakin, T. P. (1982). The Schild regression in the process of receptor classification. *Canadian Journal of Physiology and Pharmacology, 60*, 249–265.

Koek, W., Carter, L. P., Lamb, R. J., Chen, W., Wu, H., Coop, A., et al. (2005). Discriminative stimulus effects of gamma-hydroxybutyrate (GHB) in rats discriminating GHB from baclofen and diazepam. *Journal of Pharmacology and Experimental Therapeutics, 314*, 170–179.

Koek, W., Chen, W., Mercer, S. L., Coop, A., & France, C. P. (2006). Discriminative stimulus effects of gamma-hydroxybutyrate: Role of training dose. *Journal of Pharmacology and Experimental Therapeutics, 317*, 409–417.

Koek, W., Cheng, K., & Rice, K. C. (2013). Discriminative stimulus effects of the GABAB receptor-positive modulator rac-BHFF: Comparison with GABAB receptor agonists and drugs of abuse. *Journal of Pharmacology and Experimental Therapeutics, 344*, 553–560.

Koek, W., Flores, L. R., Carter, L. P., Lamb, R. J., Chen, W., Wu, H., et al. (2004). Discriminative stimulus effects of gamma-hydroxybutyrate in pigeons: Role of diazepam-sensitive and -insensitive GABA(A) and GABA(B) receptors. *Journal of Pharmacology and Experimental Therapeutics, 308*, 904–911.

Koek, W., France, C. P., Cheng, K., & Rice, K. C. (2012). Effects of the GABAB receptor-positive modulators CGP7930 and rac-BHFF in baclofen- and gamma-hydroxybutyrate-discriminating pigeons. *Journal of Pharmacology and Experimental Therapeutics, 341*, 369–376.

Koek, W., Mercer, S. L., & Coop, A. (2007). Cataleptic effects of gamma-hydroxybutyrate (GHB), its precursor gamma-butyrolactone (GBL), and GABAB receptor agonists in mice: Differential antagonism by the GABAB receptor antagonist CGP35348. *Psychopharmacology, 192*, 407–414.

Koek, W., Mercer, S. L., Coop, A., & France, C. P. (2009). Behavioral effects of gamma-hydroxybutyrate, its precursor gamma-butyrolactone, and GABA(B) receptor agonists: Time course and differential antagonism by the GABA(B) receptor antagonist 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP35348). *Journal of Pharmacology and Experimental Therapeutics, 330*, 876–883.

Koek, W., & Slangen, J. L. (1982). The role of fentanyl training dose and of the alternative stimulus condition in drug generalization. *Psychopharmacology, 76*, 149–156.

Krimmer, E. C., & Barry, H., III. (1979). Pentobarbital and chlordiazepoxide differentiated from each other and from nondrug. *Communications in Psychopharmacology, 3*, 93–99.

Lambert, N. A., & Wilson, W. A. (1993). Heterogeneity in presynaptic regulation of GABA release from hippocampal inhibitory neurons. *Neuron, 11*, 1057–1067.

Lanza, M., Fassio, A., Gemignani, A., Bonanno, G., & Raiteri, M. (1993). CGP 52432: A novel potent and selective GABAB autoreceptor antagonist in rat cerebral cortex. *European Journal of Pharmacology, 24*, 191–195.

Li, J. X., Rice, K. C., & France, C. P. (2009). Discriminative stimulus effects of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane in rhesus monkeys: Antagonism and apparent pA2 analyses. *Journal of Pharmacology and Experimental Therapeutics, 328*, 976–981.

Lobina, C., Agabio, R., Reali, R., Gessa, G. L., & Colombo, G. (1999). Contribution of GABA(A) and GABA(B) receptors to the discriminative stimulus produced by gamma-hydroxybutyric acid. *Pharmacology, Biochemistry and Behavior, 64*, 363–365.

Mansbach, R. S., & Balster, R. L. (1991). Pharmacological specificity of the phencyclidine discriminative stimulus in rats. *Pharmacology, Biochemistry and Behavior, 39*, 971–975.

Mathivet, P., Bernasconi, R., De Barry, J., Marescaux, C., & Bittiger, H. (1997). Binding characteristics of gamma-hydroxybutyric acid as a weak but selective GABAB receptor agonist. *European Journal of Pharmacology, 321*, 67–75.

McMillan, D. E., Hardwick, W. C., & Li, M. (2002). Drug discrimination under concurrent variable-ratio variable-ratio schedules. *Journal of Experimental Analysis of Behavior, 77*, 91–104.

Metcalf, B. R., Stahl, J. M., Allen, J. D., Woolfolk, D. R., & Soto, P. L. (2001). Discrimination of gamma-hydroxybutyrate and ethanol administered separately and as a mixture in rats. *Pharmacology, Biochemistry and Behavior, 70*, 31–41.

Nader, M. A., Winger, G., Woods, J. H., & Woolverton, W. L. (1991). Discriminative and reinforcing effects of brotizolam in rhesus monkeys. *Psychopharmacology, 103*, 166–171.

Nielsen, J. F., Hansen, H. J., Sunde, N., & Christensen, J. J. (2002). Evidence of tolerance to baclofen in treatment of severe spasticity with intrathecal baclofen. *Clinical Neurology and Neurosurgery, 104*, 142–145.

Nissbrandt, H., & Engberg, G. (1996). The GABAB-receptor antagonist, CGP 35348, antagonises gamma-hydroxybutyrate- and baclofen-induced alterations in locomotor activity and forebrain dopamine levels in mice. *Journal of Neural Transmission, 103*, 1255–1263.

Parker, D. A., Marino, V., Ong, J., Puspawati, N. M., & Prager, R. H. (2008). The CGP7930 analogue 2,6-di-tert-butyl-4-(3-hydroxy-2-spiropentylpropyl)-phenol (BSPP) potentiates baclofen action at $GABA_B$ autoreceptors. *Clinical and Experimental Pharmacology & Physiology, 35*, 1113–1115.

Paronis, C. A., & Bergman, J. (1999). Apparent pA2 values of benzodiazepine antagonists and partial agonists in monkeys. *Journal of Pharmacology and Experimental Therapeutics, 290*, 1222–1229.

Rush, C. R., Kelly, T. H., Hays, L. R., & Wooten, A. F. (2002). Discrimnative-stimulus effects of modafinil in cocaine-trained humans. *Drug and Alcohol Dependence, 67*, 311–322.

Sannerud, C. A., & Ator, N. A. (1995). Drug discrimination analysis of midazolam under a three-lever procedure: I. Dose-dependent differences in generalization and antagonism. *Journal of Pharmacology and Experimental Therapeutics, 272*, 100–111.

Schechter, M. D. (1997). Discrete versus cumulative dosing in dose-response discrimination studies. *European Journal of Pharmacology, 326*, 113–118.

Seabrook, G. R., Howson, W., & Lacey, M. G. (1990). Electrophysiological characterization of potent agonists and antagonists at pre- and postsynaptic GABAB receptors on neurones in rat brain slices. *British Journal of Pharmacology, 101*, 949–957.

Sell, S. L., McMahon, L. R., & France, C. P. (2003). Relative efficacy of buprenorphine, nalbuphine and morphine in opioid-treated rhesus monkeys discriminating naltrexone. *Journal of Pharmacology and Experimental Therapeutics, 306*, 1167–1173.

Shannon, H. E., & Holtzman, S. G. (1976a). Evaluation of the discriminative effects of morphine in the rat. *Journal of Pharmacology and Experimental Therapeutics, 198*, 54–65.

Shannon, H. E., & Holtzman, S. G. (1976b). Blockade of the discriminative effects of morphine in the rat by naltrexone and naloxone. *Psychopharmacology, 50*, 119–124.

Shannon, H. E., & Holtzman, S. G. (1977). Further evaluation of the discriminative effects of morphine in the rat. *Journal of Pharmacology and Experimental Therapeutics, 201*, 55–66.

Shannon, H. E., & Holtzman, S. G. (1979). Morphine training dose: A determinant of stimulus generalization to narcotic antagonists in the rat. *Psychopharmacology, 61*, 239–244.

Smith, M. A., Gergans, S. R., & Lyle, M. A. (2006). The motor-impairing effects of GABA(A) and GABA(B) agonists in gamma-hydroxybutyrate (GHB)-treated rats: Cross-tolerance to baclofen but not flunitrazepam. *European Journal of Pharmacology, 552*, 83–89.

Snodgrass, S. H., & McMillan, D. E. (1991). Effects of schedule of reinforcement on a pentobarbital discrimination in rats. *Journal of Experimental Analysis of Behavior, 56*, 313–329.

Stolerman, I. P., & Mariathasan, E. A. (1990). Discrimination of an amphetamine-pentobarbitone mixture by rats in an AND-OR paradigm. *Psychopharmacology, 102*, 557–560.

Stoops, W. W. (2006). Aripiprazole as a potential pharmacotherapy for stimulant dependence: Human laboratory studies with d-amphetamine. *Experimental and Clinical Psychopharmacology, 14*, 413–421.

Walker, E. A., Makhay, M. M., House, J. D., & Young, A. M. (1994). In vivo apparent pA2 analysis for naltrexone antagonism of discriminative stimulus and analgesic effects of opiate agonists in rats. *Journal of Pharmacology and Experimental Therapeutics, 271*, 959–968.

Walker, E. A., Richardson, T. M., & Young, A. M. (1997). Tolerance and cross-tolerance to morphine-like stimulus effects of mu opioids in rats. *Psychopharmacology, 133*, 17–28.

White, J. M., & Holtzman, S. G. (1981). Three-choice drug discrimination in the rat: Morphine, cyclazocine and saline. *Journal of Pharmacology and Experimental Therapeutics, 217*, 254–262.

Winger, G., & Herling, S. (1982). Discriminative stimulus effects of pentobarbital in rhesus monkeys: Tests of stimulus generalization and duration of action. *Psychopharmacology, 76*, 172–176.

Winter, J. C. (1981). The stimulus properties of gamma-hydroxybutyrate. *Psychopharmacology, 73*, 372–375.

Woolverton, W. L., & Nader, M. A. (1995). Effects of several benzodiazepines, alone and in combination with flumazenil, in rhesus monkeys trained to discriminate pentobarbital from saline. *Psychopharmacology, 122*, 230–236.

Yamada, K., Yu, B., & Gallagher, J. P. (1999). Different subtypes of GABAB receptors are present at pre- and postsynaptic sites within the rat dorsolateral septal nucleus. *Journal of Neurophysiology, 81*, 2875–2883.

Young, A. M. (1991). Tolerance to drugs acting as discriminative stimuli. *NIDA Research Monograph, 116*, 197–211.

Chapter 10
Targeting the $GABA_B$ Receptor for the Treatment of Epilepsy

Krutika Joshi, Miguel Angel Cortez, and O. Carter Snead

Abstract Epilepsy is a disorder of neural networks that is characterized by spontaneous recurrent seizures. The role of $GABA_B$ receptor-mediated mechanisms in the pathogenesis of seizures depends upon neural networks involved, which determine the seizure type. Generalized seizures involve diffuse, bi-hemispheric neuronal networks, while focal seizures involve regional brain networks. $GABA_B$ receptor agonists have been shown to diminish seizure activity in mouse models of both generalized convulsive and focal seizures. However, generalized non-convulsive seizures such as typical and atypical absence seizures (AASs) characteristically are exacerbated by $GABA_B$ receptor agonists and blocked by $GABA_B$ receptor antagonists. The reason for this dichotomy is the involvement of thalamic circuitry in both typical and atypical absence seizures. Thalamocortical circuitry underpins typical absence seizures (TASs) and hippocampal-thalamocortical circuitry is involved in AASs. In addition, high doses of $GABA_B$ receptor agonists active at the $GABA_B$ receptor/G-protein coupled inwardly rectifying potassium 2 (GIRK2) channel recently have been shown to induce the phenotype of a specific catastrophic epilepsy syndrome of childhood, infantile spasms, in mice. Therefore, $GABA_B$ receptor-mediated mechanisms can be pro- or anti-convulsant depending on the nature of the patho-

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logical neuronal networks involved. Although there are pre-clinical data in support of the efficacy of $GABA_B$ receptor agonists and antagonists in specific experimental models of seizures, these data have not been translated into the clinical arena because of the potential for downstream adverse effects. The therapeutic goal for the use of these compounds in epilepsy awaits a strategy that targets only those $GABA_B$ receptor for specific networks that are involved in a given pathological state.

Keywords $GABA_B$ receptor • Non-convulsive seizures • Convulsive seizures • Infantile spasms • Thalamocortical circuitry • Thalamohippocampal circuitry

10.1 Introduction

10.1.1 Seizures and Epilepsy

An epileptic seizure is a transient event of signs and/or symptoms due to excessive and/or synchronous neuronal activity (Berg et al. 2010). The seizure semiology, i.e. its clinical manifestation, reflects the almost infinite clinical repertoire of the brain since the clinical appearance of the seizure depends on the region of the brain where this paroxysmal neuronal discharge occurs. Seizure semiology ranges from the classical tonic–clonic seizure (convulsion) that results from involvement of the entire brain, to staring spells, which may involve thalamocortical (TC) and/or limbic circuitry. Generalized seizures have been defined as occurring in and rapidly engaging bilaterally distributed networks (generalized), while focal seizures occur within networks limited to one hemisphere and either discretely localized or more widely distributed (Berg et al. 2010).

Seizures are a symptom of epilepsy, but not all patients who have seizures have epilepsy. Rather, the term “epilepsy” refers to a chronic disease of the brain that is characterized by spontaneous, unprovoked, recurrent seizures. Epilepsy may be thought of as a disorder of neuronal networks or circuits (Chiang and Haneef 2014; Spencer 2002; Richardson 2012). Many people with epilepsy have more than one type of seizure and may be afflicted with a number of co-morbid conditions. An electroclinical or epilepsy syndrome is a set of clinical, signs, and symptoms that define a distinctive, recognizable clinical disorder (Berg et al. 2010).

The mechanisms of seizure generation in the brain are separate from those of epileptogenesis. The mechanisms of epileptogenesis are thought to involve molecular changes that contribute to formation of ictogenic neuronal networks, including genes regulating plasticity, cell death, proliferation, and inflamatory or immune responses (Pitkanen and Lukasiuk 2011). Mechanisms of seizures, on the other hand, involve hyperactivity of excitatory amino acid systems, insufficient GABA receptor-mediated inhibitory neurotransmission (both $GABA_A$ and $GABA_B$ receptor), and disturbances in intrinsic properties of neuronal membranes (Lason et al.

2013; Raimondo et al. 2015). Therefore, in the remainder of this chapter we will be discussing the role of $GABA_B$ receptor-mediated mechanisms in the mechanisms of seizure generation more than epileptogenesis.

10.1.2 Role of $GABA_B$ Receptor-Mediated Mechanisms in Seizures

The molecular structure and trafficking of the $GABA_B$ receptor is extensively reviewed in this volume by Benke and colleagues (See Chap. 4 of this book) and so, will be dealt with only briefly here for the purposes of completeness. The $GABA_B$ receptor is a heterodimer consisting of R1 and R2 subunits, the presence of both being essential for membrane trafficking (Han et al. 2012). Several isoforms of the $GABA_BR1$ subunit exist, but the most abundant are $GABA_BR1a$ and $GABA_BR1b$ (Han et al. 2012). The $GABA_BR1a$ subunits are predominantly found on presynaptic terminals of both excitatory and inhibitory synapses while the $GABA_BR1b$ subunit exists primarily in postsynaptic locations on excitatory synapses (Han et al. 2012).

The role of $GABA_B$ receptor-mediated mechanisms in seizures is dependent upon the seizure type and the neuronal network involved. $GABA_B$ receptor agonists provide anticonvulsant effect' in rat models of generalized convulsive seizures (Velísková et al. 1996). However, while $GABA_B$ receptor agonists may be anticonvulsant against convulsive seizures, they exacerbate non-convulsive seizures due to abnormal thalamocortical (TC) circuitry activity. Conversely, $GABA_B$ receptor antagonists are specifically anticonvulsant against non-convulsive seizures where TC circuitry is involved (Onat et al. 2013).

10.2 Generalized Non-convulsive Seizures

10.2.1 $GABA_B$ Receptor-Mediated Mechanisms and Typical Absence Seizures

10.2.1.1 Introduction

The prototypical non-convulsive seizure is the typical absence seizure (TAS) characterized by a transient, intermittent impairment of consciousness associated with a brief interruption of behavior and a simultaneous, time-locked bilaterally synchronous, symmetrical 2.5–4 Hz spike-and-wave discharges (SWDs) on EEG (Fig. 10.1a). There is no aura or warning that the seizure is about to happen, nor is there a post-ictal state, i.e. a period of altered consciousness or confusion following the seizure (Blumenfeld 2005). Rather the patient is immediately back to normal following the seizure (Blumenfeld 2005).

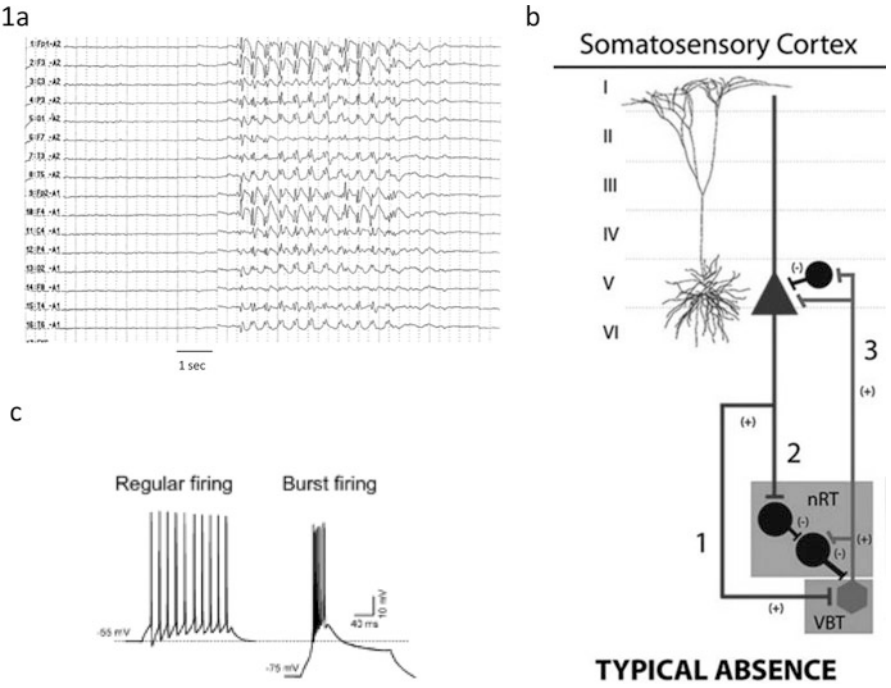

Fig. 10.1 Typical absence seizures (TASs). (**a**) An electroencephalogram (EEG) showing 3 Hz spike wave discharges in the frontal (F), frontal polar (FP), parietal (P), occipital (O), central (C), and temporal (T) lobes. "Z" refers to an electrode placed on the midline. The amplitudes are more predominant in the frontal cortex. Figure adapted from Bilgic et al. (2001). (**b**) Thalamocortical loop in TASs involving reciprocal connections between the somatosensory cortex (1), ventrobasal thamalus (VBT) (1 and 3), and reticular nucleus of the thalamus (nRT) (2). Image adapted from Han et al. (2012). (**c**) Membrane hyperpolarization leads to the activation of T-type calcium channels at −75 mV, resulting in a burst firing pattern (*right image*). This causes recurrent excitation in the thalamus and cortex. Figure adapted from Perez-Reyes (2003)

10.2.1.2 Thalamocortical Circuitry in Absence Seizures

Because of extensive animal model work over the last 20–30 years (Han et al. 2012), TC circuitry is known to be involved in the pathogenesis of generalized absence seizures. Intrathalamic neural networks serve as an intrinsic oscillatory unit within TC circuitry to drive normal and abnormal state-dependent activity in the brain. The function of TC circuitry in this regard depends upon reciprocal synaptic connectivity between excitatory TC relay neurons, excitatory corticothalamic neurons, and inhibitory thalamic GABAergic thalamic reticular neurons from the nucleus reticularis of the thalamus (nRT) and cortical interneurons (Fig. 10.1b) (Steriade 2005; Beenhakker and Huguenard 2009). TC circuitry has

the ability to control states of consciousness by firing in the tonic or oscillatory mode. In the tonic mode the EEG is desynchronized, which is associated with a state of alertness. In the oscillatory mode, the EEG shows synchrony which is associated with an altered state of consciousness which may be normal, i.e. sleep spindles, or pathological, i.e. bilaterally synchronous spike wave discharge associated with absence seizures (Han et al. 2012).

10.2.1.3 The Role of $GABA_B$ Receptor-Mediated Mechanisms in Typical Absence Seizures

The $GABA_B$ receptor-mediated link in this TC system that creates and promulgates the abnormal neuronal oscillatory activity within the circuitry, which manifests itself as SWD and TASs, is low-voltage activated (LVA) Ca^{2+} current which involves LVA Ca^{2+} channels (Crunelli and Leresche 2002). The voltage-gated Ca^{2+} channel is found in both thalamus (Khosravani and Zamponi 2006) and frontal cortex (Karameh and Massaquoi 2009), and has the property of exquisite voltage sensitivity that allows regulation of oscillatory behavior within neuronal networks. In corticothalamic circuitry, powerful $GABA_B$ receptor-mediated inhibition results in deinactivation of LVA Ca^{2+} channels with resultant LVA Ca^{2+} current, rebound burst firing, recurrent excitation of the nRT, and ventrobasal TC neurons, with resultant oscillations within the circuit (Fig. 10.1c). Thus, burst firing within the TAS circuitry depends upon LVA Ca^{2+} current which is coupled to $GABA_B$ receptor-mediated inhibition (Beenhakker and Huguenard 2009; Crunelli and Leresche 2002). Therefore, $GABA_B$ receptor-mediated mechanisms are critically involved in the pathogenesis of absence seizures. Indeed, one of the criteria for an experimental animal model of absence seizures (Table 10.1) is that the seizures be exacerbated by a $GABA_B$ receptor agonist and blocked by a $GABA_B$ receptor antagonist (Onat et al. 2013). This pharmacological profile has been demonstrated extensively in both genetic (Snead et al. 1999) and pharmacological (Snead et al. 1999) animal models of TASs.

In spite of the compelling evidence of the efficacy of $GABA_B$ receptor antagonists against experimental absence seizures, these compounds have not been used to treat this disorder in humans, because of the risk of acting on other downstream targets of $GABA_B$ receptor. Ethosuximide, which is a T-type Ca^{+2} channel blocker, is the first line of treatment for patients with TAS (Glauser et al. 2013; Callaghan et al. 1982). Blocking the T-type Ca^{+2} channel prevents the recurrent excitation of the nRT and cortex caused by membrane hyperpolarization, thereby normalizing the activity of the TC loop. Ethosuximide has been effective in over 75 % of children with TASs and has resulted in a reduction of seizure frequency (Glauser et al. 2013; Callaghan et al. 1982).

An apparent dichotomy in the mechanism-based treatment of TASs is the efficacy of benzodiazepines. These compounds are allosteric modulators of the $GABA_A$ receptor and thus based on the evidence presented above would be predicted to exacerbate, not ameliorate, absence seizures. However, the efficacy of benzodiazepines in absence

Table 10.1 Criteria for animal models of typical absence seizures in rodent

Bilaterally synchronous 7–9 Hz SWD
SWD recorded from TC circuitry
SWD not observed in hippocampus
Immobility with staring and facial myoclonus
Onset/offset of SWD time-locked with immobility, staring, and facial myoclonus
Reproducible and predictable
Quantifiable EEG and behavioral changes
Seizures blocked by ETO, VPA, BDZ
Seizures exacerbated by PHT, CBZ
Seizures exacerbated by $GABA_A$ receptor and $GABA_B$ receptor agonists
Seizures blocked by $GABA_B$ receptor antagonists

SWD spike-wave discharge, *EEG* electroencephalogram, *ETO* ethosuximide, *VPA* valproic acid, *BDC* benzodiazepines, *PHT* phenytoin, *CBZ* carbamazepine
From Snead (2002)

seizures is directly related to their specific effect upon intra-nRT GABAergic networks (Huguenard and Prince 1994). Benzodiazepines target these networks thereby increasing inhibition *within* the nRT with a resultant decrease in the inhibitory output of nRT and dampening down of the circuitry (Huguenard and Prince 1994).

To date there has been no significant linkage between $GABA_B$ receptor polymorphisms and childhood absence epilepsy (Crunelli and Leresche 2005). However, single nucleotide polymorphisms (SNPs) have been found in the *CACNA1A* gene, which codes for the T-Type Ca^{2+} channel in children with TASs (Crunelli and Leresche 2002).

Although we have focused exclusively upon $GABA_B$ receptor-mediated mechanisms in absence seizures, it should be noted that there is extensive cross talk between $GABA_B$ receptor and $GABA_A$ receptor which has a profound effect upon excitability throughout the brain and, in particular, within TC circuitry. Presynaptic $GABA_B$ receptor regulates the release of GABA and thus influences postsynaptic $GABA_A$ receptor. In addition, $GABA_B$ receptors also can regulate $GABA_A$ receptor-mediated tonic inhibitory tone by modulating $GABA_A$ receptor via postsynaptic mechanisms (Connelly et al. 2013). Specifically $GABA_B$ receptor-mediated enhancement of $GABA_A$ receptor-mediated tonic current in TC circuitry is sufficient to significantly alter the excitability of TC cells, indicating that this source of modulation may be of functional importance in both normally (sleep spindles) and abnormally (absence seizures) functioning TC circuitry (Connelly et al. 2013). Although increased extrasynaptic $GABA_A$ receptor-mediated tonic inhibition due to compromised GABA uptake by the GABA transporter GAT-1 may lead to absence seizures, the GAT-1 does not appear to be involved in $GABA_B$ receptor-mediated $GABA_A$ tonic current (Connelly et al. 2013; Cope et al. 2009).

Table 10.2 Comparison of features of typical and atypical absence seizures in rodent models and human[a]

	Rat/mouse		Human	
	(Typical)	(Atypical)	(Typical)	(Atypical)
EEG				
Bilaterally synchronous SWD	+	+	+	+
SWD frequency[b]	7–11 Hz	4–6 Hz	2.5–4 Hz	1.5–3 Hz
SWD from thalamus and cortex	+	+	+	+
SWD from hippocampus[b]	–	+	–	+
Ictal behavior				
Staring; myoclonus	+	+	+	+
Move during SSWD[b]	–	+	–	+
Precise EEG/behavioral correlation[b]	+	–	+	–
Pharmacology				
Blocked by ETO, VPA, BDZ	+	+	+	+
Exacerbated by $GABA_{A–B}$ receptor agonists	+	+	+	+
Blocked by $GABA_B$ receptor antagonists	+	+	No data	
Severe cognitive disability[b]	–	+	–	+

[a]Modified from Onat et al. (2013)

[b]Characteristics that separate atypical absence seizures from typical absence seizures. *ETO* ethosuximide, *VPA* valproic acid, *BDZ* benzodiazepines, *SWD* spike-and-wave discharge, *SSWD* slow spike-and-wave discharge

10.2.2 $GABA_B$ Receptor-Mediated Mechanisms and Atypical Absence Seizures

10.2.2.1 Introduction

Atypical absence seizures (AASs) share the same anticonvulsant drug pharmacology as TASs but differ in semiology, associated EEG abnormalities, severity, refractoriness to medical therapy, co-morbid cognitive impairment, and an association with the catastrophic pediatric epilepsy syndrome of Lennox Gastaut (Table 10.2). These differences between typical and AAS appear to be circuitry dependent. While both typical and AAS involve TC circuitry, they each engage different neuronal networks within that circuitry with AAS involving limbic-TC circuitry (Wang et al. 2009; Perez Velazquez et al. 2007) while in typical absence the limbic circuitry is minimally engaged, if at all (Onat et al. 2013).

Electrographically, AAS have an ictal EEG profile of slow spike-wave discharges (SSWDs) which occur at a frequency of 1–2 Hz and which do not always show perfect synchrony like the 3 Hz SWDs in TASs (Fig. 10.2a) (Nolan et al. 2005). Also unlike TASs, in AAS the behavioral arrest is not time-locked with the onset and offset of the SSWDs (Nolan et al. 2005). However, the pharmacological profile of TASs is the same as that of AAS. Ethosuximide and valproic acid are effective in 70 % of patients.

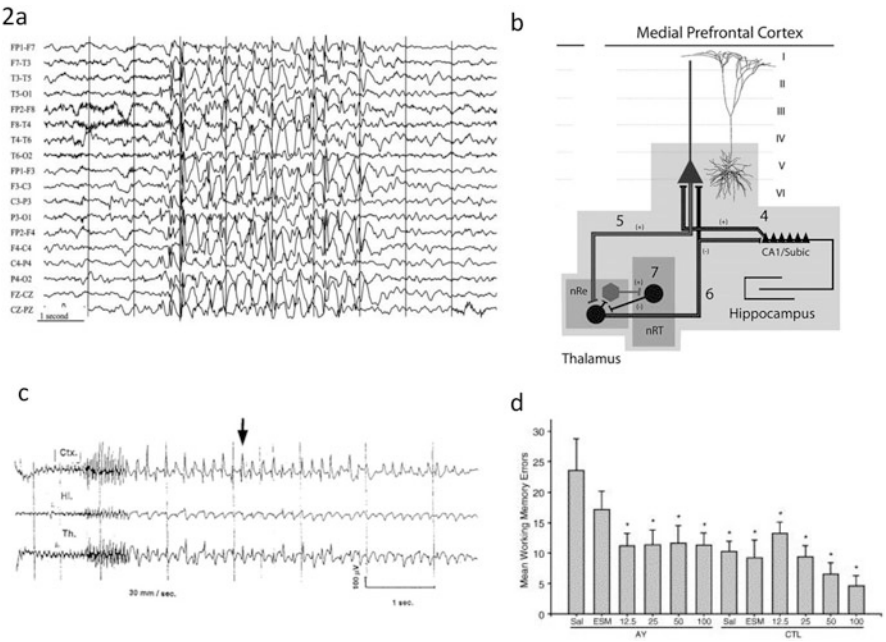

Fig. 10.2 Atypical absence seizures (AAS). (**a**) An EEG showing 1–2 Hz slow spike wave discharges in the frontal (F), frontal polar (FP), parietal (P), occipital (O), central (C), and temporal (T) lobes. "Z" refers to an electrode placed on the midline. Figure adapted from Nolan et al. (2005). (**b**) Cortico-thalamo-hippocampal loop in AAS involving reciprocal connections between the medial prefrontal cortex (5), nucleus reunions of the thamalus (nRe) (5 and 6), reticular nucleus of the thalamus (nRT) (7) and CA1 of the hippocampus (4). Figure adapted from Han et al. (2012). (**c**) Spontaneous 5 Hz spike-waves in the AY-9944 model of AAS recorded using bipolar depth electrodes. The recording shows slow spike-wave discharges (SSWDs) in the cortex (ctx), thalamus (Th), and hippocampus (Hi) of the AY-9944 rat. Figure adapted from Han et al. (2012). (**d**) Proof of hippocampal involvement in AAS. The AY-9944 rats (AY) showed a greater number of working memory errors compared to control rats (CTL) as seen in the radial arm maze task. A dose-dependent decrease in errors was seen with GABAb antagonist CGP 35348 (ESM = single dose ethosuximide). Figure adapted from Chan et al. (2006)

The pharmacological similarity of typical to AAS suggests that both seizure types arise from alteration in the activity of TC circuitry; however, unlike TASs, in AAS the deinactivation of LVA Ca^{2+} channels with resultant LVA Ca^{2+} current, rebound burst firing, and recurrent excitation of the nRT described above engages midline TC neurons, most likely the nucleus reunions (nRe) of the thalamus as well as hippocampal circuitry (Fig. 10.2b) (Wang et al. 2009). Unlike the reverberating cortico-thalamo-cortical circuitry responsible for the electrographic, behavioral, and pharmacological characteristics of TASs, the circuitry that underpins atypical seizures clearly involves thalamo-hippocampal networks, hence the atypical seizure semiology, the co-morbid intellectual disability, and the heightened propensity for the development of other catastrophic epilepsy syndromes such as Lennox Gastaut (Onat et al. 2013).

10.2.2.2 The Role of the $GABA_B$ Receptor-Mediated Mechanisms in Atypical Absence Seizures

In a manner similar to TASs, burst firing within the circuitry that underpins AAS also depends upon LVA Ca^{2+} current which is coupled to $GABA_B$ receptor-mediated inhibition. Therefore, the clinical efficacy of ethosuximide, valproic acid, and benzodiazepines observed in TASs also is seen in AAS. Similarly, in experimental animal models of AAS the seizures are exacerbated by $GABA_B$ receptor agonists and blocked by $GABA_B$ receptor antagonists (Cortez et al. 2001). There are two animal models of chronic AAS that have been fully characterized which have shed some light on the role of $GABA_B$ receptor- mediated mechanisms in AAS. Since these animal models are characterized by chronic, spontaneous, recurrent AAS, they represent animal models of atypical absence epilepsy.

The AY-9944 (AY) model of atypical absence epilepsy is a pharmacological model where developing Long-Evans hooded rats are treated with the cholesterol synthesis inhibitor *trans-N,N'-bis*[2-Chlorophenylmethyl]-1,4-cyclohexanedimethanamine dihydrochloride (AY-9944).

AY rats show cognitive impairment and bilaterally synchronous 4–5 Hz ictal SSWDs during which period, the animal sometimes moves (Cortez et al. 2001). Depth electrode recordings show the presence of SSWDs as well as high phase synchrony in the cortex, thalamus, and hippocampus (Perez Velazquez et al. 2007), which demonstrates the disinhibition of the hippocampus and its subsequent involvement in the circuitry involved in AAS (Fig. 10.2c). The involvement of the hippocampus in AY animals also has been observed. Radial arm maze testing demonstrates deficits in hippocampal-dependent learning (Fig. 10.2d) and in-vitro studies indicate altered long-term potentiation (LTP) in hippocampal CA1 neurons of these rats (Chan et al. 2006).

Cholesterol is an important component of lipid rafts which are lipid microdomains on the cell surface that are involved in protein trafficking (Huo et al. 2009). Inhibition of cholesterol synthesis by AY-9944 has been shown to alter lipid raft-associated $GABA_B$ receptor and increases the trafficking and expression levels of $GABA_B$ receptor in forebrain regions suggesting a possible $GABA_B$ receptor-mediated mechanism of epileptogenesis in this animal model (Huo et al. 2009). Additionally, a significant increase in $GABA_B$R1a and R1b mRNA and protein expression throughout the forebrain of the AY model has been described, but no change in the expression of either subunit was observed in cerebellum in the AY rats compared to sham controls (Snead et al. 2000b).

The finding that $GABA_B$R1a and R1b were overexpressed in the forebrain of the AY model of AAS gave rise to the hypothesis that overexpression of $GABA_B$R1a and R1b in mouse forebrain would result in an atypical absence epilepsy phenotype. This hypothesis was tested by creating transgenic mice that overexpressed $GABA_B$R1a in forebrain using a Calcium/Calmodulin-dependent kinase II alpha (CaMKIIa) promoter. $GABA_B$R1a transgenic mice ($R1a^{tg}$) were generated and shown to have increased $GABA_B$ receptor-mediated function in forebrain. The $R1a^{tg}$ mice showed the characteristic atypical absence epilepsy phenotype with severe, prolonged AAS, involvement of hippocampal circuitry in the SSWD, and impairment of both CA1-LTP, and hippocampal-dependent learning (Wu et al. 2007). $GABA_B$R1b trangenics also were generated and characterized. The R1b

transgenics also showed an atypical absence epilepsy phenotype, but it was not as robust as the R1a phenotype suggesting that the severity of AAS and their co-morbidities is $GABA_B$ receptor subunit dependent (Stewart et al. 2009).

From a clinical perspective, as mentioned, AAS tend to be medically refractory unlike TASs, but ethosuximide, valproate, and benzodiazepines all have some beneficial effect. $GABA_B$ receptor antagonists have not been used as a line of treatment for AAS (Nolan et al. 2005) because these compounds are not clinically available. Zonizamide, which is a weak T-type Ca^{2+} channel blocker, has shown some promise in treating AAS (Lee et al. 2010). Carbamazepine, well known to exacerbate TASs, has been shown in some studies to exacerbate AAS by upregulating $GABA_B$ receptors (Snead and Hosey 1985).

10.3 Generalized Convulsive Seizures

10.3.1 Introduction

The generalized convulsive seizure types include tonic, clonic, and tonic–clonic seizures. In a tonic seizure, the body's normal muscle tone becomes greatly enhanced and the torso, and arms exhibit sudden stiffening movements (Intusoma et al. 2013). A clonic seizure consists of rapidly alternating states of contraction and relaxation of the muscle, resulting in jerking movements (Panayiotopoulos 2005). A generalized tonic–clonic seizure or convulsion (GTC) is represented by stiffening followed by jerking movements (Libenson et al. 2010). Generalized seizures may occur in a variety of epilepsy syndromes. Other types of generalized seizures include myoclonic, which consists of brief muscle jerks, and atonic seizures which consist of a loss of tone with falling as the ictal event (Berg et al. 2010).

EEG recordings from patients with generalized convulsive seizures demonstrate multiple spike-wave complexes and spikes with increasing frequencies (i.e. paroxysmal fast wave discharges) during the tonic phase followed by rhythmic polyspikes/slow waves associated with clonic jerking movements (Kaufman 2007) (Fig. 10.3a). The spikes are associated with the jerks and the slow waves with the muscle relaxation (Fig. 10.3a). The post-ictal phase shows a depression of the EEG waveform (Fig. 10.3a) (Engel 2013).

10.3.2 The Role of $GABA_B$ Receptor-Mediated Mechanisms in Generalized Convulsive Seizures

A number of animal models have been utilized to explore the putative role of GABAergic mechanisms in generalized convulsive seizures. Kindling is a popular method of induction of chronic generalized seizures, where a sub-convulsive electrical or chemical stimulus is administered repeatedly and intermittently until full-blown convulsions are generated (Dhir 2012). There are many variations on this theme that have been used to produce chronic epilepsy models in rodents (Modebadze et al.

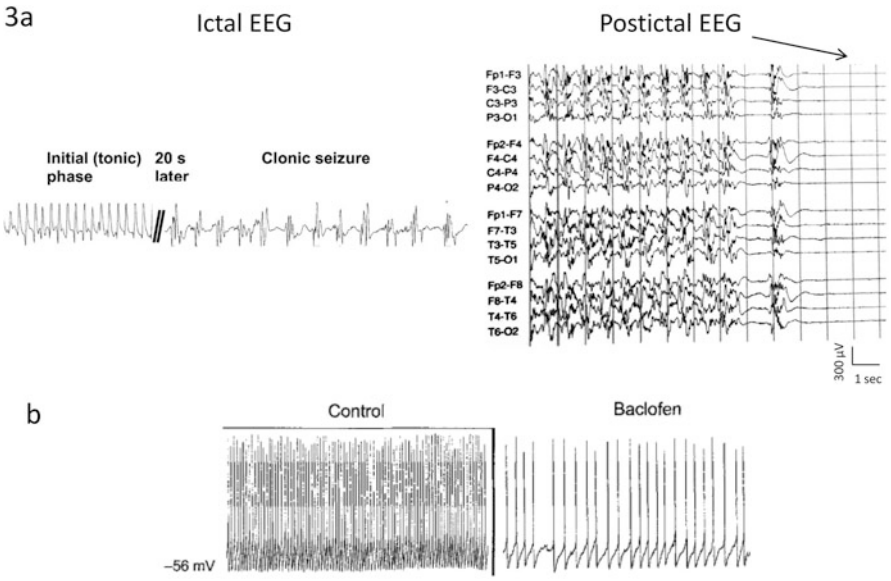

Fig. 10.3 Generalized convulsive seizures. (**a**) Ictal EEG in a patient with focal clonic seizures showing tonic and clonic seizure activity and post-ictal EEG showing the refractory period. Figure adapted from Hamer et al. (2003) and Libenson (2010). Focal onset seizures are often the source of secondary generalization. (**b**) Whole cell current clamp recordings showing baclofen reduces the frequency of action potentials without changing membrane potentials in globus pallidus neurons. Figure adapted from Chen et al. (2004)

2016). Pentylenetetrazol (PTZ) is a $GABA_A$ agonist that may be used to induce acute seizures in rodents. PTZ-induced seizures are dose-dependent. Low doses induce absence like seizures; mid-range doses induce clonic forelimb seizures which model myoclonic seizures in human, and high doses produce generalized tonic seizures (Snead et al. 2000b). As well PTZ can be used to kindle animals to chronic generalized convulsive seizures (Squires et al. 1984; Kliouéva et al. 2001; Nishida et al. 2007).

High-affinity $GABA_B$ receptor agonists such as baclofen have been shown to have anticonvulsant activity in PTZ-induced seizures in rats (Veliskova et al. 1996). Further, baclofen exacerbates post-ictal refractoriness in kindled rats (Wurpel et al. 1990). Whole—cell current—clamp recordings of neurons in the globus pallidus have shown that baclofen-applied post-PTZ induction reduces the number of ictal spikes and causes membrane hyperpolarization (Fig. 10.3b) (Chen et al. 2004). Conversely, the $GABA_B$ receptor antagonist CGP 55845A exacerbates kindling-induced seizures (Lang et al. 2014).

$GABA_B$ receptor knockout mice also have been used to study generalized convulsive seizures. Null mutants of the pre- and post-synaptic $GABA_B$ R1 subunit ($GABA_BR1^{-/-}$) display several episodes of spontaneous clonic seizures per day, and sporadic bouts of tonic–clonic seizures (Schuler et al. 2001). The ictal EEG in these mice demonstrates classic polyspike activity and spike-and-wave complexes during tonic–clonic seizures (Schuler et al. 2001). Single neuron recordings show both a pre- and post-synaptic loss of $GABA_B$ responses and loss of baclofen-induced pre and post-synaptic $GABA_B$ activation (Schuler et al. 2001).

The $GABA_B$ agonist baclofen also has been used to reduce susceptibility to audiogenic seizures in a mouse model of fragile X syndrome (Pacey et al. 2009). Fragile X syndrome occurs due to a mutation in the X-linked FMR1 gene, leading to a deficit in the fragile X mental retardation protein (FMRP). FMRP activates regulator of G protein signaling 4 (RGS4), which in turn modulates the activity of $GABA_B$ (Pacey et al. 2009). FMRP knockout mice show RGS4 suppression and group 1 metabotropic glutamate receptor (mGluR) activation. This resulting imbalance in $GABA_B$ and mGluR signaling, has been thought to be the cause of the audiogenic seizures experienced by 20 % of patients with fragile X syndrome (Pacey et al. 2009). Administration of baclofen blocks seizures in the FMRP knockout mouse model of fragile X syndrome (Pacey et al. 2009).

Studies linking $GABA_B$ polymorphisms to generalized convulsive seizures are scarce. Large genome-wide association studies have shown that the $GABA_BR1$ gene maps close to the HLA class I histocompatibility antigen, alpha chain F (HLA-F) locus on chromosome 6p21.3 which is a susceptibility locus for idiopathic generalized epilepsy with tonic–clonic seizures (Ander et al. 1999). However, there has been no indication that genetic variants of the $GABA_B$ receptor gene confer different susceptibilities to developing idiopathic generalized epilepsy (Ander et al. 1999).

Patients with paraneoplastic syndrome secondary to a tumor have presented with autoantibodies against $GABA_B$ receptor (Höftberger et al. 2013). These patients present with severe seizures, which often progress to status epilepticus (Höftberger et al. 2013). Treatment of the tumor reverses these symptoms. As well, patients are beginning to be reported with generalized seizures secondary to autoimmune encephalitis associated with $GABA_B$ receptor antibodies in the cerebrospinal fluid (Alexopoulos et al. 2014; Kruer et al. 2014).

10.4 Focal Seizures

10.4.1 Introduction

Focal epileptic seizures are conceptualized as originating within networks limited to one hemisphere. They may be discretely localized or more widely distributed. As well, focal seizures may originate in subcortical structures. For each seizure type, ictal onset is consistent from one seizure to another, with preferential propagation patterns that can involve the contralateral hemisphere (Berg et al. 2010; Bradley 2012). Focal seizures may be defined using the descriptors of "with impairment of consciousness", which used to be called partial complex, and "without impairment of consciousness", which used to be called partial simple. As well focal seizures can secondarily generalize (Berg et al. 2010) to generalized convulsive seizures.

Due to the large range of symptoms and the vast plethora of brain regions involved in focal motor or focal sensory seizures, the EEG characteristics seen in patients with focal seizures can be remarkably variable. Ictal theta waves, spikes, or

sharp waves may be localized to a small region in a single hemisphere during some focal seizures (Bare et al. 1994). In other focal seizures, such as frontal lobe seizures emanating from deep in the frontal lobe or some mesial temporal seizures, there may be little evidence on a scalp EEG of spikes or sharp waves because the ictal event does not make it to the surface of the brain to be recorded from a scalp electrode. Variable post-ictal symptoms can be seen in patients depending on the neuronal network involved in seizure initiation and propagation. For example, focal motor seizures may be followed by transient contralateral weakness and focal sensory seizures may be followed by contralateral numbness in that particular region of the body (Kelly et al. 1999). These post-ictal phenomena may be represented by slowing on the EEG with predominance of delta activity (Jan et al. 2001).

EEG characteristics of focal seizures with impairment of consciousness are quite variable, albeit affecting larger areas of a single hemisphere. The most common epilepsy syndrome that presents in this manner is mesial temporal lobe epilepsy (TLE) EEG recordings from patients with TLE show characteristic ictal theta waves and ipsilateral interictal spikes that emanate from the anterior/mid hippocampus (Seeck et al. 2005; Smith 2005).

10.4.2 The Role of $GABA_B$ Receptor-Mediated Mechanisms in Focal Seizures

One of the most commonly used animal models of focal seizures is the amygdaloid kindling animal model of limbic seizures. $GABA_B$ receptor agonists have been shown to be anticonvulsant in this model and $GABA_B$ receptor antagonists exacerbate amygdaloid kindled seizures (Wurpel et al. 1990; Karlsson et al. 1992). Another animal model for focal seizures utilizes intra-hippocampal administration of Kainic Acid (KA) which leads to acute seizures characterized behaviorally by immobility, head nodding, movements of the jaw, rearing, and falling (Levesque and Avoli 2013). The ictal EEG is characterized by paroxysmal bursts and spikes in the hippocampus (Fig. 10.4a). Often, this activity spreads to the ipsilateral amygdala, followed by the contralateral amygdala and surrounding cortical area, thus generating a secondary generalized seizure. Interictal spikes are seen in a large percentage of animals (Lévesque and Avoli 2013).

Administration of a high dose (10 mg/kg) of the $GABA_B$ receptor agonist baclofen to the KA-generated model of TLE reduces the power of gamma oscillations and the frequency of discharges in the CA3 region of the hippocampus (Dugladze et al. 2013). However, low-dose (1 mg/kg) baclofen shows the opposite effect. In-vitro studies have also shown that when high-dose (10 μM) baclofen is applied to CA3 hippocampal slices in a medium containing KA, the frequency of the burst firing in the cells reduces, but a low dose (0.5 μM) of baclofen increases the frequency of burst firing (Dugladze et al. 2013). This dual effect could be explained by the different affinities of the pre- and post-synaptic $GABA_B$ receptor to baclofen.

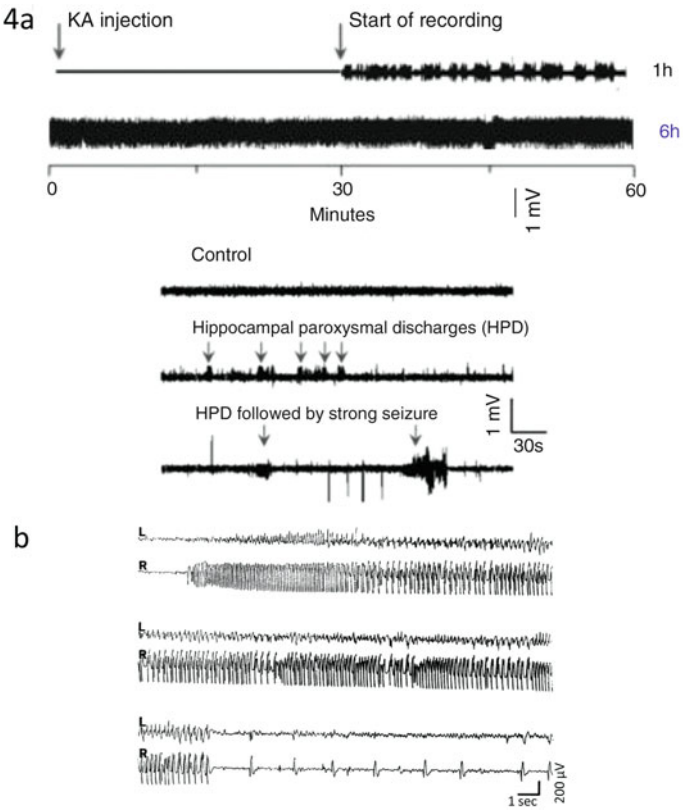

Fig. 10.4 Complex partial seizures. (**a**) Intrahippocampal injection of Kainic acid (KA) and subsequent temporal lobe epilepsy (TLE). Traces showing hippocampal paroxysmal discharge and seizure events 12 days after initial status epilepticus. Figure adapted from Jagirdar et al. (2015). (**b**) Unilateral infusion of $GABA_B$ receptor antagonist CGP 36742 in the right fronto-parietal cortex of a wild type mouse caused local polyspike waves which then migrated to the contralateral cortex becoming a secondary generalized seizure. Figure adapted from Vergnes et al. (1997)

Consistent with the evidence that high-dose baclofen can protect animals from focal seizures generated by KA and electrical kindling, intracortical infusion of $GABA_B$ receptor antagonists have been shown to induce focal seizures which secondarily generalize (Fig. 10.4b) (Dugladze et al. 2013; Vergnes et al. 1997).

From a clinical perspective, to date, no $GABA_B$ receptor agonists have been used against focal seizures. However, carbamazepine, a drug which is quite effective against partial seizures (Mattson et al. 1992), has been shown to upregulate $GABA_B$ receptors (Motohashi et al. 1989). A $GABA_B$ receptor polymorphism (G1465A) has been shown to confer susceptibility to developing TLE (French

2003). The polymorphism, which is a missense mutation, results in an amino acid substitution in the ligand binding site of the $GABA_B$ receptor (French 2003). This may alter the GABA binding affinity for its receptor, thereby leading to an imbalance of excitation and inhibition. Further evidence that altered $GABA_B$ receptor function is present in patients with TLE, comes from receptor autoradiography studies using surgically resected hippocampal tissue. Compared to neurologically normal post-mortem hippocampal tissue, TLE tissue showed a reduction in $GABA_B$ receptor density in the dentate gyrus of the hippocampus and an increase in $GABA_B$ receptor binding in the CA1 (when adjusted for neuronal loss), reflecting altered synaptic activity (Billinton et al. 2001).

10.5 Infantile Spasms

10.5.1 Introduction

As mentioned above, an epilepsy syndrome is a complex of clinical features, signs, and symptoms that together define a distinctive, recognizable clinical disorder. Infantile Spasms is a catastrophic childhood epilepsy syndrome where the seizures are severe, progressive, medically refractory, and associated with severe cognitive impairment as well as the emergence of other forms of severe seizures as the child grows older (Widjaja et al. 2015). This is the most common of the catastrophic epilepsy syndromes in childhood. The seizures in infantile spasms typically present within the first year of life, with clusters of 100–150 massive myoclonic seizures per day, which may be flexor, extensor, or mixed flexor-extensor spasms (Widjaja et al. 2015). These seizures are associated with an electroencephalographic (EEG) pattern called hypsarrythmia, which refers to a chaotic electrical pattern replete with high voltage slowing, multifocal spikes, and/or a burst suppression phenomenon also known as electrodecremental response (EDR) (Fig. 10.5a) (Blichowski et al. 2015). Another prominent feature of infantile spasms is a complete arrest of neurodevelopment commensurate with the onset of the spasms (Widjaja et al. 2015; Carmant 2002). Although refractory to all standard anticonvulsants, infantile spasms respond uniquely to adrenocorticotropic hormone (ACTH) or vigabatrin treatment (Go et al. 2012).

Down syndrome is the phenotypic manifestation of the triplication of human chromosome 21 (HSA21) and is the single most common genetic cause of intellectual disability, affecting 1 in 1000 newborn children in North America (Lott 2012). The relevance of Down syndrome to infantile spasms is that while children with Down syndrome have a much higher incidence of epilepsy than the general population, they are particularly susceptible to the development of infantile spasms (Verrotti et al. 2013). $GABA_B$ receptor-mediated mechanisms may play a role in the increased vulnerability of infants with Down syndrome to infantile spasms (Blichowski et al. 2015).

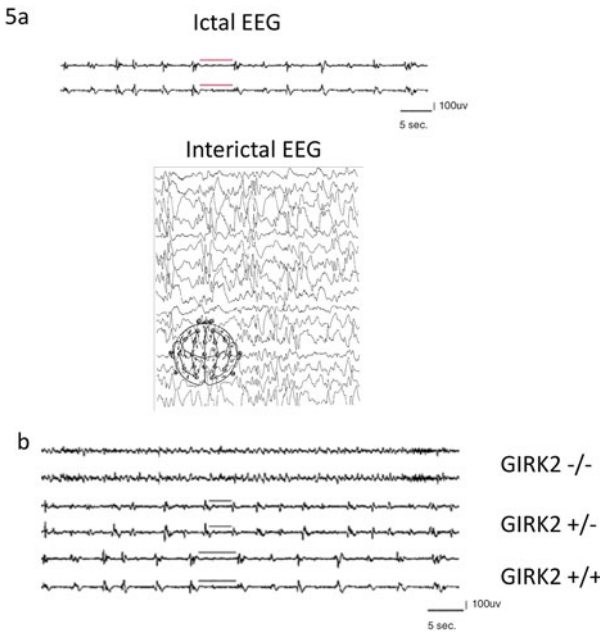

Fig. 10.5 Infantile spasms. (**a**) Ictal and interictal EEG traces in Infantile Spasms. The ictal trace taken from gamma-butyrolactone (GBL)-treated Ts65Dn mice shows 1–4 Hz spike waves and characteristic electrodecremental response (EDR). The interictal trace taken from a 4-month-old infant shows the chaotic pattern of hypsarrythmia characterized by global polyspike waves. From Blichowski et al. (2015) and Carmant (2002). (**b**) GIRK2 null mice (GIRK2$^{-/-}$) are resistant to GBL-induced infantile spasms phenotype. Image adapted from Blichowski et al. (2015)

10.5.2 The Role of GABA$_B$ Receptor-Mediated Mechanisms in Infantile Spasms in Down Syndrome

The most commonly used and widely studied animal model of Down syndrome is the Ts(17^{16})65Dn (Ts65Dn) mouse which is segmentally trisomic for the distal end of murine chromosome 16. The triplicated region of chromosome16 in this mutant animal is predicted to contain ~132 known genes that are homologous to HSA21 in human (Davisson et al. 1990), the "Down syndrome critical region" thought to be sufficient to produce the DS phenotype (Belichenko et al. 2009). The *Kcnj6* gene that encodes for the G protein-coupled inward rectifying potassium (K^+) channel subunit 2 (GIRK2), which is coupled to the GABA$_B$ receptor (Liu et al. 2012), resides within this triplicated region of chromosome16 in the Ts65Dn mouse model. As a result the GABA$_B$ receptor-coupled GIRK2 channel is overexpressed in Ts65Dn brain with a resultant increase in GABA$_B$ receptor-induced potassium current (Best et al. 2007). These GIRK2 data are relevant to infantile spasms, because Ts65Dn mice are exquisitely sensitive to an infantile spasms phenotype induced by very low doses of GABA$_B$ receptor agonists that are active at the GIRK2 channel,

i.e. baclofen and γ-hydroxybutyrate (GHB) (Cortez et al. 2009). Specifically, very low doses of the prodrug of GHB, γ-butyrolactone (GBL) induces extensor spasms associated with bursts of epileptiform activity on the EEG, separated by an EDR similar to that seen in the hypsarrythmic EEG associated with infantile spasms in human (Fig. 10.5a). As well, the $GABA_B$ receptor-agonist-induced infantile spasms phenotype in Ts65Dn mice also has the pharmacological signature of human infantile spasms since the seizures are blocked or attenuated by ACTH and vigabatrin as well as a $GABA_B$ receptor antagonist. Interestingly, GIRK2 null mice fail to show any response to supramaximal doses of GBL (Fig. 10.5b). These very high doses of GBL induce a similar infantile spasms phenotype in wild type mice, but the smaller doses of GBL that induce the infantile spasms phenotype in Ts65Dn mice have no effect in wild type mice.

10.6 Conclusions

$GABA_B$ receptor mediates slow inhibitory neurotransmission throughout the brain and therefore plays an important role in the mechanisms of seizures in a wide variety of seizure types and epilepsy syndromes. Whether $GABA_B$ receptor-mediated mechanisms are pro- or anticonvulsant in this regard depends upon the pathological networks involved in a given pathological process. This chapter has highlighted these network-dependent effects of $GABA_B$ receptor agonists and antagonists in the mechanism of seizures. However, when it comes to translating the anticonvulsant effects of $GABA_B$ receptor agonists and antagonists in specific seizure types and epileptic disorders, the problem remains that considerable side effects of these compounds, both proven and suspected, have hindered their clinical use in epilepsy. As Benke has pointed out it is unlikely to avoid unwanted effects with systemically administered $GABA_B$ receptor agonists and antagonists because $GABA_B$ receptor are involved in a variety of brain functions. Therefore, to indiscriminately stimulate or antagonize them would be to invite significant neurological and psychiatric adverse effects. The aim then should be to target only those $GABA_B$ receptor involved in a given pathological state, such as Down syndrome for example. This strategy awaits the clinical development of appropriate $GABA_B$ receptor antagonists (Benke 2013).

References

Alexopoulos, H., Dagklis, I. E., Akrivou, S., Bostantjopoulou, S., & Dalakas, M. C. (2014). Autoimmune encephalitis with GABAB antibodies, thymoma, and GABAB receptor thymic expression. *Neurology: Neuroimmunology & Neuroinflammation, 1*(4), e39.

Bare, M. A., Burnstine, T. H., Fisher, R. S., & Lesser, R. P. (1994). Electroencephalographic changes during simple partial seizures. *Epilepsia, 35*(4), 715–720.

Beenhakker, M. P., & Huguenard, J. R. (2009). Neurons that fire together also conspire together: Is normal sleep circuitry hijacked to generate epilepsy? *Neuron, 62*(5), 612–632.

Belichenko, N. P., Belichenko, P. V., Kleschevnikov, A. M., Salehi, A., Reeves, R. H., & Mobley, W. C. (2009). The "Down syndrome critical region" is sufficient in the mouse model to confer behavioral, neurophysiological, and synaptic phenotypes characteristic of Down syndrome. *Journal of Neuroscience, 29*(18), 5938–5948.

Benke, D. (2013). GABAB receptor trafficking and interacting proteins: Targets for the development of highly specific therapeutic strategies to treat neurological disorders? *Biochemical Pharmacology, 86*(11), 1525–1530.

Berg, A. T., Berkovic, S. F., Brodie, M. J., Buchhalter, J., Cross, J. H., Van Emde Boas, W., et al. (2010). Revised terminology and concepts for organization of seizures and epilepsies: Report of the ILAE Commission on Classification and Terminology 2005-2009. *Epilepsia, 51*(4), 676–685.

Best, T. K., Siarey, R. J., & Galdzicki, Z. (2007). Ts65Dn, a mouse model of Down syndrome, exhibits increased GABAB-induced potassium current. *Journal of Neurophysiology, 97*(1), 892–900.

Bilgic, B., Baykan, B., Gurses, C., & Gokyigit, A. (2001). Perioral myoclonia with absence seizures: A rare epileptic syndrome. *Epileptic Disorders, 3*(1), 23–27.

Billinton, A., Baird, V. H., Thom, M., Duncan, J. S., Upton, N., & Bowery, N. G. (2001). GABAB receptor autoradiography in hippocampal sclerosis associated with human temporal lobe epilepsy. *British Journal of Pharmacology, 132*(2), 475–480.

Blichowski, M., Shephard, A., Armstrong, J., Shen, L., Cortez, M. A., Eubanks, J. H., et al. (2015). The GIRK2 subunit is involved in ISs-like seizures induced by GABAB receptor agonists. *Epilepsia, 56*(7), 1081–1087.

Blumenfeld, H. (2005). Consciousness and epilepsy: Why are patients with absence seizures absent? *Progress in Brain Research, 150*, 271–286.

Bradley, W. G. (Ed.). (2012). *Bradley's neurology in clinical practice* (6th ed.). Philadelphia, PA: Elsevier/Saunders. Chapter 67.

Callaghan, N., O'Hare, J., O'Driscoll, D., O'Neill, B., & Daly, M. (1982). Comparative study of ethosuximide and sodium valproate in the treatment of typical absence seizures (petit mal). *Developmental Medicine and Child Neurology, 24*(6), 830–836.

Carmant, L. (2002). Infantile spasms: West syndrome. *Archives of Neurology, 59*(2), 317–318.

Chan, K. F., Burnham, W. M., Jia, Z., Cortez, M. A., & Snead, O. C., 3rd. (2006). GABAB receptor antagonism abolishes the learning impairments in rats with chronic atypical absence seizures. *European Journal of Pharmacology, 541*(1–2), 64–72.

Chen, L., Chan, Y. S., & Yung, W. H. (2004). GABA-B receptor activation in the rat globus pallidus potently suppresses pentylenetetrazol-induced tonic seizures. *Journal of Biomedical Science, 11*(4), 457–464.

Chiang, S., & Haneef, Z. (2014). Graph theory findings in the pathophysiology of temporal lobe epilepsy. *Clinical Neurophysiology, 125*(7), 1295–1305.

Connelly, W. M., Fyson, S., Errington, A. C., McCafferty, C. P., Cope, D. W., Di Giovanni, G., et al. (2013). GABAB receptors regulate extrasynaptic GABAA receptors. *Journal of Neuroscience, 33*(9), 3780–3785.

Cope, D. W., Di Giovanni, G., Fyson, S. J., Orban, G., Errington, A. C., Lorincz, M. L., et al. (2009). Enhanced tonic GABAA inhibition in typical absence epilepsy. *Nature Medicine, 15*(12), 1392–1398.

Cortez, M. A., McKerlie, C., & Snead, O. C., III. (2001). A model of atypical absence seizures: EEG, pharmacology, and developmental characterization. *Neurology, 56*(3), 341–349.

Cortez, M. A., Shen, L., Wu, Y., Aleem, I. S., Trepanier, C. H., Sadeghnia, H. R., et al. (2009). Infantile spasms and Down syndrome: A new animal model. *Pediatric Research, 65*(5), 499–503.

Crunelli, V., & Leresche, N. (2002). Childhood absence epilepsy: Genes, channels, neurons, and networks. *Nature Reviews Neuroscience, 3*(5), 371–382.

Davisson, M. T., Schmidt, C., & Akeson, E. C. (1990). Segmental trisomy of murine chromosome 16: A new system for studying Down syndrome. In D. Patteron & C. J. Epstein (Eds.),

Molecular genetics of chromosome 21 and Down syndrome (pp. 263–280). New York: Wiley-Liss.

Dhir, A. (2012). Pentylenetetrazol (PTZ) kindling model of epilepsy. *Current Protocols in Neuroscience, 9*(37), 1–9.

Dugladze, T., Maziashvili, N., Börgers, C., Gurgenidze, S., Haussler, U., Winkelmann, A., et al. (2013). GABA(B) autoreceptor-mediated cell type-specific reduction of inhibition in epileptic mice. *Proceedings of the National Academy of Sciences of the United States of America, 110*(37), 15073–15078.

Engel, J. (2013). Chapter 9: Periictal phenomena. In *Seizures and epilepsy* (pp. 320–341). New York, NY: Oxford University Press.

French, J. (2003). A gene polymorphism associated with temporal lobe epilepsy? *Epilepsy Currents, 3*(4), 123–124.

Glauser, T. A., Cnaan, A., Shinnar, S., Hirtz, D. G., Dlugos, D., Masur, D., et al. (2013). Ethosuximide, valproic acid, and lamotrigine in childhood absence epilepsy: Initial monotherapy outcomes at 12 months. *Epilepsia, 54*(1), 141–155.

Go, C. Y., Mackay, M. T., Weiss, S. K., Stephens, D., Adams-Webber, T., Ashwal, S., et al. (2012). Evidence based guideline update: Medical treatment of infantile spasms. Report of the Guideline Development Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology, 78*(24), 1974–1980.

Hamer, H. M., Lüders, H. O., Knake, S., Fritsch, B., Oertel, W. H., & Rosenow, F. (2003). Electrophysiology of focal clonic seizures in humans: A study using subdural and depth electrodes. *Brain, 126*(Pt 3), 547–555.

Han, H. A., Cortez, M. A., & Snead, O. C., III. (2012). GABAB receptor and absence epilepsy. In J. L. Noebels, M. Avoli, M. A. Rogawski, R. W. Olsen, & A. V. Delgado-Escueta (Eds.), *Jasper's basic mechanisms of the epilepsies [Internet]* (4th ed., pp. 242–256). Bethesda, MD: National Center for Biotechnology Information (US).

Höftberger, R., Titulaer, M. J., Sabater, L., Dome, B., Rozsas, A., Hegedus, B., et al. (2013). Encephalitis and GABAB receptor antibodies: Novel findings in a new case series of 20 patients. *Neurology, 81*(17), 1500–1506.

Huguenard, J. R., & Prince, D. A. (1994). Clonazepam suppresses GABAB-mediated inhibition in thalamic relay neurons through effects in nucleus reticularis. *Journal of Neurophysiology, 71*(6), 2576–2581.

Huo, J. Z., Cortez, M. A., & Snead, O. C. (2009). GABA receptor proteins within lipid rafts in the AY-9944 model of atypical absence seizures. *Epilepsia, 50*(4), 776–788.

Intusoma, U., Abbott, D. F., Masterton, R. A., Stagnitti, M. R., Newton, M. R., Jackson, G. D., et al. (2013). Tonic seizures of Lennox-Gastaut syndrome: Periictal single-photon emission computed tomography suggests a corticopontine network. *Epilepsia, 54*(12), 2151–2157.

Jagirdar, R., Drexel, M., Kirchmair, E., Tasan, R. O., & Sperk, G. (2015). Rapid changes in expression of class I and IV histone deacetylases during epileptogenesis in mouse models of temporal lobe epilepsy. *Experimental Neurology, 273*, 92–104.

Karameh, F. N., & Massaquoi, S. G. (2009). Intracortical augmenting responses in networks of reduced compartmental models of tufted layer 5 cells. *Journal of Neurophysiology, 101*(1), 207–233.

Karlsson, G., Klebs, K., Hafner, T., Schmutz, M., & Olpe, H. R. (1992). Blockade of GABAB receptors accelerates amygdala kindling development. *Experientia, 48*(8), 748–751.

Kaufman, D. M. (2007). Epilepsy. In D. M. Kaufman (Ed.), *Clinical neurology for psychiatrists* (pp. 203–240). Philadelphia, PA: Saunders Elsevier.

Kelly, K. M., Valeriano, J. P., & Solot, J. A. (1999). Chapter 12: Seizures. In S. M. Shah & K. M. Kelly (Eds.), *Emergency neurology: Principles and practice* (pp. 154–172). Cambridge, UK: Cambridge University Press.

Kenyon, K., Mintzer, S., & Nei, M. (2014). Carbamazepine treatment of generalized tonic-clonic seizures in idiopathic generalized epilepsy. *Seizure, 23*(3), 234–236.

Khosravani, H., & Zamponi, G. W. (2006). Voltage-gated calcium channels and idiopathic generalized epilepsies. *Physiological Reviews, 86*(3), 941–966.

Kliouеva, I. A., van Luijtelaar, E. L., Chepurnova, N. E., & Chepurnov, S. A. (2001). PTZ-induced seizures in rats: Effects of age and strain. *Physiology & Behavior, 72*(3), 421–426.

Kruer, M. C., Hoeftberger, R., Lim, K. Y., Coryell, J. C., Svoboda, M. D., Woltjer, R. L., et al. (2014). Aggressive course in encephalitis with opsoclonus, ataxia, chorea, and seizures: The first pediatric case of GABA(B) receptor autoimmunity. *JAMA Neurology, 71*(5), 620–623.

Lang, M., Moradi-Chameh, H., Zahid, T., Gane, J., Wu, C., et al. (2014). Regulating hippocampal hyperexcitability through GABAB receptors. *Physiological Reports, 2*(4), e00278.

Lason, W., Chlebicka, M., & Rejdak, K. (2013). Research advances in basic mechanisms of seizures and antiepileptic drug action. *Pharmacological Reports, 65*(4), 787–801.

Lee, Y. J., Kang, H. C., Seo, J. H., Lee, J. S., & Kim, H. D. (2010). Efficacy and tolerability of adjunctive therapy with zonisamide in childhood intractable epilepsy. *Brain and Development, 32*(3), 208–212.

Lévesque, M., & Avoli, M. (2013). The kainic acid model of temporal lobe epilepsy. *Neuroscience & Biobehavioral Reviews, 37*, 2887–2899.

Libenson, M. H. (2010). Chapter 10: The EEG in epilepsy. In M. H. Libenson (Ed.), *Practical approach to electroencephalography*. Philadelphia, PA: Saunders Elsevier.

Liu, Z. L., Ma, H., Xu, R. X., Dai, Y. W., Zhang, H. T., Yao, X. Q., et al. (2012). Potassium channels underlie postsynaptic but not presynaptic GABAB receptor-mediated inhibition on ventrolateral periaqueductal gray neurons. *Brain Research Bulletin, 88*(5), 529–533.

Lott, I. T. (2012). Neurological phenotypes for Down syndrome across the life span. *Progress in Brain Research, 197*, 101–121.

Mattson, R. H., Cramer, J. A., & Collins, J. F. (1992). A comparison of valproate with carbamazepine for the treatment of complex partial seizures and secondarily generalized tonic-clonic seizures in adults. The Department of Veterans Affairs Epilepsy Cooperative Study No. 264 Group. *New England Journal of Medicine, 327*(11), 765–771.

Modebadze, T., Morgan, N. H., Pérès, I. A., Hadid, R. D., Amada, N., Hill, C., et al. (2016). A low mortality, high morbidity reduced intensity status epilepticus (RISE) model of epilepsy and epileptogenesis in the rat. *PLoS One, 11*(2), e0147265.

Motohashi, N., Ikawa, K., & Kariya, T. (1989). GABAB receptors are up-regulated by chronic treatment with lithium or carbamazepine. GABA hypothesis of affective disorders? *European Journal of Pharmacology, 166*(1), 95–99.

Nishida, N., Huang, Z. L., Mikuni, N., Miura, Y., Urade, Y., & Hashimoto, N. (2007). Deep brain stimulation of the posterior hypothalamus activates the histaminergic system to exert antiepileptic effect in rat pentylenetetrazol model. *Experimental Neurology, 205*(1), 132–144.

Nolan, M., Bergazar, M., Chu, B., Cortez, M. A., & Snead, O. C., 3rd. (2005). Clinical and neurophysiologic spectrum associated with atypical absence seizures in children with intractable epilepsy. *Journal of Child Neurology, 20*(5), 404–410.

Onat, F. Y., van Luijtelaar, G., Nehllig, A., & Snead, O. C. (2013). The involvement of limbic structures in typical and atypical absence epilepsy. *Epilepsy Research, 103*(2–3), 111–123.

Pacey, L. K., Heximer, S. P., & Hampson, D. R. (2009). Increased GABA(B) receptor-mediated signaling reduces the susceptibility of fragile X knockout mice to audiogenic seizures. *Molecular Pharmacology, 76*(1), 18–24.

Panayiotopoulos, C. P. (2005). Chapter 10: Idiopathic generalized epilepsies. In C. P. Panayiotopoulos (Ed.), *The epilepsies: Seizures, syndromes and management*. Bladon Medical: Oxfordshire, UK.

Perez-Reyes, E. (2003). Molecular physiology of low voltage activated T-type calcium channels. *Physiological Reviews, 83*(1), 117–161.

Perez-Velazquez, J. L., Huo, J. Z., Garcia Dominguez, L. G., Leshchenko, Y., & Snead, O. C., III. (2007). Typical versus atypical absence seizures: Network mechanisms of the spread of paroxysms. *Epilepsia, 48*(8), 1585–1593.

Pitkanen, A., & Lukasiuk, K. (2011). Mechanisms of epileptogenesis and potential treatment targets. *Lancet Neurology, 10*(2), 173–186.

Raimondo, J. V., Burman, R. J., Katz, A. A., & Akerman, C. J. (2015). Ion dynamics during seizures. *Frontiers in Cellular Neuroscience, 9*, 419.

Richardson, M. P. (2012). Large scale models of epilepsy: Dynamics meets connectomics. *Journal of Neurology, Neurosurgery & Psychiatry, 83*(12), 1238–1248.

Sander, T., Peters, C., Kämmer, G., Samochowiec, J., Zirra, M., Mischke, D., et al. (1999). Association analysis of exonic variants of the gene encoding the GABAB receptor and idiopathic generalized epilepsy. *American Journal of Medical Genetics, 88*(4), 305–310.

Schuler, V., Lüscher, C., Blanchet, C., Klix, N., Sansig, G., Klebs, K., et al. (2001). Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic GABA(B) responses in mice lacking GABA(B(1)). *Neuron, 31*(1), 47–58.

Seeck, M., Schomer, D. L., & Niedermeyer, E. (2005). Chapter 33: Intracranial monitoring, depth, subdural, and foramen ovale electrodes. In E. Niedermeyer & F. L. De Silva (Eds.), *Electroencephalography: Basic principles, clinical applications and related fields* (5th ed., pp. 677–714). Baltimore, MD: Lippincott, Williams and Wilkins.

Smith, S. J. (2005). EEG in the diagnosis, classification, and management of patients with epilepsy. *Journal of Neurology, Neurosurgery & Psychiatry, 76*(Suppl 2), ii2–ii7.

Snead, O. C. (2002). γ-Hydroxybutyrate and absence seizure activity. In G. Tunnicliff & C. D. Cash (Eds.), *Gamma-hydroxybutyrate: Molecular, functional, and clinical aspects* (pp. 132–149). New York: Taylor Francis.

Snead, O. C., Banerjee, P. K., Burnham, M., & Hampson, D. (2000). Modulation of absence seizures by the GABA(A) receptor: A critical role for metabotropic glutamate receptor (mGLUR4). *Journal of Neuroscience, 20*(16), 6218–6224.

Snead, O. C., Cortez, M. A., Francis, J., & Eubanks, J. (2000). GABAB receptor gene expression is altered in an animal model of atypical absence epilepsy. *Epilepsia, 41*(Suppl 7), 23.

Snead, O. C., Depaulis, A., Vergnes, M., & Marescaux, C. (1999). Absence epilepsy: Advances in experimental animal models. *Advances in Neurology, 79*, 253–278.

Snead, O. C., 3rd, & Hosey, L. C. (1985). Exacerbation of seizures in children by carbamazepine. *New England Journal of Medicine, 313*(15), 916–921.

Spencer, S. S. (2002). Neural networks in human epilepsy: Evidence of and implications for treatment. *Epilepsia, 43*(3), 219–227.

Squires, R. F., Saederup, E., Crawley, J. N., Skolnick, P., & Paul, S. M. (1984). Convulsant potencies of tetrazoles are highly correlated with actions on GABA/benzodiazepine/picrotoxin receptor complexes in brain. *Life Sciences, 35*(14), 1439–1444.

Steriade, M. (2005). Sleep, epilepsy, and thalamic reticular inhibitory neurons. *Trends in Neurosciences, 28*(6), 317–324.

Stewart, L. S., Wu, Y., Eubanks, J. H., Han, H., Leschenko, Y., Perez Velazquez, J. L., et al. (2009). Severity of atypical absence phenotype in GABAB transgenic mice is subunit specific. *Epilepsy & Behavior, 14*(4), 577–581.

Velísková, J., Velísek, L., & Moshé, S. L. (1996). Age-specific effects of baclofen on pentylenetetrazol-induced seizures in developing rats. *Epilepsia, 37*(8), 718–722.

Vergnes, M., Boehrer, A., Simler, S., Bernasconi, R., & Morescaux, C. (1997). Opposite effects of GABAB receptor antagonists on absences and convulsive seizures. *European Journal of Pharmacology, 332*(3), 245–255.

Verrotti, A., Cusmai, R., Nicita, F., Pizzolorusso, A., Elia, M., Zamponi, N., et al. (2013). Electroclinical features and long term outcome of cryptogenic epilepsy children with Down syndrome. *Journal of Pediatrics, 163*(6), 1754–1758.

Wang, X., Stewart, L., Cortez, M. A., Wu, Y., Velazquez, J. L., Liu, C. C., et al. (2009). The circuitry of atypical absence seizures in GABA(B)R1a transgenic mice. *Pharmacology, Biochemistry and Behavior, 94*(1), 124–130.

Widjaja, E., Go, C., McCoy, B., & Snead, O. C., 3rd. (2015). Neurodevelopmental outcome of infantile spasms: A systematic review and meta-analysis. *Epilepsy Research, 109*, 155–162.

Wu, Y., Chan, K. F., Eubanks, J. H., Guin Ting Wong, C., Cortez, M. A., Shen, L., et al. (2007). Transgenic mice over-expressing GABA(B)R1a receptors acquire an atypical absence epilepsy-like phenotype. *Neurobiology of Disease, 26*(2), 439–451.

Wurpel, J. N., Sperber, E. F., & Moshé, S. L. (1990). Baclofen inhibits amygdala kindling in immature rats. *Epilepsy Research, 5*(1), 1–7.

Chapter 11
Targeting the $GABA_B$ Receptor for the Treatment of Pain

Sam J. Enna and Kenneth E. McCarson

Abstract Pharmacological and neurobiological data indicate that γ-aminobutyric acid (GABA) is involved in pain processing and perception, with both basic and clinical studies demonstrating that selective activation of GABAergic transmission yields a nociceptive response. This is particularly true for agents that stimulate $GABA_B$ receptors. While these findings are in accord with the neuroanatomical localization of $GABA_B$ receptors on nociceptive pathways, such work has yet to yield a clinically useful analgesic. Some reasons for this failure are the side effects associated with $GABA_B$ agonists and the tolerance that develops to their therapeutic effects. Described in this chapter are the neuroanatomical localization and function of GABAergic neurons as they relate to nociception and to the antinociceptive responses to $GABA_B$ receptor agonists. Particular emphasis is placed on detailing possible reasons why GABAergic compounds, especially orthosteric receptor agonists, display limited clinical efficacy as analgesics. Among these are the variations in GABA receptor expression and function that occur with the persistent receptor activation associated with a painful stimulus and the chronic administration of orthosteric compounds. Strategies are described for developing GABAergic drugs, such as allosteric $GABA_B$ receptor modulators, that by selectively activating sites associated with pain pathways provoke fewer side effects and less tolerance than orthosteric agents.

Keywords $GABA_B$ receptors • Nociception • Neuropathic pain • $GABA_B$ pharmacology

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11.1 Introduction

The concentration and ubiquitous distribution of γ-aminobutyric acid (GABA) throughout the neuraxis suggests it is the primary inhibitory neurotransmitter in the spinal cord and brain. For this reason, the GABAergic system has long been considered a prime target for the development of novel therapeutics for the treatment of a variety of conditions, including pain (Bowery and Enna 2000; Enna and McCarson 2006; Mohler 2001; McCarson and Enna 2014).

The antinociceptive properties of GABAergic agents in animal models of acute, inflammatory, and neuropathic pain have been the subject of investigation for decades (Kendall et al. 1982; Levy and Proudfit 1977; Malan et al. 2002; Shafizadeh et al. 1997; Smith et al. 1994). Rigid GABA analogs, such as THIP and muscimol, were initially used to define this receptor system and the relationship between GABAergic transmission and pain. However, despite the fact that orthosteric GABA receptor agonists, as well as inhibitors of GABA uptake and metabolism, display antinociceptive activity in a variety of animal pain models, such work has yet to yield a new drug for the management of pain. As it is known that the antinociceptive responses to GABAergic compounds vary with the duration and intensity of a painful stimulus (Smith et al. 1994; Mohler et al. 1997, 2004), differences between well-characterized preclinical pain models and the human condition are undoubtedly responsible for the lack of success in identifying human pain states that are consistently responsive to such agents. Nonetheless, efforts continue to define the structural, molecular, pharmacological, and biochemical properties of GABA receptors with the aim of identifying compounds with the requisite selectivity, efficacy, and safety needed for the management of pain.

Detailed in this chapter are historical and recent data on the anatomical localization and function of GABA neurons and receptors as they relate to the perception and processing of pain, with emphasis on the molecular and pharmacological properties of the $GABA_B$ site. Evidence is presented that spinal cord and brain $GABA_B$ receptors contribute to the origination, transmission, and modification of pain impulses. These data indicate that GABA plays a crucial role in nociceptive processing and support the notion that pharmacological manipulation of $GABA_B$ receptors is a viable approach for the clinical management of pain.

11.2 $GABA_B$ Receptor Structure and Function

Responses to GABA are mediated by two pharmacologically and molecularly distinct receptors, $GABA_A$ and $GABA_B$. Unlike the $GABA_A$ receptor, which is a pentameric ligand-gated ion channel, $GABA_B$ is a heterodimeric, metabotropic, class III G-protein-coupled site consisting of $GABA_{B1}$ and $GABA_{B2}$ subunits (Pinard et al. 2010) (Fig. 11.1). The production of each of the two subunits appears to be independently controlled. While experiments indicate that the

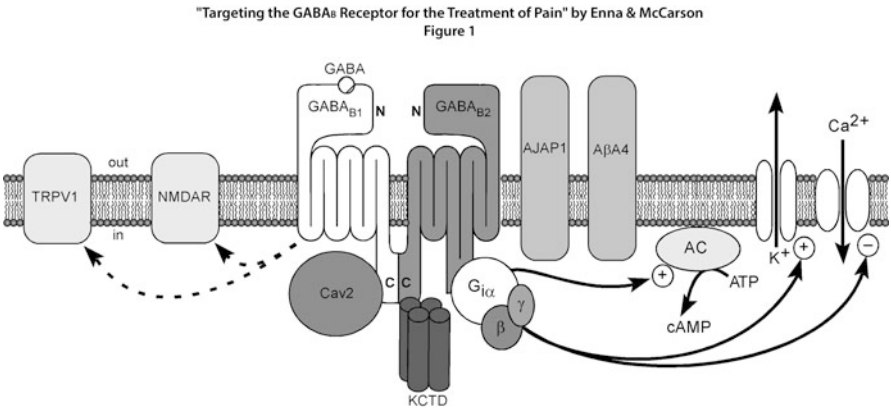

Fig. 11.1 Heterodimeric structure of the $GABA_B$ receptor. Full receptor function requires the heterodimeric assembly of $GABA_{B1}$ and $GABA_{B2}$ subunits. Each subunit has a G-protein-coupled receptor-like structure with seven transmembrane domains. The two subunits are linked by protein interactions between their intracellular C-terminal domains. The GABA recognition site is located on the extracellular domain of the $GABA_{B1}$ subunit, while the $GABA_{B2}$ subunit interacts with the G proteins, thereby influencing second messenger production. When stimulated, the receptor complex also activates inwardly rectifying potassium channels and inhibits the opening of calcium channels. Trafficking and function of the $GABA_B$ receptor complex is regulated via interactions with several membrane-associated proteins (e.g., KCTD, AJAP1, amyloid β A4, Cav 2.2). $GABA_B$ receptors also interact directly or indirectly with NMDA and TRPV'1 receptors in the mediation of nociceptive signaling. Adapted from McCarson and Enna (2014). *AC* adenylyl cyclase, *KCTD* potassium channel tetramerization domain proteins, *AJAP1* adherens junctions associated protein 1, *Aβ A4* amyloid β A4 precursor protein, *Cav2* caveolin 2, *NMDAR N*-methyl-D-aspartate receptor, *TRPV1* transient receptor potential cation channel subfamily V member 1

$GABA_{B1}$ subunit alone is capable of translocating to the cell membrane and forming a functional receptor (Gassmann et al. 2004; Baloucoune et al. 2012), the heterodimer is needed for a fully functional site (Chronwall et al. 2001; Enna 2001a; Jones et al. 1998; Kaupmann et al. 1998). There are several protein constituents that appear to be needed for trafficking the subunits to the cell membrane, for dimer assembly, and for receptor function (Schwenk et al. 2010; see also Chap. 4 of this volume). These include four K^+ channel tetramerization domain (KCTD) proteins in the receptor complex core as well as peripheral constituents such as Cav2, AJAP1, and amyloid-β A4 proteins associated with sushi domains of the $GABA_{B1a}$ subunit (Schwenk et al. 2016) (Fig. 11.1). This complexity suggests additional possibilities for pharmacologically manipulating the production and function of these receptors.

It also appears that the $GABA_{B1}$ receptor subunit interacts independently with TRPV1 channels to inhibit their sensitization by inflammatory stimuli. This modulation of signaling by a pain-mediating ion channel, while not associated with a functional $GABA_B$ receptor, requires a juxtaposition of $GABA_{B1a}$-containing neurons and TRPV1 (Hanack et al. 2015). Such a non-canonical $GABA_{B1a}$ signaling pathway may also serve as target for novel analgesics.

The attachment of GABA to the $GABA_{B1}$ subunit causes a conformational change in the $GABA_{B2}$ protein partner which, in turn, activates G_i or G_o proteins (Fig. 11.1). Given the duality of the G protein coupling, activation of $GABA_B$ receptors may either stimulate or inhibit the formation of cyclic AMP, depending upon the types of adenylyl cyclase in the cell and the availability of $G\alpha_s$ subunits (Bowery and Enna 2000; Enna 2001b). Presynaptic $GABA_B$ auto- and heteroreceptors regulate transmitter release by decreasing the availability of calcium (Brenowitz et al. 1998). The inhibition of GABA release in the spinal cord dorsal horn is regulated by $GABA_B$ receptors coupled to P/Q type calcium channels (Yang et al. 2015). Activation of postsynaptic $GABA_B$ receptors causes hyperpolarization by increasing potassium conductance.

Besides modulating the presynaptic release of glutamate, $GABA_B$ receptors directly influence glutamatergic transmission by altering the expression and activity of *N*-methyl D-aspartate (NMDA) receptors. This regulation is reciprocal, with NMDA receptor activity influencing $GABA_B$ receptor subunit production and function by increasing both the cell surface expression and recycling of internalized $GABA_B$ subunits. It has been demonstrated in the spinal cord that the postsynaptic sensitivity of dorsal horn neurons is regulated, at least in part, by the balance between glutamate receptor activation and $GABA_B$ receptor-mediated neuronal inhibition (Derjean et al. 2003). This indicates the expression and function of at least some $GABA_B$ and NMDA receptors are subject to a reciprocal regulation between these two neurotransmitter systems (Kantamneni et al. 2014; see also Chap. 7 of this volume). This is an example of how glutamate, the major excitatory neurotransmitter in brain, and GABA, the major inhibitor transmitter, interact to maintain homeostasis.

11.3 Localization of GABA Receptors in Pain Pathways

In brain, $GABA_B$ subunits are present in the thalamus (Ulrich and Bettler 2007), with activation or inhibition of those in the ventrobasal area provoking an antinociceptive response in the formalin model of inflammatory pain (Potes et al. 2006) (Fig. 11.2). In addition, stimulation of $GABA_B$ receptors on ventroposterior medial thalamic neurons inhibits trigeminal nociceptive transmission (Andreou et al. 2010). Other GABAergic projections in brain include those from the ventral tegmentum and substantia nigra to the descending modulatory control centers in the periaqueductal gray and dorsal medullary raphe nuclei (Kirouac et al. 2004) (Fig. 11.2). Nociceptive facilitation also results from activation of inhibitory projections from the rostral ventral medulla to the dorsal horn (Gilbert and Franklin 2001), with an enhancement of descending inhibition in the spinal cord following an increase in GABA activity in the rostral agranular insular cortex. In contrast, selective activation of $GABA_B$ receptors in this brain region causes hyperalgesia, perhaps via projections to the amygdale (Jasmin et al. 2003). It is known that $GABA_B$ receptors play an important role in the

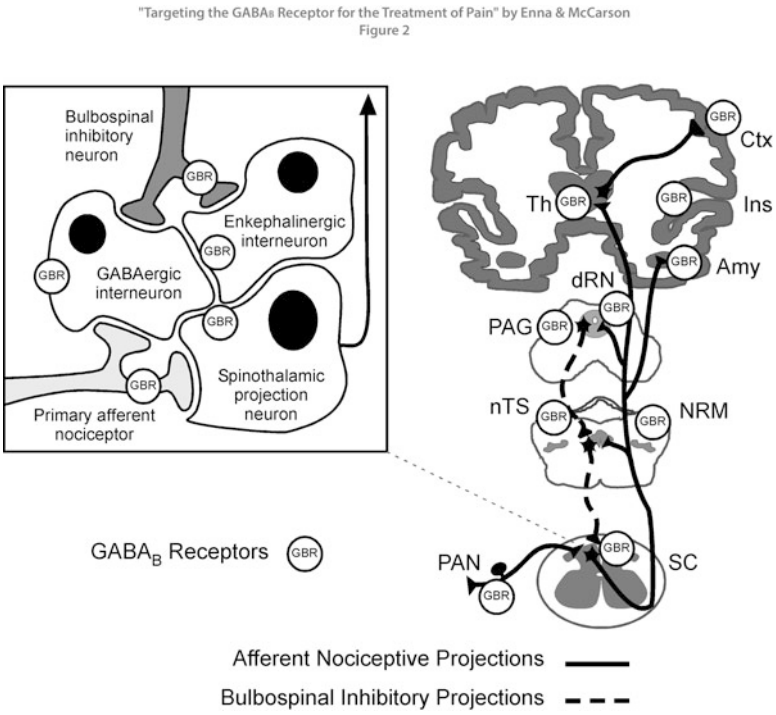

Fig. 11.2 Localization of $GABA_B$ receptors in the central and peripheral nervous systems. $GABA_B$ receptors are located throughout the nervous system and contribute to the processing of pain sensation. Displayed are the general locations of $GABA_B$ receptors within ascending and descending systems that are particularly relevant to pain processing. Depicted in the inset are the locations of GABA receptors within particular areas of the spinal cord dorsal horn circuitry. Adapted from McCarson and Enna (2014). *GBR* $GABA_B$ receptor, *CTX* primary sensory cortex, *Ins* insular cortex, *Th* thalamus, *Amy* Amygdala, *dRN* dorsal reticular nuclei, *PAG* periaqueductal gray, *nTS* nucleus tractus solitarius, *NRM* medullary raphe nucleus, *SC* dorsal horn of the spinal cord, *PAN* primary afferent nociceptor

disinhibition of descending pro-nociceptive projections from the dorsal reticular nucleus, as lentiviral knockdown of $GABA_{B1a}$ subunits or administration of CGP35348, a $GABA_B$ receptor antagonist, decreases late-phase behaviors in the formalin model of persistent inflammatory pain (Martins et al. 2015). In this study, retrograde labeling of the dorsal reticular nucleus revealed GABAergic inputs from insular, somatosensory, and motor cortices.

It has also been reported that activation of parasympathetic reflexes by vagal nerve stimulation is abolished by the microinjection of baclofen, the prototypical orthosteric $GABA_B$ receptor agonist, into the nucleus tractus solitarius (nTS). The microinjection of CGP35348, a $GABA_B$ receptor antagonist, into the nTS reduces the inhibition of this reflex by noxious trigeminal activation, indicating that the modulatory effects of nociception are mediated by $GABA_B$ receptors in this brain region (Ishii and Izumi 2012).

The excitatory inputs from the pontine parabrachial nucleus to the lateral division of the central amygdala are under the control of presynaptic $GABA_B$ receptors that inhibit N-type calcium channels (Delaney and Crane 2016). Transcranial magnetic stimulation is reported to activate $GABA_B$ receptor-mediated cortical inhibition, providing relief for patients with chronic pain (Barr et al. 2013). Moreover, it has been proposed that $GABA_B$ receptors are involved in the analgesic response to electro-acupuncture in patients with neuropathic pain (Kim et al. 2013). The presence of $GABA_B$-mediated potentiation of inwardly rectifying potassium currents has been demonstrated in satellite glial cells, suggesting a mechanism for regulating neuronal GABA release in the presence of noxious stimuli (Takeda et al. 2013).

Within the spinal cord, $GABA_B$ receptors are scattered throughout the gray matter. They are most highly concentrated in laminae I and II of the dorsal horn on both pre- and postsynaptic sites located on Aδ and C fiber terminations and on the first synapse in the pain pathway (Carlton et al. 1999; Desarmenien et al. 1984; Price et al. 1984) (Fig. 11.2). Generally, facilitation of GABAergic transmission in the spinal cord raises the nociceptive threshold, with activation of presynaptic $GABA_B$ receptors resulting in the inhibition of substance P and glutamate release from primary afferent neurons (Malcangio and Bowery 1994; Yang et al. 2002). This contrasts with the response to stimulation of presynaptic $GABA_B$ receptors located on the descending opioid-containing neurons (Mahmoudi and Zarrindast 2002) and on the inhibitory serotonergic or noradrenergic terminals (Yang et al. 2002), activation of which tends to lower the pain threshold.

Stimulation of $GABA_B$ receptors in sensory-related systems affects neurotransmission by reducing presynaptic transmitter release or by hyperpolarizing postsynaptic neurons. In this way, $GABA_B$ receptors regulate the activity of a wide variety of excitatory and inhibitory neurotransmitter systems known to be involved in pain processing. For example, supraspinal $GABA_B$ receptor activation facilitates noradrenergic- and opioid-mediated antinociception (Nguyen et al. 1997; Thomas et al. 1995). The response to these supraspinal effects are concentration-dependent, with the placement of lower amounts of baclofen into the medullary raphe nucleus and the nucleus reticularis gigantocellularis pars alpha increasing thermal hypoalgesia, while higher concentrations cause hyperalgesia (Thomas et al. 1995).

Optogenetic studies on dorsal root ganglia C-fiber nociceptive neurons indicate that baclofen inhibits all populations of nociceptors, while the response to morphine is restricted to select sub-populations. This suggests that activation of $GABA_B$ receptors inhibits afferent nociceptive signaling across a broader array of stimuli than do opioids (Honsek et al. 2015).

It has also been reported that $GABA_B$ receptors on peripheral nociceptors modulate low-voltage-activated calcium currents through a redox-dependent signal, providing another potential target for novel analgesics (Huang et al. 2015).

In the spinal cord dorsal horn, A-fiber primary inputs drive polysynaptic interneuronal pathways culminating in $GABA_B$-mediated inhibition of C-fiber nociceptive inputs (Melin et al. 2013).

It appears the long-term analgesic effects of oxycodone are mediated in part by upregulation of $GABA_B$ receptor expression in primary afferent nociceptors and by the trafficking of the receptor subunits to the central terminals. These $GABA_B$ receptors are required to maintain the anti-allodynic effects of oxycodone in vincristine-evoked neuropathic pain. This oxycodone effect is not observed with repeated administration of morphine (Thibault et al. 2014).

It has been found that the regulation of neuronal calcium flux by alpha-conotoxin requires the expression of both $GABA_{B1}$ and $GABA_{B2}$ subunits (Cuny et al. 2012). The $GABA_B$ receptor-mediation of alpha-conotoxin-induced analgesia appears to be dependent on the presence of certain SRC phosphorylation sites on the C-terminal tail region of neuronal Cav2.3 calcium channels (Berecki et al. 2014). Moreover, the alpha-conotoxins Vc1.1 and Rg1A are reported to modulate calcium currents through activation of $GABA_B$ receptor-mediated G protein signaling (Callaghan et al. 2008). Such agents are now being developed as treatments for neuropathic pain.

The antinociceptive actions of systemic baclofen are thought to be mediated mainly by spinal $GABA_B$ receptor activation because they are blocked by intrathecal, but not supraspinal (ventromedial medulla), administration of CGP 35348, a selective $GABA_B$ receptor antagonist (Thomas et al. 1996). It has also been reported that the spinal antinociceptive effects of $GABA_B$ receptor activation are inhibited by muscarinic (Zorn and Enna 1987) and cannabinoid (Naderi et al. 2005) receptor antagonists, and that intrathecal administration of a $GABA_B$ receptor antagonist blocks muscarine- and neostigmine-evoked anti-allodynia (Chen and Pan 2003). Moreover, the antinociceptive effects of subcutaneous nicotine in the tail-immersion and hot-plate assays are abolished by baclofen and potentiated by 2-hydroxysaclofen, a $GABA_B$ receptor antagonist (Varani et al. 2014). These findings suggest an interrelationship between cholinergic transmission and endocannabinoids in the antinociceptive responses to $GABA_B$ receptor agonists. It appears, therefore, that the effects of $GABA_B$ agonists on pain perception are dependent upon the anatomical pathways involved and the ability of other neurotransmitter systems to respond appropriately to $GABA_B$ receptor activation. The variability in the antinociceptive effects of $GABA_B$ receptor agonists are most likely related, at least in part, to this dependency on the functional integrity of other transmitter systems.

11.4 $GABA_B$ Receptor Deletion Phenotypes

Selective deletion of either the $GABA_{B1}$ or $GABA_{B2}$ subunit results in mechanical and thermal hyperalgesia (Gassmann et al. 2004; Magnaghi et al. 2008; Schuler et al. 2001), indicating the overall nociceptive threshold is regulated by $GABA_B$ receptor tone. Morphological changes in $GABA_{B1}$ knock-out mice include an increased number of small, thinly myelinated neurons in the lumbar dorsal root ganglia (Magnaghi et al. 2008). In contrast to the results obtained with knock-out animals, the conditional deletion of $GABA_{B1}$ receptor genes from peripheral

nociceptors has no effect on nociception and the antinociceptive responses to systemic baclofen. This indicates the importance of central, most likely spinal cord, $GABA_B$ receptors in mediating and modulating the response to painful stimuli (Gangadharan et al. 2009).

Deletion of GINIP, a $G\alpha_i$-interacting protein, results in an exaggerated mechanical hypersensitivity, suggesting that the level of $GABA_B$ receptor signaling in peripheral nociceptive neurons is dependent upon the availability of this component of the second messenger system (Gaillard et al. 2014).

Data suggest that interactions between $GABA_A$ and $GABA_B$ receptor systems may contribute to their effects on nociception. For example, while inactivation of the $GABA_A\beta_3$ subunit-expressing gene reduces nociceptive threshold and the antinociceptive effects of THIP, a $GABA_A$ agonist, it also diminishes the antinociceptive response to baclofen (Ugarte et al. 2000). This suggests a continuous GABA receptor tone controlling nociceptive threshold and indicates an interaction between $GABA_A$ and $GABA_B$ receptor systems might be important for mediating the antinociceptive effects of $GABA_B$ agonists. Alternatively, these data might indicate there is an undefined transmitter system or neuronal protein that is crucial for mediating the response to baclofen that is affected by the deletion of the $GABA_A$ receptor β_3 subunit gene (Rudolph and Mohler 2004).

11.5 $GABA_B$ Receptors and Nerve Injury

Studies in animal models indicate a loss of spinal GABA-positive neurons, a decrease in GABA synthesis, and abnormalities in GABA transport are associated with neuropathic pain (Drew et al. 2004; Gwak et al. 2006; Ibuki et al. 1997; Meisner et al. 2010; Miletic et al. 2003; Moore et al. 2002; Somers and Clemente 2002; Zhang et al. 1994). While there is debate about whether a decline in GABAergic function is sufficient to produce the hyperalgesia associated with neuropathic pain (Polgar et al. 2003), there are data indicating a facilitation of sub-threshold primary afferent nociceptor activity when GABAergic inhibitory tone is diminished (Lu et al. 2008). Moreover, the reduction of mechanical allodynia in rats following sciatic nerve injury is accompanied by an increase in the expression of $GABA_{B1}$ subunits in the dorsal root ganglion (Vallejo et al. 2013). Taken together, these findings indicate that a decline in $GABA_B$ receptor activity results in hyperalgesia and allodynia, whereas an increase in GABAergic tone diminishes these symptoms (Drew et al. 2004; Gwak et al. 2006).

Baclofen induces an antinociceptive response in neuropathic pain models whether administered intrathecally (Hwang et al. 2001; Hwang and Yaksh 1997; von Heijne et al. 2001), intraperitoneally (Smith et al. 1994), intracerebroventricularly (Zarrindast et al. 2000; Zarrindast and Mahmoudi 2001), or subcutaneously (Deseure et al. 2003; Franek et al. 2004; Nowak et al. 2013; Whitehead et al. 2012). Furthermore, the intrathecal administration of baclofen enhances the anti-allodynia evoked by spinal cord stimulation (Cui et al. 1996) and offsets the diabetic neuropathy-driven upregulation

of NMDA receptors and phosphorylated cAMP-responsive element-binding protein (CREB) (Bai et al. 2014). Moreover, the intracerebroventricular administration of a $GABA_B$ receptor antagonist blocks imipramine-induced antinociception, suggesting a role for this transmitter receptor in the analgesic response to this antidepressant (Zarrindast et al. 2000). These findings indicate that activation of pathways involving $GABA_B$ receptors reduces the pain associated with nerve injury.

Although the reduction in the neuropathic pain response resulting from the intrathecal administration of GABA diminishes over time (Eaton et al. 1999), the anti-allodynic response to baclofen is the same in a model of trigeminal pain regardless of when it is administered with respect to the insult (Deseure et al. 2003). This suggests no time-dependent change in $GABA_B$ receptor sensitivity with this condition. Changes over time in $GABA_B$ receptor sensitivity have been noted under certain conditions in animal models of neuropathic pain. For example, the antinociceptive effects of baclofen in the sciatic nerve ligation (SNL) model declines in the ipsilateral, but not contralateral, limb (Franek et al. 2004). In addition, a reduction has been reported in the presynaptic $GABA_B$ receptor response to baclofen, with a consequent increase in glutamate release from primary afferent nociceptors, in a rat model of diabetic neuropathy (Wang et al. 2007). The nociception-induced decline in $GABA_B$ receptor responsiveness may be due to a decrease in the expression of the $GABA_{B1}$ subunits in the spinal cord dorsal horn (Wang et al. 2011). As part of the endogenous disinhibition that contributes to central sensitization in models of sciatic nerve injury, modification of $GABA_B$ chaperone protein binding regulates $GABA_B$ dimer dissociation and baclofen antinociceptive efficacy (Laffray et al. 2012). This suggests that compounds capable of disrupting the chaperone–$GABA_B$ receptor interactions might be of value in maintaining $GABA_B$ receptor function in chronic nerve injury (Benke 2013). The variations in results obtained when examining time-dependent changes in $GABA_B$ receptor sensitivity associated with neuropathic pain may to some extent be due to differences in the animal models or in the route or timing of drug administration. On the other hand, these inconsistencies might suggest that baclofen may not be generally effective as a treatment for neuropathic pain.

There have also been contradictory findings with respect to the effects of $GABA_B$ receptor antagonists in neuropathic pain models. Whereas intrathecal administration of baclofen diminishes nerve-injury-induced allodynia (Hwang and Yaksh 1997) and suppresses diabetic neuropathy-evoked increases in spinal expression of pCREB and NR2B (Liu et al. 2014), spinal or medullary delivery of CGP 35348, a $GABA_B$ receptor antagonist, has no effect on nociceptive thresholds (Thomas et al. 1996). Although this implies that basal $GABA_B$ receptor tone is low in regions associated with nociceptive processing, the intrathecal administration to normal animals of the $GABA_B$ receptor antagonist phaclofen produces tactile allodynia and hyperalgesia (Malan et al. 2002), indicating that a $GABA_B$ receptor tone helps maintain nociceptive thresholds. These apparently disparate findings might be due to the use of different $GABA_B$ antagonists, or to the fact that in one case the antagonist was administered to an animal suffering from neuropathic pain, whereas pain-free animals were tested in the other study.

The apparent difference in responses to the antagonists quite possibly reflects differences in $GABA_B$ receptor activity as a result of the underlying pathology.

11.6 Plasticity of the GABA Receptor System

The variability in the antinociceptive response to $GABA_B$ receptor agonists and antagonists may be associated with the plasticity of the $GABA_B$ receptor system. For example, the GABAergic input to dorsal horn neurons, which affects endogenous glutamate release, is rapidly suppressed by nociceptive stimulation of primary afferent neurons (Zhou et al. 2007). Indeed, there is a great deal of evidence indicating that regulation of $GABA_B$ receptor gene expression and function is modified by persistent inflammatory and neuropathic pain. In monoarthritic animal models, the number of $GABA_B$-containing neurons increases in the brainstem and the number of $GABA_B$ receptors declines in the spinal cord (Pinto et al. 2003). In addition, chronic administration of baclofen, inflammatory nociception, and repeated mechanical stimulation all increase spinal cord $GABA_{B1}$ and $GABA_{B2}$ subunit protein and gene expression (Malcangio et al. 1995; McCarson and Enna 1999; Sands et al. 2003). Tolerance to the analgesic effects of baclofen is associated with a decrease in the functional activity of $GABA_B$ receptors, and an attenuation of pain-induced increases in spinal neurokinin receptor gene expression (Enna et al. 1998; Sands et al. 2003; McCarson and Enna 1996). Moreover, in a neuropathic pain model, spinal cord $GABA_B$ receptor subunit expression and sensitivity increase in tandem with a decrease in thermal and mechanical pain thresholds (McCarson et al. 2005).

These reports suggest that $GABA_B$ receptor function decreases with continuous activation, and indicate a possible association between the development of neuropathic pain and a decline in spinal cord GABAergic activity. It seems likely that non-genomic mechanisms also play a role in these changes given the lack of a strict correlation between $GABA_B$ receptor subunit expression, the functional coupling of the receptor, and analgesic efficacy (Laffray et al. 2012; Zhu et al. 2012; Schwenk et al. 2010). These findings also support the possibility that $GABA_B$ subunit proteins may have additional functions unrelated to GABAergic transmission, such as the aforementioned interaction between the $GABA_{B1}$ subunit and TRPV1 channels (McCarson et al. 2005; Sands et al. 2003; Hanack et al. 2015). Most importantly, the finding that $GABA_B$ receptor expression and function varies over time in the presence of persistent pain or during administration of orthosteric agonists provides an explanation for the inconsistent clinical and preclinical results obtained when examining the analgesic properties of these agents.

11.7 Orthosteric GABAergic Agents

While it is clear that the $GABA_B$ receptor-mediated effects on the pain threshold varies among brain regions, results are consistent in indicating that systemic administration of $GABA_B$ receptor agonists generally produces antinociception in animal

models of acute and persistent pain (Kendall et al. 1982; Levy and Proudfit 1977; Malan et al. 2002; Shafizadeh et al. 1997; Smith et al. 1994; Thomas et al. 1996). The most convincing data supporting a role for GABA in the transmission and perception of pain are derived from clinical and preclinical pharmacological studies. The findings indicate that the antinociceptive response varies as a function of dose, route and site of administration, the type of GABAergic agent, and the nature, intensity and duration of the pain stimulus.

For years baclofen has been employed clinically as a treatment for spasticity and for managing certain types of pain (Fromm 1994; Loubser and Akman 1996; Sindrup and Jensen 2002; Slonimski et al. 2004). Its effectiveness as an analgesic, although limited by side effects, provides proof of an involvement of $GABA_B$ receptors in regulating pain in humans. In patch-clamp studies, it has been shown that baclofen preferentially inhibits noxious vs innocuous mechanical stimulus-evoked activity in substantia gelatinosa neurons (Fukuhara et al. 2013). With animal pain models, orthosteric $GABA_B$ receptor agonists, whether administered systemically or intrathecally, are generally effective in reducing the thermal hyperalgesia associated with acute or inflammatory pain (Dirig and Yaksh 1995; Enna et al. 1998; Green and Dickenson 1997; Hammond and Drower 1984). While it was initially believed that gabapentin, a GABA analog used for the treatment of neuropathic pain, was a $GABA_B$ receptor agonist, it is now known that its effectiveness, along with its structural analog pregabalin, is due to a direct interaction with the $\alpha_2\delta$ subunit of neuronal calcium channels rather than to an interaction with the $GABA_B$ site (Gee et al. 1996).

Orthosteric $GABA_B$ receptor agonists are thought to reduce pain by receptor-mediated presynaptic inhibition of the release of tachykinins, glutamate, and other spinal neurotransmitters (Enna et al. 1998; Li et al. 2002; Riley et al. 2001) and by regulation of potassium flux at postsynaptic sites (Reis and Duarte 2006). While it was once believed that the antinociceptive and sedative effects of $GABA_B$ receptor agonists are closely related, CGP 35024, an orthosteric $GABA_B$ receptor agonist is reported to reduce nerve injury-evoked mechanical allodynia and hyperalgesia at non-sedating doses (Patel et al. 2001). This suggests the possible existence of $GABA_B$ receptor subtypes having molecularly distinct recognition sites.

The fact that orthosteric $GABA_B$ receptor antagonists, such as phaclofen, 2-hydroxysaclofen, CGP 35348 and CGP 55845, typically lower the nociceptive threshold is taken as further evidence that $GABA_B$ receptor systems tonically inhibit pain pathways. This notion is reinforced by the finding that CGP 35348 administration prolongs late-phase formalin-induced nociceptive behaviors (Green and Dickenson 1997) and by the discovery that the antagonist CGP 55845 enhances noxious mechanical stimulus-evoked nociceptive activity in the substantia gelatinosa (Fukuhara et al. 2013). It has also been reported that the antinociceptive effect resulting from tonic spinal cord stimulation to reduce cranial nerve firing in rat is blocked by the $GABA_B$ receptor antagonist CGP 35348 (Crosby et al. 2015). Thus, the responses to systemically administered $GABA_B$ receptor orthosteric agonists and antagonists are consistent with the notion that stimulation of the $GABA_B$ receptor system reduces nociception, and that $GABA_B$ systems exert tonic control over pain pathways.

The development of tolerance to the antinociceptive response is a major limitation for the routine use of baclofen as an analgesic. Preclinical studies indicate that baclofen-tolerant animals become hyperalgesic with respect to inflammatory nociception, limiting further its long-term clinical use for reducing pain (Enna et al. 1998). Unlike baclofen, analgesic tolerance is not reported for CGP 44532, a $GABA_B$ receptor agonist (Enna et al. 1998). The reason for this difference between the two agents is unknown, although it may once again indicate regionally distinct subtypes of $GABA_B$ receptors. Indeed, there is evidence of both pre- and post-synaptic populations of $GABA_B$ receptors in the periaqueductal grey that retain the ability to mediate inhibition despite prolonged administration of baclofen (Liu et al. 2013). Nevertheless, because tolerance limits the clinical utility of systemically administered orthosteric $GABA_B$ receptor agonists, attempts are being made to activate these sites more selectively in other ways.

11.8 Allosteric GABAergic Agents

Allosteric modulation has been known for some time to be a mechanism for regulating the function of ligand-gated ion channels, in particular nicotinic cholinergic and $GABA_A$ receptors. In recent years it was found that it is also possible to allosterically regulate seven transmembrane receptors, including the $GABA_B$ site (Brusberg et al. 2009; Carai et al. 2004a; Castelli et al. 2012; Frankowska et al. 2007; Gjoni and Urwyler 2008; Hensler et al. 2012; Slattery et al. 2005; Urwyler et al. 2003). These studies indicate a number of allosteric targets on $GABA_B$ subunits. Work with CGP7930, a $GABA_B$ receptor positive allosteric modulator, indicates it selectively attaches to the $GABA_{B2}$ protein, enhancing the affinity of the $GABA_{B1}$ component for GABA and increasing the efficacy of the receptor-mediated signal transduction (Carai et al. 2004b; see also Chap. 18 of this volume). It was also found that CGP7930 prevents the $GABA_B$ receptor desensitization that occurs with repeated activation of the site, suggesting that the response to this modulator will not diminish over time (Gjoni and Urwyler 2008). With regard to pain, it is reported that CGP7930 reduces mechanical nociceptive responsiveness in a visceral pain model (Brusberg et al. 2009). In addition, CGP7930 has been shown to reduce anxiety (Frankowska et al. 2007; see also Chap. 12 of this volume) and self-administration of drugs of abuse (Slattery et al. 2005; see also Chaps. 14 and 15 of this volume). These pharmacological effects are similar to those reported for orthosteric $GABA_B$ receptor agonists, supporting the notion that CGP7930 selectively activates this receptor system. In fact, in vitro studies demonstrate that CGP7930 interacts selectively with only a` subset of $GABA_B$ receptors in distinct brain regions, suggesting that such positive allosteric modulators may provoke fewer side effects than orthosteric agents. This hypothesis is supported by the report that BHF177, a $GABA_B$ receptor positive allosteric modulator, has less of an effect on locomotor activity and memory, and is less sedating, than baclofen (Li et al. 2013).

A number of systemically active $GABA_B$ receptor positive allosteric modulators have been developed, including COR627 and COR628 (Castelli et al. 2012). The positive allosteric action of these agents is supported by the finding that they potentiate baclofen-induced sedation/hypnosis and loss of righting reflex (Mugnaini et al. 2013).

The GABA receptor-positive allosteric modulator rac-BHFF blocks the prepulse inhibition (PPI) deficits induced by NMDA receptor blockade or that occur spontaneously in DBA/2J mice (Frau et al. 2014). While changes in PPI are most commonly associated with a response to antipsychotics, this regulation of sensorimotor gating could also suggest a more generalized effect on sensory function.

Administration of the selective $GABA_B$ receptor-positive allosteric modulator ADX71441 is superior to baclofen in improving functional measures of micturition in two animal models of overactive bladder (Kalinichev et al. 2014). It has also been proposed that $GABA_B$ receptor-positive allosteric modulators affect gastrointestinal sensory function in a manner similar to baclofen, an orthosteric agonist (Hyland and Golubeva 2015). Overall, these data indicate that allosteric regulation of $GABA_B$ receptor activity is a viable way to selectively influence these sites and that this mechanistic approach could yield drugs that are superior to orthosteric agents with respect to safety and the development of tolerance.

11.9 Conclusions

There is no question that systemic administration of a $GABA_B$ receptor agonist can provoke an antinociceptive response in laboratory animals and humans. This is not surprising given the anatomical localization of these receptors on neural pathways associated with the perception and transmission of pain, and the neurochemical responses to activation of these sites. It is disappointing, therefore, that efforts to exploit these findings for therapeutic gain have so far proven futile. Research on this issue suggests various reasons why this may be the case. Chief among these is the fact that repeated administration of orthosteric $GABA_B$ receptor agonists downregulates this site, diminishing the response to these agents and to GABA itself. In addition, the discovery that the expression and function of $GABA_B$ receptors is modified by chronic pain suggests that the antinociceptive response to an orthosteric agonist could vary widely among patients, and even for a particular individual, depending on when it is administered relative to the duration of the symptom. Another factor limiting the utility of orthosteric $GABA_B$ receptor agonists is their lack of selectivity for the population of sites most involved in pain processing and perception. The widespread distribution of these receptors throughout the neuraxis ensures that nonselective activation will yield a plethora of responses, and therefore side effects, that limit their use as analgesics. It is also possible there are more redundancies built into the human pain processing system than in laboratory animals such that the enhancement of one antinociceptive system in patients is overridden by the activation of other nociceptive pathways.

At least some of these limitations may be overcome with positive allosteric $GABA_B$ receptor modulators. By design such agents act only on select populations of $GABA_B$ receptors, opening the possibility of discovering compounds that interact primarily with those sites associated with pain pathways. Such selectivity is consistent with the reports indicating that side effects and tolerance are less evident with positive allosteric modulators than with orthosteric $GABA_B$ receptor agonists. The lessening of side effects could be explained by the greater receptor selectivity of the allosteric compounds, while the decline in tolerance to the pharmacological effect could reflect the fact that allosteric agents do not directly stimulate the site, but rather enhance the responsiveness to the endogenous transmitter. By acting in this manner, positive allosteric modulators are less capable of over-stimulating, and therefore downregulating, the site.

It remains to be seen whether these mechanistic differences between allosteric and orthosteric agents will yield a clinically viable analgesic. It is conceivable that, like orthosteric compounds, allosteric agents will display variability in antinociceptive efficacy because of the modifications in $GABA_B$ receptor expression, sensitivity and function that occur in association with the underlying pathology. Nonetheless, the demonstrated ability of positive allosteric modulators to selectively potentiate the responsiveness of $GABA_B$ receptors gives new impetus for exploring further the possibility of developing novel analgesics that target this site.

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Bibliography

Andreou, A. P., Shields, K. G., & Goadsby, P. J. (2010). GABA and valproate modulate trigeminovascular nociceptive transmission in the thalamus. *Neurobiology of Disease, 37*(2), 314–323. doi:10.1016/j.nbd.2009.10.007.

Bai, H. P., Liu, P., Wu, Y. M., Guo, W. Y., Guo, Y. X., & Wang, X. L. (2014). Activation of spinal GABAB receptors normalizes N-methyl-D-aspartate receptor in diabetic neuropathy. *Journal of the Neurological Sciences, 341*(1–2), 68–72. doi:10.1016/j.jns.2014.04.002.

Baloucoune, G. A., Chun, L., Zhang, W., Xu, C., Huang, S., Sun, Q., et al. (2012). GABAB receptor subunit GB1 at the cell surface independently activates ERK1/2 through IGF-1R transactivation. *PLoS One, 7*(6), e39698. doi:10.1371/journal.pone.0039698.

Barr, M. S., Farzan, F., Davis, K. D., Fitzgerald, P. B., & Daskalakis, Z. J. (2013). Measuring GABAergic inhibitory activity with TMS-EEG and its potential clinical application for chronic pain. *Journal of Neuroimmune Pharmacology, 8*(3), 535–546. doi:10.1007/s11481-012-9383-y.

Benke, D. (2013). GABAB receptor trafficking and interacting proteins: Targets for the development of highly specific therapeutic strategies to treat neurological disorders? *Biochemical Pharmacology, 86*(11), 1525–1530. doi:10.1016/j.bcp.2013.09.016.

Berecki, G., McArthur, J. R., Cuny, H., Clark, R. J., & Adams, D. J. (2014). Differential Cav2.1 and Cav2.3 channel inhibition by baclofen and alpha-conotoxin Vc1.1 via GABAB receptor activation. *The Journal of General Physiology, 143*(4), 465–479. doi:10.1085/jgp.201311104.

Bowery, N. G., & Enna, S. J. (2000). Gamma-aminobutyric acid(B) receptors: First of the functional metabotropic heterodimers. *The Journal of Pharmacology and Experimental Therapeutics, 292*(1), 2–7.

Brenowitz, S., David, J., & Trussell, L. (1998). Enhancement of synaptic efficacy by presynaptic GABA(B) receptors. *Neuron, 20*(1), 135–141.

Brusberg, M., Ravnefjord, A., Martinsson, R., Larsson, H., Martinez, V., & Lindstrom, E. (2009). The GABA(B) receptor agonist, baclofen, and the positive allosteric modulator, CGP7930, inhibit visceral pain-related responses to colorectal distension in rats. *Neuropharmacology, 56*(2), 362–367. doi:10.1016/j.neuropharm.2008.09.006.

Callaghan, B., Haythornthwaite, A., Berecki, G., Clark, R. J., Craik, D. J., & Adams, D. J. (2008). Analgesic alpha-conotoxins Vc1.1 and Rg1A inhibit N-type calcium channels in rat sensory neurons via GABAB receptor activation. *The Journal of Neuroscience, 28*(43), 10943–10951. doi:10.1523/jneurosci.3594-08.2008.

Carai, M. A., Colombo, G., Froestl, W., & Gessa, G. L. (2004a). In vivo effectiveness of CGP7930, a positive allosteric modulator of the GABAB receptor. *European Journal of Pharmacology, 504*(3), 213–216. doi:10.1016/j.ejphar.2004.10.008.

Carai, M. A., Vacca, G., Serra, S., Colombo, G., Froestl, W., & Gessa, G. L. (2004b). Suppression of GABA(B) receptor function in vivo by disulfide reducing agent, DL-dithiothreitol (DTT). *Psychopharmacology, 174*(2), 283–290. doi:10.1007/s00213-003-1737-y.

Carlton, S. M., Zhou, S., & Coggeshall, R. E. (1999). Peripheral GABA(A) receptors: Evidence for peripheral primary afferent depolarization. *Neuroscience, 93*(2), 713–722.

Castelli, M. P., Casu, A., Casti, P., Lobina, C., Carai, M. A., Colombo, G., et al. (2012). Characterization of COR627 and COR628, two novel positive allosteric modulators of the GABAB receptor. *The Journal of Pharmacology and Experimental Therapeutics, 340*(3), 529–538. doi:10.1124/jpet.111.186460.

Chen, S. R., & Pan, H. L. (2003). Spinal GABAB receptors mediate antinociceptive actions of cholinergic agents in normal and diabetic rats. *Brain Research, 965*(1–2), 67–74.

Chronwall, B. M., Davis, T. D., Severidt, M. W., Wolfe, S. E., McCarson, K. E., Beatty, D. M., et al. (2001). Constitutive expression of functional GABA(B) receptors in mIL-tsA58 cells requires both GABA(B(1)) and GABA(B(2)) genes. *Journal of Neurochemistry, 77*(5), 1237–1247.

Crosby, N. D., Weisshaar, C. L., Smith, J. R., Zeeman, M. E., Goodman-Keiser, M. D., & Winkelstein, B. A. (2015). Burst and tonic spinal cord stimulation differentially activate GABAergic mechanisms to attenuate pain in a rat model of cervical radiculopathy. *IEEE Transactions on Bio-medical Engineering, 62*(6), 1604–1613. doi:10.1109/tbme.2015.2399374.

Cui, J. G., Linderoth, B., & Meyerson, B. A. (1996). Effects of spinal cord stimulation on touch-evoked allodynia involve GABAergic mechanisms. An experimental study in the mononeuropathic rat. *Pain, 66*(2–3), 287–295.

Cuny, H., de Faoite, A., Huynh, T. G., Yasuda, T., Berecki, G., & Adams, D. J. (2012). gamma-Aminobutyric acid type B (GABAB) receptor expression is needed for inhibition of N-type (Cav2.2) calcium channels by analgesic alpha-conotoxins. *The Journal of Biological Chemistry, 287*(28), 23948–23957. doi:10.1074/jbc.M112.342998.

Delaney, A. J., & Crane, J. W. (2016). Presynaptic GABAB receptors reduce transmission at parabrachial synapses in the lateral central amygdala by inhibiting N-type calcium channels. *Scientific Reports, 6*, 19255. doi:10.1038/srep19255.

Derjean, D., Bertrand, S., Le Masson, G., Landry, M., Morisset, V., & Nagy, F. (2003). Dynamic balance of metabotropic inputs causes dorsal horn neurons to switch functional states. *Nature Neuroscience, 6*(3), 274–281. doi:10.1038/nn1016.

Desarmenien, M., Feltz, P., Occhipinti, G., Santangelo, F., & Schlichter, R. (1984). Coexistence of GABAA and GABAB receptors on A delta and C primary afferents. *British Journal of Pharmacology, 81*(2), 327–333.

Deseure, K., Koek, W., Adriaensen, H., & Colpaert, F. C. (2003). Continuous administration of the 5-hydroxytryptamine1A agonist (3-Chloro-4-fluoro-phenyl)-[4-fluoro-4-[[(5-methyl-pyridin-2-ylmethyl)-amino]-methyl]piperidin-1-yl]-methadone (F 13640) attenuates allodynia-like behavior in a rat model of trigeminal neuropathic pain. *The Journal of Pharmacology and Experimental Therapeutics, 306*(2), 505–514. doi:10.1124/jpet.103.050286.

Dirig, D. M., & Yaksh, T. L. (1995). Intrathecal baclofen and muscimol, but not midazolam, are antinociceptive using the rat-formalin model. *The Journal of Pharmacology and Experimental Therapeutics, 275*(1), 219–227.

Drew, G. M., Siddall, P. J., & Duggan, A. W. (2004). Mechanical allodynia following contusion injury of the rat spinal cord is associated with loss of GABAergic inhibition in the dorsal horn. *Pain, 109*(3), 379–388. doi:10.1016/j.pain.2004.02.007.

Eaton, M. J., Martinez, M. A., & Karmally, S. (1999). A single intrathecal injection of GABA permanently reverses neuropathic pain after nerve injury. *Brain Research, 835*(2), 334–339.

Enna, S. J. (2001a). A GABA(B) mystery: The search for pharmacologically distinct GABA(B) receptors. *Molecular Interventions, 1*(4), 208–218.

Enna, S. J. (2001b). GABAB receptor signaling pathways. In H. Mohler (Ed.), *Pharmacology of GABA and glycine neurotransmission* (pp. 329–342). Berlin: Springer.

Enna, S. J., Harstad, E. B., & McCarson, K. E. (1998). Regulation of neurokinin-1 receptor expression by GABA(B) receptor agonists. *Life Sciences, 62*(17–18), 1525–1530.

Enna, S. J., & McCarson, K. E. (2006). The role of GABA in the mediation and perception of pain. *Advances in Pharmacology, 54*, 1–27.

Franek, M., Vaculin, S., & Rokyta, R. (2004). GABA(B) receptor agonist baclofen has non-specific antinociceptive effect in the model of peripheral neuropathy in the rat. *Physiological Research, 53*(3), 351–355.

Frankowska, M., Filip, M., & Przegalinski, E. (2007). Effects of GABAB receptor ligands in animal tests of depression and anxiety. *Pharmacological Reports, 59*(6), 645–655.

Frau, R., Bini, V., Pillolla, G., Malherbe, P., Pardu, A., Thomas, A. W., et al. (2014). Positive allosteric modulation of GABAB receptors ameliorates sensorimotor gating in rodent models. *CNS Neuroscience & Therapeutics, 20*(7), 679–684. doi:10.1111/cns.12261.

Fromm, G. H. (1994). Baclofen as an adjuvant analgesic. *Journal of Pain and Symptom Management, 9*(8), 500–509.

Fukuhara, K., Katafuchi, T., & Yoshimura, M. (2013). Effects of baclofen on mechanical noxious and innocuous transmission in the spinal dorsal horn of the adult rat: In vivo patch-clamp analysis. *The European Journal of Neuroscience, 38*(10), 3398–3407. doi:10.1111/ejn.12345.

Gaillard, S., Lo Re, L., Mantilleri, A., Hepp, R., Urien, L., Malapert, P., et al. (2014). GINIP, a Galphai-interacting protein, functions as a key modulator of peripheral GABAB receptor-mediated analgesia. *Neuron, 84*(1), 123–136. doi:10.1016/j.neuron.2014.08.056.

Gangadharan, V., Agarwal, N., Brugger, S., Tegeder, I., Bettler, B., Kuner, R., et al. (2009). Conditional gene deletion reveals functional redundancy of GABAB receptors in peripheral nociceptors in vivo. *Molecular Pain, 5*, 68. doi:10.1186/1744-8069-5-68.

Gassmann, M., Shaban, H., Vigot, R., Sansig, G., Haller, C., Barbieri, S., et al. (2004). Redistribution of GABAB(1) protein and atypical GABAB responses in GABAB(2)-deficient mice. *The Journal of Neuroscience, 24*(27), 6086–6097. doi:10.1523/JNEUROSCI.5635-03.2004.

Gee, N. S., Brown, J. P., Dissanayake, V. U., Offord, J., Thurlow, R., & Woodruff, G. N. (1996). The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. *The Journal of Biological Chemistry, 271*(10), 5768–5776.

Gilbert, A. K., & Franklin, K. B. (2001). GABAergic modulation of descending inhibitory systems from the rostral ventromedial medulla (RVM). Dose-response analysis of nociception and neurological deficits. *Pain, 90*(1–2), 25–36.

Gjoni, T., & Urwyler, S. (2008). Receptor activation involving positive allosteric modulation, unlike full agonism, does not result in GABAB receptor desensitization. *Neuropharmacology, 55*(8), 1293–1299. doi:10.1016/j.neuropharm.2008.08.008.

Green, G. M., & Dickenson, A. (1997). GABA-receptor control of the amplitude and duration of the neuronal responses to formalin in the rat spinal cord. *European Journal of Pain, 1*(2), 95–104.

Gwak, Y. S., Tan, H. Y., Nam, T. S., Paik, K. S., Hulsebosch, C. E., & Leem, J. W. (2006). Activation of spinal GABA receptors attenuates chronic central neuropathic pain after spinal cord injury. *Journal of Neurotrauma, 23*(7), 1111–1124. doi:10.1089/neu.2006.23.1111.

Hammond, D. L., & Drower, E. J. (1984). Effects of intrathecally administered THIP, baclofen and muscimol on nociceptive threshold. *European Journal of Pharmacology, 103*(1–2), 121–125.

Hanack, C., Moroni, M., Lima, W. C., Wende, H., Kirchner, M., Adelfinger, L., et al. (2015). GABA blocks pathological but not acute TRPV1 pain signals. *Cell, 160*(4), 759–770. doi:10.1016/j.cell.2015.01.022.

Hensler, J. G., Advani, T., Burke, T. F., Cheng, K., Rice, K. C., & Koek, W. (2012). GABAB receptor-positive modulators: Brain region-dependent effects. *The Journal of Pharmacology and Experimental Therapeutics, 340*(1), 19–26. doi:10.1124/jpet.111.186577.

Honsek, S. D., Seal, R. P., & Sandkuhler, J. (2015). Presynaptic inhibition of optogenetically identified VGluT3+ sensory fibres by opioids and baclofen. *Pain, 156*(2), 243–251. doi:10.1097/01.j.pain.0000460304.63948.40.

Huang, D., Huang, S., Peers, C., Du, X., Zhang, H., & Gamper, N. (2015). GABAB receptors inhibit low-voltage activated and high-voltage activated Ca(2+) channels in sensory neurons via distinct mechanisms. *Biochemical and Biophysical Research Communications, 465*(2), 188–193. doi:10.1016/j.bbrc.2015.07.137.

Hwang, J. H., Hwang, K. S., Kim, J. U., Choi, I. C., Park, P. H., & Han, S. M. (2001). The interaction between intrathecal neostigmine and GABA receptor agonists in rats with nerve ligation Injury. *Anesthesia and Analgesia, 93*(5), 1297–1303.

Hwang, J. H., & Yaksh, T. L. (1997). The effect of spinal GABA receptor agonists on tactile allodynia in a surgically-induced neuropathic pain model in the rat. *Pain, 70*(1), 15–22.

Hyland, N. P., & Golubeva, A. V. (2015). GABA receptors in the bladder and bowel: Therapeutic potential for positive allosteric modulators? *British Journal of Pharmacology, 171*, 995–1006. doi:10.1111/bph.12617.

Ibuki, T., Hama, A. T., Wang, X. T., Pappas, G. D., & Sagen, J. (1997). Loss of GABA-immunoreactivity in the spinal dorsal horn of rats with peripheral nerve injury and promotion of recovery by adrenal medullary grafts. *Neuroscience, 76*(3), 845–858.

Ishii, H., & Izumi, H. (2012). GABAB receptors in the NTS mediate the inhibitory effect of trigeminal nociceptive inputs on parasympathetic reflex vasodilation in the rat masseter muscle. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology, 302*(6), R776–R784. doi:10.1152/ajpregu.00569.2011.

Jasmin, L., Rabkin, S. D., Granato, A., Boudah, A., & Ohara, P. T. (2003). Analgesia and hyperalgesia from GABA-mediated modulation of the cerebral cortex. *Nature, 424*(6946), 316–320. doi:10.1038/nature01808.

Jones, K. A., Borowsky, B., Tamm, J. A., Craig, D. A., Durkin, M. M., Dai, M., et al. (1998). GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2. *Nature, 396*(6712), 674–679. doi:10.1038/25348.

Kalinichev, M., Palea, S., Haddouk, H., Royer-Urios, I., Guilloteau, V., Lluel, P., et al. (2014). ADX71441, a novel, potent and selective positive allosteric modulator of the GABA(B) receptor, shows efficacy in rodent models of overactive bladder. *British Journal of Pharmacology, 171*(4), 995–1006. doi:10.1111/bph.12517.

Kantamneni, S., Gonzalez-Gonzalez, I. M., Luo, J., Cimarosti, H., Jacobs, S. C., Jaafari, N., et al. (2014). Differential regulation of GABAB receptor trafficking by different modes of N-methyl-D-aspartate (NMDA) receptor signaling. *The Journal of Biological Chemistry, 289*(10), 6681–6694. doi:10.1074/jbc.M113.487348.

Kaupmann, K., Malitschek, B., Schuler, V., Heid, J., Froestl, W., Beck, P., et al. (1998). GABA(B)-receptor subtypes assemble into functional heteromeric complexes. *Nature, 396*(6712), 683–687. doi:10.1038/25360.

Kendall, D. A., Browner, M., & Enna, S. J. (1982). Comparison of the antinociceptive effect of gamma-aminobutyric acid (GABA) agonists: Evidence for a cholinergic involvement. *The Journal of Pharmacology and Experimental Therapeutics, 220*(3), 482–487.

Kim, W., Kim, S. K., & Min, B. I. (2013). Mechanisms of electroacupuncture-induced analgesia on neuropathic pain in animal model. *Evidence-Based Complementary and Alternative Medicine, 2013*, 436913. doi:10.1155/2013/436913.

Kirouac, G. J., Li, S., & Mabrouk, G. (2004). GABAergic projection from the ventral tegmental area and substantia nigra to the periaqueductal gray region and the dorsal raphe nucleus. *The Journal of Comparative Neurology, 469*(2), 170–184. doi:10.1002/cne.11005.

Laffray, S., Bouali-Benazzouz, R., Papon, M. A., Favereaux, A., Jiang, Y., Holm, T., et al. (2012). Impairment of GABAB receptor dimer by endogenous 14-3-3zeta in chronic pain conditions. *The EMBO Journal, 31*(15), 3239–3251. doi:10.1038/emboj.2012.161.

Levy, R. A., & Proudfit, H. K. (1977). The analgesic action of baclofen [beta-(4-chlorophenyl)-gamma-aminobutyric acid]. *The Journal of Pharmacology and Experimental Therapeutics, 202*(2), 437–445.

Li, D. P., Chen, S. R., Pan, Y. Z., Levey, A. I., & Pan, H. L. (2002). Role of presynaptic muscarinic and GABA(B) receptors in spinal glutamate release and cholinergic analgesia in rats. *The Journal of Physiology, 543*(Pt 3), 807–818.

Li, X., Risbrough, V. B., Cates-Gatto, C., Kaczanowska, K., Finn, M. G., Roberts, A. J., et al. (2013). Comparison of the effects of the GABAB receptor positive modulator BHF177 and the GABAB receptor agonist baclofen on anxiety-like behavior, learning, and memory in mice. *Neuropharmacology, 70*, 156–167. doi:10.1016/j.neuropharm.2013.01.018.

Liu, P., Guo, W. Y., Zhao, X. N., Bai, H. P., Wang, Q., Wang, X. L., et al. (2014). Intrathecal baclofen, a GABAB receptor agonist, inhibits the expression of p-CREB and NR2B in the spinal dorsal horn in rats with diabetic neuropathic pain. *Canadian Journal of Physiology and Pharmacology, 92*(8), 655–660. doi:10.1139/cjpp-2013-0463.

Liu, J., Ren, Y., Li, G., Liu, Z. L., Liu, R., Tong, Y., et al. (2013). GABAB receptors resist acute desensitization in both postsynaptic and presynaptic compartments of periaqueductal gray neurons. *Neuroscience Letters, 543*, 146–151. doi:10.1016/j.neulet.2013.03.035.

Loubser, P. G., & Akman, N. M. (1996). Effects of intrathecal baclofen on chronic spinal cord injury pain. *Journal of Pain and Symptom Management, 12*(4), 241–247.

Lu, Y., Zheng, J., Xiong, L., Zimmermann, M., & Yang, J. (2008). Spinal cord injury-induced attenuation of GABAergic inhibition in spinal dorsal horn circuits is associated with down-regulation of the chloride transporter KCC2 in rat. *The Journal of Physiology, 586*(Pt 23), 5701–5715. doi:10.1113/jphysiol.2008.152348.

Magnaghi, V., Ballabio, M., Camozzi, F., Colleoni, M., Consoli, A., Gassmann, M., et al. (2008). Altered peripheral myelination in mice lacking GABAB receptors. *Molecular and Cellular Neurosciences, 37*(3), 599–609. doi:10.1016/j.mcn.2007.12.009.

Mahmoudi, M., & Zarrindast, M. R. (2002). Effect of intracerebroventricular injection of GABA receptor agents on morphine-induced antinociception in the formalin test. *Journal of Psychopharmacology, 16*(1), 85–91.

Malan, T. P., Mata, H. P., & Porreca, F. (2002). Spinal GABA(A) and GABA(B) receptor pharmacology in a rat model of neuropathic pain. *Anesthesiology, 96*(5), 1161–1167.

Malcangio, M., & Bowery, N. G. (1994). Spinal cord SP release and hyperalgesia in monoarthritic rats: Involvement of the GABAB receptor system. *British Journal of Pharmacology, 113*(4), 1561–1566.

Malcangio, M., Libri, V., Teoh, H., Constanti, A., & Bowery, N. G. (1995). Chronic (-)baclofen or CGP 36742 alters GABAB receptor sensitivity in rat brain and spinal cord. *Neuroreport, 6*(2), 399–403.

Martins, I., Carvalho, P., de Vries, M. G., Teixeira-Pinto, A., Wilson, S. P., Westerink, B. H., et al. (2015). GABA acting on GABAB receptors located in a medullary pain facilitatory area enhances nociceptive behaviors evoked by intraplantar formalin injection. *Pain, 156*(8), 1555–1565. doi:10.1097/j.pain.0000000000000203.

McCarson, K. E., & Enna, S. J. (1996). Relationship between GABAB receptor activation and neurokinin receptor expression in spinal cord. *Pharmacology Research Communications, 8*, 191–194.

McCarson, K. E., & Enna, S. J. (1999). Nociceptive regulation of GABA(B) receptor gene expression in rat spinal cord. *Neuropharmacology, 38*(11), 1767–1773.

McCarson, K. E., & Enna, S. J. (2014). GABA pharmacology: The search for analgesics. *Neurochemical Research, 39*(10), 1948–1963. doi:10.1007/s11064-014-1254-x.

McCarson, K. E., Ralya, A., Reisman, S. A., & Enna, S. J. (2005). Amitriptyline prevents thermal hyperalgesia and modifications in rat spinal cord GABA(B) receptor expression and function in an animal model of neuropathic pain. *Biochemical Pharmacology, 71*(1–2), 196–202. doi:10.1016/j.bcp.2005.10.026.

Meisner, J. G., Marsh, A. D., & Marsh, D. R. (2010). Loss of GABAergic interneurons in laminae I-III of the spinal cord dorsal horn contributes to reduced GABAergic tone and neuropathic pain after spinal cord injury. *Journal of Neurotrauma, 27*(4), 729–737. doi:10.1089/neu.2009.1166.

Melin, C., Jacquot, F., Dallel, R., & Artola, A. (2013). Segmental disinhibition suppresses C-fiber inputs to the rat superficial medullary dorsal horn via the activation of GABAB receptors. *The European Journal of Neuroscience, 37*(3), 417–428. doi:10.1111/ejn.12048.

Miletic, G., Draganic, P., Pankratz, M. T., & Miletic, V. (2003). Muscimol prevents long-lasting potentiation of dorsal horn field potentials in rats with chronic constriction injury exhibiting decreased levels of the GABA transporter GAT-1. *Pain, 105*(1–2), 347–353.

Mohler, H. (2001). Functions of GABAA-receptor: Pharmacology and pathophysiology. In H. Mohler (Ed.), *Pharmacology of GABA and glycine neurotransmission* (pp. 101–116). Berlin: Springer.

Mohler, H. B. J., Benson, B., Luscher, B., Rudolph, U., & Fritschy, J. M. (1997). Diversity in structure, pharmacology, and regulation of GABAA receptors. In S. J. Enna & N. G. Bowery (Eds.), *The GABA receptors* (pp. 11–36). Totowa: Humana Press.

Mohler, H., Fritschy, J. M., Crestani, F., Hensch, T., & Rudolph, U. (2004). Specific GABA(A) circuits in brain development and therapy. *Biochemical Pharmacology, 68*(8), 1685–1690. doi:10.1016/j.bcp.2004.07.025.

Moore, K. A., Kohno, T., Karchewski, L. A., Scholz, J., Baba, H., & Woolf, C. J. (2002). Partial peripheral nerve injury promotes a selective loss of GABAergic inhibition in the superficial dorsal horn of the spinal cord. *The Journal of Neuroscience, 22*(15), 6724–6731.

Mugnaini, C., Pedani, V., Casu, A., Lobina, C., Casti, A., Maccioni, P., et al. (2013). Synthesis and pharmacological characterization of 2-(acylamino)thiophene derivatives as metabolically stable, orally effective, positive allosteric modulators of the GABAB receptor. *Journal of Medicinal Chemistry, 56*(9), 3620–3635. doi:10.1021/jm400144w.

Naderi, N., Shafaghi, B., Khodayar, M. J., & Zarindast, M. R. (2005). Interaction between gamma-aminobutyric acid GABAB and cannabinoid CB1 receptors in spinal pain pathways in rat. *European Journal of Pharmacology, 514*(2–3), 159–164. doi:10.1016/j.ejphar.2005.03.037.

Nguyen, T. T., Matsumoto, K., & Watanabe, H. (1997). Involvement of supraspinal GABA-ergic systems in clonidine-induced antinociception in the tail-pinch test in mice. *Life Sciences, 61*(11), 1097–1103.

Nowak, P., Kowalinska-Kania, M., Nowak, D., Kostrzewa, R. M., & Malinowska-Borowska, J. (2013). Antinociceptive effects of H(3) (R-methylhistamine) and GABA(B) (baclofen)-receptor ligands in an orofacial model of pain in rats. *Neurotoxicity Research, 24*(2), 258–264. doi:10.1007/s12640-013-9385-4.

Patel, S., Naeem, S., Kesingland, A., Froestl, W., Capogna, M., Urban, L., et al. (2001). The effects of GABA(B) agonists and gabapentin on mechanical hyperalgesia in models of neuropathic and inflammatory pain in the rat. *Pain, 90*(3), 217–226.

Pinard, A., Seddik, R., & Bettler, B. (2010). GABAB receptors: Physiological functions and mechanisms of diversity. *Advances in Pharmacology, 58*, 231–255. doi:10.1016/S1054-3589(10)58010-4.

Pinto, M., Lima, D., Castro-Lopes, J., & Tavares, I. (2003). Noxious-evoked c-fos expression in brainstem neurons immunoreactive for GABAB, mu-opioid and NK-1 receptors. *The European Journal of Neuroscience, 17*(7), 1393–1402.

Polgar, E., Hughes, D. I., Riddell, J. S., Maxwell, D. J., Puskar, Z., & Todd, A. J. (2003). Selective loss of spinal GABAergic or glycinergic neurons is not necessary for development of thermal hyperalgesia in the chronic constriction injury model of neuropathic pain. *Pain, 104*(1–2), 229–239.

Potes, C. S., Neto, F. L., & Castro-Lopes, J. M. (2006). Inhibition of pain behavior by GABA(B) receptors in the thalamic ventrobasal complex: Effect on normal rats subjected to the formalin test of nociception. *Brain Research, 1115*(1), 37–47. doi:10.1016/j.brainres.2006.07.089.

Price, G. W., Wilkin, G. P., Turnbull, M. J., & Bowery, N. G. (1984). Are baclofen-sensitive GABAB receptors present on primary afferent terminals of the spinal cord? *Nature, 307*(5946), 71–74.

Reis, G. M., & Duarte, I. D. (2006). Baclofen, an agonist at peripheral GABAB receptors, induces antinociception via activation of TEA-sensitive potassium channels. *British Journal of Pharmacology, 149*(6), 733–739. doi:10.1038/sj.bjp.0706898.

Riley, R. C., Trafton, J. A., Chi, S. I., & Basbaum, A. I. (2001). Presynaptic regulation of spinal cord tachykinin signaling via GABA(B) but not GABA(A) receptor activation. *Neuroscience, 103*(3), 725–737.

Rudolph, U., & Mohler, H. (2004). Analysis of GABAA receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. *Annual Review of Pharmacology and Toxicology, 44*, 475–498. doi:10.1146/annurev.pharmtox.44.101802.121429.

Sands, S. A., McCarson, K. E., & Enna, S. J. (2003). Differential regulation of GABA B receptor subunit expression and function. *The Journal of Pharmacology and Experimental Therapeutics, 305*(1), 191–196. doi:10.1124/jpet.102.046342.

Schuler, V., Luscher, C., Blanchet, C., Klix, N., Sansig, G., Klebs, K., et al. (2001). Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic GABA(B) responses in mice lacking GABA(B(1)). *Neuron, 31*(1), 47–58.

Schwenk, J., Metz, M., Zolles, G., Turecek, R., Fritzius, T., Bildl, W., et al. (2010). Native GABA(B) receptors are heteromultimers with a family of auxiliary subunits. *Nature, 465*(7295), 231–235. doi:10.1038/nature08964.

Schwenk, J., Perez-Garci, E., Schneider, A., Kollewe, A., Gauthier-Kemper, A., Fritzius, T., et al. (2016). Modular composition and dynamics of native GABAB receptors identified by high-resolution proteomics. *Nature Neuroscience, 19*(2), 233–242. doi:10.1038/nn.4198.

Shafizadeh, M., Semnanian, S., Zarrindast, M. R., & Hashemi, B. (1997). Involvement of GABAB receptors in the antinociception induced by baclofen in the formalin test. *General Pharmacology, 28*(4), 611–615.

Sindrup, S. H., & Jensen, T. S. (2002). Pharmacotherapy of trigeminal neuralgia. *The Clinical Journal of Pain, 18*(1), 22–27.

Slattery, D. A., Markou, A., Froestl, W., & Cryan, J. F. (2005). The GABAB receptor-positive modulator GS39783 and the GABAB receptor agonist baclofen attenuate the reward-facilitating effects of cocaine: Intracranial self-stimulation studies in the rat. *Neuropsychopharmacology, 30*(11), 2065–2072. doi:10.1038/sj.npp.1300734.

Slonimski, M., Abram, S. E., & Zuniga, R. E. (2004). Intrathecal baclofen in pain management. *Regional Anesthesia and Pain Medicine, 29*(3), 269–276.

Smith, G. D., Harrison, S. M., Birch, P. J., Elliott, P. J., Malcangio, M., & Bowery, N. G. (1994). Increased sensitivity to the antinociceptive activity of (+/-)-baclofen in an animal model of chronic neuropathic, but not chronic inflammatory hyperalgesia. *Neuropharmacology, 33*(9), 1103–1108.

Somers, D. L., & Clemente, F. R. (2002). Dorsal horn synaptosomal content of aspartate, glutamate, glycine and GABA are differentially altered following chronic constriction injury to the rat sciatic nerve. *Neuroscience Letters, 323*(3), 171–174.

Takeda, M., Ikeda, M., Takahashi, M., Kanazawa, T., Nasu, M., & Matsumoto, S. (2013). Suppression of ATP-induced excitability in rat small-diameter trigeminal ganglion neurons by activation of GABAB receptor. *Brain Research Bulletin, 98*, 155–162. doi:10.1016/j.brainresbull.2013.08.005.

Thibault, K., Calvino, B., Rivals, I., Marchand, F., Dubacq, S., McMahon, S. B., et al. (2014). Molecular mechanisms underlying the enhanced analgesic effect of oxycodone compared to morphine in chemotherapy-induced neuropathic pain. *PLoS One, 9*(3), e91297. doi:10.1371/journal.pone.0091297.

Thomas, D. A., McGowan, M. K., & Hammond, D. L. (1995). Microinjection of baclofen in the ventromedial medulla of rats: Antinociception at low doses and hyperalgesia at high doses. *The Journal of Pharmacology and Experimental Therapeutics, 275*(1), 274–284.

Thomas, D. A., Navarrete, I. M., Graham, B. A., McGowan, M. K., & Hammond, D. L. (1996). Antinociception produced by systemic R(+)-baclofen hydrochloride is attenuated by CGP 35348 administered to the spinal cord or ventromedial medulla of rats. *Brain Research, 718*(1–2), 129–137.

Ugarte, S. D., Homanics, G. E., Firestone, L. L., & Hammond, D. L. (2000). Sensory thresholds and the antinociceptive effects of GABA receptor agonists in mice lacking the beta3 subunit of the GABA(A) receptor. *Neuroscience, 95*(3), 795–806.

Ulrich, D., & Bettler, B. (2007). GABA(B) receptors: Synaptic functions and mechanisms of diversity. *Current Opinion in Neurobiology, 17*(3), 298–303. doi:10.1016/j.conb.2007.04.001.

Urwyler, S., Pozza, M. F., Lingenhoehl, K., Mosbacher, J., Lampert, C., Froestl, W., et al. (2003). N, N′-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) and structurally related compounds: Novel allosteric enhancers of gamma-aminobutyric acidB receptor function. *The Journal of Pharmacology and Experimental Therapeutics, 307*(1), 322–330. doi:10.1124/jpet.103.053074.

Vallejo, R., Tilley, D. M., Williams, J., Labak, S., Aliaga, L., & Benyamin, R. M. (2013). Pulsed radiofrequency modulates pain regulatory gene expression along the nociceptive pathway. *Pain Physician, 16*(5), E601–E613.

Varani, A. P., Aso, E., Maldonado, R., & Balerio, G. N. (2014). Baclofen and 2-hydroxysaclofen modify acute hypolocomotive and antinociceptive effects of nicotine. *European Journal of Pharmacology, 738*, 200–205. doi:10.1016/j.ejphar.2014.05.039.

von Heijne, M., Hao, J. X., Sollevi, A., & Xu, X. J. (2001). Effects of intrathecal morphine, baclofen, clonidine and R-PIA on the acute allodynia-like behaviours after spinal cord ischaemia in rats. *European Journal of Pain, 5*(1), 1–10. doi:10.1053/eujp.2000.0212.

Wang, X. L., Zhang, H. M., Chen, S. R., & Pan, H. L. (2007). Altered synaptic input and GABAB receptor function in spinal superficial dorsal horn neurons in rats with diabetic neuropathy. *The Journal of Physiology, 579*(Pt 3), 849–861. doi:10.1113/jphysiol.2006.126102.

Wang, X. L., Zhang, Q., Zhang, Y. Z., Liu, Y. T., Dong, R., Wang, Q. J., et al. (2011). Downregulation of GABAB receptors in the spinal cord dorsal horn in diabetic neuropathy. *Neuroscience Letters, 490*(2), 112–115. doi:10.1016/j.neulet.2010.12.038.

Whitehead, R. A., Puil, E., Ries, C. R., Schwarz, S. K., Wall, R. A., Cooke, J. E., et al. (2012). GABA(B) receptor-mediated selective peripheral analgesia by the non-proteinogenic amino acid, isovaline. *Neuroscience, 213*, 154–160. doi:10.1016/j.neuroscience.2012.04.026.

Yang, K., Ma, W. L., Feng, Y. P., Dong, Y. X., & Li, Y. Q. (2002). Origins of GABA(B) receptor-like immunoreactive terminals in the rat spinal dorsal horn. *Brain Research Bulletin, 58*(5), 499–507.

Yang, K., Ma, R., Wang, Q., Jiang, P., & Li, Y. Q. (2015). Optoactivation of parvalbumin neurons in the spinal dorsal horn evokes GABA release that is regulated by presynaptic GABAB receptors. *Neuroscience Letters, 594*, 55–59. doi:10.1016/j.neulet.2015.03.050.

Zarrindast, M. R., & Mahmoudi, M. (2001). GABA mechanisms and antinociception in mice with ligated sciatic nerve. *Pharmacology & Toxicology, 89*(2), 79–84.

Zarrindast, M., Valizadeh, S., & Sahebgharani, M. (2000). GABA(B) receptor mechanism and imipramine-induced antinociception in ligated and non-ligated mice. *European Journal of Pharmacology, 407*(1–2), 65–72.

Zhang, A. L., Hao, J. X., Seiger, A., Xu, X. J., Wiesenfeld-Hallin, Z., Grant, G., et al. (1994). Decreased GABA immunoreactivity in spinal cord dorsal horn neurons after transient spinal cord ischemia in the rat. *Brain Research, 656*(1), 187–190.

Zhou, H. Y., Zhang, H. M., Chen, S. R., & Pan, H. L. (2007). Increased nociceptive input rapidly modulates spinal GABAergic transmission through endogenously released glutamate. *Journal of Neurophysiology, 97*(1), 871–882. doi:10.1152/jn.00964.2006.

Zhu, Y., Dua, S., & Gold, M. S. (2012). Inflammation-induced shift in spinal GABA(A) signaling is associated with a tyrosine kinase-dependent increase in GABA(A) current density in nociceptive afferents. *Journal of Neurophysiology, 108*(9), 2581–2593. doi:10.1152/jn.00590.2012.

Zorn, S. H., & Enna, S. J. (1987). The GABA agonist THIP, attenuates antinociception in the mouse by modifying central cholinergic transmission. *Neuropharmacology, 26*(5), 433–437.

Chapter 12
Targeting the $GABA_B$ Receptor for the Treatment of Depression and Anxiety Disorders

Daniela Felice, Olivia F. O'Leary, and John F. Cryan

Abstract The $GABA_B$ receptor is a functional heterodimer comprising the $GABA_{B1}$ and $GABA_{B2}$ subunits, with the $GABA_{B1}$ subunit displaying two major isoforms, $GABA_{B(1a)}$ and $GABA_{B(1b)}$. Since the discovery of the $GABA_B$ receptor by Bowery in 1980, preclinical and clinical findings have strongly implicated the $GABA_B$ receptor in depression and anxiety disorders. Indeed, postmortem and clinical studies have highlighted a key role for the $GABA_B$ receptor in mood disorders. In parallel, pharmacological and genetic preclinical studies have confirmed that the $GABA_B$ receptor can modulate anxiety and depression-related behaviours. Despite the literature clearly linking $GABA_B$ receptor dysfunction with depression and anxiety disorders, $GABA_B$ receptor-based drugs have not yet been approved for their treatment. One of the main reasons for this is that drugs targeting the whole $GABA_B$ receptor may induce several major side effects. However, positive allosteric modulators and more recently, negative allosteric modulators of the $GABA_B$ receptor have been developed, and these could represent future therapeutic approaches for depression and anxiety disorders. Moreover, recent studies suggest that proteins interacting with the $GABA_B$ receptor could also be valid targets for the development of $GABA_B$ receptor-based drugs. This chapter will review the preclinical and clinical evidence for the $GABA_B$ receptor as a therapeutic target in the treatment of depression and anxiety disorders, and will outline some of the challenges that need to be overcome before $GABA_B$ receptor-based drugs can be used for this purpose in the clinic.

Keywords $GABA_B$ receptor • Depression • Anxiety • Antidepressant • Neurogenesis

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12.1 Introduction

Anxiety disorders and depression are serious health problems in today society. In 2011, it was estimated that brain disorders affected 38.2 % of the total population (aged 18–65) in Europe (Wittchen et al. 2011). This corresponds to an estimated 164.7 million persons suffering from mental disorders. The most prevalent brain disorders are depression and anxiety disorders which affect 30.3 million and 69.1 million people in Europe, respectively (Wittchen et al. 2011). The estimated cost burden of these disorders in Europe is €113.4 billion for mood disorders and €74.4 billion for anxiety disorders (Gustavsson et al. 2011). Despite the high impact of depression and anxiety disorders on society and the economy, current therapeutic options are suboptimal (Gaynes et al. 2009; Felice et al. 2015; O'Leary et al. 2015). Indeed, the recent large multi-centre effectiveness trial, Sequenced Treatment Alternatives to relieve Depression (STAR*D), revealed that approximately 47 % of patients failed to respond to a first-line antidepressant treatment (the selective serotonin reuptake inhibitor (SSRI), citalopram), and 1 in 3 did not achieve remission after four different consecutive interventions (Trivedi et al. 2006; Warden et al. 2007).

In addition, current antidepressants including the SSRIs which are the most commonly prescribed first-line treatments have a slow onset of action requiring several weeks or months of treatment before clinical improvement is reported (Artigas 2015; Felice et al. 2015; O'Leary et al. 2015). Most of the currently available antidepressants target the serotonin and/or noradrenaline neurotransmitter systems but it is becoming increasingly clear that novel therapeutic targets need to be identified in order to develop more effective antidepressants with a rapid onset of action (Artigas 2015; Felice et al. 2015; O'Leary et al. 2015).

Current treatments of anxiety disorders include benzodiazepines and SSRIs (Cryan and Sweeney 2011). Benzodiazepines allosterically enhance $GABA_A$ receptor activation. Importantly, benzodiazepines are effective in the acute treatment of generalized anxiety disorder (GAD) and social anxiety disorder (SAD) but have limited to no efficacy in other anxiety conditions (Baldwin et al. 2010). Moreover, long-term use of benzodiazepines present several unwanted effects including sedation, memory disturbance, tolerance, and dependence (Nemeroff 2003a; Hoffman and Mathew 2008). Such limitations have led to a renewed interest in developing new therapeutic approaches for anxiety disorders (Griebel and Holmes 2013).

Accumulating evidence suggests that the $GABA_B$ receptor may be one such novel therapeutic target for the treatment of depression and anxiety disorders. This chapter will outline the clinical and preclinical evidence supporting a role for $GABA_B$ receptors in the pathophysiology of depression and anxiety disorders and will discuss the preclinical evidence of the antidepressant and anxiolytic effects of pharmacological and genetic modulation of $GABA_B$ receptor activity.

12.2 $GABA_B$ Receptors: Structure and Function

$GABA_B$ receptors belong to the family of G-protein-coupled receptors (GPCRs) (Kaupmann et al. 1997) and consist of two subunits, namely $GABA_{B(1)}$ and $GABA_{B(2)}$, which heterodimerise to form the functional receptor (Bettler et al. 2004) (Fig. 12.1). The $GABA_{B(1)}$ receptor subunit has the site for orthosteric ligand binding (Bettler et al. 2004; Gassmann and Bettler 2012), while the $GABA_{B(2)}$ subunit is responsible for G-protein activation (Galvez et al. 2001) and contains binding sites for positive allosteric modulators (PAMs) (Binet et al. 2004). The two main isoforms of the $GABA_{B(1)}$ receptor subunit are $GABA_{B(1a)}$ and $GABA_{B(1b)}$ (Fig. 12.1). In most neurons, one of these two isoforms combines with the $GABA_{B(2)}$ receptor subunit to form functional heteromeric receptors, $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ (Mohler and Fritschy 1999). Structurally, the $GABA_{B(1a)}$ and $GABA_{B(1b)}$ receptor subunit isoforms differ only by the presence of a *sushi* domain in the N-terminal ectodomain of the $GABA_{B(1a)}$ receptor subunit isoform (Bettler et al. 2004). The discovery of differences in the $GABA_{B(1)}$ receptor subunit isoforms is important from a pharmacological point of view because heterogeneity in the $GABA_B$ receptor system could allow the development of more specific compounds targeted at the $GABA_B$ receptor. Although in vitro studies have reported that the two isoforms do not show any pharmacological or functional differences (Brauner-Osborne and Krogsgaard-Larsen 1999), phenotypic characterization of mice lacking these subunit isoforms (Fig. 12.1b) demonstrates differences in stress resilience, cognition and anxiety (Bettler et al. 2004; Jacobson et al. 2006, 2007a, b; Shaban et al. 2006; O'Leary et al. 2014).

12.3 Evidence of a Role for the $GABA_B$ Receptor in Antidepressant Action and the Pathophysiology of Depression and Anxiety Disorders

Clinical evidence strongly supports GABAergic dysfunction in major depression (Cryan and Slattery 2010). Postmortem studies have reported reduced GABA levels in the cerebral cortex, plasma and cerebrospinal fluid (CSF) in depressed patients (Sanacora et al. 2000; Krystal et al. 2002). In addition, a reduced number of calbindin-immunoreactive GABAergic neurons in the prefrontal cortex (Rajkowska et al. 2007) and occipital cortex (Karolewicz et al. 2010) has been reported in depression. Similarly, several studies have reported that the expression of the GABA synthesizing enzyme glutamic acid decarboxylase (GAD)-67 is reduced in the prefrontal cortex in depressed patients (Gos et al. 2009; Karolewicz et al. 2010). Moreover, several clinical studies suggest a role for the $GABA_B$ receptor in the pathophysiology of depression. Indeed, β-chlorophenyl-GABA (baclofen)-induced

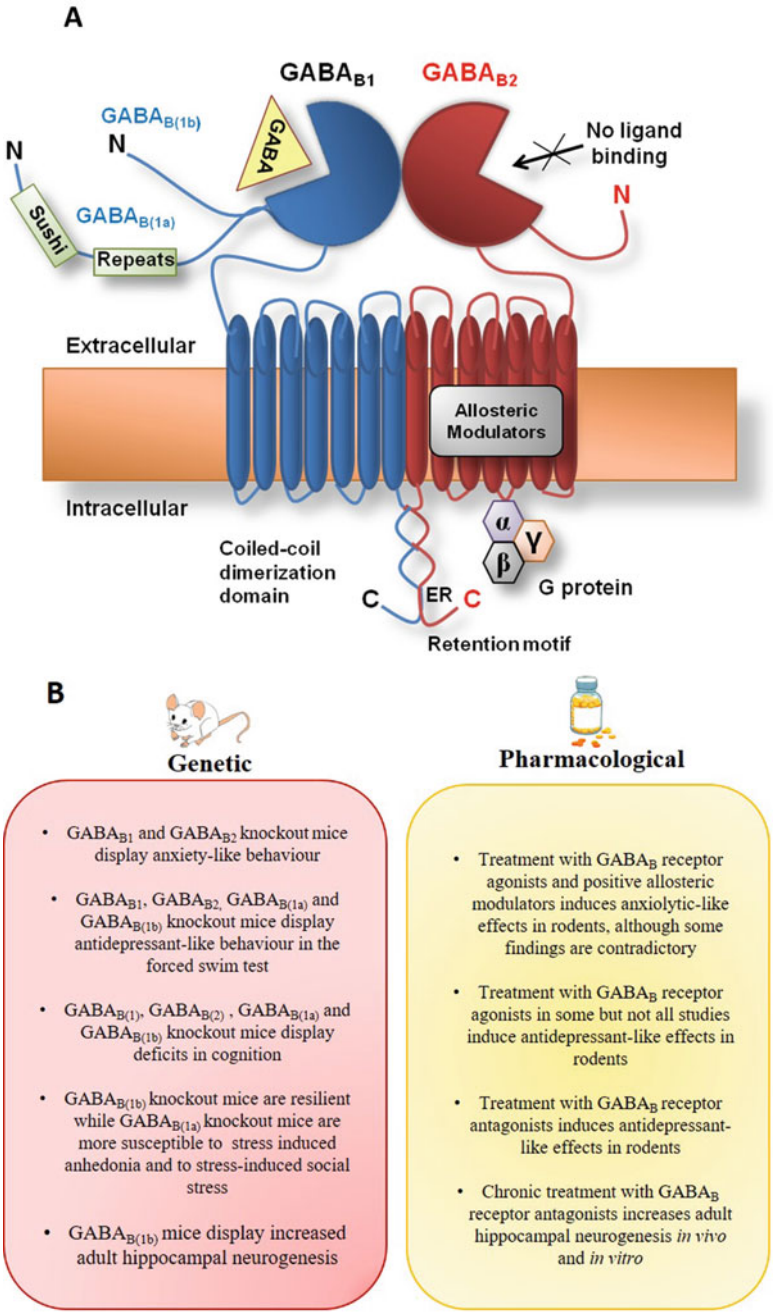

Fig. 12.1 (**a**) GABA$_B$ receptors are composed of GABA$_{B(1)}$ and GABA$_{B(2)}$ receptor subunits, that form an active heterodimer. The GABA$_{B(1)}$ receptor subunit is essential for the binding of GABA and GABA$_B$ receptor agonists and antagonists. The GABA$_{B(2)}$ receptor subunit interacts with $G_{\beta\gamma}$ subunits of the activated G proteins. 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); (**b**) Genetic and pharmacological evidence of the involvement of GABA$_B$ receptor in depression and anxiety disorders

growth hormone release is blunted in depressed patients (Marchesi et al. 1991; O'Flynn and Dinan 1993), thus suggesting $GABA_B$ receptor dysfunction in major depression. Furthermore, postmortem studies have revealed significant changes in $GABA_B$ receptor subunit expression in brains from depressed suicide victims (Klempan et al. 2009) and depressed patients (Ghose et al. 2011). In depressed suicide victims, the $GABA_{B(2)}$ receptor subunit was reported to be upregulated compared with non-depressed suicide victims in cortical and subcortical brain regions (Klempan et al. 2009). Another small postmortem study revealed that depressed patients displayed a 30 % decrease in $GABA_{B(1a)}$ gene expression and a 50 % increase of $GABA_{B(2)}$ gene expression in the hippocampus (Ghose et al. 2011). Interestingly, the hippocampus is one of just a few areas of the mammalian brain where neurogenesis, the birth of new neurons, occurs throughout life (Altman 1962; Altman and Das 1965). Several studies suggest that adult hippocampal neurogenesis is required for antidepressant action (Santarelli et al. 2003; David et al. 2009; O'Leary and Cryan 2014; Miller and Hen 2015) and the modulation of stress responses (Snyder et al. 2011; Levone et al. 2015). Moreover, recent studies have shown that adult hippocampal neurogenesis is increased by $GABA_B$ receptor antagonists (Felice et al. 2012; Giachino et al. 2014) and in mice lacking $GABA_{B(1)}$ (Giachino et al. 2014) or $GABA_{B(1b)}$ receptor subunits (O'Leary et al. 2014). Specifically, experimental evidence from our group has demonstrated that chronic treatment with a $GABA_B$ receptor antagonist increased adult hippocampal cell proliferation in mice (Felice et al. 2012) and that $GABA_{B(1b)}$ receptor knockout mice display increased adult hippocampal cell proliferation (O'Leary et al. 2014). Interestingly, these increases in adult hippocampal cell proliferation occurred specifically in the ventral rather than the dorsal hippocampus (Felice et al. 2012; O'Leary et al. 2014). This finding is interesting in light of evidence suggesting that the hippocampus is functionally segregated along its longitudinal axis into dorsal and ventral regions in rodents whereby the ventral hippocampus rather than the dorsal hippocampus preferentially regulates anxiety and the stress response (Moser and Moser 1998; Bannerman et al. 2004; O'Leary and Cryan 2014).

Several studies suggest that $GABA_B$ receptor expression and function is affected by antidepressant treatments thus suggesting that the $GABA_B$ receptor may be important in the mechanism of action of some antidepressant drugs. Specifically antidepressant treatment (tranylcypromine, phenelzine, desipramine, fluoxetine) increased $GABA_B$ receptor expression in the rat hippocampus (Sands et al. 2004) and the rat spinal cord (McCarson et al. 2006). On the other hand, another study reported that chronic fluoxetine treatment decreased $GABA_B$ receptor function in socially stressed rats (Cornelisse et al. 2007). Interestingly, $GABA_B$ receptor antagonists have been shown to elevate brain-derived neurotophic factor (BDNF) protein and mRNA levels in various brain regions in rodents (Heese et al. 2000; Enna et al. 2006). BDNF is a neurotrophin involved in the mechanism of action of antidepressants (Duman et al. 1997; Altar 1999; Nestler et al. 2002; Saarelainen et al. 2003; Castren 2004; O'Leary and Castren 2010), adult hippocampal neurogenesis (Li et al. 2008; Vilar and Mira 2016) and it is required for antidepressant-induced

increases in adult hippocampal neurogenesis and antidepressant-like behaviour (Sairanen et al. 2005; Taliaz et al. 2010).

In contrast to depression, the role of $GABA_B$ receptor in the pathophysiology and treatment of anxiety disorders is relatively less explored. Nonetheless, clinical evidence strongly supports a key role for the GABAergic neurotransmitter system in anxiety disorders. Indeed, anxiety disorders are characterized by a deficiency in GABAergic neurotransmission (Nemeroff 2003b) and benzodiazepines, which act through $GABA_A$ receptors, are among the most widely prescribed anxiolytic drugs. Baclofen, a $GABA_B$ receptor agonist, has been shown to reverse anxiety associated with several conditions (Cryan and Kaupmann 2005) such as alcohol withdrawal (Addolorato et al. 2002), post-traumatic stress (Drake et al. 2003; Manteghi et al. 2014), and panic disorder (Breslow et al. 1989). A recent study conducted by Rocha and colleagues demonstrated that patients with temporal lobe epilepsy (TLE) and comorbid anxiety and/or depression display reduced $GABA_B$-induced G-protein activation in spite of elevated $GABA_B$ binding, and lower tissue content of GABA when compared to autopsy controls in neocortex (Rocha et al. 2014) thus suggesting that $GABA_B$ receptor dysfunction might also be associated with anxiety. Currently, the strongest evidence supporting a role for the $GABA_B$ receptor in the modulation of anxiety comes from preclinical studies whereby mice lacking either the $GABA_{B(1)}$ or $GABA_{B(2)}$ receptor subunit display anxiety-like behaviours in some paradigms (Mombereau et al. 2005).

Taken together, clinical and preclinical evidence (Fig. 12.1b) strongly suggests that the $GABA_B$ receptor plays a role in antidepressant action and in the pathophysiology of depression and anxiety disorders.

12.4 The Effects of $GABA_B$ Receptor Agonists on Anxiety and Depression-Like Behaviours

The history of the $GABA_B$ receptor began with Bowery in 1980, who described for the first time a bicucculine- and isoguvacine-insensitive receptor, which was later named the $GABA_B$ receptor (Hill and Bowery 1981). This receptor was activated by baclofen, a compound that is inactive at classical available $GABA_A$ receptor sites. Currently, baclofen is the only $GABA_B$ receptor modulating drug in clinical use and is prescribed primarily for use as a muscle relaxant in the treatment of spasticity and skeletal muscle rigidity, but is also used intrathecally to treat neuropathic pain and to attenuate pain after spinal cord injury or stroke [reviewed by Froestl (2010)]. Since the discovery of baclofen, several $GABA_B$ receptor agonists have been synthesized. Several preclinical studies have assessed the effects of $GABA_B$ receptor agonists on anxiety- and depression-related behaviours in rodents as summarized in Tables 12.1 and 12.2 and as described below.

Table 12.1 Effects of $GABA_B$ receptor agonists on anxiety-related behaviours

Agonists	Dose/length	Subjects	Paradigm	Finding	References
Baclofen	0.375, 3 mg/kg	BALB/cOlaHs mice	EPM	↔[a]	Dalvi and Rodgers (1996)
	2.5, 5 mg/kg (acute)	Hooded Lister rat; DW-induced anxiety		↓	File et al. (1991)
	1.25, 2,5 mg/kg (acute)	Hooded Lister rat DW-induced anxiety		↓	File et al. (1992)
	0.05, 0.1, 0.2 μg/rat icv or 1, 2, and 4 mg/kg	Wistar rat		↔	Zarrindast et al. (2001)
	0.5, 1, 2 mg/kg (acute)	Swiss Webster mice; Nicotine-induced anxiety		↔	Varani and Balerio (2012)
	0.5, 1, 2 mg/kg (acute)	Swiss Webster mice; Nicotine-induced anxiolysis		↔	
	128 and 256 pmol/0.2 microl/side (injection in AcbSh)	Wistar rat		↓[b]	Lopes et al. (2007)
	32, 64 ng/side (injection in AcbSh)	Wistar rat		↓[b]	Lopes et al. (2012)
	0.5, 1.5, and 2.5 mg/kg (acute)	C57BL/6J mice		↔	Li et al. (2013)
	2 mg/kg (PND 14–28)	BALB/cOlaHsd mice		↑	Sweeney et al. (2014)
	2.5, 5.1 mg/kg (acute)	Hooded Lister rat; DW-induced anxiety	SI	↓	File et al. (1991)
	1.25, 2, 5 mg/kg (acute)	Hooded Lister rat; DW-induced anxiety		↓	File et al. (1992)
	Microinjection in BLA	Wistar rat		↔	Sanders and Shekhar (1995)
	1.25, 2.5, 5 mg/kg	Sprague-Dawley rats; repeated stress and ethanol withdrawal		↓	Knapp et al. (2007)
	0.5, 1.5, 2.5 mg/kg (acute)	C57BL/6J mice	LDB	↔	Li et al. (2013)
	0.5, 1.5, 2.5 mg/kg(acute)	C57BL/6J mice	FC	↔	Li et al. (2013)
	2 mg/kg (PND 14-28)	BALB/cOlaHsd mice	DMB	↔	Sweeney et al. (2014)

(continued)

Table 12.1 (continued)

Agonists	Dose/length	Subjects	Paradigm	Finding	References
	2 mg/kg (PND 14-28)	BALB/cOlaHsd mice	SIH	↔	Sweeney et al. (2014)
	0.4, 0.9, 1.25 mg/kg (acute)	Wistar rat		Sedative	Li et al. (2015)
	0.4, 0.9, 1.25 mg/kg (acute)	Wistar rat	LES	Sedative	Li et al. (2015)
	0.4, 0.9, 1.25 mg/kg (acute)	Wistar rat	FPS	Sedative	Li et al. (2015)
	0.5, 1.5, and 2.5 mg/kg (acute)	C57BL/6J mice	VCT	↔[c]	Li et al. (2013)
	1.25 and 2.5 mg/kg (acute)	Wistar rat		↔	Agmo et al. (1991)
	0.46, 1 mg/kg (acute)	Wistar rat		↓	Ketelaars et al. (1988)
	0.25 mg/kg (acute)	Wistar rat	EZM	↔	Frankowska et al. (2007)
SKF97541	0.01, 0.05 mg/kg (acute)	Wistar rat	EZM	↔	Frankowska et al. (2007)
CGP44532	0.125 mg/kg (acute)	Swiss mice	Four-plate test	↑	Partyka et al. (2007)

PND postnatal day, *icv* intracerebroventricular injection, *AcbSh* nucleus accumbens shell, *BLA* basolateral amygdala, *DMB* defensive marbles burying, *DW* drug withdrawal, *EPM* elevated plus maze, *EZM* elevated zero maze, *FC* fear conditioning, *FPS* fear-potentiated startle, *LES* light-enhanced startle, *LDB* light–dark box, *SI* social interaction, *SIH* stress-induced hyperthermia, ↑= increased anxiety-like behaviour, *VCT* vogel conflict test, ↓= decreased anxiety-like behaviour, ↔= no effects

[a]Baclofen (3.0 mg/kg) increased time spent in the centre of EPM and open/closed arm entries but decreased total arm entries

[b]In the EPM were decreased the number of risk assessment behavior but not number of entries or time spent in open/closed arms

[c]The increase of number of licks in the Vogel conflict test may be due to analgesic effect of baclofen

Table 12.2 Effects of $GABA_B$ receptor agonists on depression-related behaviours

Agonists	Dose (mg/kg)/length	Subjects	Paradigm	Finding	References
Baclofen	0.5, 1 (acute)	Wistar Albino mice	FST	↓	Aley and Kulkarni (1989)
	0.5, 1 (acute)	Wistar Albino mice		↓	Aley and Kulkarni (1990)
	10 (acute)	CD-COBS rat		↔	Borsini et al. (1986)
	2, 4 (acute)	Wistar rat treated with antidepressants		↑	Nakagawa et al. (1996c)
	0.25 (acute)	Wistar rat (females)		↓	Car and Wisniewska (2006)
	0.125 (acute)	Wistar rat, discontinuation of cocaine self-administration		↓	Frankowska et al. (2010)
	0.25 (acute)	Wistar rat		↓	Frankowska et al. (2007)
	0.01, 0.05 (acute)	NMRI mice		↓	Khan et al. (2016)
SKF97541	0.01, 0.05 (acute)	Wistar rat		↓	Frankowska et al. (2007)
Baclofen	4 (14 days)	Wistar rat; LH	LH	↑	Nakagawa et al. (1996b)
	1.2 (14 days)	Wistar rat; LH, following treatment with desipramine		↑	Nakagawa et al. (1996a)

FST forced swim test, *LH* learned helplessness, ↑= increased depression-related behaviours, ↓= decreased depression-related behaviours, ↔= no effects

12.4.1 *Effects of $GABA_B$ Receptor Agonists on Anxiety-Like Behaviours*

The effects of $GABA_B$ receptor agonists on anxiety-like behaviours are summarized in Table 12.1. In rats, acute systemic treatment with baclofen has been shown to induce anxiolytic-like effects in the elevated plus maze (EPM) in a model of drug withdrawal (File et al. 1991, 1992). Curiously however intracerebroventricular (icv) administration of baclofen did not induce anxiolytic effects in the rat EPM (Zarrindast et al. 2001) but baclofen was effective at reducing anxiety in this test when it was injected into the shell of the nucleus accumbens (AbcSh) (Lopes et al. 2007, 2012). However, in the latter two studies baclofen reduced the number of risk assessment behaviour but not number of entries or time spent in open/close arms (Lopes et al. 2007, 2012). Accordingly, baclofen has been shown to increase social interaction in a rat model of drug-withdrawal, ethanol-withdrawal or repeated stress combined with ethanol-withdrawal, thus further supporting an anxiolytic profile (File et al. 1991, 1992; Knapp et al. 2007). In contrast however, one study reported that administration of baclofen into the rat basolateral amygdala (BLA) did not affect social interaction (Sanders and Shekhar 1995). Similarly, there are contradictory findings on the effects of baclofen in the Vogel conflict test in rats. While one study reported that baclofen increased the number of punishments accepted by rats suggesting anxiolytic-like effects (Ketelaars et al. 1988), another study reported no such effect (Agmo et al. 1991). Importantly, the latter study also demonstrated that higher doses of baclofen induced motor deficits and reduced the number of licks in rats (Agmo et al. 1991). The authors suggest that the reduced number of licks in rats may be due to motor deficiency (Agmo et al. 1991). Similarly, Li and colleagues have reported sedative effects of baclofen in several rat behavioural tests including stress-induced hyperthermia (SIH), light-enhanced startle (LES) and fear-potentiated startle (FPS) test (Li et al. 2015). Motor impairing and hypothermic effects are characteristic side effects of $GABA_B$ receptor agonists and this could confound the interpretation of anxiety-related behavioural tests (such as EPM, Vogel conflict-motor impairing or SIH-hypothermia) (Cryan et al. 2004).

Although several (but not all) studies suggest that baclofen can exert anxiolytic-like effects in rats, this does not seem to be the case in mice (Dalvi and Rodgers 1996; Varani and Balerio 2012; Li et al. 2013). Accordingly, baclofen was not effective in several tests of anxiety-related behaviour in mice including the EPM, fear conditioning and light dark box (Dalvi and Rodgers 1996; Varani and Balerio 2012; Li et al. 2013). In one study, baclofen increased the number of licks in the Vogel conflict test which would be indicative of an anxiolytic effect, but the authors suggest that this finding may be due to analgesic effects of baclofen (Li et al. 2013). Moreover, another study reported that the $GABA_B$ receptor agonist CGP44532 actually induced anxiogenic-like effects in the four-plate test (Partyka et al. 2007) although a higher dose of this drug was inactive in this test. Intriguingly, chronic treatment of mice with R-baclofen during early postnatal life (postnatal day (PND) 14–28) induced anxiety-like behaviour in adulthood as assessed by the EPM test

(Sweeney et al. 2014). However, no effects were observed in other behavioural tests of anxiety including SIH and marble burying tests. These findings suggest that during early life $GABA_B$ receptor signalling might play a functional role in programming anxiety behaviour in adulthood (Sweeney et al. 2014), although these effects might also be test-specific.

In summary, many studies in rats using the EPM and social interaction tests suggest that baclofen can have anxiolytic effects although negative findings have also been reported particularly with other behavioural tests. However, most of the studies in mice suggest that baclofen does not affect anxiety behaviour in adulthood. Nevertheless, a recent study suggests that treatment with baclofen during early postnatal life can influence anxiety behaviour in mice when they reach adulthood. Taken together, the effects of $GABA_B$ receptor agonists on anxiety-like behaviours remain unclear but may be species- and test-specific.

12.4.2 Effects of $GABA_B$ Receptor Agonists on Depression-Like Behaviours

Several studies have also investigated the potential antidepressant-like effects of $GABA_B$ receptor agonists in rodents. Overall, preclinical findings suggest that $GABA_B$ receptor agonists display antidepressant-like activity in the forced swim test (FST) (Aley and Kulkarni 1989, 1990; Car and Wisniewska 2006; Frankowska et al. 2007; Khan et al. 2016). Baclofen induced antidepressant-like behaviour in the FST in both mice and rats (Aley and Kulkarni 1989, 1990; Car and Wisniewska 2006; Frankowska et al. 2007; Khan et al. 2016). Similarly, the $GABA_B$ receptor agonist SKF97541 was also active in the rat FST (Frankowska et al. 2007). However, one study reported that baclofen did not exert antidepressant-like activity in Charles River Sprague-Dawley-Derived (CD-COBS) rats when tested in the FST (Borsini et al. 1986). Interestingly, baclofen treatment reduced time spent immobile in the FST in rats following discontinuation of cocaine self-administration, further suggesting that the antidepressant effects of baclofen is not limited to naïve rodents (Frankowska et al. 2010). Moreover, baclofen has been shown to induce antidepressant-like effects in female rats when tested in the FST (Car and Wisniewska 2006). This is an interesting finding in light of the fact that women are twice more likely to develop depression than men (Weissman and Klerman 1977) and yet there is a paucity of preclinical studies investigating putative antidepressant compounds in both females and males.

In contrast to the antidepressant-like effects of acute baclofen treatment in the FST, some studies have reported that baclofen may induce depression-related behaviours. Specifically, chronic treatment with baclofen (14 days) exacerbated helplessness in rats (Nakagawa et al. 1996b). Moreover, baclofen attenuated the behavioural effects of the antidepressants desipramine, mianserin and buspirone in the rat FST, and that of desipramine in the learned helplessness rat model (Nakagawa

et al. 1996a, c) thus suggesting that baclofen may negatively modulate antidepressant-like action.

Taken together, most preclinical findings report that acute treatment with baclofen induces antidepressant-like effects in the FST. However, baclofen also attenuates the behavioural effects of antidepressant drugs in naïve rats and in the learned helplessness rat model. Moreover, chronic administration of baclofen may exacerbate helplessness in rats. Recent structure–activity studies have led to the discovery of a novel $GABA_B$ receptor agonist, namely 3-(4-pyridyl)methyl ether derivative 9d that has 25- to 50-fold greater functional potency than R-baclofen at human and rodent $GABA_B$ receptors in vitro (Xu et al. 2011). Studies investigating the effects of this $GABA_B$ receptor agonist on anxiety/depression-related behaviours are largely awaited as the increased potency of this $GABA_B$ receptor agonist might induce greater effects.

12.5 The Effects of $GABA_B$ Receptor-Positive Allosteric Modulators on Anxiety and Depression-Like Behaviours

$GABA_B$ receptor agonists such as baclofen can induce several unwanted effects such as sedation or somnolence, excessive weakness, hypothermia and vertigo (Agabio et al. 2013). Furthermore, repeated administration of GPCR agonists can induce receptor tolerance which will lead to a decline in the response to treatment (Lehmann et al. 2003). Positive allosteric modulators of $GABA_B$ receptors may be clinically important in this regard because such compounds could potentially have fewer side effects and reduced risk for receptor densitization/tolerance when compared with classical $GABA_B$ receptor agonists (Gjoni and Urwyler 2008, 2009). This potential improved side effect profile is linked to the fact that allosteric modulators act only on endogenously activated receptors and not on the total receptor population (Pin et al. 2001; Urwyler et al. 2003). Recently, several positive allosteric modulators of $GABA_B$ receptors have been developed including GS39783 (Urwyler et al. 2003), CGP7930 (Urwyler et al. 2001), rac-BHFF (Malherbe et al. 2008) and BHF177 (compound #27 in (Guery et al. 2007) (see also Chaps. 3 and 18 of this book)). Importantly, such positive allosteric modulators do not exhibit the motor-impairing and hypothermic effects characteristic of $GABA_B$ receptor agonists (Cryan et al. 2004). The effects of these positive allosteric modulators in anxiety and depression-like behaviour are summarized in Table 12.3 and are described below.

The effects of $GABA_B$ receptor allosteric modulators on anxiety-related behaviour have been investigated in many studies, but their effects on depression-like behaviours are relatively unexplored. Overall, preclinical studies suggest that positive allosteric modulators induce anxiolytic-like effects in rat and mice in several behavioural tests. However, as observed for $GABA_B$ receptor agonists, the effects

Table 12.3 Effects of $GABA_B$ receptor allosteric-positive modulators on anxiety/depression-related behaviours

Anxiety-like behavioural tests					
PAM	Dose (mg/kg)/length	Subjects	Paradigm	Finding	References
GS39783	0.1, 1, 10, 100 (acute)	Sprague-Dawley rats	EPM	↓	Cryan et al. (2004)
CGP7930	3, 300 (acute)	OF1/IC mice		↔	Jacobson and Cryan (2008)
	20, 40 (acute)	Wistar rats		↔	Mares et al. (2013)
BHF177	10, 20, 40 (acute)	C57BL/6J		↔	Li et al. (2013)
GS39783	10, 30 (acute)	OF1/IC mice	EZM	↔	Cryan et al. (2004)
	3, 10, 30 (acute)	Sprague-Dawley rats		↓	Cryan et al. (2004)
	10 (acute)	BALB/c		↓	Mombereau et al. (2004)
CGP7930	1, 3 (acute)	Wistar rat		↓	Frankowska et al. (2007)
	3, 300 (acute)	OF1/IC mice		↓	Jacobson and Cryan (2008)
GS39783	0.1, 1, 10, 100 (acute)	Sprague-Dawley rats	SIH	↓	Cryan et al. (2004)
CGP7930	100 (acute)	OF1/IC mice		↓	Jacobson and Cryan (2008)
rac-BHFF	100 (acute)	NMRI mice		↓	Malherbe et al. (2008)
BHF177	10, 20, 40 (acute)	Wistar rat		↓	Li et al. (2015)
	10, 20, 40 (acute)	Wistar rat	VCT	↔	Li et al. (2013)
	10, 20, 40 (acute)	C57BL/6J	FC	↔	Li et al. (2013)
GS39783	10, 30 (acute)	BALB/cOlaHsd		↔	Sweeney et al. (2013)
	0.3, 30 (acute)	BALB/c	LDB	↓	Mombereau et al. (2004)
	10 (chronic)	BALB/c		↓	Mombereau et al. (2004)
BHF177	10, 20, 40	C57BL/6J		↔	Li et al. (2013)
CGP7930	100, 300 (acute)	OF1/IC mice	Staircase	↓	Jacobson and Cryan (2008)
BHF177	10, 20, 40 (acute)	Wistar rat	LES	↔	Li et al. (2015)
	10, 20, 40 (acute)	Wistar rat	FPS	↔	Li et al. (2015)

(continued)

Table 12.3 (continued)

Depression-like behavioural test					
PAM	Dose (mg/kg)/length	Strain	Paradigm	Finding	References
GS39783	10 (chronic)	BALB/c	FST	↔	Mombereau et al. (2004)
	10,20,40 (acute)	Sprague-Dawley rats		↔	Slattery et al. (2005a)
CGP7930	1, 3 (acute)	Wistar rat		↓	Frankowska et al. (2007)

EPM elevated plus maze, *EZM* elevated zero maze, *FC* fear conditioning, *FPS* fear-potentiated startle, *FST* forced swim test, *LDB* light–dark box test, *LES* light-enhanced startle, *SIH* stress-induced hyperthermia, *VCT* Vogel conflict test, ↑= increased anxiety/depression-related behaviours, ↓= decreased anxiety/depression-related behaviours, ↔= no effects

of positive allosteric modulators on anxiety-like behaviour can be test-specific whereby positive allosteric modulators seem to have anxiolytic effects in tests of innate but not conditioned anxiety. In the SIH test (a physiological test of anxiety) several positive allosteric modulators including GS39783, CGP7930, rac-BHFF and BHF177 induced anxiolytic-like effects in both mice and rats (Cryan et al. 2004; Jacobson and Cryan 2008; Malherbe et al. 2008; Li et al. 2015). Similarly, several positive allosteric modulators including CGP7930 and GS39783 induced anxiolytic-like effects in both rats and mice in the elevated zero maze (Cryan et al. 2004; Mombereau et al. 2004; Frankowska et al. 2007; Jacobson and Cryan 2008). In the EPM, GS39783 also induced anxiolytic-like effects in rats (Cryan et al. 2004) while CGP7930 and BHF177 were inactive in both mice and rats (Jacobson and Cryan 2008; Li et al. 2013; Mares et al. 2013). Similarly, GS39783 was also effective in the light dark box in mice while BHF177 was without effect (Mombereau et al. 2004; Li et al. 2013). In the staircase test in mice, CGP7930 induced anxiolytic-like effects (Jacobson and Cryan 2008) while BHF177 induced anxiolytic-like effects on LES in high, but not low, LES responding rats (Li et al. 2015). Taken together, it is clear that positive allosteric modulators of the $GABA_B$ receptor have anxiolytic effects in tests of innate anxiety. In tests of conditioned anxiety however, these positive allosteric modulators do not seem to be effective. For examples, BHF177 did not affect conditioned fear responses in the FPS test in rats (Li et al. 2015) and was not effective 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, positive allosteric modulators of the $GABA_B$ receptor are effective in tests of innate anxiety but not in conditioned anxiety.

As mentioned above, few studies have assessed the effects of these positive allosteric modulators on depression-like and antidepressant-like behaviours. Interestingly, acute treatment with CGP7930 but not GS39783 induced antidepressant-like effects in the rat FST (Slattery et al. 2005b; Frankowska et al. 2007). Moreover, a recent study showed that acute treatment with the positive allosteric modulator CGP 7930 counteracted the cocaine discontinuation-induced enhancement of immobility in the rat FST (Frankowska et al. 2010). Future studies should evaluate the effects of $GABA_B$ receptor positive modulators in animal models of depression such as chronic mild stress (Willner 2005), chronic corticosterone treatment (David et al. 2009), chronic social defeat stress (Fano et al. 2001; Berton et al. 2006), and learned helplessness (Maier and Watkins 2005).

In summary, $GABA_B$ receptor-positive allosteric modulators have been shown to modulate anxiety and depression-related behaviours. Specifically, preclinical evidence suggests that $GABA_B$ receptor allosteric modulation induces anxiolytic and antidepressant-like effects in rodents. However, the anxiolytic effects of $GABA_B$ receptor-positive allosteric modulators may be test-specific occurring in behavioural tests of innate rather than conditioned anxiety. Importantly, $GABA_B$ receptor-positive allosteric modulators exhibit receptor orthologue selectivity as well as downstream intracellular signal transduction specificity in cell lines expressing rat or human $GABA_B$ receptors (Li et al. 2015). This may hamper the development of

$GABA_B$ receptor-positive allosteric modulators for medical use, since positive allosteric modulators may induce different effects between rodent species, and between humans and rodents.

12.6 The Effects of $GABA_B$ Receptor Antagonists in Anxiety and Depression-Like Behaviours

In the 1980s, several $GABA_B$ receptor antagonists (phaclofen, saclofen, 2-hydroxysaclofen, CGP35348, CGP36742, and SCH50911) were synthesized (see Chap. 2 of this book) and these drugs have allowed further characterization of $GABA_B$ receptor function (Kerr et al. 1987; Bolser et al. 1995; Enna 2001). Although these first compounds exhibited good selectivity for $GABA_B$ receptors, their pharmaceutical use was limited by their low affinity (Kerr et al. 1987; Bolser et al. 1995) and poor availability in the brain after peripheral administration (Kerr and Ong 1992). In the last few years, the introduction of new high-affinity receptor antagonists, such as CGP56433A, CGP55845A, CGP44532, CGP 36742, and CGP 51176, has been a significant advancement in $GABA_B$ receptor pharmacology (see Chap. 2 of this book). These compounds resulted from the addition of a 3,4-dichlorobenzyl or a 3-carboxybenzyl substituent onto the phosphinic acid group of the pharmacophore (Froestl and Mickel 1997). These new generation $GABA_B$ receptor antagonists are more potent and selective and have been widely used in research to investigate the role of the $GABA_B$ receptor in depression and anxiety-related behaviours as summarized in Tables 12.4 and 12.5 and as described below.

Most of the published studies have focused on the effects of $GABA_B$ receptor antagonists on depression-like behaviours rather than anxiety-like behaviours. The majority of studies suggest that $GABA_B$ receptor antagonists exert antidepressant-like effects in rodents. Specifically, the $GABA_B$ receptor antagonists CGP56433A, CGP51176, CGP5633A, CGP36742 and SCH50911 have all been shown to induce antidepressant-like behaviour in the FST in rodents (Mombereau et al. 2004; Slattery et al. 2005a; Nowak et al. 2006; Frankowska et al. 2007; Felice et al. 2012). The $GABA_B$ receptor antagonist SCH 50911 (0.3 mg/kg) counteracted the cocaine discontinuation-induced enhancement of immobility in the rat FST (Frankowska et al. 2010). Importantly, the $GABA_B$ receptor agonist CGP 44532 has been shown to offset the antidepressant-like effects of CGP 51176 in the rat FST (Nowak et al. 2006) thus showing that the effects observed were specifically due to inhibition of $GABA_B$ receptor. $GABA_B$ receptor antagonists are also effective in other rodent models of depression and antidepressant activity including the olfactory bulbectomy model (CGP 36742 and CGP 51176) (Nowak et al. 2006), chronic mild stress (CGP 51176) (Nowak et al. 2006) and learned helplessness model (CGP36742) (Nakagawa et al. 1999).

Given the role of GABAergic dysfunction in the pathophysiology of anxiety and that $GABA_B$ receptor modulation by agonists and positive allosteric modulators

Table 12.4 Effects of $GABA_B$ receptor antagonists on depression-related behaviours

Antagonists	Dose (mg/kg)/length	Subjects	Paradigm	Finding	References
CGP56433A	3 (chronic)	BALB/c	FST	↓	Mombereau et al. (2004)
	10, 30 (acute)	BALB/c		↓	Mombereau et al. (2004)
	10 (acute)	Sprague-Dawley rats		↓	Slattery et al. (2005a)
CGP44532	0.12, 0.250 (acute)	Albino Swiss mice		↔	Nowak et al. (2006)
CGP 36742	10, 30 (acute)	Albino Swiss mice		↓	Nowak et al. (2006)
CGP 51176	8, 10, 12 (acute)	Albino Swiss mice		↓	Nowak et al. (2006)
CGP55845A	3–10 (acute)	Sprague-Dawley rats		↔	Slattery et al. (2005a)
SCH 50911	1, 3 (acute)	Wistar rat		↓	Frankowska et al. (2007)
CGP 52432	3, 10 (acute and chronic)	BALB/cOlaHsd mice		↓	Felice et al. (2012)
CGP 52432	10, 30 (PND 14–28)	BALB/cOlaHsd mice		↔	Sweeney et al. (2014)
CGP56433A	10, 30 (acute)	BALB/c	TST	↔	Mombereau et al. (2004)
CGP51776	3, 30 (chronic)	Wistar rat; CMS	Sucrose consumption	↓	Nowak et al. (2006)
	30, 100 (14 days)	Wistar rat	LH	↓	Nakagawa et al. (1999)

PND postnatal day, *CMS* chronic mild stress, *FST* forced swim test, *LH* learned helplessness, *TST* tail suspension test, ↑= increased depression-related behaviour, ↓= decreased depression-related behaviour, ↔= no effects

Table 12.5 Effects of $GABA_B$ receptor antagonists on anxiety-related behaviours

Antagonists	Dose (mg/kg)/length	Subjects	Paradigm	Finding	References
CGP35348	25, 200 (acute)	DBA/2 mice	EPM	↔	Dalvi and Rodgers (1996)
	5, 10, 30	Wistar rat		↓	Zarrindast et al. (2001)
CGP 36742	30 (acute)	Wistar rat		↓	Partyka et al. (2007)
CGP 52432	10, 30 (PND 14–28)	BALB/cOlaHsd mice		↔	Sweeney et al. (2014)
2OH-Saclofen	1 (acute)	Swiss Webster mice-Nicotine-induced anxiety		↓	Varani and Balerio (2012)
	1 (acute)	Swiss Webster mice; Nicotine-induced anxiolytic effects		↓	
	1,5, 3 μg injection in AcbSh	Wistar rats		↔	Lopes et al. (2012)
SCH 50911	1, 3 (acute)	Wistar rat	EZM	↓	Frankowska et al. (2007)
CGP56433A	3 (chronic)	BALB/c	LDB	↔	Mombereau et al. (2004)
CGP 52432	10, 30 (PND 14–28)	BALB/cOlaHsd mice		↔	Sweeney et al. (2014)
	10, 30 (PND 14–28)	BALB/cOlaHsd mice	DMB	↔	Sweeney et al. (2014)
CGP44532	0.065, 0.125, 0.25 (acute)	Wistar rat	LES	Sedative	Li et al. (2015)
	0.065, 0.125, 0.25 (acute)	Wistar rat	FPS	Sedative	Li et al. (2015)
	0.065, 0.125, 0.25 (acute)	Wistar rat	SIH	Sedative	Li et al. (2015)
CGP 52432	10, 30 (PND 14–28)	BALB/cOlaHsd mice		↔	Sweeney et al. (2014)
CGP51776	3 (14 days)	Wistar rat; OB	Passive avoidance	↓	Nowak et al. (2006)
CGP 36742	10 (14 days)	Wistar rat; OB		↓	Nowak et al. (2006)
	30 (acute)	Wistar rat	VCT	↓	Partyka et al. (2007)
	30 (acute)	Swiss mice	Four-plate test	↓	Partyka et al. (2007)
CGP51776	5, 8 (acute)	Swiss mice		↔	Partyka et al. (2007)
Saclofen	Micorinjection BLA	Wistar rats	SI	↔	Sanders and Shekhar (1995)

PND postnatal day, *OB* olfactory bulbectomy, *AcbSh* nucleus accumbens shell, *BLA* basolateral amygdala, *EPM* elevated plus maze, *EZM* elevated zero maze, *DMB* defensive marble burying, *FPS* fear-potentiated startle, *LES* light-enhanced startle, *SIH* stress-induced hyperthermia, *VCT* Vogel conflict test, ↑= increased anxiety-related behaviour, ↓= decreased anxiety-related, ↔= no effects

affects anxiety-like behaviours in rodents, some studies have assessed the effects of $GABA_B$ receptor antagonists on anxiety-related behaviours. In contrast to the clear antidepressant-like effects of $GABA_B$ receptor antagonists, the effects of these drugs on anxiety are less clear. Indeed, in some studies $GABA_B$ receptor antagonists induced anxiolytic-like effects in rodents (Zarrindast et al. 2001; Frankowska et al. 2007; Partyka et al. 2007), whereas in other studies they did not alter anxiety-like behaviours (Dalvi and Rodgers 1996; Mombereau et al. 2004). Overall, the findings suggest that $GABA_B$ receptor antagonists induce anxiolytic-like effects in rats (Zarrindast et al. 2001; Nowak et al. 2006; Partyka et al. 2007) but not in mice (Dalvi and Rodgers 1996; Mombereau et al. 2004; Partyka et al. 2007; Sweeney et al. 2013). However, $GABA_B$ receptor antagonists did not induce anxiolytic-like effects when locally administered into the basolateral amygdala or the shell of the nucleus accumbens of rats (Sanders and Shekhar 1995; Lopes et al. 2012). In addition, the $GABA_B$ receptor antagonist CGP44532 induced sedative effects in the SIH, LES and FPS in rats (Li et al. 2015). Sedative effects may be a confounding factor for the effects of $GABA_B$ receptor antagonists on anxiety-like behaviours since the observed results may be due to motor deficits. On the other hand, the $GABA_B$ receptor antagonist CGP36742 induced 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). Taken together, $GABA_B$ receptor antagonists consistently induce antidepressant-like behavioural effects in rodents, but the effects on anxiety are less clear. Moreover, the development of negative allosteric modulators will be important since such compounds may be devoid of the side effects associated with antagonists including sedation, nausea, dizziness, motor impairment, drowsiness and mental confusions (Ong and Kerr 2005). In 2014, the first negative allosteric modulators were synthetized by Chen and colleagues and designed from the positive allosteric modulator CGP7930 (Chen et al. 2014). The $GABA_B$ receptor NAM CLH304a (also named Compound 14) has been shown to bind at a different site to GABA and act on the heptahelical domain of $GABA_{B2}$ subunits (Sun et al. 2016). This finding suggests that there may be novel therapeutic approaches to inhibit $GABA_B$ receptor activity.

12.7 $GABA_B$ Receptor Genetically Modified Mice

Genetically modified mice are powerful tools to investigate genes which have been sequenced but whose function is not yet known. To elucidate the role of specific $GABA_B$ receptor subunits, mice with a loss of function of the $GABA_{B(1)}$ or $GABA_{B(2)}$ receptor subunits have been developed and characterized in terms of anxiety and depression-like behaviour (Bettler et al. 1998; Bowery and Enna 2000; Couve et al. 2000; Mombereau et al. 2004, 2005; Jacobson et al. 2007a; Cryan and Slattery 2010).

$GABA_{B(1)}$ receptor knockout mice do not exhibit pre- and postsynaptic biochemical and electrophysiological $GABA_B$ receptor responses (Prosser et al. 2001; Schuler et al. 2001), thus suggesting that the $GABA_{B(1)}$ receptor subunit is an essential component of a functional $GABA_B$ receptor. In addition, $GABA_{B(1)}$ receptor knockout mice are also characterized by retarded growth and premature death (Prosser et al. 2001). The development of mice with altered $GABA_B$ receptor function has confirmed a clear role for this metabotropic receptor in anxiety. Mice lacking either the $GABA_{B(1)}$ or $GABA_{B(2)}$ receptor subunits exhibit an anxious phenotype (Mombereau et al. 2004, 2005). Specifically, $GABA_{B(1)}$-deficient mice exhibit increased anxiety in the light–dark box and staircase test as well as in many other tests of anxiety behaviour (Mombereau et al. 2004). Mutant mice lacking either the $GABA_{B(1)}$ or the $GABA_{B(2)}$ receptor subunit exhibit antidepressant-like behaviour in the FST (Mombereau et al. 2004, 2005). However, $GABA_{B(1)}$ or $GABA_{B(2)}$ receptor subunit knockout mice display spontaneous seizures, hyperalgesia and hyperlocomotor activity (Prosser et al. 2001; Schuler et al. 2001). $GABA_{B(1)}$ or $GABA_{B(2)}$ receptor subunit knockout mice also display severe memory impairment (Prosser et al. 2001; Schuler et al. 2001; Heaney and Kinney 2016).

Recently, mice differentially expressing one of the two $GABA_{B(1)}$ receptor subunit isoforms have been generated ($GABA_{B(1a)}^{-/-}$ or $GABA_{B(1b)}^{-/-}$ mice) (Vigot et al. 2006; Gassmann and Bettler 2012). Using these mice, it is now possible to investigate the molecular and behavioural functions of these two isoforms in vivo. These mice have revealed that $GABA_{B(1)}$ receptor subunit isoforms display differential subcellular distributions. The $GABA_{B(1a)}$ receptor subunit isoform is expressed predominantly in dendritic spines opposing glutamate release sites (Vigot et al. 2006) while the $GABA_{B(1b)}$ receptor subunit isoform is principally localized at glutamatergic terminals (Vigot et al. 2006). The two isoforms are also differentially expressed during brain development. The $GABA_{B(1a)}$ receptor subunit isoform is expressed mainly during development while the $GABA_{B(1b)}$ receptor subunit isoform is the principal isoform of the adult brain (Cryan and Kaupmann 2005). Moreover, the two $GABA_{B(1)}$ receptor subunit isoforms exhibit differences in some behavioural paradigms and are involved in different neurophysiological processes. $GABA_{B(1b)}^{-/-}$ mice are hyperactive in a novel environment when compared with $GABA_{B(1a)}^{-/-}$ mice (Jacobson et al. 2006). Both $GABA_{B(1a)}^{-/-}$ and $GABA_{B(1b)}^{-/-}$ mice exhibit cognitive impairments (Jacobson et al. 2006). $GABA_{B(1a)}^{-/-}$ mice are unable to acquire conditioned taste aversive (CTA), whereas $GABA_{B(1b)}^{-/-}$ mice are able to acquire CTA but cannot extinguish aversive taste memories (Jacobson et al. 2006). Interestingly, overexpression of $GABA_{B(1a)}$ and $GABA_{B(1b)}$ receptor subunit in mice leads to cognitive impairment (Wu et al. 2007; Stewart et al. 2009). $GABA_{B(1a)}^{-/-}$ and $GABA_{B(1b)}^{-/-}$ mice exhibit a panic-like response in the elevated zero maze (Mombereau et al. 2004) as well as alterations in conditioning aversive learning tasks (Shaban et al. 2006). In addition, these mice also display antidepressant-like behaviour (O'Leary et al. 2014). $GABA_{B(1b)}^{-/-}$ mice are more resilient to the anhedonic-like effects of early-life stress and to stress-induced social stress, while those lacking the $GABA_{B(1a)}$ receptor isoform are more sensitive than the $GABA_{B(1b)}^{-/-}$

mice (O'Leary et al. 2014). Intriguingly, the helpless H/Rouen mice, a mouse model of depression, display increased hippocampal $GABA_{B(1b)}$ mRNA expression compared with nonhelpless counterparts (O'Leary et al. 2014), suggesting that increased hippocampal $GABA_{B(1b)}$ mRNA expression is associated with a depression-like phenotype. On the other hand, as indicated with the $GABA_{B(1b)}^{-/-}$ mice, reducing $GABA_{B(1b)}$ expression has antidepressant-like effects. Interestingly, another study has reported that treatment with antidepressants selectively increased the expression of the $GABA_{B(1a)}$ receptor subunit in the hippocampus, but had no consistent effects on the $GABA_{B(1b)}$ or $GABA_{(B2)}$ expression (Sands et al. 2004).

Overall, blockade or loss of function of the $GABA_B$ receptor induces antidepressant-like effects. By contrast, the effects of pharmacological and genetic studies on the effects of the $GABA_B$ receptor on anxiety-like behaviour are not so clear. Indeed, deletion of $GABA_B$ receptors induces anxiety-like behaviour, whereas treatment with $GABA_B$ receptor antagonists induces anxiolytic-like effects or no changes. Taken together, the preclinical findings from $GABA_B$ receptor genetically modified mice have confirmed a key role of $GABA_B$ receptors in anxiety/depression-related disorders (Fig. 12.1b).

12.8 Is the $GABA_B$ Receptor a Potential Therapeutic Target for Psychiatric Disorders?

Both preclinical and clinical studies support the hypothesis that the $GABA_B$ receptor is a potential target for the development of new drugs for the treatment of depression and anxiety disorders. However, so far the only $GABA_B$ receptor drug approved for clinical use is baclofen, a $GABA_B$ receptor agonist, used for the treatment of severe spasticity due to multiple sclerosis or spinal cord injury or disease (see Chap. 17 of this book). SGS742 (CGP36742) is the only $GABA_B$ receptor antagonist that progressed to Phase II of clinical trials as potential treatment for cognitive deficits (Froestl et al. 2004; Ghose et al. 2011). This Phase II clinical trial was conducted in 110 patients with mild cognitive impairment and SGS742 was well tolerated by the patients and induced a significant improvement in cognitive tasks (Froestl et al. 2004). However, a larger follow-up trial with 271 patients with mild to moderate Alzheimer disease (Sabbagh 2009) reported that SGS742 was only effective in patients with mild Alzheimer disease (Sabbagh 2009; Froestl 2010). Nevertheless, SGS742 will shortly progress to clinical trials investigating its utility as a treatment for succinic semialdehyde dehydrogenase (SSADH)-deficient patients (Vogel et al. 2012). SSADH is a rare recessive autosomal disorder of GABA metabolism. Core symptoms include developmental delay, hypotonia, expressive language deficit and mental retardation (Pearl et al. 2009). Taken together, there is clinical evidence that both a $GABA_B$ receptor agonist and antagonist are safe and well-tolerated in humans and thus the $GABA_B$ receptor remains to be a clinically relevant target for new drug development.

The development of drugs targeting the $GABA_B$ receptor has been mainly hampered by its potential side effects. Indeed, the $GABA_B$ receptor is widely distributed both in the CNS and in the periphery and its dysregulation is not only involved in depression and anxiety disorders but also in the modulation of spasticity, pain, food intake, cognition, drug addiction and epilepsy (Bowery 2006). Potential side effects of the modulation of the $GABA_B$ receptor include hypotension, seizures, hallucinations, nausea, muscle weakness, drowsiness, dizziness and mental confusion (Bowery 2006). In addition, the $GABA_B$ receptor plays dual role on the modulation of anxiety and depression-related behaviours. $GABA_B$ receptor agonists exhibit anxiolytic-like action, whereas $GABA_B$ receptor antagonists induce antidepressant-like effects (Mombereau et al. 2004; Cryan and Slattery 2010). On the other hand, $GABA_{B(1)}$ and $GABA_{B(2)}$ knockout mice display both anxiogenic and antidepressant-like behaviour (Mombereau et al. 2004, 2005). Given the overlap of $GABA_B$ receptor function on the modulation of both anxiety- and depression-related behaviours, a $GABA_B$ receptor-based compound could induce antidepressant-like effects with anxiogenic side effects or vice versa. The discovery that heterodimerization is required for functional $GABA_B$ receptors and that the $GABA_B$ receptor has limited subunits has strongly hampered the discovery of drugs targeting $GABA_B$ receptors. Indeed, orthosteric binding sites for GABA modulators are localized exclusively in the $GABA_{B(1)}$ subunits and there is limited variability among orthosteric binding sites which make unlikely the discovery of pharmacological distinguishable sites. Given those limitations, it is clear why the development of $GABA_B$ receptor-based drugs has been held back.

Nevertheless, preclinical evidence suggests differential roles for two $GABA_{B(1)}$ receptor subunits isoforms. Indeed, $GABA_{B(1a)}$ and $GABA_{B(1b)}$ play a differential role in response to stress (O'Leary et al. 2014) with $GABA_{B(1b)}^{-/-}$ mice being more resilient to stress-induced anhedonia and stress-induced reductions in hippocampal cell proliferation compared with $GABA_{B(1a)}^{-/-}$ mice and wild-type mice (O'Leary et al. 2014). In addition, a recent study in mice reported that epigenetic-mediated increases in $GABA_{B(1a)}$ receptor expression in the dorsal raphe nucleus is associated with isolation-induced abnormal responses to social stimulation (Araki et al. 2016). Thus, targeting specific $GABA_{B(1)}$ subunits could represent a novel therapeutic approach in the modulation of behaviour. However, both $GABA_{B(1a)}$ and $GABA_{B(1b)}$ receptor subunit isoforms are transcribed from the same *Gabbr1* gene and are structurally identical except for a *sushi* motif in the N-terminal domain of the $GABA_{B(1a)}$ receptor subunit. Therefore, the only potential target to discriminate between the two isoforms is represented by the *sushi* motif (Blein et al. 2004). However, it is realistically difficult to develop drugs specifically targeting the $GABA_{B(1a)}$ or $GABA_{B(1b)}$ receptor subunit isoforms given their structural similarity. More feasible strategies to specifically target those isoforms could rely on specific proteins with which these isoforms interact (Marshall 2005; Pinard et al. 2010) such as regulators of G-protein signalling (RGS) and the auxiliary receptor subunit proteins of the KCTD family (named after their K+-channel tetramerization domain) (Dafoe et al. 1985; Ni et al. 1999; Metz et al. 2011; Stratinaki et al. 2013; Kim et al. 2014; Cathomas et al. 2015; Jung et al. 2016).

RGS are proteins that assemble at the C-terminal domain of the $GABA_B$ receptor that could represent new pharmacological targets to differentiate the $GABA_B$ receptor responses (Pinard et al. 2010; Lujan and Ciruela 2012). Among the RGS proteins, RGS4 has received the most attention in the context of stress-related psychiatric disorders. Acute and chronic stress decreases RGS4 expression in the paraventricular nucleus and in the prefrontal cortex in rodents (Ni et al. 1999; Kim et al. 2010, 2014; Jung et al. 2016). Importantly, RGS4 is co-localized with the $GABA_{B(2)}$ receptor subunit in those brain regions in mice (Kim et al. 2014). Mice with local knockdown of RGS4 in the PVN via in vivo electroporation of RGS4 short-hairpin RNA (shRNA) facilitated the recovery of blood coricosterone basal levels in mice (Jung et al. 2016), modulating $GABA_B$ receptor stress responses. Postmortem brain analysis revealed that RGS4 is upregulated in the nucleus accumbens in patients treated with monoaminergic antidepressants (Stratinaki et al. 2013). In parallel, preclinical studies showed that RGS4 is a positive modulator of monoaminergic antidepressant drugs, but acts as a negative modulator of non-monoaminergic drugs such as ketamine (Stratinaki et al. 2013). Specifically, RGS4 knockdown mice exhibit no response to monoaminergic drugs but greater responses to ketamine (Stratinaki et al. 2013). Taken together, RGS4 may represent a valid target for pharmacological intervention for the treatment of depression.

A proteomic analysis revealed the existence of the KCTD protein family which consists of four sequence-related cytosolic proteins (KCTD8, KCTD12, KCTD12b and KCTD16) that bind as tetramers to the C-terminal domain of the $GABA_{B(2)}$ subunit, influencing the pharmacology and kinetics of the receptor response (Pinard et al. 2010). A recent study showed that KCTD proteins display differential temporal and spatial distribution in the mouse brain (Metz et al. 2011). KCTD12 downregulation has been associated with psychiatric disorders including bipolar disorder (Lee et al. 2011; Cathomas et al. 2015). Knockout mice for KTCD12 display increased fear learning of a discrete auditory-conditioned stimulus and altered neuronal excitability (Cathomas et al. 2015). Clinical and preclinical findings suggest that KCTD proteins may be a potential target for the treatment of psychiatric disorders.

Taken together, preclinical evidence suggests that pharmacological or genetic manipulation of the $GABA_B$ receptor regulates anxiety and depression-like behaviours. Thus, the $GABA_B$ receptor may be an important novel therapeutic target. However, challenges remain in developing effective $GABA_B$ receptor-based drugs with limited side-effect profiles. Based on preclinical evidence, it would be desirable to develop drugs targeting specific subunits and subunit isoforms of the $GABA_B$ receptor or specific proteins that interact with the receptor. This will be crucial for the development of novel therapeutic approaches to treat depression and anxiety disorders, drugs for which have largely targeted the serotonin system. Although within the pharmaceutical industry there has been a move away from single target pharmacology towards multimodal drugs (Felice et al. 2015; Millan et al. 2015a, b; O'Leary et al. 2015), there is still much hope for the discovery of an effective $GABA_B$ receptor drug devoid of side effects, and with that hope, the legacy of the great $GABA_B$ receptor chemist Froestl (2010, 2011) who passed away in 2015 lives on.

References

Addolorato, G., Armuzzi, A., Gasbarrini, G., De Lorenzi, G., Ancona, C., Abenavoli, L., et al. (2002). Pharmacological approaches to the management of alcohol addiction. *European Review for Medical and Pharmacological Sciences, 6*, 89–97.

Agabio, R., Preti, A., & Gessa, G. L. (2013). Efficacy and tolerability of baclofen in substance use disorders: A systematic review. *European Addiction Research, 19*, 325–345.

Agmo, A., Pruneda, R., Guzman, M., & Gutierrez, M. (1991). GABAergic drugs and conflict behavior in the rat: Lack of similarities with the actions of benzodiazepines. *Naunyn-Schmiedeberg's Archives of Pharmacology, 344*, 314–322.

Aley, K. O., & Kulkarni, S. K. (1989). GABA-mediated modification of despair behavior in mice. *Naunyn-Schmiedeberg's Archives of Pharmacology, 339*, 306–311.

Aley, K. O., & Kulkarni, S. K. (1990). Effect of baclofen, a GABAB-agonist, on forced swimming-induced immobility in mice. *Archives Internationales de Pharmacodynamie et de Thérapie, 307*, 18–31.

Altar, C. A. (1999). Neurotrophins and depression. *Trends in Pharmacological Sciences, 20*, 59–61.

Altman, J. (1962). Are new neurons formed in the brains of adult mammals? *Science, 135*, 1127–1128.

Altman, J., & Das, G. D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *Journal of Comparative Neurology, 124*, 319–335.

Araki, R., Hiraki, Y., Nishida, S., Kuramoto, N., Matsumoto, K., & Yabe, T. (2016). Epigenetic regulation of dorsal raphe GABA(B1a) associated with isolation-induced abnormal responses to social stimulation in mice. *Neuropharmacology, 101*, 1–12.

Artigas, F. (2015). Developments in the field of antidepressants, where do we go now? *European Neuropsychopharmacology, 25*, 657–670.

Baldwin, D. S., Ajel, K. I., & Garner, M. (2010). Pharmacological treatment of generalized anxiety disorder. *Current Topics in Behavioral Neurosciences, 2*, 453–467.

Bannerman, D. M., Rawlins, J. N., McHugh, S. B., Deacon, R. M., Yee, B. K., Bast, T., et al. (2004). Regional dissociations within the hippocampus—Memory and anxiety. *Neuroscience & Biobehavioral Reviews, 28*, 273–283.

Berton, O., McClung, C. A., Dileone, R. J., Krishnan, V., Renthal, W., Russo, S. J., et al. (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science, 311*, 864–868.

Bettler, B., Kaupmann, K., & Bowery, N. (1998). GABAB receptors: Drugs meet clones. *Current Opinion in Neurobiology, 8*, 345–350.

Bettler, B., Kaupmann, K., Mosbacher, J., & Gassmann, M. (2004). Molecular structure and physiological functions of GABA(B) receptors. *Physiological Reviews, 84*, 835–867.

Binet, V., Brajon, C., Le Corre, L., Acher, F., Pin, J. P., & Prezeau, L. (2004). The heptahelical domain of GABA(B2) is activated directly by CGP7930, a positive allosteric modulator of the GABA(B) receptor. *Journal of Biological Chemistry, 279*, 29085–29091.

Blein, S., Ginham, R., Uhrin, D., Smith, B. O., Soares, D. C., Veltel, S., et al. (2004). Structural analysis of the complement control protein (CCP) modules of GABA(B) receptor 1a: Only one of the two CCP modules is compactly folded. *Journal of Biological Chemistry, 279*, 48292–48306.

Bolser, D. C., Blythin, D. J., Chapman, R. W., Egan, R. W., Hey, J. A., Rizzo, C., et al. (1995). The pharmacology of SCH 50911: A novel, orally-active GABA-beta receptor antagonist. *The Journal of Pharmacology and Experimental Therapeutics, 274*, 1393–1398.

Borsini, F., Evangelista, S., & Meli, A. (1986). Effect of GABAergic drugs in the behavioral 'despair' test in rats. *European Journal of Pharmacology, 121*, 265–268.

Bowery, N. G. (2006). GABAB receptor: A site of therapeutic benefit. *Current Opinion in Pharmacology, 6*, 37–43.

Bowery, N. G., & Enna, S. J. (2000). Gamma-aminobutyric acid(B) receptors: First of the functional metabotropic heterodimers. *The Journal of Pharmacology and Experimental Therapeutics, 292*, 2–7.

Brauner-Osborne, H., & Krogsgaard-Larsen, P. (1999). Functional pharmacology of cloned heterodimeric GABAB receptors expressed in mammalian cells. *British Journal of Pharmacology, 128*, 1370–1374.

Breslow, M. F., Fankhauser, M. P., Potter, R. L., Meredith, K. E., Misiaszek, J., & Hope, D. G., Jr. (1989). Role of gamma-aminobutyric acid in antipanic drug efficacy. *The American Journal of Psychiatry, 146*, 353–356.

Car, H., & Wisniewska, R. J. (2006). Antidepressant-like effects of baclofen and LY367385 in the forced swim test in rats. *Pharmacological Reports, 58*, 758–764.

Castren, E. (2004). Neurotrophic effects of antidepressant drugs. *Current Opinion in Pharmacology, 4*, 58–64.

Cathomas, F., Stegen, M., Sigrist, H., Schmid, L., Seifritz, E., Gassmann, M., et al. (2015). Altered emotionality and neuronal excitability in mice lacking KCTD12, an auxiliary subunit of GABAB receptors associated with mood disorders. *Translational Psychiatry, 5*, e510.

Chen, L. H., Sun, B., Zhang, Y., Xu, T. J., Xia, Z. X., Liu, J. F., et al. (2014). Discovery of a negative allosteric modulator of GABAB receptors. *ACS Medicinal Chemistry Letters, 5*, 742–747.

Cornelisse, L. N., Van der Harst, J. E., Lodder, J. C., Baarendse, P. J., Timmerman, A. J., Mansvelder, H. D., et al. (2007). Reduced 5-HT1A- and GABAB receptor function in dorsal raphe neurons upon chronic fluoxetine treatment of socially stressed rats. *Journal of Neurophysiology, 98*, 196–204.

Couve, A., Moss, S. J., & Pangalos, M. N. (2000). GABAB receptors: A new paradigm in G protein signaling. *Molecular and Cellular Neurosciences, 16*, 296–312.

Cryan, J. F., & Kaupmann, K. (2005). Don't worry 'B' happy!: A role for GABA(B) receptors in anxiety and depression. *Trends in Pharmacological Sciences, 26*, 36–43.

Cryan, J. F., Kelly, P. H., Chaperon, F., Gentsch, C., Mombereau, C., Lingenhoehl, K., et al. (2004). Behavioral characterization of the novel GABAB receptor-positive modulator GS39783 (N, N′-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine): Anxiolytic-like activity without side effects associated with baclofen or benzodiazepines. *The Journal of Pharmacology and Experimental Therapeutics, 310*, 952–963.

Cryan, J. F., & Slattery, D. A. (2010). GABAB receptors and depression. Current status. *Advances in Pharmacology, 58*, 427–451.

Cryan, J. F., & Sweeney, F. F. (2011). The age of anxiety: Role of animal models of anxiolytic action in drug discovery. *British Journal of Pharmacology, 164*, 1129–1161.

Dafoe, D. C., Campbell, D. A., Jr., Marks, W. H., Borgstrom, A., Lloyd, R. V., & Turcotte, J. G. (1985). Association of inclusion of the donor spleen in pancreaticoduodenal transplantation with rejection. *Transplantation, 40*, 579–584.

Dalvi, A., & Rodgers, R. J. (1996). GABAergic influences on plus-maze behaviour in mice. *Psychopharmacology, 128*, 380–397.

David, D. J., Samuels, B. A., Rainer, Q., Wang, J. W., Marsteller, D., Mendez, I., et al. (2009). Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron, 62*, 479–493.

Drake, R. G., Davis, L. L., Cates, M. E., Jewell, M. E., Ambrose, S. M., & Lowe, J. S. (2003). Baclofen treatment for chronic posttraumatic stress disorder. *Annals of Pharmacotherapy, 37*, 1177–1181.

Duman, R. S., Heninger, G. R., & Nestler, E. J. (1997). A molecular and cellular theory of depression. *Archives of General Psychiatry, 54*, 597–606.

Enna, S. J. (2001). A GABA(B) mystery: The search for pharmacologically distinct GABA(B) receptors. *Molecular Interventions, 1*, 208–218.

Enna, S. J., Reisman, S. A., & Stanford, J. A. (2006). CGP 56999A, a GABA(B) receptor antagonist, enhances expression of brain-derived neurotrophic factor and attenuates dopamine depletion

in the rat corpus striatum following a 6-hydroxydopamine lesion of the nigrostriatal pathway. *Neuroscience Letters, 406*, 102–106.

Fano, E., Sanchez-Martin, J. R., Arregi, A., Castro, B., Alonso, A., Brain, P., et al. (2001). Social stress paradigms in male mice: Variations in behavior, stress and immunology. *Physiology & Behavior, 73*, 165–173.

Felice, D., O'Leary, O. F., Cryan, J. F., Dinan, T. G., Gardier, A. M., Sanchez, C., et al. (2015). When ageing meets the blues: Are current antidepressants effective in depressed aged patients? *Neuroscience & Biobehavioral Reviews, 55*, 478–497.

Felice, D., O'Leary, O. F., Pizzo, R. C., & Cryan, J. F. (2012). Blockade of the GABA(B) receptor increases neurogenesis in the ventral but not dorsal adult hippocampus: Relevance to antidepressant action. *Neuropharmacology, 63*, 1380–1388.

File, S. E., Zharkovsky, A., & Gulati, K. (1991). Effects of baclofen and nitrendipine on ethanol withdrawal responses in the rat. *Neuropharmacology, 30*, 183–190.

File, S. E., Zharkovsky, A., & Hitchcott, P. K. (1992). Effects of nitrendipine, chlordiazepoxide, flumazenil and baclofen on the increased anxiety resulting from alcohol withdrawal. *Progress in Neuropsychopharmacology and Biological Psychiatry, 16*, 87–93.

Frankowska, M., Filip, M., & Przegalinski, E. (2007). Effects of GABAB receptor ligands in animal tests of depression and anxiety. *Pharmacological Reports, 59*, 645–655.

Frankowska, M., Golda, A., Wydra, K., Gruca, P., Papp, M., & Filip, M. (2010). Effects of imipramine or GABA(B) receptor ligands on the immobility, swimming and climbing in the forced swim test in rats following discontinuation of cocaine self-administration. *European Journal of Pharmacology, 627*, 142–149.

Froestl, W. (2010). Chemistry and pharmacology of GABAB receptor ligands. *Advances in Pharmacology, 58*, 19–62.

Froestl, W. (2011). An historical perspective on GABAergic drugs. *Future Medicinal Chemistry, 3*, 163–175.

Froestl, W., Gallagher, M., Jenkins, H., Madrid, A., Melcher, T., Teichman, S., et al. (2004). SGS742: The first GABA(B) receptor antagonist in clinical trials. *Biochemical Pharmacology, 68*, 1479–1487.

Froestl, W., & Mickel, S. J. C. (1997). Chemistry of GABAB modulators. In S. J. Enna & N. G. Bowery (Eds.), *The GABA receptors* (pp. 271–296). Totowa, NJ: Humana Press.

Galvez, T., Duthey, B., Kniazeff, J., Blahos, J., Rovelli, G., Bettler, B., et al. (2001). Allosteric interactions between GB1 and GB2 subunits are required for optimal GABA(B) receptor function. *EMBO Journal, 20*, 2152–2159.

Gassmann, M., & Bettler, B. (2012). Regulation of neuronal GABA(B) receptor functions by subunit composition. *Nature Reviews Neuroscience, 13*, 380–394.

Gaynes, B. N., Warden, D., Trivedi, M. H., Wisniewski, S. R., Fava, M., & Rush, A. J. (2009). What did STAR*D teach us? Results from a large-scale, practical, clinical trial for patients with depression. *Psychiatric Services, 60*, 1439–1445.

Ghose, S., Winter, M. K., McCarson, K. E., Tamminga, C. A., & Enna, S. J. (2011). The GABAβ receptor as a target for antidepressant drug action. *British Journal of Pharmacology, 162*, 1–17.

Giachino, C., Barz, M., Tchorz, J. S., Tome, M., Gassmann, M., Bischofberger, J., et al. (2014). GABA suppresses neurogenesis in the adult hippocampus through GABAB receptors. *Development, 141*, 83–90.

Gjoni, T., & Urwyler, S. (2008). Receptor activation involving positive allosteric modulation, unlike full agonism, does not result in GABAB receptor desensitization. *Neuropharmacology, 55*, 1293–1299.

Gjoni, T., & Urwyler, S. (2009). Changes in the properties of allosteric and orthosteric GABAB receptor ligands after a continuous, desensitizing agonist pretreatment. *European Journal of Pharmacology, 603*, 37–41.

Gos, T., Gunther, K., Bielau, H., Dobrowolny, H., Mawrin, C., Trubner, K., et al. (2009). Suicide and depression in the quantitative analysis of glutamic acid decarboxylase-Immunoreactive neuropil. *Journal of Affective Disorders, 113*, 45–55.

Griebel, G., & Holmes, A. (2013). 50 years of hurdles and hope in anxiolytic drug discovery. *Nature Reviews Drug Discovery, 12*, 667–687.

Guery, S., Floersheim, P., Kaupmann, K., & Froestl, W. (2007). Syntheses and optimization of new GS39783 analogues as positive allosteric modulators of GABA B receptors. *Bioorganic & Medicinal Chemistry Letters, 17*, 6206–6211.

Gustavsson, A., Svensson, M., Jacobi, F., Allgulander, C., Alonso, J., Beghi, E., et al. (2011). Cost of disorders of the brain in Europe 2010. *European Neuropsychopharmacology, 21*, 718–779.

Heaney, C. F., & Kinney, J. W. (2016). Role of GABAB receptors in learning and memory and neurological disorders. *Neuroscience & Biobehavioral Reviews, 63*, 1–28.

Heese, K., Otten, U., Mathivet, P., Raiteri, M., Marescaux, C., & Bernasconi, R. (2000). GABA(B) receptor antagonists elevate both mRNA and protein levels of the neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) but not neurotrophin-3 (NT-3) in brain and spinal cord of rats. *Neuropharmacology, 39*, 449–462.

Hill, D. R., & Bowery, N. G. (1981). 3H-baclofen and 3H-GABA bind to bicuculline-insensitive GABA B sites in rat brain. *Nature, 290*, 149–152.

Hoffman, E. J., & Mathew, S. J. (2008). Anxiety disorders: A comprehensive review of pharmacotherapies. *Mount Sinai Journal of Medicine, 75*, 248–262.

Jacobson, L. H., Bettler, B., Kaupmann, K., & Cryan, J. F. (2007a). Behavioral evaluation of mice deficient in GABA(B(1)) receptor isoforms in tests of unconditioned anxiety. *Psychopharmacology, 190*, 541–553.

Jacobson, L. H., & Cryan, J. F. (2008). Evaluation of the anxiolytic-like profile of the GABAB receptor positive modulator CGP7930 in rodents. *Neuropharmacology, 54*, 854–862.

Jacobson, L. H., Kelly, P. H., Bettler, B., Kaupmann, K., & Cryan, J. F. (2006). GABA(B(1)) receptor isoforms differentially mediate the acquisition and extinction of aversive taste memories. *Journal of Neuroscience, 26*, 8800–8803.

Jacobson, L. H., Kelly, P. H., Bettler, B., Kaupmann, K., & Cryan, J. F. (2007b). Specific roles of GABA(B(1)) receptor isoforms in cognition. *Behavioural Brain Research, 181*, 158–162.

Jung, S., Son, H., Lee, D. H., Roh, G. S., Kang, S. S., Cho, G. J., et al. (2016). Decreased levels of RGS4 in the paraventricular nucleus facilitate GABAergic inhibition during the acute stress response. *Biochemical and Biophysical Research Communications, 472*, 276–280.

Karolewicz, B., Maciag, D., O'Dwyer, G., Stockmeier, C. A., Feyissa, A. M., & Rajkowska, G. (2010). Reduced level of glutamic acid decarboxylase-67 kDa in the prefrontal cortex in major depression. *International Journal of Neuropsychopharmacology, 13*, 411–420.

Kaupmann, K., Huggel, K., Heid, J., Flor, P. J., Bischoff, S., Mickel, S. J., et al. (1997). Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. *Nature, 386*, 239–246.

Kerr, D. I., & Ong, J. (1992). GABA agonists and antagonists. *Medicinal Research Reviews, 12*, 593–636.

Kerr, D. I., Ong, J., Prager, R. H., Gynther, B. D., & Curtis, D. R. (1987). Phaclofen: A peripheral and central baclofen antagonist. *Brain Research, 405*, 150–154.

Ketelaars, C. E., Bollen, E. L., Rigter, H., & Bruinvels, J. (1988). GABA-B receptor activation and conflict behaviour. *Life Sciences, 42*, 933–942.

Khan, M. I., Ostadhadi, S., Zolfaghari, S., Ejtemaei Mehr, S., Hassanzadeh, G., & Dehpour, A. R. (2016). The involvement of NMDA receptor/NO/cGMP pathway in the antidepressant like effects of baclofen in mouse force swimming test. *Neuroscience Letters, 612*, 52–61.

Kim, G., Jung, S., Son, H., Kim, S., Choi, J., Lee, D. H., et al. (2014). The GABAB receptor associates with regulators of G-protein signaling 4 protein in the mouse prefrontal cortex and hypothalamus. *BMB Reports, 47*, 324–329.

Kim, G., Lee, Y., Jeong, E. Y., Jung, S., Son, H., Lee, D. H., et al. (2010). Acute stress responsive RGS proteins in the mouse brain. *Molecules and Cells, 30*, 161–165.

Klempan, T. A., Sequeira, A., Canetti, L., Lalovic, A., Ernst, C., ffrench-Mullen, J., et al. (2009). Altered expression of genes involved in ATP biosynthesis and GABAergic neurotransmission in the ventral prefrontal cortex of suicides with and without major depression. *Molecular Psychiatry, 14*, 175–189.

Knapp, D. J., Overstreet, D. H., & Breese, G. R. (2007). Baclofen blocks expression and sensitization of anxiety-like behavior in an animal model of repeated stress and ethanol withdrawal. *Alcoholism, Clinical and Experimental Research, 31*, 582–595.

Krystal, J. H., Sanacora, G., Blumberg, H., Anand, A., Charney, D. S., Marek, G., et al. (2002). Glutamate and GABA systems as targets for novel antidepressant and mood-stabilizing treatments. *Molecular Psychiatry, 7*(Suppl 1), S71–S80.

Lee, M. T., Chen, C. H., Lee, C. S., Chen, C. C., Chong, M. Y., Ouyang, W. C., et al. (2011). Genome-wide association study of bipolar I disorder in the Han Chinese population. *Molecular Psychiatry, 16*, 548–556.

Lehmann, A., Mattsson, J. P., Edlund, A., Johansson, T., & Ekstrand, A. J. (2003). Effects of repeated administration of baclofen to rats on GABAB receptor binding sites and subunit expression in the brain. *Neurochemical Research, 28*, 387–393.

Levone, B. R., Cryan, J. F., & O'Leary, O. F. (2015). Role of adult hippocampal neurogenesis in stress resilience. *Neurobiology of Stress, 1*, 147–155.

Li, X., Kaczanowska, K., Finn, M. G., Markou, A., & Risbrough, V. B. (2015). The GABA(B) receptor positive modulator BHF177 attenuated anxiety, but not conditioned fear, in rats. *Neuropharmacology, 97*, 357–364.

Li, Y., Luikart, B. W., Birnbaum, S., Chen, J., Kwon, C. H., Kernie, S. G., et al. (2008). TrkB regulates hippocampal neurogenesis and governs sensitivity to antidepressive treatment. *Neuron, 59*, 399–412.

Li, X., Risbrough, V. B., Cates-Gatto, C., Kaczanowska, K., Finn, M. G., Roberts, A. J., et al. (2013). Comparison of the effects of the GABAB receptor positive modulator BHF177 and the GABAB receptor agonist baclofen on anxiety-like behavior, learning, and memory in mice. *Neuropharmacology, 70*, 156–167.

Lopes, A. P., da Cunha, I. C., Steffens, S. M., Ferraz, A., Vargas, J. C., de Lima, T. C., et al. (2007). GABAA and GABAB agonist microinjections into medial accumbens shell increase feeding and induce anxiolysis in an animal model of anxiety. *Behavioural Brain Research, 184*, 142–149.

Lopes, A. P., Ganzer, L., Borges, A. C., Kochenborger, L., Januario, A. C., Faria, M. S., et al. (2012). Effects of GABA ligands injected into the nucleus accumbens shell on fear/anxiety-like and feeding behaviours in food-deprived rats. *Pharmacology, Biochemistry, and Behavior, 101*, 41–48.

Lujan, R., & Ciruela, F. (2012). GABAB receptors-associated proteins: Potential drug targets in neurological disorders? *Current Drug Targets, 13*, 129–144.

Maier, S. F., & Watkins, L. R. (2005). Stressor controllability and learned helplessness: The roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor. *Neuroscience & Biobehavioral Reviews, 29*, 829–841.

Malherbe, P., Masciadri, R., Norcross, R. D., Knoflach, F., Kratzeisen, C., Zenner, M. T., et al. (2008). Characterization of (R, S)-5,7-di-tert-butyl-3-hydroxy-3-trifluoromethyl-3H-benzofuran-2-one as a positive allosteric modulator of GABAB receptors. *British Journal of Pharmacology, 154*, 797–811.

Manteghi, A. A., Hebrani, P., Mortezania, M., Haghighi, M. B., & Javanbakht, A. (2014). Baclofen add-on to citalopram in treatment of posttraumatic stress disorder. *Journal of Clinical Psychopharmacology, 34*, 240–243.

Marchesi, C., Chiodera, P., De Ferri, A., De Risio, C., Dasso, L., Menozzi, P., et al. (1991). Reduction of GH response to the GABA-B agonist baclofen in patients with major depression. *Psychoneuroendocrinology, 16*, 475–479.

Mares, P., Ticha, K., & Mikulecka, A. (2013). Anticonvulsant and behavioral effects of GABA(B) receptor positive modulator CGP7930 in immature rats. *Epilepsy & Behavior, 28*, 113–120.

Marshall, F. H. (2005). Is the GABA B heterodimer a good drug target? *Journal of Molecular Neuroscience, 26*, 169–176.

McCarson, K. E., Duric, V., Reisman, S. A., Winter, M., & Enna, S. J. (2006). GABA(B) receptor function and subunit expression in the rat spinal cord as indicators of stress and the antinociceptive response to antidepressants. *Brain Research, 1068*, 109–117.

Metz, M., Gassmann, M., Fakler, B., Schaeren-Wiemers, N., & Bettler, B. (2011). Distribution of the auxiliary GABAB receptor subunits KCTD8, 12, 12b, and 16 in the mouse brain. *Journal of Comparative Neurology, 519*, 1435–1454.

Millan, M. J., Goodwin, G. M., Meyer-Lindenberg, A., & Ogren, S. O. (2015a). 60 years of advances in neuropsychopharmacology for improving brain health, renewed hope for progress. *European Neuropsychopharmacology, 25*, 591–598.

Millan, M. J., Goodwin, G. M., Meyer-Lindenberg, A., & Ove Ogren, S. (2015b). Learning from the past and looking to the future: Emerging perspectives for improving the treatment of psychiatric disorders. *European Neuropsychopharmacology, 25*, 599–656.

Miller, B. R., & Hen, R. (2015). The current state of the neurogenic theory of depression and anxiety. *Current Opinion in Neurobiology, 30*, 51–58.

Mohler, H., & Fritschy, J. M. (1999). GABAB receptors make it to the top—As dimers. *Trends in Pharmacological Sciences, 20*, 87–89.

Mombereau, C., Kaupmann, K., Froestl, W., Sansig, G., van der Putten, H., & Cryan, J. F. (2004). Genetic and pharmacological evidence of a role for GABA(B) receptors in the modulation of anxiety- and antidepressant-like behavior. *Neuropsychopharmacology, 29*, 1050–1062.

Mombereau, C., Kaupmann, K., Gassmann, M., Bettler, B., van der Putten, H., & Cryan, J. F. (2005). Altered anxiety and depression-related behaviour in mice lacking GABAB(2) receptor subunits. *Neuroreport, 16*, 307–310.

Moser, M. B., & Moser, E. I. (1998). Functional differentiation in the hippocampus. *Hippocampus, 8*, 608–619.

Nakagawa, Y., Ishima, T., Ishibashi, Y., Tsuji, M., & Takashima, T. (1996a). Involvement of GABAB receptor systems in action of antidepressants. II: Baclofen attenuates the effect of desipramine whereas muscimol has no effect in learned helplessness paradigm in rats. *Brain Research, 728*, 225–230.

Nakagawa, Y., Ishima, T., Ishibashi, Y., Tsuji, M., & Takashima, T. (1996b). Involvement of GABAB receptor systems in experimental depression: Baclofen but not bicuculline exacerbates helplessness in rats. *Brain Research, 741*, 240–245.

Nakagawa, Y., Ishima, T., Ishibashi, Y., Yoshii, T., & Takashima, T. (1996c). Involvement of GABA(B) receptor systems in action of antidepressants: Baclofen but not bicuculline attenuates the effects of antidepressants on the forced swim test in rats. *Brain Research, 709*, 215–220.

Nakagawa, Y., Sasaki, A., & Takashima, T. (1999). The GABA(B) receptor antagonist CGP36742 improves learned helplessness in rats. *European Journal of Pharmacology, 381*, 1–7.

Nemeroff, C. B. (2003a). Anxiolytics: Past, present, and future agents. *The Journal of Clinical Psychiatry, 64*(Suppl 3), 3–6.

Nemeroff, C. B. (2003b). The role of GABA in the pathophysiology and treatment of anxiety disorders. *Psychopharmacology Bulletin, 37*, 133–146.

Nestler, E. J., Barrot, M., DiLeone, R. J., Eisch, A. J., Gold, S. J., & Monteggia, L. M. (2002). Neurobiology of depression. *Neuron, 34*, 13–25.

Ni, Y. G., Gold, S. J., Iredale, P. A., Terwilliger, R. Z., Duman, R. S., & Nestler, E. J. (1999). Region-specific regulation of RGS4 (Regulator of G-protein-signaling protein type 4) in brain by stress and glucocorticoids: In vivo and in vitro studies. *Journal of Neuroscience, 19*, 3674–3680.

Nowak, G., Partyka, A., Palucha, A., Szewczyk, B., Wieronska, J. M., Dybala, M., et al. (2006). Antidepressant-like activity of CGP 36742 and CGP 51176, selective GABAB receptor antagonists, in rodents. *British Journal of Pharmacology, 149*, 581–590.

O'Flynn, K., & Dinan, T. G. (1993). Baclofen-induced growth hormone release in major depression: Relationship to dexamethasone suppression test result. *The American Journal of Psychiatry, 150*, 1728–1730.

O'Leary, O. F., & Castren, E. (2010). Neurotrophic factors and antidepressant action: Recent advances. In J. F. Cryan & B. E. Leonard (Eds.), *Depression: Psychopathology to pharmacotherapy* (Mod Trends Pharmacopsychiatry). Basel: Karger.

O'Leary, O. F., & Cryan, J. F. (2014). A ventral view on antidepressant action: Roles for adult hippocampal neurogenesis along the dorsoventral axis. *Trends in Pharmacological Sciences, 35*, 675–687.

O'Leary, O. F., Dinan, T. G., & Cryan, J. F. (2015). Faster, better, stronger: Towards new antidepressant therapeutic strategies. *European Journal of Pharmacology, 753*, 32–50.

O'Leary, O. F., Felice, D., Galimberti, S., Savignac, H. M., Bravo, J. A., Crowley, T., et al. (2014). GABAB(1) receptor subunit isoforms differentially regulate stress resilience. *Proceedings of the National Academy of Sciences of the United States of America, 111*, 15232–15237.

Ong, J., & Kerr, D. I. (2005). Clinical potential of GABAB receptor modulators. *CNS Drug Reviews, 11*, 317–334.

Partyka, A., Klodzinska, A., Szewczyk, B., Wieronska, J. M., Chojnacka-Wojcik, E., Librowski, T., et al. (2007). Effects of GABAB receptor ligands in rodent tests of anxiety-like behavior. *Pharmacological Reports, 59*, 757–762.

Pearl, P. L., Gibson, K. M., Cortez, M. A., Wu, Y., Carter Snead, O., III, Knerr, I., et al. (2009). Succinic semialdehyde dehydrogenase deficiency: Lessons from mice and men. *Journal of Inherited Metabolic Disease, 32*, 343–352.

Pin, J. P., Parmentier, M. L., & Prezeau, L. (2001). Positive allosteric modulators for gamma-aminobutyric acid(B) receptors open new routes for the development of drugs targeting family 3 G-protein-coupled receptors. *Molecular Pharmacology, 60*, 881–884.

Pinard, A., Seddik, R., & Bettler, B. (2010). GABAB receptors: Physiological functions and mechanisms of diversity. *Advances in Pharmacology, 58*, 231–255.

Prosser, H. M., Gill, C. H., Hirst, W. D., Grau, E., Robbins, M., Calver, A., et al. (2001). Epileptogenesis and enhanced prepulse inhibition in GABA(B1)-deficient mice. *Molecular and Cellular Neurosciences, 17*, 1059–1070.

Rajkowska, G., O'Dwyer, G., Teleki, Z., Stockmeier, C. A., & Miguel-Hidalgo, J. J. (2007). GABAergic neurons immunoreactive for calcium binding proteins are reduced in the prefrontal cortex in major depression. *Neuropsychopharmacology, 32*, 471–482.

Rocha, L., Alonso-Vanegas, M., Martinez-Juarez, I. E., Orozco-Suarez, S., Escalante-Santiago, D., Feria-Romero, I. A., et al. (2014). GABAergic alterations in neocortex of patients with pharmacoresistant temporal lobe epilepsy can explain the comorbidity of anxiety and depression: The potential impact of clinical factors. *Frontiers in Cellular Neuroscience, 8*, 442.

Saarelainen, T., Hendolin, P., Lucas, G., Koponen, E., Sairanen, M., MacDonald, E., et al. (2003). Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. *Journal of Neuroscience, 23*, 349–357.

Sabbagh, M. N. (2009). Drug development for Alzheimer's disease: Where are we now and where are we headed? *The American Journal of Geriatric Pharmacotherapy, 7*, 167–185.

Sairanen, M., Lucas, G., Ernfors, P., Castren, M., & Castren, E. (2005). Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. *Journal of Neuroscience, 25*, 1089–1094.

Sanacora, G., Mason, G. F., & Krystal, J. H. (2000). Impairment of GABAergic transmission in depression: New insights from neuroimaging studies. *Critical Reviews in Neurobiology, 14*, 23–45.

Sanders, S. K., & Shekhar, A. (1995). Regulation of anxiety by GABAA receptors in the rat amygdala. *Pharmacology, Biochemistry, and Behavior, 52*, 701–706.

Sands, S. A., Reisman, S. A., & Enna, S. J. (2004). Effect of antidepressants on GABA(B) receptor function and subunit expression in rat hippocampus. *Biochemical Pharmacology, 68*, 1489–1495.

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., et al. (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science, 301*, 805–809.

Schuler, V., Luscher, C., Blanchet, C., Klix, N., Sansig, G., Klebs, K., et al. (2001). Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic GABA(B) responses in mice lacking GABA(B(1)). *Neuron, 31*, 47–58.

Shaban, H., Humeau, Y., Herry, C., Cassasus, G., Shigemoto, R., Ciocchi, S., et al. (2006). Generalization of amygdala LTP and conditioned fear in the absence of presynaptic inhibition. *Nature Neuroscience, 9*, 1028–1035.

Slattery, D. A., Desrayaud, S., & Cryan, J. F. (2005a). GABAB receptor antagonist-mediated antidepressant-like behavior is serotonin-dependent. *The Journal of Pharmacology and Experimental Therapeutics, 312*, 290–296.

Slattery, D. A., Markou, A., Froestl, W., & Cryan, J. F. (2005b). The GABAB receptor-positive modulator GS39783 and the GABAB receptor agonist baclofen attenuate the reward-facilitating effects of cocaine: Intracranial self-stimulation studies in the rat. *Neuropsychopharmacology, 30*, 2065–2072.

Snyder, J. S., Soumier, A., Brewer, M., Pickel, J., & Cameron, H. A. (2011). Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. *Nature, 476*, 458–461.

Stewart, L. S., Wu, Y., Eubanks, J. H., Han, H., Leschenko, Y., Perez Velazquez, J. L., et al. (2009). Severity of atypical absence phenotype in GABAB transgenic mice is subunit specific. *Epilepsy & Behavior, 14*, 577–581.

Stratinaki, M., Varidaki, A., Mitsi, V., Ghose, S., Magida, J., Dias, C., et al. (2013). Regulator of G protein signaling 4 [corrected] is a crucial modulator of antidepressant drug action in depression and neuropathic pain models. *Proceedings of the National Academy of Sciences of the United States of America, 110*, 8254–8259.

Sun, B., Chen, L., Liu, L., Xia, Z., Pin, J. P., Nan, F., et al. (2016). A negative allosteric modulator modulates GABAB-receptor signalling through GB2 subunits. *Biochemical Journal, 473*, 779–787.

Sweeney, F. F., O'Leary, O. F., & Cryan, J. F. (2013). GABAB receptor ligands do not modify conditioned fear responses in BALB/c mice. *Behavioural Brain Research, 256*, 151–156.

Sweeney, F. F., O'Leary, O. F., & Cryan, J. F. (2014). Activation but not blockade of GABAB receptors during early-life alters anxiety in adulthood in BALB/c mice. *Neuropharmacology, 81*, 303–310.

Taliaz, D., Stall, N., Dar, D. E., & Zangen, A. (2010). Knockdown of brain-derived neurotrophic factor in specific brain sites precipitates behaviors associated with depression and reduces neurogenesis. *Molecular Psychiatry, 15*, 80–92.

Trivedi, M. H., Rush, A. J., Wisniewski, S. R., Nierenberg, A. A., Warden, D., Ritz, L., et al. (2006). Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: Implications for clinical practice. *The American Journal of Psychiatry, 163*, 28–40.

Urwyler, S., Mosbacher, J., Lingenhoehl, K., Heid, J., Hofstetter, K., Froestl, W., et al. (2001). Positive allosteric modulation of native and recombinant gamma-aminobutyric acid(B) receptors by 2,6-Di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and its aldehyde analog CGP13501. *Molecular Pharmacology, 60*, 963–971.

Urwyler, S., Pozza, M. F., Lingenhoehl, K., Mosbacher, J., Lampert, C., Froestl, W., et al. (2003). N, N′-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) and structurally related compounds: Novel allosteric enhancers of gamma-aminobutyric acidB receptor function. *The Journal of Pharmacology and Experimental Therapeutics, 307*, 322–330.

Varani, A. P., & Balerio, G. N. (2012). GABA(B) receptors involvement in the effects induced by nicotine on anxiety-related behaviour in mice. *Pharmacological Research, 65*, 507–513.

Vigot, R., Barbieri, S., Brauner-Osborne, H., Turecek, R., Shigemoto, R., Zhang, Y. P., et al. (2006). Differential compartmentalization and distinct functions of GABAB receptor variants. *Neuron, 50*, 589–601.

Vilar, M., & Mira, H. (2016). Regulation of neurogenesis by neurotrophins during adulthood: Expected and unexpected roles. *Frontiers in Neuroscience, 10*, 26.

Vogel, K. R., Pearl, P. L., Theodore, W. H., McCarter, R. C., Jakobs, C., & Gibson, K. M. (2012). *Thirty years beyond discovery—Clinical trials in succinic semialdehyde dehydrogenase deficiency, a disorder of GABA metabolism*. J Inherit Metab Dis.

Warden, D., Rush, A. J., Trivedi, M. H., Fava, M., & Wisniewski, S. R. (2007). The STAR*D project results: A comprehensive review of findings. *Current Psychiatry Reports, 9*, 449–459.

Weissman, M. M., & Klerman, G. L. (1977). Sex differences and the epidemiology of depression. *Archives of General Psychiatry, 34*, 98–111.

Willner, P. (2005). Chronic mild stress (CMS) revisited: Consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology, 52*, 90–110.

Wittchen, H. U., Jacobi, F., Rehm, J., Gustavsson, A., Svensson, M., Jonsson, B., et al. (2011). The size and burden of mental disorders and other disorders of the brain in Europe 2010. *European Neuropsychopharmacology, 21*, 655–679.

Wu, Y., Chan, K. F., Eubanks, J. H., Guin Ting Wong, C., Cortez, M. A., Shen, L., et al. (2007). Transgenic mice over-expressing GABA(B)R1a receptors acquire an atypical absence epilepsy-like phenotype. *Neurobiology of Disease, 26*, 439–451.

Xu, F., Peng, G., Phan, T., Dilip, U., Chen, J. L., Chernov-Rogan, T., et al. (2011). Discovery of a novel potent GABA(B) receptor agonist. *Bioorganic & Medicinal Chemistry Letters, 21*, 6582–6585.

Zarrindast, M., Rostami, P., & Sadeghi-Hariri, M. (2001). GABA(A) but not GABA(B) receptor stimulation induces antianxiety profile in rats. *Pharmacology, Biochemistry, and Behavior, 69*, 9–15.

Chapter 13
Targeting the $GABA_B$ Receptor in Fragile X Syndrome and Autism Spectrum Disorders

Aileen Healy

Abstract Fragile X syndrome (FXS) was long considered an immutable condition until the 1991 discovery of the causative genetic mutation, which enabled the basic research necessary to define the molecular pathophysiology. Understanding the disease biology was an essential step in identifying potential targeted treatments that are disease modifying. In FXS, the regulation of protein synthesis at the neuronal synapse is now thought to be a central pathway for pharmacologic intervention. Anecdotal evidence from a patient who received the $GABA_B$ agonist, baclofen, for the drug's antispasmodic properties and found benefit for their autism lead to research focused on developing baclofen for the treatment of autism and FXS. Arbaclofen, the active enantiomer of baclofen, was tested in the FXS mouse model and shown to reverse many of the abnormal neuronal functions associated with FXS including reversing the abnormally elevated protein synthesis. The existing safety data on baclofen facilitated clinical development, and arbaclofen was tested in autistic and FXS patients in multiple large, controlled clinical trials. The results of these studies failed to show clinical benefit in the chosen outcome measures; however, the arbaclofen clinical trials contributed to the reevaluation of what success will look like for FXS patients. The FXS community has a renewed focus in developing sensitive and reliable outcome measures for future trials, with the ultimate goal of developing disease-modifying treatments.

Keywords Fragile X syndrome • Autism • $GABA_B$ • Baclofen • Synaptic plasticity • Protein synthesis • Disease-modifying treatments and clinical trials

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13.1 Introduction

Fragile X syndrome (FXS) is the leading inherited cause of intellectual disability (ID). A rare, monogenic disease FXS is also the single most prevalent known cause of autism (Garber et al. 2008; Hagerman et al. 2009). Therefore, therapies developed as disease-modifying agents for FXS may also prove to be effective treatments for the core disease pathologies of autism.

Individuals with FXS exhibit cognitive and behavioral deficits. The neurological manifestations are heterogeneous and present at different developmental stages. Most persist throughout adulthood. For example, cognitive deficits are evident from infancy and social deficits emerge within the first 2–3 years of life. Seizures may emerge in early childhood, but are usually manageable and often outgrown before adulthood (Kidd et al. 2014). Social withdrawal, attention deficits and hyperactivity, and stereotyped behaviors all emerge early in development. Irritability and aggressive behaviors in adults often replace childhood hyperactivity. In addition, individuals can show hypersensitivity to tactile stimulation or other environmental stimuli and perseverative speech are typical. FXS is the most prevalent known genetic cause of autism, accounting for 3–5 % of autism cases, with the majority of FXS individuals meeting the criteria for autism or autism spectrum disorder (ASD) (Harris et al. 2008; Kaufmann et al. 2004). More recent work is beginning to reveal a distinct developmental profile for infants with FXS when compared with typically developing or autistic infants (Roberts et al. 2016). Nonneurologic manifestations include distinct facial characteristics, macroorchidism, connective tissue abnormalities affecting joints and cardiac valve function, strabismus, recurrent ear infections, and gastroesophageal reflux disorder.

The prevalence of FXS is ~1:5000 males and ~1:8000 females (Coffee et al. 2009), with females being generally less affected than males due to the mosaicism of X-chromosome inactivation. Females show a spectrum of neurologic impairments from mild learning disabilities to ID.

There are close associations between FXS and autism. Individuals with autism exhibit impairments in behavior, communication, and social skills. The neurological manifestations are heterogeneous and vary in severity. The presence of symptoms in all three domains, with sufficient severity, results in the full diagnosis of autistic disorder. Patients with symptoms of lesser severity, or without significant symptoms in one or two domains, may be diagnosed with ASD. Impairments in behavior manifest as repetitive behaviors and restricted interests. Secondary or associated symptoms of ASD include irritable and aggressive behaviors, self-injury, hypersensitivity to visual, auditory, tactile, or other sensory stimuli, apparent inattention, and sleep difficulties. Anxiety is thought to underlie irritable, aggressive, or self-injurious behaviors (Talisa et al. 2014).

The prevalence of autism is currently estimated at 1:68 in the United States with a higher prevalence in boys (1 in 42 boys and 1 in 189 girls) (Zablotsky et al. 2014). Educational and behavioral therapies are the mainstay of treatment for the core symptoms of ASD. There are no approved therapeutics for the core symptoms of

ASD, though risperidone and aripiprazole are approved for the treatment of the associated symptom of irritability. The clinical and genetic heterogeneities of autism have been a major challenge in defining etiology and identifying therapeutic targets. Several neurodevelopmental disabilities caused by single gene mutations, including FXS, have a high prevalence of autism, and research in these syndromic autisms may provide insight into the molecular pathophysiology of idiopathic autism.

13.2 FXS Etiology

FXS is caused by a DNA triplet repeat expansion (CpG) in the 5′ noncoding region of the fragile X mental retardation 1 gene (*FMR1*). Small CpG expansions up to 50 repeats occur in the general population and are not associated with disease. Premutation expansions between 55 and 200 repeats were initially characterized as nonpathogenic; however, it is now understood that the premutation expansions cause premature ovarian insufficiency (POI) in female carriers and fragile X tremor-associated ataxias (FXTAS) in male and to a lesser extent in female carriers (Brown and Stanfield 2015). POI and FXTAS are the result of dysregulated expression of *FMR1* causing a toxic gain-of-function condition. The premutation is almost ten times more prevalent than FXS and can also be associated with lifelong mood and anxiety disorders (Bourgeois et al. 2011). A diagnosis of FXS is confirmed with CpG triplet expansions greater than 200 repeats. The CpG expansions are chemically modified by methylation, which prevents transcription of FMR1 and creates the loss-of-function condition. Repeat expansions can become quite large; however, there is no correlation between disease severity and triplet repeat expansions beyond the 200 CpG threshold that silences transcription. Loss of function of the FMR1 gene product negatively affects synaptic function and circuitry throughout the brain.

There are no approved disease-modifying therapies for FXS. Current treatment modalities focus on relieving symptoms of the disease and evidence may be limited for the best practices to care for FXS psychiatric manifestations. The risks of long-term use and polypharmacy have not been fully assessed for patients with FXS and autism. However, there is a general consensus on current treatment modalities for the use of psychotropic medications for FXS (Wadell et al. 2013; Wheeler et al. 2016). Behavioral symptoms are significant and vary widely both between individuals and over the course of the disease in a single patient. FXS patients with attention deficit and hyperactivity, in general, respond well to stimulants (methylphenidate and dextroamphetamine) and alpha-2 adrenergic agonists (clonidine, guanfacine). The antipsychotics, risperidone and aripiprazole, may also treat ADHD in FXS. The anxiety disorder rate is high in FXS and treatment with antidepressants (selective serotonin reuptake inhibitors (SSRIs), citalopram, and fluoxetine and atypical antidepressants) help manage daily activities. Psychoactive treatments such as single dose benzodiazepine can aid in the management of highly stressful activities, for example, those triggered by unfamiliar environments. Mood instability and aggression are quite common in FXS, and Valproic acid and Lithium

are often prescribed to aid mood disorders. Mild aggressive behavior is prevalent in the majority of adult FXS patients, with a smaller percentage demonstrating severe aggressive behavior (Wheeler et al. 2016). Treatments include SSRIs, antipsychotics, and Lithium. Patient medical management often requires a trial and error approach to establish maximum benefit.

13.3 The Molecular Pathophysiology of FXS

The gene associated with a cytologic "fragile site" of the X chromosome was identified in 1991 (Verkerk et al. 1991). This finding enabled over two decades of work that defined fragile X mental retardation protein (FMRP) function and the pathophysiology resulting from FMR1 mutations. FMRP is a multifunctional protein. Experimental evidence suggests FMRP functions during nuclear binding and export of newly transcribed RNAs, mediates active transport of cargo mRNAs through the cytoplasm to neuronal dendritic spines (the receiving terminals for synaptic transmissions), and locally regulates protein synthesis in response to neurotransmitter signals (Kim et al. 2009; Fridell et al. 1996; Eberhart et al. 1996).

13.4 Protein Synthesis in Cognitive Processes

Learning associated with long-term memory requires protein synthesis (Bailey et al. 1996). Neuronal connections are constantly being modified and synaptic plasticity enables the continual strengthening or weakening of these connections based on synaptic activation. Newly synthesized proteins maintain the plastic response to synaptic activation. FMRP is highly expressed throughout the brain and is enriched at excitatory synapses where it regulates the activity-dependent protein synthesis required for synaptic plasticity. FMRP represses mRNA translation by binding to specific mRNA elements and stalling ribosome translocation along the target mRNAs. FMRP targets hundreds of mRNAs (Vasilyev and Serganov 2015; Darnell 2013). In FXS, the absence of FMRP causes dysregulation of synaptic transcripts (Darnell 2013) leading to a quantifiable increase in synaptic protein synthesis as detected in synapse-enriched tissue preparations and in whole brain (Qin et al. 2015; Greenough et al. 2001). The uncoupling of synaptic protein synthesis from excitatory neurotransmission is proposed to be causative of the cognitive and behavioral impairments in FXS (Bear et al. 2004; Huber et al. 2002).

The long-term potentiation (LTP) or depression (LTD) of synaptic transmissions is a well-characterized mechanism shaping neural circuitry (Malenka and Bear 2004). In hippocampus, the activation of group I, metabotropic glutamate receptors (mGluR1/5), triggers LTD in excitatory synapses. Hippocampal mGluR-LTD requires the de novo protein synthesis of preexisting mRNAs located in dendritic spines (Bear et al. 2004). In the *fmr1*-knockout mouse, a model of FXS, mGluR-LTD,

is independent of protein synthesis and this synaptic signal persists longer than the wild-type signal (Huber et al. 2002). This finding led to the "mGluR theory of FXS," which proposes that glutamate signaling through the mGluRs contributes significantly to the neurologic manifestations of FXS and suggests that countering mGluR signaling would reverse disease phenotypes (Bear et al. 2004). Since the "mGluR theory of FXS" was first proposed, the central hypothesis has been widely tested in multiple laboratories using various genetic and pharmacologic methods and measures of synaptic structure, function, and neural circuitry. The first evidence came from the genetic reduction of mGluR5 in *fmr1*-knockout mice and demonstrated correction of ocular dominance plasticity, dendritic spine density, inhibitory avoidance extinction—a hippocampus dependent memory, audiogenic seizures, and exaggerated hippocampal protein synthesis (Dolen et al. 2007). Additional evidence supporting the theory stems from the pharmacologic reduction of mGluR5 using the selective inhibitor 2-chloro-4-[2-[2,5-dimethyl-1-[4-(trifluoromethoxy)phenyl]-1H-imidazol-4-yl]ethynyl]pyridine (CTEP), which also corrected dendritic spine density, inhibitory avoidance extinction, audiogenic seizures, and exaggerated hippocampal protein synthesis (Michalon et al. 2012). The inhibition of signal transduction elements downstream of mGlur5 also reverses multiple FXS-like phenotypes in mice. For example, lovastatin, which inhibits Ras-Erk1/2 signaling, corrects both the persistent mGluR-LTD and the excessive hippocampal protein synthesis (Osterweil et al. 2010). The inhibition of the MAP kinase, ERK1/2, also inhibits audiogenic seizures in *fmr1*-knockout mice and synaptic protein synthesis in *fmr1*-knockout hippocampal slices (Osterweil et al. 2010). Genetic reduction of the translation initiation and elongation factor, p70S6-kinase, also reduces the exaggerated mGluR-LTD, dendritic spine morphology and restores select synaptic protein synthesis (Bhattacharya et al. 2012). These studies supported reversal of uncontrolled synaptic protein synthesis as a therapeutic approach to correct the core pathologies associated with FXS.

13.5 $GABA_B$ Signaling in FXS

The heightened excitatory signaling in *fmr1*-knockout mice also results from reduced inhibition. Reduced GABA signaling is evidenced by changes in the ligand-receptor expression, synaptic signaling, and circuit function. For example, GABA type A ($GABA_A$) receptor subunits and GABA synthetic enzyme levels are reduced in *fmr1*-knockout mice when compared to wild-type mice (Adusei et al. 2010; D'Hulst et al. 2006; El Idrissi et al. 2005). Defects in cortical inhibitory synaptic transmission were identified in inhibitory cortical neurons in juvenile *fmr1*-knockout mice (Gibson et al. 2008). Reduced amygdalar inhibition observed in *fmr1*-knockout mice (Olmos-Serrano et al. 2010), may model the excessive amygdalar activation during gaze processing observed in boys with FXS (Watson et al. 2008). These deficiencies in GABA-mediated inhibitory neurotransmission and the excessive activation in brain regions controlling emotion, behavior, and learning is proposed

to underlie the social anxiety and social avoidance behaviors of FXS. Based on this evidence, targeting GABA receptors may be therapeutic by directly restoring inhibitory tone in the FXS brain. Of particular interest is the metabotropic GABA type B ($GABA_B$) receptor, which regulates cell excitability through pre- and postsynaptic mechanisms (Isaacson and Hille 1997; Sohn et al. 2007). *Fmr1*-knockout mice treated with the $GABA_B$ receptor agonist baclofen showed reduced susceptibility to audiogenic seizures (Pacey et al. 2009). Presynaptic $GABA_B$ receptors on glutamatergic terminals inhibit glutamate release (Isaacson and Hille 1997) and therefore may reduce synaptic protein synthesis by acting upstream of postsynaptic mGluR5.

13.6 Disease-Modifying Effects of Arbaclofen in FXS Mice

The $GABA_B$ receptor agonist arbaclofen was tested in a similar battery of assays as those assessing the effects of limiting mGluR5 signaling in *fmr1*-knockout mice (Dolen et al. 2007). The goal of this work was to determine whether $GABA_B$ activation modifies the core pathophysiology of FXS. In the *fmr1*-knockout mouse, arbaclofen corrected dendritic spine density, audiogenic seizures, and exaggerated hippocampal protein synthesis (Henderson et al. 2012). The effect of arbaclofen on regional rates of protein synthesis in live *fmr1*-knockout mice was also corrected by acute arbaclofen treatment (Qin et al. 2015). Arbaclofen fully restored normal dendritic spine density in *fmr1*-knockout mice without significantly reducing spine density in wild-type littermates. During development, large numbers of spines are formed and pruned so that the density of dendritic spines is higher during development as compared with adults. Abnormal spine density and morphology have been reported in FXS brains examined postmortem and in other neurodevelopmental disorders including Down syndrome and schizophrenia (Suetsugu and Mehraein 1980; Garey et al. 1998). The increased density and immature morphology of dendritic spines in the *fmr1*-knockout mouse has led to the hypothesis that aspects of the disease may lie in the pruning or shaping of the neuronal network, the results of which persist throughout adulthood. The correction of the spine phenotype with arbaclofen occurred in mice post-weaning and after the peak of spinogenesis in the neocortex, suggesting it may be possible to correct an already established disease phenotype, an important goal for developing targeted treatments.

13.7 Clinical Data

Initial clinical assessments of arbaclofen indicated it is generally safe and well tolerated (Erickson et al. 2014). In addition, the significant safety data that existed with racemic baclofen supported testing arbaclofen in controlled trials including children and adolescents. The safety, tolerability, and efficacy of arbaclofen in FXS patients were first addressed in a Phase 2 randomized, double-blind, and placebo-controlled

study sponsored by Seaside Therapeutics. Arbaclofen was tested for improvement in behavior measured on the Aberrant Behavior Checklist-Irritability (ABC-I) subscale, a measure of irritable mood, aggression, and self-injury (ClinicalTrials.gov Identifier: NCT00788073). The endpoint was chosen based on regulatory precedent. Sixty-three FXS patients between the ages of 12 and 40 years (subsequently lowered to 6–40 years) with a molecular diagnosis of FXS were enrolled. Inclusion criteria were moderate or higher problem behavior based on the Clinical Global Impression-Severity (CGI-S) score and moderate to severe scores on the ABC-I. The treatment period with the optimal tolerated dose was 4 weeks, with titration periods before and after the 4-week period. Although the primary endpoint was not achieved (Berry-Kravis et al. 2012), an exploratory analysis of the study results using a revised ABC scoring algorithm, which was developed using a factor analysis specific for FXS, showed a drug benefit in one FXS subscale (Sansone et al. 2012). Within the ABC-Community Fragile X (C_{FX}) scale is an added measure for active social avoidance (SA). Here, a significant effect ($P = 0.01$) of arbaclofen treatment was observed in the intent-to-treat population using the ABC-SA scale, but not on other subscales of the aberrant behavior checklist-community fragile X (ABC-C_{FX}) scale (Berry-Kravis et al. 2012). Additional exploratory analyses suggested that FXS patients with more severe social impairments (ABC-Lethargy/Social Withdrawal (LSW) scores ≥8 at screening) showed greater improvement on the ABC-SA and the Vineland-Socialization measure of adaptive function, as well as on global assessments.

13.7.1 Arbaclofen for ASD and FXS

The safety, tolerability, and efficacy of arbaclofen in ASD patients was also addressed in a Phase 2 randomized, double-blind, and placebo-controlled study. Arbaclofen was tested for improvements in social withdrawal, using the ABC-LSW subscale (NCT01288716). One hundred and fifty ASD patients between the ages of 5 and 21 years with a diagnosis of ASD were enrolled. Inclusion criteria were reduced social functioning (as defined by an ABC-LSW subscale ≥8). The treatment period was 12 weeks, with titration periods before and after the optimal treatment period. There was no benefit of drug over placebo in the primary endpoint, ABC/LSW; however, there was a significant improvement with arbaclofen in a secondary measure, the CGI-S (presented in abstract by the American Academy of Pediatrics: Delahunty C et al. (2013)). An exploratory analysis suggested there was a significant improvement in the Vineland Adaptive Behavior Socialization (VABS) subscale, which appeared more pronounced in higher functioning participants. The noted improvements in social functioning in the FXS and ASD studies suggested revised thinking was required around suitable outcome measures to advance arbaclofen in clinical development.

Based on the findings and observations from the arbaclofen trials, two additional double-blind, placebo-controlled clinical trials tested arbaclofen for the treatment of

social withdrawal in adults and children with FXS (NCT01282268 and NCT01325220, respectively). One hundred and twenty-five FXS patients between the ages of 12 and 50 years with a molecular diagnosis of FXS were enrolled in a double-blind, placebo-controlled trial. The primary outcome was the ABC-SA subscale. A second, double-blind, placebo-controlled study of 172 FXS patients between the ages of 5 and 11 years was enrolled during the same period between 2011 and 2013. The primary outcome was the ABC-LSW subscale. Each trial tested an 8-week treatment period. It was notable that there were no exclusion criteria in either trial based on behavior. Neither study showed a benefit of drug over placebo in the primary behavioral endpoints. The detailed results of the arbaclofen efficacy studies and subsequent open-label extension studies have not been reported.

An mGluR5 antagonist, mavoglurant, sponsored by Novartis Pharmaceuticals, was concurrently being evaluated in large-scale controlled clinical trials (2011–2014) (Berry-Kravis et al. 2016). In these studies, improvements in behavior were measured using the ABC-C_{FX} as the primary endpoint. The first study enrolled 175 adult FXS patients between 18 and 45 years ((NCT01348087) and a second study enrolled 139 adolescent FXS patients between 12 and 17 years of age (NCT01433354). Based on the post hoc analysis of a preceding Phase 2b study, inclusion criteria stratified patients based on molecular evidence of a fully methylated gene, a proposed novel biomarker of responders (Jacquemont et al. 2011). Treatment duration was 12 weeks. Neither study showed a benefit of drug over placebo in the primary endpoint, ABC-C_{FX} (Berry-Kravis et al. 2016). An exploratory analysis considering data from the adult and adolescent trials and including open-label extension data examined the global improvement narratives from the clinical global impression scales. The goal of this analysis was to glean information that was not captured in some of the other instruments including anxiety, behavior and mood, and communication (Bailey et al. 2016). The analysis of the narratives did not reveal a specific drug effect in any of the improvement domains examined (Bailey et al. 2016).

A second mGluR5 antagonist, basimglurant, sponsored by Roche Pharmaceuticals, was also tested for efficacy between 2012 and 2014 (NCT01517698). One hundred and eighty-five FXS patients between the ages of 14 and 50 years with a molecular diagnosis of FXS were enrolled in a double-blind, placebo-controlled trial. The primary outcome was the change in Anxiety Depression and Mood Scale. Patients were randomized to receive treatment for 12 weeks. Results of this study were not made available; however, the Roche program was halted suggesting there were no specific beneficial drug effects noted.

Many questions remain unanswered following the $GABA_B$ and mGluR trials for FXS. For example, the doses selected for the $GABA_B$ and mGluR trials were not based on pharmacodynamics measures of effect and therefore leave open the question of whether the optimal dose regimen was achieved. The use of concomitant medications in all trials may also have confounded results. Another concern is that the blinded arms of the treatment periods were brief when considering that FXS phenotypes develop over the course of years, and therefore the treatment periods may not have had sufficient time to affect the FXS neural circuitry. Moreover, the critical period for learning and memory in FXS patients is not completely understood

and treatments may need to be tested in much younger individuals. This raises challenges for study design because safety and efficacy assessments must be completed in adults prior to initiating studies in the pediatric population.

13.8 Developing Objective Outcome Measures for FXS

The heterogeneous nature of FXS phenotypes has contributed to the recent clinical failures. In the aftermath of these studies, an understanding has grown that success will be achieved after objective and specific outcome measures are developed. A common anecdotal benefit often noted in the arbaclofen trial was improved language skills. Although anecdotal evidence of effect is often misleading, recent efforts are focused on language competence (Channell et al. 2015). Improvements in expressive language may reflect a positive effect on cognition or on behavior, either result would yield a meaningful benefit to patients. Many of the rating scales used as outcome measures for the FXS trials were initially developed as diagnostics and in hindsight may not have been sensitive to change during the short course of treatment. Validating outcome measures specific to FXS, like the ABC-C_{FX}, and also sensitive to change over a short duration are also key. EEG coherence, eye tracking, and transcranial magnetic resonance (TMS) are all being explored as potential measures for FXS and other ASDs (Devitt et al. 2015; Farzin et al. 2011; Oberman et al. 2010).

The broad spectrum of phenotypes, the relatively short duration of double-blind clinical trials, and existing outcome measures are all now understood to be challenges that must be addressed to advance new therapies for FXS. As we continue to test new therapies affecting multiple neural circuits in FXS and ASD, it is likely that a host of targets will require modulation to affect change, and biomarkers of disease will be important to advance a more personalized treatment regime for FXS patients.

References

Adusei, D. C., Adusei, D. C., Pacey, L. K., Chen, D., & Hampson, D. R. (2010). Early developmental alterations in GABAergic protein expression in fragile X knockout mice. *Neuropharmacology, 59*(3), 167–171.

Bailey, D. B., Jr., Berry-Kravis, E., Wheeler, A., Raspa, M., Merrien, F., Ricart, J., et al. (2016). Mavoglurant in adolescents with fragile X syndrome: Analysis of clinical global impression-improvement source data from a double-blind therapeutic study followed by an open-label, long-term extension study. *Journal of Neurodevelopmental Disorders, 8*, 1.

Berry-Kravis, E., Des Portes, V., Hagerman, R., Jacquemont, S., Charles, P., Visootsak, J., et al. (2016). Mavoglurant in fragile X syndrome: Results of two randomized, double-blind, placebo-controlled trials. *Science Translational Medicine, 8*(321), 321ra5.

Berry-Kravis, E. M., Hessl, D., Zarevics, P., Cherubini, M., Walton-Bowen, K., Mu, Y., et al. (2012). Effects of STX209 (arbaclofen) on neurobehavioral function in children and adults with fragile X syndrome: A randomized, controlled, phase 2 trial. *Science Translational Medicine, 4*(152), 152ra127.

Bhattacharya, A., Kaphzan, H., Alvarez-Dieppa, A. C., Murphy, J. P., Pierre, P., & Klann, E. (2012). Genetic removal of p70 S6 kinase 1 corrects molecular, synaptic, and behavioral phenotypes in fragile X syndrome mice. *Neuron, 76*(2), 325–337.

Bourgeois, J. A., Seritan, A. L., Casillas, E. M., Hessl, D., Schneider, A., Yang, Y., et al. (2011). Lifetime prevalence of mood and anxiety disorders in fragile X premutation carriers. *Journal of Clinical Psychiatry, 72*(2), 175–182.

Channell, M. M., McDuffie, A. S., Bullard, L. M., & Abbeduto, L. (2015). Narrative language competence in children and adolescents with Down syndrome. *Frontiers in Behavioral Neuroscience, 9*, 283.

Coffee, B., Keith, K., Albizua, I., Malone, T., Mowrey, J., Sherman, S. L., & Warren, S. T. (2009). Incidence of fragile X syndrome by newborn screening for methylated FMR1 DNA. *American Journal of Human Genetics, 85*(4), 503–514.

D'Hulst, C., De Geest, N., Reeve, S. P., Van Dam, D., De Deyn, P. P., et al. (2006). Decreased expression of the GABAA receptor in fragile X syndrome. *Brain Research, 1121*(1), 238–245.

Dolen, G., Osterweil, E., Rao, B. S., Auerbach, B. D., Chattarji, S., & Bear, M. F. (2007). Correction of fragile X syndrome in mice. *Neuron, 56*(6), 955–962.

Eberhart, D. E., Malter, H. E., Feng, Y., & Warren, S. T. (1996). The fragile X mental retardation protein is a ribonucleoprotein containing both nuclear localization and nuclear export signals. *Human Molecular Genetics, 5*(8), 1083–1091.

El Idrissi, A., Ding, X. H., Scalia, J., Trenkner, E., Brown, W. T., & Dobkin, C. (2005). Decreased GABA(A) receptor expression in the seizure-prone fragile X mouse. *Neuroscience Letters, 377*(3), 141–146.

Erickson, C. A., Veenstra-Vanderweele, J. M., Melmed, R. D., McCracken, J. T., Ginsberg, L. D., Sikich, L., et al. (2014). STX209 (arbaclofen) for autism spectrum disorders: An 8-week open-label study. *Journal of Autism and Developmental Disorders, 44*(4), 958–964.

Farzin, F., Scaggs, F., Hervey, C., Berry-Kravis, E., & Hessl, D. (2011). Reliability of eye tracking and pupillometry measures in individuals with fragile X syndrome. *Journal of Autism and Developmental Disorders, 41*(11), 1515–1522.

Fridell, R. A., Benson, R. E., Hua, J., Bogerd, H. P., & Cullen, B. R. (1996). A nuclear role for the fragile X mental retardation protein. *The EMBO Journal, 15*(19), 5408–5414.

Garey, L. J., Ong, W. Y., Patel, T. S., Kanani, M., Davis, A., et al. (1998). Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. *Journal of Neurology, Neurosurgery, and Psychiatry, 65*(4), 446–453.

Gibson, J. R., Bartley, A. F., Hays, S. A., & Huber, K. M. (2008). Imbalance of neocortical excitation and inhibition and altered UP states reflect network hyperexcitability in the mouse model of fragile X syndrome. *Journal of Neurophysiology, 100*(5), 2615–2626.

Greenough, W. T., Klintsova, A. Y., Irwin, S. A., Galvez, R., Bates, K. E., et al. (2001). Synaptic regulation of protein synthesis and the fragile X protein. *Proceedings of the National Academy of Sciences of the United States of America, 98*(13), 7101–7106.

Hagerman, R. J., Berry-Kravis, E., Kaufmann, W. E., Ono, M. Y., Tartaglia, N., et al. (2009). Advances in the treatment of fragile X syndrome. *Pediatrics, 123*(1), 378–390.

Harris, S. W., Hessl, D., Goodlin-Jones, B., Ferranti, J., Bacalman, S., Barbato, I., et al. (2008). Autism profiles of males with fragile X syndrome. *American Journal of Mental Retardation, 113*(6), 427–438.

Henderson, C., Wijetunge, L., Kinoshita, M. N., Shumway, M., Hammond, R. S., Postma, F. R., et al. (2012). Reversal of disease-related pathologies in the fragile X mouse model by selective activation of GABAB receptors with arbaclofen. *Science Translational Medicine, 4*(152), 152ra128.

Huber, K. M., Gallagher, S. M., Warren, S. T., & Bear, M. F. (2002). Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proceedings of the National Academy of Sciences of the United States of America, 99*(11), 7746–7750.

Jacquemont, S., Curie, A., des Portes, V., Torrioli, M. G., Berry-Kravis, E., Hagerman, R. J., et al. (2011). Epigenetic modification of the FMR1 gene in fragile X syndrome is associated with

differential response to the mGluR5 antagonist AFQ056. *Science Translational Medicine, 3*(64), 64ra1.

Kaufmann, W. E., Cortell, R., Kau, A. S., Bukelis, I., Tierney, E., Gray, R. M., et al. (2004). Autism spectrum disorder in fragile X syndrome: Communication, social interaction, and specific behaviors. *American Journal of Medical Genetics. Part A, 129A*(3), 225–234.

Kidd, S. A., Lachiewicz, A., Barbouth, D., Blitz, R. K., Delahunty, C., McBrien, D., et al. (2014). Fragile X syndrome: A review of associated medical problems. *Pediatrics, 134*(5), 995–1005.

Michalon, A., Sidorov, M., Ballard, T. M., Ozmen, L., Spooren, W., Wettstein, J. G., et al. (2012). Chronic pharmacological mGlu5 inhibition corrects fragile X in adult mice. *Neuron, 74*(1), 49–56.

Oberman, L., Ifert-Miller, F., Najib, U., Bashir, S., Woollacott, I., Gonzalez-Heydrich, J., et al. (2010). Transcranial magnetic stimulation provides means to assess cortical plasticity and excitability in humans with fragile x syndrome and autism spectrum disorder. *Frontiers in Synaptic Neuroscience, 2*, 26.

Olmos-Serrano, J. L., Paluszkiewicz, S. M., Martin, B. S., Kaufmann, W. E., Corbin, J. G., & Huntsman, M. M. (2010). Defective GABAergic neurotransmission and pharmacological rescue of neuronal hyperexcitability in the amygdala in a mouse model of fragile X syndrome. *The Journal of Neuroscience, 30*(29), 9929–9938.

Osterweil, E. K., Krueger, D. D., Reinhold, K., & Bear, M. F. (2010). Hypersensitivity to mGluR5 and ERK1/2 leads to excessive protein synthesis in the hippocampus of a mouse model of fragile X syndrome. *The Journal of Neuroscience, 30*(46), 15616–15627.

Qin, M., Huang, T., Kader, M., Krych, L., Xia, Z., Burlin, T., et al. (2015). R-baclofen reverses a social behavior deficit and elevated protein synthesis in a mouse model of fragile X syndrome. *The International Journal of Neuropsychopharmacology, 18*(9).

Roberts, J. E., McCary, L., Shinkareva, S. V., & Bailey, D. B., Jr. (2016). Infant development in fragile X syndrome: Cross-syndrome comparisons. *Journal of Autism and Developmental Disorders, 46*(6), 2088–2099.

Sansone, S. M., Widaman, K. F., Hall, S. S., Reiss, A. L., Lightbody, A., Kaufmann, W. E., et al. (2012). Psychometric study of the aberrant behavior checklist in fragile X syndrome and implications for targeted treatment. *Journal of Autism and Developmental Disorders, 42*(7), 1377–1392.

Sohn, J. W., Lee, D., Cho, H., Lim, W., Shin, H. S., Lee, S. H., et al. (2007). Receptor-specific inhibition of GABAB-activated K+ currents by muscarinic and metabotropic glutamate receptors in immature rat hippocampus. *The Journal of Physiology, 580*(Pt. 2), 411–422.

Talisa, V. B., Boyle, L., Crafa, D., & Kaufmann, W. E. (2014). Autism and anxiety in males with fragile X syndrome: An exploratory analysis of neurobehavioral profiles from a parent survey. *American Journal of Medical Genetics. Part A, 164A*(5), 1198–1203.

Verkerk, A. J., Pieretti, M., Sutcliffe, J. S., Fu, Y. H., Kuhl, D. P., Pizzuti, A., et al. (1991). Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell, 65*(5), 905–914.

Wadell, P. M., Hagerman, R. J., & Hessl, D. R. (2013). Fragile X syndrome: Psychiatric manifestations, assessment and emerging therapies. *Current Psychiatry Reviews, 9*(1), 53–58.

Watson, C., Hoeft, F., Garrett, A. S., Hall, S. S., & Reiss, A. L. (2008). Aberrant brain activation during gaze processing in boys with fragile X syndrome. *Archives of General Psychiatry, 65*(11), 1315–1323.

Wheeler, A. C., Raspa, M., Bishop, E., & Bailey, D. B., Jr. (2016). Aggression in fragile X syndrome. *Journal of Intellectual Disability Research, 60*(2), 113–125.

Zablotsky, B., Black, L. I., Maenner, M. J., Schieve, L. A., & Blumberg, S. J. (2014). Estimated prevalence of autism and other developmental disabilities following questionnaire changes in the 2014 National Health Interview Survey. *National Health Statistics Reports, 2015*(87), 1–20.

Chapter 14
Targeting the $GABA_B$ Receptor for the Treatment of Substance Use Disorder

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Abstract This chapter reviews the current preclinical research on the significance of central γ-amino butyric acid $(GABA)_B$ receptors in substance use disorder (SUD) and the SUD potential pharmacotherapy based on $GABA_B$ receptor ligands. We focused on the role of $GABA_B$ receptors in the effects of psychostimulants, opioids, and nicotine in various preclinical behavioral models used in drug addiction research. We provide an overview of the neurochemical mechanisms underlying the interactions between $GABA_B$ receptors localized to the mesocorticolimbic dopamine system and drugs of abuse. Finally we discuss the efficacy of $GABA_B$ receptor agonists in treatment of human drug addicts. On the whole, the presented data provide compelling preclinical evidence that $GABA_B$ receptor agonists and positive allosteric modulators may have efficacy in the treatment of SUD, with $GABA_B$ receptor positive allosteric modulators having a better pharmacological profile than orthosteric agonists. There is the need for additional clinical studies to ascertain whether preclinical data translate to the human.

Keywords $GABA_B$ receptor ligands • Preclinical research • Clinical trials • Psychostimulants • Opioids • Nicotine

14.1 Introduction

In the twenty-first century one of the unsolved medical problems is substance use disorder (SUD), also known as "drug addiction." SUD is a brain disease in which psychoactive drug(s)—being either legal drugs approved for therapeutic use or used for non-medical purposes or illegal drugs that evoke clinically significant impairments or distress due to out-of-control drug use, despite negative consequences. This brain disease develops from repeated drug administration and results

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in withdrawal upon cessation of drug use. According to the current classification found in the Diagnostic and Manual of Mental Disorders (DSM 5), SUD means substance abuse and substance dependence/substance addiction. As indicated by the Global Burden of Disease Study in 2013 drug use disorders resulted in 127,000 deaths. During 1990 until 2013 the highest increase in the number of deaths resulted from opioid use disorder (187 %), cocaine use disorder (77 %), amphetamine use disorder (80 %), and other drug use disorders, including alcohol and nicotine use disorders (123 %) (Vos et al. 2015).

14.2 Psychostimulants, Opioids, and Nicotine: Mechanisms of Action to Develop Abuse and Addiction

Among several legal and illegal substances, three different classes are currently most widely used: psychostimulants, opioids, and nicotine. Psychostimulants [e.g., cocaine and amphetamines, like methamphetamine and 3,4-methylenedioxymetha mphetamine (MDMA=ecstasy)] are drugs that in humans temporarily enhance the activity of the brain with the feeling of euphoria. The most prominent pharmacological mechanism of psychostimulants is the facilitation of dopaminergic neurotransmission; however, these drugs also enhance noradrenaline and/or serotonin activity (for review Fleckenstein et al. 2007; Sulzer 2011). Psychostimulants are either "releasers" (amphetamines) or "uptake blockers" (cocaine) based on the mechanism of their acute effect on neurotransmitter flux. Among releasers that induce stimulation-independent dopamine release (Sitte et al. 1998; Riddle et al. 2002), amphetamine and methamphetamine mainly reverse the transport across the dopamine transporter (in lower extracellular concentrations; Sitte et al. 1998), diffuse into the cell due to lipophilicity (Sulzer et al. 1995; Kahlig et al. 2005), or inhibit the dopamine transporter and the monoamine transporter 2 (VMAT-2). Other releasers, like MDMA, act either as a dopamine releaser in mice (Rothman and Baumann 2003) or as a serotonin (5-HT) neurotoxin in nonhuman primates and rats due to enhanced 5-HT release and depletion of 5-HT terminal markers (reviewed by Green et al. 2003; see Fig. 14.1a).

Cocaine, being the representative of the uptake blockers, binds to the dopamine transporter, inhibits dopamine reuptake, and results in an increase in synaptic and extrasynaptic dopamine levels (Brown et al. 2001; see Fig. 14.1a). As shown recently, cocaine in low (1–10 nM) concentrations is an allosteric agonist at dopamine receptors (Ferraro et al. 2010). Apart from being the dopamine transporter blocker, cocaine inhibits the noradrenaline and 5-HT transporters, as well (Gatley and Volkow 1998).

Opioids (morphine, heroin, oxycodone, methadone, pentanyl, tramadol, pethidine, dextropropoxyphene.) in humans induce feelings of well-being and pain relief, while their long-term repeated use leads to tolerance and addiction. Opiate intake is linked with a strong withdrawal symptom that includes both somatic and affective components. Opioids acts as μ, δ, and k opioid receptor agonists, and the former two receptors are linked with drug pleasure (see Fig. 14.1b).

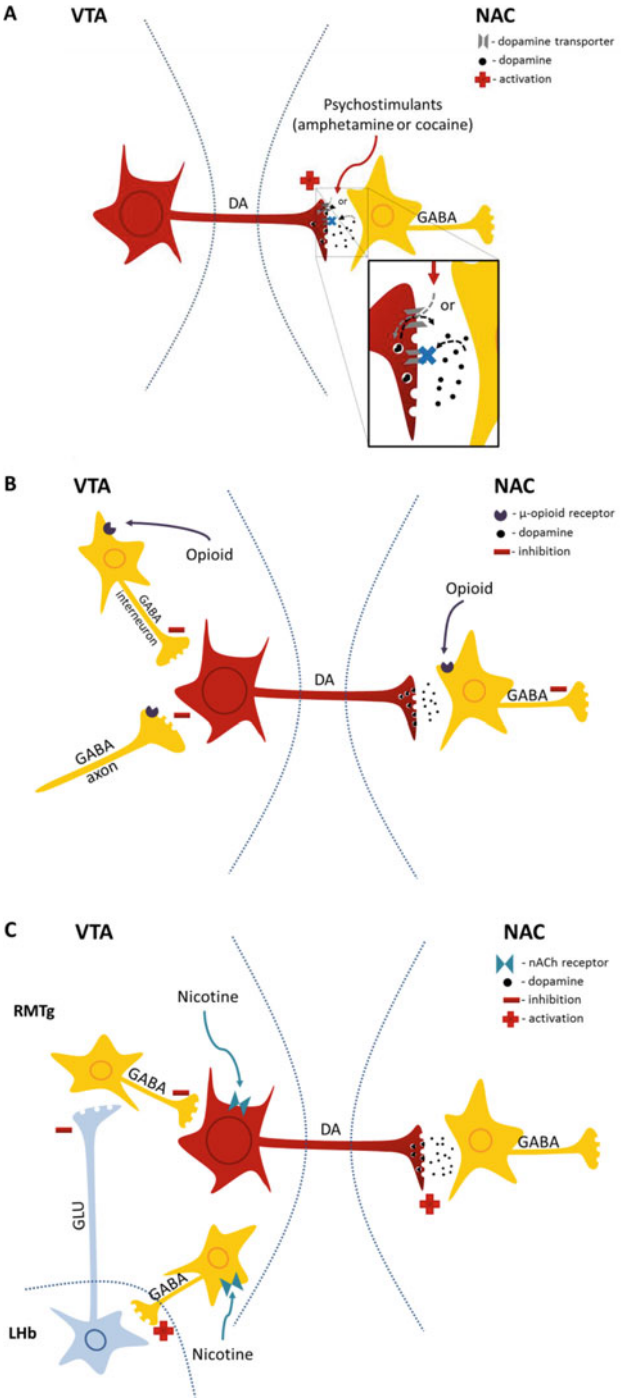

Fig. 14.1 Primary mechanism of action for psychostimulants (**a**), opioids (**b**), and nicotine (**c**). Please refer Sect. 14.2 for detailed explanation. *NAC* nucleus accumbens, *LHb* lateral habenula, *RMTg* rostromedial tegmental nucleus, *VTA* ventral tegmental area

Nicotine intake by humans is linked with its reinforcing efficacy, increasing vigilance, learning abilities, and memory performance (Zaniewska et al. 2009). The mechanism of the pharmacological action of nicotine is based on the stimulation of ionotropic nicotinic acetylcholine (nACh) receptors, especially those composed of α4ß2, α6ß2, or α7 subunits which play an important role in the reward-related effects of nicotine. The above nACh receptors are either somatodendritic or presynaptic heteroreceptors [localized on the 5-HT and dopamine terminals in the striatum, on glutamatergic terminals in the midbrain, and on γ-amino butyric acid (GABA)-ergic terminals in the ventral tegmental area (VTA)] in which stimulation elevates the release of different neurotransmitters. The nACh receptors are found also postsynaptically where they are present on dopamine and GABA cell bodies in the VTA; their local activation mediates fast excitation (see Fig. 14.1c).

The aforementioned abused drugs, regardless of their primary mechanism of action, activate the brain reward pathway within the mesolimbic circuitry of the brain; these effects are seen after acute (see above) or chronic drug exposure (Filip et al. 2010; Sulzer 2011). The reward pathway is composed of dopaminergic neurons that project from the VTA to several cortical and subcortical structures. The key structure that executes motivation and learning processes related to reward-forming habits and is implicated in the addictive effects of the drugs, is the nucleus accumbens, the terminal of the dopaminergic mesoaccumbal pathway (Di Chiara et al. 1999). As found, psychostimulants acting directly on dopaminergic terminals enhance the release of dopamine (by use of amphetamines) or inhibit the reuptake of this neurotransmitter (by use of cocaine) in the nucleus accumbens (Fig. 14.1a). Following opioid exposure enhanced dopamine extrasynaptic levels in the nucleus accumbens results from their indirect effect leading to the disinhibition of VTA dopaminergic neurons by inhibiting local GABA interneurons or GABA terminals that comprise opioid receptors or by the direct action at the end of the reward pathway via activation of opioid receptors localized to accumbal cholinergic or GABA neurons (reviewed in Wise 1987; Sulzer 2011) (Fig. 14.1b). Nicotine activates the VTA dopamine cell bodies and prolongs the dopamine neuron firing via stimulation of nACh receptors containing α4 and β2 subunits, being localized on dopamine neurons or on GABA-ergic interneurons in the VTA. The latter stimulation may lead to disinhibition of dopamine neurons due to (a) desensitization of nACh receptors on the GABA neuron followed by reduced GABA-ergic neuronal activity (Mansvelder et al. 2002), (b) activation of the β2 subunit of nACh receptors localized to GABA-ergic neurons in which stimulation inhibits the lateral habenula glutamatergic inputs to the rostromedial VTA nucleus (Hong et al. 2011; Lecca et al. 2011; Tolu et al. 2013) (Fig. 14.1c).

Data from the literature (pre-clinical studies and clinical trials) of recent years have provided several lines of evidence that GABA neurotransmission and its $GABA_B$ receptors play a inhibitory role in relation to tegmental dopamine neurons, what functionally may counteract the behavioral readouts of drugs of abuse and could be beneficial in the treatment of SUD (Filip and Frankowska 2008; Filip et al. 2015).

14.3 $GABA_B$ Receptors and Drugs of Abuse

Several selective and potent $GABA_B$ receptor orthosteric ligands (agonists, antagonists) and positive allosteric modulators have been discovered (see Chaps. 2, 3, and 18 of this book). Orthosteric ligands connect to the $GABA_{B1}$ subunit, binding to N-terminal domain in a binding site, which leads to the activation of G-proteins in $GABA_{B2}$ subunit on the cytoplasmic side of the transmembrane domains (see Gassmann and Bettler 2012). The $GABA_{B2}$ subunit contains a binding site for positive allosteric modulators for which binding pocket is located in transmembrane domain of the $GABA_{B2}$ subunit (see Pin and Prézeau 2007; see also Chap. 4 of this book). Positive allosteric modulators have little or no agonistic activity of their own, but induced changes in the receptor protein and affect both potency and efficacy of orthosteric agonists of $GABA_B$ receptors (see Chaps. 3 and 18 of this book).

14.3.1 Preclinical Methods and Procedures to Study SUD

Preclinical behavioral studies of the addictive drugs are performed in animal models, in which psychomotor stimulatory, subjective, and rewarding effects of the drugs can be demonstrated. For example, some addictive drugs, particularly psychostimulants, increase locomotor activity, usually monitored as horizontal activity in mice and rats. At the same time repeated, intermittent exposure to drugs of abuse is known to induce sensitization characterized by an increase in the number of behavioral events, including locomotor hyperactivity when a challenge dose of the drug is readministered after the repeated treatment regimen was discontinued. It has been suggested that the sensitization paradigm models drug craving (Robinson and Berridge 2001) though some objections to such conclusion have been raised (Steketee and Kalivas 2011).

Drugs of abuse produce an interoceptive stimulus that allows animals to distinguish the drug from its vehicle in the drug discrimination test. In this model, an animal is trained to perform a certain instrumental reaction (e.g., lever pressing) in response to a conditioned stimulus, signaling availability of the reinforcer (e.g., water in water-deprived animals). After a long training, animals discriminate between the training substance (e.g., the psychostimulant) and its vehicle. Interestingly, the use of different psychostimulants (or other drugs of abuse) can resemble to some extent the addiction cycle in humans with the alternating phases of maintenance and reinstatement. In this model the substitution or combination (augmentation or antagonism) tests can be performed.

Intracranial self-stimulation (ICSS) is a method used to study the neural mechanisms of reinforcement. In this model, animals are trained to deliver electrical stimulation (eliciting positive reinforcement) to certain regions of the brain by performing an operant response (e.g., lever pressing). ICSS can be measured

as self-stimulation rate (responses/min) or current threshold. Many studies reported that drugs of abuse facilitated the self-stimulation responding with electrodes placed in several brain structures, including the substantia nigra, nucleus accumbens, locus coeruleus, dorsal noradrenergic bundle, and medial forebrain bundle of the lateral hypothalamus (Fibiger and Phillips 1974; Wise 1978; Aulakh et al. 1979; Markou and Koob 1991).

Conditioned place preference (CPP) is a model for testing the addictive reward properties of the psychostimulants or other drugs of abuse. In the first phase of CPP (pretest), animals are allowed a 15–25 min access to a two-compartment apparatus. In the second phase (conditioning) animals are given repeated injections of the drug in one chamber and vehicle in the other. In a postconditioning phase, animals are tested for expression of CPP by allowing them full access to both compartments in the absence of the drug (preference posttest; retrieval trial). Following the posttest, animals can be introduced to the extinction sessions during which they are given vehicle injection and are placed in the apparatus with access to both compartments. After reaching "no-preference" criterion (Fricks-Gleason and Marshall 2008), reinstatement tests (induced by the drug or stress) can be conducted.

The most frequently used test to examine drugs of abuse is the drug self-administration model. In this technique, an animal is introduced into the chamber to self-administer intravenously a drug using operant responding (a lever press or nose poke). There are different operant responding of drug self-administration including fixed ratio (FR, completion of the FR requirement, usually ranging from 1 to 5, results in drug infusion) or progressive ratio (PR, drug infusion is contingent on exponential increase in response requirements) schedules, with the latter method used to measure the motivating effect of drug reinforcement. The drug self-administration paradigm consists of several experimental phases: acquisition, maintenance, extinction, and reinstatement. As far as maintenance phase allows us to study the rewarding properties of the drugs of abuse, the reinstatement of drug-seeking behavior—precipitated by the administration of the priming dose of the addictive drug, presentation of drug-associated cues or stress—models some aspects of relapse.

14.3.2 $GABA_B$ Receptors and Psychostimulants

14.3.2.1 Locomotor Responses

There are only a few data on the effect of $GABA_B$ receptor agonists or positive allosteric modulators on sensitization to the locomotor stimulant effect of cocaine. Thus, Frankowska et al. (2009) have shown that baclofen and SKF 97541, the $GABA_B$ receptor agonists, attenuated both development and expression of the cocaine-induced sensitization in rats, whereas Lhuillier et al. (2007) have demonstrated that GS39783, the $GABA_B$ positive allosteric modulator, inhibited acquisition, but not expression, of sensitization in mice.

Similar effects were found in the case of the sensitization to the hyperlocomotion induced by amphetamine. Actually, Bartoletti et al. (2004, 2005) as well as Cedillo and Miranda (2013) have shown that baclofen prevented both development and expression of the amphetamine sensitization in rats. Moreover, the latter authors have also observed that CGP7930 increased antisensitizing effect of baclofen. Importantly, the above effects of the $GABA_B$ stimulants were observed after their administration in doses which did not affect basal locomotor activity, but attenuated cocaine- or amphetamine-induced locomotor hyperactivity (Bartoletti et al. 2004; Lhuillier et al. 2007; Frankowska et al. 2009). It should also be underlined that the antisensitizing effects of $GABA_B$ stimulants were not examined in animals pretreated with $GABA_B$ receptor antagonists.

14.3.2.2 Drug Discrimination

In contrast to sensitization, $GABA_B$ receptor ligands did not affect discriminative stimulus properties of psychostimulants in drug discrimination paradigm. In fact, it has been reported that the $GABA_B$ receptor antagonist (SCH 50911), agonists (baclofen, SKF 97541), or positive allosteric modulator (CGP7930) neither mimics cocaine or methamphetamine discriminative effects in substitution studies nor modifies the effect of the psychostimulant in combination experiments (Filip et al. 2007; Munzar et al. 2007).

At the same time Miranda et al. (2009) examined the drug discriminative effects of amphetamine using conditioned taste aversion procedure. They found that baclofen did not substitute for the psychostimulant but in combination studies it decreased amphetamine-induced discriminative cue. Moreover, they also found that the above inhibitory effect of baclofen was blocked by the $GABA_B$ receptor antagonist, 2-hydroxysalcofen.

14.3.2.3 Reward and Reinforcement

Inhibitory effect of $GABA_B$ stimulants on rewarding activity of psychostimulants has been demonstrated in CPP paradigm. In fact, Li et al. (2001), using a biased 8-day schedule of conditioning, have shown that baclofen attenuated the development and expression of methamphetamine-induced CPP in rats. The expression of CPP produced by the psychostimulant has also been reduced by two $GABA_B$ positive allosteric modulators, GS39783 and CGP7930, which were administered for 2 days during the early withdrawal phase after 6-day methamphetamine conditioning (Voigt et al. 2011a). Finally, Halbout et al. (2011) have recently reported that baclofen and GS39783 blocked the expression of amphetamine CPP without affecting locomotor activity. At the same time Voigt et al. (2011b) have demonstrated that baclofen facilitated the extinction of methamphetamine-induced CPP in rats. Again, the above effects of the $GABA_B$ stimulants were not examined in the presence of $GABA_B$ receptor antagonists.

Some other reports have shown that $GABA_B$ receptor antagonists (CGP56433A, SCH 50911) do not affect cocaine intake in self-administration paradigm (Brebner et al. 2002; Filip et al. 2007), indicating that tonic activation of these receptors is not involved in rewarding activity of the psychostimulant. On the other hand, several data indicate that $GABA_B$ receptor agonists and positive allosteric modulators attenuate cocaine and amphetamines self-administration in rodents with different schedules of reinforcement (FR or PR) used to examine particular aspects of the psychostimulants self-administration. In fact, baclofen, CGP44532, SKF 97541, CGP7930, and GS39783 produce suppression of cocaine or amphetamines intake in FR schedule (Brebner et al. 2000a; Brebner et al. 2005; Filip et al. 2007; Roberts et al. 1996; Shoaib et al. 1998; Ranaldi and Poeggel 2002; Campbell et al. 1999; Smith et al. 2004). Since it has also been shown that in FR schedule baclofen shifts the cocaine dose–response curve downward, particularly at lower unit injection dose of the psychostimulant, the above results indicate that the activation of $GABA_B$ receptors reduce rewarding activity of the psychostimulants (Brebner et al. 2000a). Such a conclusion is further supported by results of the experiments in PR schedule in which baclofen, CGP44532, and CGP35024 have been shown to decrease cocaine-reinforced break points regardless of the unit injection dose of the psychostimulant (Roberts et al. 1996; Brebner et al. 1999). Two other points should be raised in context of the inhibitory effect of $GABA_B$ stimulants on reinforcing effects of the psychostimulants. First, such inhibitory effects of baclofen, SKF 97541, and CGP7930 were blocked by the $GABA_B$ receptor antagonists SCH 50911 or CGP56433A, showing that the effects of the above drugs do depend on the activation of $GABA_B$ receptors (Brebner et al. 2002; Filip et al. 2007). Second, whereas $GABA_B$ receptor agonists are characterized by relatively low selectivity for motivational behaviors (psychostimulants versus food), positive allosteric modulators of these receptors do not affect food self-administration (Brebner et al. 1999; Filip et al. 2007).

It is also very well known that psychostimulants lower current threshold in ICSS model. Interestingly, Slattery et al. (2005) have found that baclofen and GS39783 attenuated threshold lowering effect of cocaine for ICSS of the medial forebrain bundle, indicating their inhibitory effect on rewarding activity of the psychostimulant. It should be underlined, however, that as far as GS39783 had no intrinsic effect on ICSS reward threshold, baclofen dose-dependently elevated the threshold (Slattery et al. 2005; Willick and Kokkinidis 1995).

14.3.2.4 Seeking Behavior

It has also been shown that $GABA_B$ receptor ligands affect the reinstatement of cocaine-seeking behavior evoked by the psychostimulant priming or by cocaine-associated cue in self-administration model. Accordingly, only one paper demonstrates that the $GABA_B$ receptor antagonist SCH 50911, having no effect on food-seeking behavior, attenuated cocaine- or cue-induced reinstatement of

cocaine seeking (Filip and Frankowska 2007). Moreover, the specificity of the antagonistic effect of SCH 50911 on cocaine seeking is further supported by the observations that it does not affect discriminative stimulus of cocaine or locomotor activity (Filip et al. 2007).

In concordance, there are several reports (Campbell et al. 1999; Di Ciano and Everitt 2003; Filip and Frankowska 2007; Froger-Colléaux and Castagné 2016) demonstrating that cocaine-seeking behavior was reduced or blocked by $GABA_B$ receptor agonists (baclofen and SKF 97541) or positive allosteric modulator (CGP7930). Interestingly, cocaine seeking induced by the psychostimulant priming was prevented by the $GABA_B$ receptor agonists administered in doses two to three times lower than that necessary to antagonize cocaine seeking evoked by the conditioned stimulus, whereas the positive allosteric modulator was more efficient in preventing cue-induced cocaine seeking (Filip and Frankowska 2007). It should also be underlined that as far as the $GABA_B$ receptor agonists prevented food-seeking behavior—though in doses much higher than those needed to antagonize cocaine seeking—the positive allosteric modulator was devoid such an activity (Filip and Frankowska 2007).

It is unclear why antagonists of $GABA_B$ receptors from one the side and $GABA_B$ receptor agonists or positive allosteric modulators on the other side induce to some extent similar inhibitory effects on cocaine seeking. Nevertheless it cannot be excluded (Filip and Frankowska 2007) that the $GABA_B$ antagonist acting on presynaptic $GABA_B$ autoreceptors located on GABA terminals in the substantia nigra (Boyes and Bolam 2003) or in the striatum (Lacey et al. 2005) may release GABA, which stimulate postsynaptic $GABA_B$ receptors.

14.3.3 $GABA_B$ Receptors and Opioids

14.3.3.1 Locomotor Responses

The administration of opioids produces a robust enhancement of locomotor activity in mice (Contet et al. 2008) and delay in time hyperactivity in rats (Smith et al. 2009). The morphine-enhancing locomotor activity was dose dependently inhibited the $GABA_B$ receptor agonist baclofen in rodents (Woo et al. 2001; Fu et al. 2012). The inhibitory action of baclofen was also present in morphine sensitization regimen in which the $GABA_B$ receptor agonist was given during several days in combination with morphine (development of sensitization) or acutely before the morphine challenge dose (expression of sensitization) in rats (Bartoletti et al. 2007; Fu et al. 2012). Interestingly, the neuroanatomical target of the above interaction is the VTA as baclofen given into this structure during morphine treatment blocked the acquisition of morphine-induced motor sensitization which neurochemically correlated with the reduction of the activation of accumbal shell neurons (Leite-Morris et al. 2004).

14.3.3.2 Drug Discrimination

To date, $GABA_B$ receptors have received little attention in the discriminative properties of opioids. As evidenced, the stimulation of $GABA_B$ receptor by baclofen (in doses which do not evoke behavioral disruption) did not alter the discriminative stimulus effects of heroin in rats (Solecki et al. 2005). The latter authors have also reported the lack of baclofen effects in combination with heroin as the $GABA_B$ receptor agonist did not change the discriminative stimulus of the training drug. On the other hand, acute or chronic baclofen pretreatment (in much lower doses than that used in the above studies) in rats trained to discriminate morphine from vehicle attenuated drug-lever responding and response rate (Bartoletti et al. 2010). However one caveat could be the methodology used including rat strain, type of reinforce, and reinforcement schedule that may explain the differences in baclofen efficacy to counteract or not the morphine subjective properties.

14.3.3.3 Reward and Reinforcement

Several groups have shown that the $GABA_B$ receptor agonist baclofen administered repeatedly blocked the rewarding effects of morphine measured by acquisition of CPP in mice, ferrets, and rats (Kaplan et al. 2003; Suzuki et al. 2005;Sahraei et al. 2005). Additionally, Heinrichs and coworkers (2010) observed that (a) baclofen injected during extinction training disrupted reconsolidation of conditioned reward memory, (b) repeated baclofen treatment after CPP sessions during development phase significantly and in a dose-dependent manner facilitated extinction and reduced conditioned sensitization of morphine in mice. Similar inhibitory effects of post-session baclofen were observed during development of morphine CPP in stressed mice (Meng et al. 2014).

The brain regions linked with the $GABA_B$ receptor control over morphine CPP are from the VTA or to the dorsal hippocampus. In fact, local microinjections of baclofen into these brain areas dose dependently suppressed morphine-induced CPP in male (Tsuji et al. 1996; Zarrindast et al. 2006) or female (Sahraei et al. 2009) rats. In full agreement with these findings intradorsal hippocampus microinjections of the $GABA_B$ receptor antagonist phaclofen increased the acquisition of morphine-induced CPP in rats (Zarrindast et al. 2006). Interestingly, intra-VTA administration of the $GABA_B$ receptor antagonist CGP35348 had displayed the same effects as baclofen on morphine-induced CPP, reducing the acquisition and/or expression of CPP in rats (Sahraei et al. 2009).

Several findings have shown that the $GABA_B$ receptor signaling is critically involved in opioid-rewarding properties measured in self-administration procedures. Thus, baclofen potently blocked morphine- or heroin-induced self-administration which was associated with the inhibition of dopamine release in the nucleus accumbens (see Xi and Stein 1999, 2000, 2002; Fadda et al. 2003; Yoon et al. 2007; Vlachou and Markou 2010). The inhibitory action of baclofen was reduced or reversed by the $GABA_B$ receptor antagonist 2-hydroxysaclofen (Xi and

Stein 1999) or SCH 50911 (Yoon et al. 2007), which alone did not alter (2-hydroxysaclofen, SCH 50911) (Xi and Stein 1999; Yoon et al. 2007) or increase (phaclofen) opioid self-administration (Ramshini et al. 2013). To further support the $GABA_B$ receptor control over opioid reward properties, the coadministration of baclofen with heroin or morphine prevented the development and reduced the maintenance of drugs self-administration in rats under an FR1 schedule of reinforcement (Xi and Stein 1999; Ramshini et al. 2013).

14.3.3.4 Seeking Behavior

There is strong evidence that the acute injection of baclofen decreases cue- and/or drug-induced reinstatement of opioid-seeking behavior in rats extinguished from morphine or heroin self-administration (Xi and Stein 1999; Di Ciano and Everitt 2003; Spano et al. 2007). The latter effects of baclofen were linked neither with its own sedative actions nor with the reduction of locomotor behaviors of heroin (Spano et al. 2007).

14.3.3.5 Withdrawal

Baclofen was able to prevent the morphine withdrawal syndrome in female as well as in male mice (Diaz et al. 2001, 2004, 2006; Kemmling et al. 2002; Niu et al. 2008). In male rats systematic or intralocus coeruleus injection of baclofen dose dependently attenuated morphine withdrawal signs and then inhibition being reversed by the $GABA_B$ receptor antagonists CGP46381 and CGP35348 (Bexis et al. 2001; Riahi et al. 2009).

14.3.4 $GABA_B$ Receptors and Nicotine

14.3.4.1 Locomotor Responses

A separate study indicates that $GABA_B$ receptor PAMs GS39783 and CGP7930 effectively suppress the locomotor hyperactivity induced by nicotine (Lobina et al. 2011) what supports the involvement of $GABA_B$ receptors in control this nicotine behavioral outcome.

14.3.4.2 Reward and Reinforcement

Numerous preclinical studies suggest the engagement of $GABA_B$ receptor stimulation in nicotine maintaining conditioned and operant tasks. Thus, baclofen was effective to reduce expression of nicotine CPP (Le Foll et al. 2008; Varani et al. 2014). Other reports with acute administration of baclofen demonstrated that this $GABA_B$ receptor

agonist in rats or mice effectively reduced nicotine self-administration under FR and PR schedules of reinforcements (Corrigall et al. 2000; Fattore et al. 2002; Paterson et al. 2004). The inhibitory effects toward nicotine self-administration were seen also for another $GABA_B$ receptor agonist CGP44532 and for several positive allosteric modulators (CGP7930, BHF177, and GS39783) (Paterson et al. 2005, 2008; Vlachou et al. 2011). Both $GABA_B$ receptor orthosteric agonist and positive allosteric modulators seem to share similar mechanism as they (i.e., GS39783 and CGP44532), given in combination in submaximal doses, show additive enhancement of inhibitory potency in rats self-administering nicotine (Paterson et al. 2008). The use of FR (to measure the reinforcing drug properties) and PR (to detect drug motivation) protocols in procedures using nicotine self-administration allows us to conclude that the enhancement of $GABA_B$ receptor signaling decreases nicotine reward and motivation. Interestingly, repeated administration with either CGP44532 (Paterson et al. 2005) or BHF177 (Vlachou et al. 2011) decreased nicotine rewarding properties with no tolerance to the inhibitory effects. The inhibitory actions of $GABA_B$ receptor agonists or positive allosteric modulators in nicotine self-administration procedures were seen in drug doses devoid of unwanted locomotor side effects (motor incoordination or sedation) typically linked with $GABA_B$ receptor stimulation (Corrigall et al. 2000; Fattore et al. 2002; Paterson et al. 2004, 2005, 2008; Vlachou et al. 2011). Furthermore $GABA_B$ positive allosteric modulators (but not orthosteric stimulants) did not significantly influence daily food intake, which may indicate their potential usefulness in nicotine addicts. The brain area engaged in the baclofen-induced inhibition of nicotine reward is the ventral tegmental area as evidenced with the local drug microinjection technique (Corrigall et al. 2000).

The $GABA_B$ positive allosteric modulator, BHF177, but not the $GABA_B$ receptor orthosteric agonist CGP44532, reduced the reward-enhancing effects of nicotine demonstrating blockade of lowered ICSS thresholds in rats treated non-contingently with nicotine and implanted with electrodes in the posterior lateral hypothalamus (Paterson et al. 2008). Concurrently, the highest dose of BHF177 per se significantly elevated ICSS thresholds interpreted as nonselective effects on the motivational properties of nicotine.

14.3.4.3 Seeking Behavior

Consistently, data show that the enhancement of $GABA_B$ receptor function by direct agonists or positive allosteric modulators attenuate nicotine-seeking behavior. Thus, the acute injection with $GABA_B$ receptor agonists baclofen and CGP44532 reduced nicotine-induced or cue-induced reinstatement in rats extinguished from nicotine self-administration in rats (Paterson et al. 2005; Fattore et al. 2009). Baclofen was also shown to reduce the reinstatement of nicotine CPP in mice (Fattore et al. 2009). A separate report indicated the inhibitory effects of $GABA_B$ positive allosteric modulator BHF177 on the cue-induced nicotine seeking (Vlachou et al. 2011). The latter effects of BHF177 influenced neither food-seeking nor animal-motor performance suggesting the specificity of its anti-seeking actions.

14.3.4.4 Withdrawal

Nicotine withdrawal is precipitated by the use of substances to remove the drug pharmacologically. Nicotine removal in rats evokes the anhedonia—seen as the rise of the basal ICSS thresholds—in rats with stimulating electrodes implanted in the lateral hypothalamus (Kenny and Markou 2006; Vlachou et al. 2011). Nicotine withdrawal effect was exacerbated by the $GABA_B$ receptor agonist CGP44532, positive allosteric modulator BHF177, and the antagonist CGP56433A which means that $GABA_B$ receptor agonism and antagonism control nicotine withdrawal in rats. The lack of antidepressant-like effects of $GABA_B$ receptor stimulants in the nicotine withdrawal/ICSS model warrants using $GABA_B$ receptor stimulants for the treatment of the depression-like state/anhedonia associated with nicotine withdrawal. On the other hand, baclofen prevented mecamylamine- or naloxone-induced behavioral outcomes of nicotine withdrawal syndrome in mice (Varani et al. 2013, 2014). These observations as well as a recent finding in $GABA_{B1}$ knockout mice (Varani et al. 2015) confirm $GABA_B$ receptor signaling to control the global withdrawal effect and/or the anxiety-like effect of nicotine withdrawal.

14.4 Clinical Trials

There are a few reports on the effect of baclofen on cocaine use disorder. Some trends toward reduced cocaine craving and its self-reported consumption have been observed by Ling et al. (1998) in a small group cocaine-dependent subjects treated with baclofen (20 mg t.i.d.) in an open-label study. On the other hand, the above observations were not confirmed in randomized placebo-controlled or double-blind clinical trials by Shoptaw et al. (2003) and Kahn et al. (2009), respectively, though the former authors observed a trend toward reduced cocaine use in subjects with heavier cocaine use. Small treatment effect of baclofen compared with placebo has been reported in methamphetamine dependence (Addolorato et al. 2012).

Similarly, few studies have examined the efficacy of baclofen in human opioid addicts (see Tyacke et al. 2010; Agabio and Colombo 2015). As shown, daily treatment of baclofen (range of doses 40–80 mg/day) increased the number of patients who achieved abstinence which indicates baclofen as an abstinence-promoting pharmacotherapy (Akhondzadeh et al. 2000; Assadi et al. 2003). The same authors failed to demonstrate a significant efficacy of baclofen to reduce opioid consumption as compared to placebo; however, some trend in the decreasing of opioid craving was observed (Akhondzadeh et al. 2000; Assadi et al. 2003).

There is also a single report showing the acute effects of baclofen (20 mg) in tobacco smokers (Cousins et al. 2001). In this study baclofen did not change the number of cigarettes smoked by the subjects nor did it change ratings of nicotine craving, but it increased ratings of 'harsh' and decreased ratings of 'like cigarette's effects.' The latter effects indicate that the $GABA_B$ receptor agonist produces some mood-altering effects and changes in sensory aspects of smoking that may facilitate smoking cessation.

14.5 Mechanisms of $GABA_B$ Receptor: Drugs of Abuse Interaction Within the Mesocorticolimbic Circuit

The mesocorticolimbic circuit is important for motivational processing and reward-related learning (Kalivas 2009). The task of these events primarily comes from the VTA to the nucleus accumbens and to the prefrontal cortex signaling with critical role for dopamine transmission (see Fig. 14.2a). In addition to dopamine neurons, the VTA has a large network of GABA-ergic neurons (Margolis et al. 2006), a recently discovered small population of glutamate neurons and several afferent and efferent connections. The afferent connections are GABA-ergic, glutamatergic, 5-HT-ergic, and cholinergic axon terminals derived mainly from the ventral pallidum, prefrontal cortex, raphe nuclei, and pedunculopontine-laterodorsal tegmentum, respectively (see Fig. 14.2a). A very recent paper shows that the bed nucleus of the striatal terminalis glutamatergic and GABA-ergic projections to the VTA preferentially innervate local GABA neurons (Jennings et al. 2013). The VTA efferent connections are dopamine and GABA projections which target—among others—the nucleus accumbens and the prefrontal cortex. In the nucleus accumbens, VTA dopamine neurons modulate GABA-ergic medium-sized spine neurons and cholinergic interneurons while VTA GABA neurons target mainly cholinergic interneurons (Brown et al. 2012; Moss and Bolam 2008; Omelchenko and Sesack 2006). In addition, the nucleus accumbens comprises glutamatergic terminals from the prefrontal cortex, amygdala, and hippocampus that also alter local network.

Tonic extracellular dopamine and phasic dopamine neuron spike activity is under control of VTA, intrinsic accumbal and accumbal-VTA feedback, and prefrontal loops. Under physiological conditions the VTA has a facilitatory influence over phasic dopaminergic neurotransmission exerted by glutamatergic projections from the prefrontal cortex and hippocampus, by cholinergic projections from the pedunculopontine and laterodorsal tegmentum, and by GABA-ergic medium-sized spine neurons that send projections back to the VTA leading to the inhibition of VTA GABA cells and thus disinhibiting DA neurons (Bocklisch et al. 2013; Xia et al. 2011; see Fig. 14.2a).

On the other hand, inhibitory control of dopaminergic neurons is represented by GABA acting via (a) VTA GABA interneurons and (b) long-range GABA projections from the VTA to the nucleus accumbens that selectively target accumbal cholinergic interneurons to reduce their firing and local cholinergic tone what tunes down their facilitatory influence of the dopamine release from its terminals (Cachope et al. 2012; Cragg and Rice 2004). Furthermore, by using optogenetic manipulations it was shown that VTA GABA neurons under physiological conditions may bidirectionally modulate activity of local dopamine neurons processing either reward or aversion (cf. Creed et al. 2014).

All drugs of abuse, including psychostimulants, opioids, and nicotine, remodel this complex network (see Fig. 14.2b) to bring adverse behavioral consequences such as pathological salience attributed to drug-associated cues and compulsive drug use despite negative consequences. Although the rewarding effects of drugs of

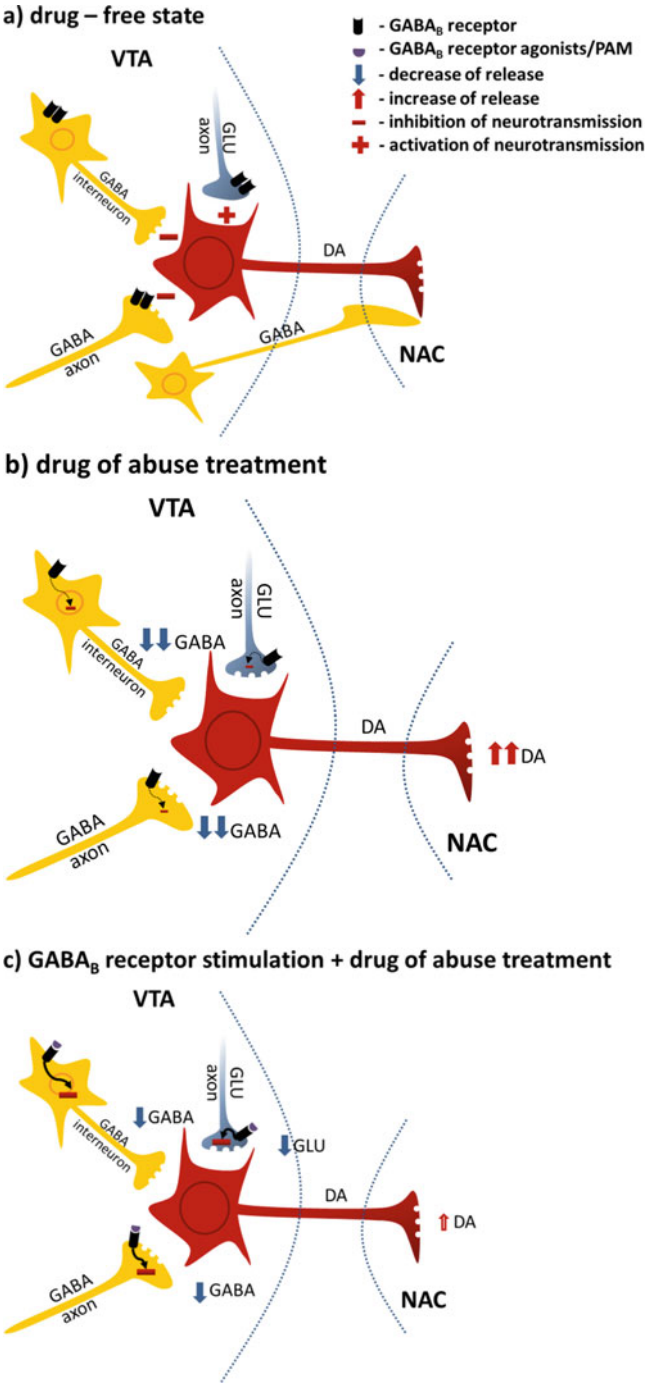

Fig. 14.2 The mechanism by which $GABA_B$ receptor orthosteric agonists and positive allosteric modulators counteract the enhancement of dopamine neurotransmission and its related rewarding stimuli and motivational processing of drugs of abuse. Please see Sect. 14.5 for more explanation. *NAC* nucleus accumbens, *VTA* ventral tegmental area. (**a**) Drug-free state, (**b**) drug of abuse treatment, (**c**) $GABA_B$ receptor stimulation + drug of abuse treatment

abuse have mostly been linked to enhanced dopamine release in the nucleus accumbens (Lüscher and Ungless 2006), psychostimulants, opioids, and nicotine induce characteristic forms of neuroplasticity on VTA dopamine neurons within a day following an acute exposure (Lüscher and Malenka 2011). The enhanced dopaminergic transmission results also from drugs of abuse-induced potentiation of the inhibitory input onto VTA GABA cells through GABA-ergic back projections from the accumbal medium-sized spine neurons (Bocklisch et al. 2013; Xia et al. 2011) followed by suppression of inhibitory transmission from the VTA back to the nucleus accumbens (Ishikawa et al. 2013; see Fig. 14.2b).

Drugs of abuse alter not only GABA neurotransmission but also $GABA_B$ receptor function. $GABA_B$ receptors are widely distributed in several areas of the brain including the reward pathway where both the transcript and protein are localized (for review see Filip and Frankowska 2008; Filip et al. 2015; see also Chap. 5 of this book). $GABA_B$ receptors act as heteroreceptors on dopaminergic and glutamatergic neurons in the VTA (Bowery et al. 1987; Liang et al. 2000; Wirtshafter and Sheppard 2001). $GABA_B$ receptors control the excitability of VTA dopamine neurons since the receptor antagonism enhances drug of abuse-induced increase of firing frequency, while the activation of VTA $GABA_B$ receptors results in a decreased dopamine release in the nucleus accumbens and prefrontal cortex (Erhardt et al. 2002; see also Chap. 8 of this book). In the VTA, a positive allosteric modulator enhances the $GABA_B$ receptor-mediated inhibition of dopaminergic neuron firing activity (Chen et al. 2005). The behavioral outcome from the above changes in neurochemistry of $GABA_B$ receptors is reduction in reward, reinforcement, and reinstatement of drug seeking seen after peripheral (Fu et al. 2012; Laviolette and van der Kooy 2003; Sagara et al. 2008; Xi et al. 2009; Yang et al. 2009) and intra-VTA (Brebner et al. 2000b; Shoaib et al. 1998) injections with $GABA_B$ receptor stimulants (see Fig. 14.2c).

Molecular analyses further confirm the specificity of $GABA_B$ receptors in the molecular events generated by long-term drug of abuse exposure. Thus, cocaine self-administration results in decreased $GABA_B$ receptor binding in several regions, including the nucleus accumbens, amygdala, and prefrontal cortex (Frankowska et al. 2008a), while reinstatement of cocaine-seeking behavior increases $GABA_B$ receptor binding in the nucleus accumbens and prefrontal cortex (Frankowska et al. 2008b). To support the above findings, cocaine administration decreases $GABA_B$-GIRK signaling and GIRK channels in the VTA, which means decreased inhibitory signaling (Arora et al. 2011). Molecular tools revealed allelic variation in $GABA_B$ gene expression resulting from nicotine dependence (Beuten et al. 2005; but see also Agrawal et al. 2008). $GABA_B$ receptor stimulation with positive allosteric modulators also counteracts long-lasting DeltaFosB (a marker of cell activation to drugs of abuse) accumulation in the mesolimbic system in response to chronic cocaine (Lhuillier et al. 2007) or chronic nicotine (Mombereau et al. 2007) as well as it reduces the expression of cAMP-response element-binding protein (CREB) and dopamine-and-cAMP-regulated-phosphoprotein of 32 kDa (DARPP-32) in the nucleus accumbens of rats exposed to cocaine (Lhuillier et al. 2007).

14.6 Conclusions

There is massive preclinical evidence that supports a role for $GABA_B$ receptors to control over SUD. In addition to the preclinical experience, there are initial clinical trials showing that $GABA_B$ receptor stimulants may be considered as anti-abuse and anti-relapse agents. In some animal studies these drugs influence food behavior and/or reinstatement of food seeking; however, the explanation whether $GABA_B$ receptor positive allosteric modulators control motivational and conditioned aspects of consummatory behaviors requires further analyses. Within $GABA_B$ receptor stimulants, $GABA_B$ receptor positive allosteric modulators act more physiologically than orthosteric agonists and they have better pharmacological profile. In fact, $GABA_B$ receptor positive allosteric modulators—in doses blocking addictive properties of drugs—do not evoke locomotor side effects, hypothermia, amnestic-like action, or tolerance after repeated drug treatment. $GABA_B$ receptor positive allosteric modulators show also efficacy to control depression and anxiety responses, but whether these drugs regulate the above emotional states or other impulsiveness and aggression that often occur in SUD is an open question. To conclude, $GABA_B$ receptor activation may be an attractive target for SUD therapy, however, more medical reports dealing with human addicts are required.

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Conflict of Interest None.

References

Addolorato, G., Leggio, L., Hopf, F. W., Diana, M., & Bonci, A. (2012). Novel therapeutic strategies for alcohol and drug addiction: Focus on GABA, ion channels and transcranial magnetic stimulation. *Neuropsychopharmacology, 37*, 163–177.

Agabio, R., & Colombo, G. (2015). $GABA_B$ receptor as therapeutic target for drug addiction: From baclofen to positive allosteric modulators. *Psychiatria Polska, 49*, 215–223.

Agrawal, A., Pergadia, M. L., Saccone, S. F., Hinrichs, A. L., Lessov-Schlaggar, C. N., Saccone, N. L., et al. (2008). Gamma-aminobutyric acid receptor genes and nicotine dependence: Evidence for association from a case-control study. *Addiction, 103*, 1027–1038.

Akhondzadeh, S., Ahmadi-Abhari, S. A., Assadi, S. M., Shabestari, O. L., Kashani, A. R., & Farzanehgan, Z. M. (2000). Double-blind randomized controlled trial of baclofen vs. clonidine in the treatment of opiates withdrawal. *Journal of Clinical Pharmacy and Therapeutics, 25*, 347–353.

Arora, D., Hearing, M., Haluk, D. M., Mirkovic, K., Fajardo-Serrano, A., Wessendorf, M. W., et al. (2011). Acute cocaine exposure weakens GABA(B) receptor-dependent G-protein-gated inwardly rectifying K+ signaling in dopamine neurons of the ventral tegmental area. *Journal of Neuroscience, 31*, 12251–12257.

Assadi, S. M., Radgoodarzi, R., & Ahmadi-Abhari, S. A. (2003). Baclofen for maintenance treatment of opioid dependence: A randomized double-blind placebo-controlled clinical trial. *BMC Psychiatry, 3*, 16.

Aulakh, C. S., Ghosh, B., & Pradhan, S. N. (1979). Actions and interactions of cocaine on self-stimulation behavior in rats. *Psychopharmacology, 63*, 75–79.

Bartoletti, M., Colantoni, A., De Luca, V., & Gaiardi, M. (2010). Single and repeated baclofen treatment attenuates the discriminative stimulus effects of morphine in rats. *Pharmacology, Biochemistry and Behavior, 97*, 279–283.

Bartoletti, M., Gubellini, C., Ricci, F., & Gaiardi, M. (2004). The $GABA_B$ agonist baclofen blocks the expression of sensitisation to the locomotor stimulant effect of amphetamine. *Behavioral Pharmacology, 15*, 397–401.

Bartoletti, M., Gubellini, C., Ricci, F., & Gaiardi, M. (2005). Baclofen blocks the development of sensitization to the locomotor stimulant effect of amphetamine. *Behavioral Pharmacology, 16*, 553–558.

Bartoletti, M., Ricci, F., & Gaiardi, M. (2007). A GABA(B) agonist reverses the behavioral sensitization to morphine in rats. *Psychopharmacology, 192*, 79–85.

Beuten, J., Ma, J. Z., Payne, T. J., Dupont, R. T., Crews, K. M., Somes, G., et al. (2005). Single- and multilocus allelic variants within the GABA(B) receptor subunit 2 (GABAB2) gene are significantly associated with nicotine dependence. *The American Journal of Human Genetics, 76*, 859–864.

Bexis, S., Ong, J., & White, J. (2001). Attenuation of morphine withdrawal signs by the GABA(B) receptor agonist baclofen. *Life Sciences, 70*, 395–401.

Bocklisch, C., Pascoli, V., Wong, J. C. Y., House DRC, Yvon, C., de Roo, M., et al. (2013). Cocaine disinhibits dopamine neurons by potentiation of GABA transmission in the ventral tegmental area. *Science, 341*, 1521–1525.

Bowery, N. G., Hudson, A. L., & Price, G. W. (1987). $GABA_A$ and $GABA_B$ receptor site distribution in the rat central nervous system. *Neuroscience, 20*, 365–383.

Boyes, J., & Bolam, J. P. (2003). The subcellular localization of GABA(B) receptor subunits in the rat substantia nigra. *European Journal of Neuroscience, 18*, 3279–3293.

Brebner, K., Ahn, S., & Phillips, A. G. (2005). Attenuation of d-amphetamine self-administration by baclofen in the rat: Behavioral and neurochemical correlates. *Psychopharmacology, 177*, 409–417.

Brebner, K., Froestl, W., Andrews, M., Phelan, R., & Roberts, D. C. (1999). The GABA(B) agonist CGP 44532 decreases cocaine self-administration in rats: Demonstration using a progressive ratio and a discrete trials procedure. *Neuropharmacology, 38*, 1797–1804.

Brebner, K., Froestl, W., & Roberts, D. C. (2002). The GABA(B) antagonist CGP56433A attenuates the effect of baclofen on cocaine but not heroin self-administration in the rat. *Psychopharmacology, 160*, 49–55.

Brebner, K., Phelan, R., & Roberts, D. C. (2000a). Effect of baclofen on cocaine self-administration in rats reinforced under fixed-ratio 1 and progressive-ratio schedules. *Psychopharmacology, 148*, 314–321.

Brebner, K., Phelan, R., & Roberts, D. C. (2000b). Intra-VTA baclofen attenuates cocaine self-administration on a progressive ratio schedule of reinforcement. *Pharmacology, Biochemistry and Behavior, 66*, 857–862.

Brown, J. M., Hanson, G. R., & Fleckenstein, A. E. (2001). Cocaine-induced increases in vesicular dopamine uptake: Role of dopamine receptors. *Journal of Pharmacol and Experimental Therapeutics, 298*, 1150–1153.

Brown, M. T. C., Tan, K. R., O'Connor, E. C., Nikonenko, I., Muller, D., & Lüscher, C. (2012). Ventral tegmental area GABA projections pause accumbal cholinergic interneurons to enhance associative learning. *Nature, 492*, 452–456.

Cachope, R., Mateo, Y., Mathur, B. N., Irving, J., Wang, H. L., Morales, M., et al. (2012). Selective activation of cholinergic interneurons enhances accumbal phasic dopamine release: Setting the tone for reward processing. *Cell Reports, 2*, 33–41.

Campbell, U. C., Lac, S. T., & Carroll, M. E. (1999). Effects of baclofen on maintenance and reinstatement of intravenous cocaine self-administration in rats. *Psychopharmacology, 143*, 209–214.

Cedillo, L. N., & Miranda, F. (2013). Effects of co-administration of the GABAB receptor agonist baclofen and a positive allosteric modulator of the $GABA_B$ receptor, CGP7930, on the development and expression of amphetamine-induced locomotor sensitization in rats. *Pharmacological Reports, 65*, 1132–1143.

Chen, Y., Phillips, K., Minton, G., & Sher, E. (2005). GABA(B) receptor modulators potentiate baclofen-induced depression of dopamine neuron activity in the rat ventral tegmental area. *British Journal of Pharmacology, 144*, 926–932.

Contet, C., Filliol, D., Matifas, A., & Kieffer, B. L. (2008). Morphine-induced analgesic tolerance, locomotor sensitization and physical dependence do not require modification of mu opioid receptor, cdk5 and adenylate cyclase activity. *Neuropharmacology, 54*, 475–486.

Corrigall, W. A., Coen, K. M., Adamson, K. L., Chow, B. L., & Zhang, J. (2000). Response of nicotine self-administration in the rat to manipulations of mu-opioid and gamma-aminobutyric acid receptors in the ventral tegmental area. *Psychopharmacology, 149*, 107–114.

Cousins, M. S., Stamat, H. M., & de Wit, H. (2001). Effects of a single dose of baclofen on self-reported subjective effects and tobacco smoking. *Nicotine and Tobacco Research, 3*, 123–129. Erratum in: Nicotine Tob Res 3:409.

Cragg, S. J., & Rice, M. E. (2004). Dancing past the DAT at a DA synapse. *Trends in Neurosciences, 27*, 270–277.

Creed, M. C., Ntamati, N. R., & Tan, K. R. (2014). VTA GABA neurons modulate specific learning behaviors through the control of dopamine and cholinergic systems. *Frontiers in Behavioral Neuroscience, 8*, 8.

Di Chiara, G., Tanda, G., Bassareo, V., Pontieri, F., Acquas, E., Fenu, S., et al. (1999). Drug addiction as a disorder of associative learning. Role of nucleus accumbens shell/extended amygdala dopamine. *The Annals of the New York Academy of Sciences, 877*, 461–485.

Di Ciano, P., & Everitt, B. J. (2003). The GABA(B) receptor agonist baclofen attenuates cocaine- and heroin-seeking behavior by rats. *Neuropsychopharmacology, 28*, 510–518.

Diaz, S. L., Barros, V. G., Antonelli, M. C., Rubio, M. C., & Balerio, G. N. (2006). Morphine withdrawal syndrome and its prevention with baclofen: Autoradiographic study of mu-opioid receptors in prepubertal male and female mice. *Synapse, 60*, 132–134.

Diaz, S. L., Kemmling, A. K., Bonavita, C. D., Rubio, M. C., & Balerio, G. N. (2004). Baclofen reestablishes micro-opioid receptor levels modified by morphine withdrawal syndrome in either sex. *Synapse, 54*, 24–29.

Diaz, S. L., Kemmling, A. K., Rubio, M. C., & Balerio, G. N. (2001). Lack of sex-related differences in the prevention by baclofen of the morphine withdrawal syndrome in mice. *Behavioral Pharmacology, 12*, 75–79.

Erhardt, S., Mathé, J. M., Chergui, K., Engberg, G., & Svensson, T. H. (2002). GABA(B) receptor-mediated modulation of the firing pattern of ventral tegmental area dopamine neurons in vivo. *Naunyn-Schmiedeberg's Archives of Pharmacology, 365*, 173–180.

Fadda, P., Scherma, M., Fresu, A., Collu, M., & Fratta, W. (2003). Baclofen antagonizes nicotine-, cocaine-, and morphine-induced dopamine release in the nucleus accumbens of rat. *Synapse, 50*, 1–6.

Fattore, L., Cossu, G., Martellotta, M. C., & Fratta, W. (2002). Baclofen antagonizes intravenous self-administration of nicotine in mice and rats. *Alcohol and Alcoholism, 37*, 495–498.

Fattore, L., Spano, M. S., Cossu, G., Scherma, M., Fratta, W., & Fadda, P. (2009). Baclofen prevents drug-induced reinstatement of extinguished nicotine-seeking behaviour and nicotine place preference in rodents. *European Neuropsychopharmacology, 19*, 487–498.

Ferraro, L., Beggiato, S., Marcellino, D., Frankowska, M., Filip, M., Agnati, L. F., et al. (2010). Nanomolar concentrations of cocaine enhance D2-like agonist-induced inhibition of the K^+-evoked [^{3}H]-dopamine efflux from rat striatal synaptosomes: A novel action of cocaine. *Journal of Neural Transmission, 17*, 593–597.

Fibiger, H. C., & Phillips, A. G. (1974). Role of dopamine and norepinephrine in the chemistry of reward. *Journal of Psychiatric Research, 11*, 135–143.

Filip, M., Alenina, N., Bader, M., & Przegaliński, E. (2010). Behavioral evidence for the significance of serotoninergic (5-HT) receptors in cocaine addiction. *Addiction Biology, 15*, 227–249.

Filip, M., & Frankowska, M. (2007). Effects of GABA(B) receptor agents on cocaine priming, discrete contextual cue and food induced relapses. *European Journal of Pharmacology, 571*, 166–173.

Filip, M., & Frankowska, M. (2008). GABA(B) receptors in drug addiction. *Pharmacological Reports, 60*, 755–770.

Filip, M., Frankowska, M., & Przegaliński, E. (2007). Effects of GABA(B) receptor antagonist, agonists and allosteric positive modulator on the cocaine-induced self-administration and drug discrimination. *European Journal of Pharmacology, 574*, 148–157.

Filip, M., Frankowska, M., Sadakierska-Chudy, A., Suder, A., Szumiec, L., Mierzejewski, P., et al. (2015). $GABA_B$ receptors as a therapeutic strategy in substance use disorders: Focus on positive allosteric modulators. *Neuropharmacology, 88*, 36–47.

Fleckenstein, A. E., Volz, T. J., Riddle, E. L., Gibb, J. W., & Hanson, G. R. (2007). New insights into the mechanism of action of amphetamines. *Annual Review of Pharmacology and Toxicology, 47*, 681–698.

Frankowska, M., Nowak, E., & Filip, M. (2009). Effects of $GABA_B$ receptor agonists on cocaine hyperlocomotor and sensitizing effects in rats. *Pharmacological Reports, 61*, 1042–1049.

Frankowska, M., Wydra, K., Faron-Górecka, A., Zaniewska, M., Kuśmider, M., & Dziedzicka-Wasylewska, M. (2008a). Neuroadaptive changes in the rat brain GABA(B) receptors after withdrawal from cocaine self-administration. *European Journal of Pharmacology, 599*, 58–64.

Frankowska, M., Wydra, K., Faron-Górecka, A., Zaniewska, M., Kuśmider, M., & Dziedzicka-Wasylewska, M. (2008b). Alterations in gamma-aminobutyric acid(B) receptor binding in the rat brain after reinstatement of cocaine-seeking behavior. *Pharmacological Reports, 60*, 834–843.

Fricks-Gleason, A. N., & Marshall, J. F. (2008). Post-retrieval beta-adrenergic receptor blockade: Effects on extinction and reconsolidation of cocaine-cue memories. *Learning and Memory, 15*, 643–648.

Froger-Colléaux, C., & Castagné, V. (2016). Effects of baclofen and raclopride on reinstatement of cocaine self-administration in the rat. *European Journal of Pharmacology, 777*, 147–155.

Fu, Z., Yang, H., Xiao, Y., Zhao, G., & Huang, H. (2012). The γ-aminobutyric acid type B ($GABA_B$) receptor agonist baclofen inhibits morphine sensitization by decreasing the dopamine level in rat nucleus accumbens. *Behavioral and Brain Functions, 8*, 20.

Gassmann, M., & Bettler, B. (2012). Regulation of neuronal $GABA_B$ receptor functions by subunit composition. *Nature Reviews Neuroscience, 13*, 380–394.

Gatley, S. J., & Volkow, N. D. (1998). Addiction and imaging of the living human brain. *Drug and Alcohol Dependence, 511*, 97–108.

Green, A. R., Mechan, A. O., Elliott, J. M., O'Shea, E., & Colado, M. I. (2003). The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *Pharmacological Reviews, 55*, 463–508.

Halbout, B., Quarta, D., Valerio, E., Heidbreder, C. A., & Hutcheson, D. M. (2011). The GABA-B positive modulator GS39783 decreases psychostimulant conditioned-reinforcement and conditioned-reward. *Addiction Biology, 16*, 416–427.

Heinrichs, S. C., Leite-Morris, K. A., Carey, R. J., & Kaplan, G. B. (2010). Baclofen enhances extinction of opiate conditioned place preference. *Behavioural Brain Research, 207*, 353–359.

Hong, S., Jhou, T. C., Smith, M., Saleem, K. S., & Hikosaka, O. (2011). Negative reward signals from the lateral habenula to dopamine neurons are mediated by rostromedial tegmental nucleus in primates. *Journal of Neuroscience, 31*, 11457–11471.

Ishikawa, M., Otaka, M., Neumann, P. A., Wang, Z., Cook, J. M., Schlüter, O. M., et al. (2013). Exposure to cocaine regulates inhibitory synaptic transmission from the ventral tegmental area to the nucleus accumbens. *Journal of Physiology, 591*, 4827–4841.

Jennings, J. H., Sparta, D. R., Stamatakis, A. M., Ung, R. L., Pleil, K. E., & Kash, T. L. (2013). Distinct extended amygdala circuits for divergent motivational states. *Nature, 496*, 224–228.

Kahlig, K. M., Binda, F., Khoshbouei, H., Blakely, R. D., McMahon, D. G., & Javitch, J. A. (2005). Amphetamine induces dopamine efflux through a dopamine transporter channel. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 3495–3500.

Kahn, R., Biswas, K., Childress, A. R., Shoptaw, S., Fudala, P. J., Gorgon, L., et al. (2009). Multicenter trial of baclofen for abstinence initiation in severe cocaine-dependent individuals. *Drug and Alcohol Dependence, 103*, 59–64.

Kalivas, P. W. (2009). The glutamate homeostasis hypothesis of addiction. *Nature Review Neuroscience, 10*, 561–572.

Kaplan, G. B., Leite-Morris, K. A., Joshi, M., Shoeb, M. H., & Carey, R. J. (2003). Baclofen inhibits opiate-induced conditioned place preference and associated induction of Fos in cortical and limbic regions. *Brain Research, 987*, 122–125.

Kemmling, A. K., Rubio, M. C., & Balerio, G. N. (2002). Baclofen prevents morphine withdrawal irrespective of seasonal variation. *Behavioral Pharmacology, 13*, 87–92.

Kenny, P. J., & Markou, A. (2006). Nicotine self-administration acutely activates brain reward systems and induces a long-lasting increase in reward sensitivity. *Neuropsychopharmacology, 31*, 1203–1211.

Lacey, C. J., Boyes, J., Gerlach, O., Chen, L., Magill, P. J., & Bolam, J. P. (2005). GABA(B) receptors at glutamatergic synapses in the rat striatum. *Neuroscience, 136*, 1083–1095.

Laviolette, S. R., & van der Kooy, D. (2003). Blockade of mesolimbic dopamine transmission dramatically increases sensitivity to the rewarding effects of nicotine in the ventral tegmental area. *Molecular Psychiatry, 8*, 50–59.

Le Foll, B., Wertheim, C. E., & Goldberg, S. R. (2008). Effects of baclofen on conditioned rewarding and discriminative stimulus effects of nicotine in rats. *Neuroscience Letters, 443*, 236–240.

Lecca, S., Melis, M., Luchicchi, A., Ennas, M. G., Castelli, M. P., & Muntoni, A. L. (2011). Effects of drugs of abuse on putative rostromedial tegmental neurons, inhibitory afferents to midbrain dopamine cells. *Neuropsychopharmacology, 36*, 589–602.

Leite-Morris, K. A., Fukudome, E. Y., Shoeb, M. H., & Kaplan, G. B. (2004). GABA(B) receptor activation in the ventral tegmental area inhibits the acquisition and expression of opiate-induced motor sensitization. *Journal of Pharmacol and Experimental Therapeutics, 308*, 667–678.

Lhuillier, L., Mombereau, C., Cryan, J. F., & Kaupmann, K. (2007). GABA(B) receptor-positive modulation decreases selective molecular and behavioral effects of cocaine. *Neuropsychopharmacology, 32*, 388–398.

Li, S. M., Yin, L. L., Ren, Y. H., Pan, L. S., & Zheng, J. W. (2001). GABA(B) receptor agonist baclofen attenuates the development and expression of d-methamphetamine-induced place preference in rats. *Life Sciences, 70*, 349–356.

Liang, F., Hatanaka, Y., Saito, H., Yamamori, T., & Hashikawa, T. (2000). Differential expression of gamma-aminobutyric acid type B receptor-1a and-1b mRNA variants in GABA and non-GABAergic neurons of the rat brain. *Journal of Comparative Neurology, 416*, 475–495.

Ling, W., Shoptaw, S., & Majewska, D. (1998). Baclofen as a cocaine anti-craving medication: A preliminary clinical study. *Neuropsychopharmacology, 18*, 403–404.

Lobina, C., Carai, M. A. M., Froestl, W., Mugnaini, C., Pasquini, S., Corelli, F., et al. (2011). Activation of the GABA(B) receptor prevents nicotine-induced locomotor stimulation in mice. *Frontiers in Psychiatry, 2*, 76.

Lüscher, C., & Malenka, R. C. (2011). Drug-evoked synaptic plasticity in addiction: From molecular changes to circuit remodeling. *Neuron, 69*, 650–663.

Lüscher, C., & Ungless, M. A. (2006). The mechanistic classification of addictive drugs. *PLoS Medicine, 3*, e437.

Mansvelder, H. D., Keath, J. R., & McGehee, D. S. (2002). Synaptic mechanisms underlie nicotine-induced excitability of brain reward areas. *Neuron, 33*, 905–919.

Margolis, E. B., Lock, H., Hjelmstad, G. O., & Fields, H. L. (2006). The ventral tegmental area revisited: Is there an electrophysiological marker for dopaminergic neurons? *Journal of Physiology, 577*, 907–924.

Markou, A., & Koob, G. F. (1991). Postcocaine anhedonia. An animal model of cocaine withdrawal. *Neuropsychopharmacology, 4*, 17–26.

Meng, S., Quan, W., Qi, X., Su, Z., & Yang, S. (2014). Effect of baclofen on morphine-induced conditioned place preference, extinction, and stress-induced reinstatement in chronically stressed mice. *Psychopharmacology, 231*, 27–36.

Miranda, F., Jiménez, J. C., Cedillo, L. N., Sandoval-Sánchez, A., Millán-Mejía, P., & Sánchez-Castillo, H. (2009). The GABA-B antagonist 2-hydroxysaclofen reverses the effects of baclofen on the discriminative stimulus effects of D-amphetamine in the conditioned taste aversion procedure. *Pharmacology, Biochemistry and Behavior, 93*, 25–30.

Mombereau, C., Lhuillier, L., Kaupmann, K., & Cryan, J. F. (2007). $GABA_B$ receptor-positive modulation-induced blockade of the rewarding properties of nicotine is associated with a reduction in nucleus accumbens DeltaFosB accumulation. *Journal of Pharmacol and Experimental Therapeutics, 321*, 172–177.

Moss, J., & Bolam, J. P. (2008). A dopaminergic axon lattice in the striatum and its relationship with cortical and thalamic terminals. *Journal of Neuroscience, 28*, 11221–11230.

Munzar, P., Kutkat, S. W., Miller, C. R., & Goldberg, S. R. (2007). Failure of baclofen to modulate discriminative-stimulus effects of cocaine or methamphetamine in rats. *European Journal of Pharmacology, 408*, 169–174.

Niu, H., Shang, B., Sun, N., Li, L., Guan, Y., Yu, H., et al. (2008). Baclofen inhibited the morphine-induced conditioned place preference and withdrawal syndromes. *Zoological Research, 29*, 621–626.

Omelchenko, N., & Sesack, S. R. (2006). Cholinergic axons in the rat ventral tegmental area synapse preferentially onto mesoaccumbens dopamine neurons. *Journal of Comparative Neurology, 494*, 863–875.

Paterson, N. E., Froestl, W., & Markou, A. (2004). The $GABA_B$ receptor agonists baclofen and CGP44532 decreased nicotine self-administration in the rat. *Psychopharmacology, 172*, 179–186.

Paterson, N. E., Froestl, W., & Markou, A. (2005). Repeated administration of the $GABA_B$ receptor agonist CGP44532 decreased nicotine self-administration, and acute administration decreased cue-induced reinstatement of nicotine-seeking in rats. *Neuropsychopharmacology, 30*, 119–128.

Paterson, N. E., Vlachou, S., Guery, S., Kaupmann, K., Froestl, W., & Markou, A. (2008). Positive modulation of GABA(B) receptors decreased nicotine self-administration and counteracted nicotine-induced enhancement of brain reward function in rats. *Journal of Pharmacol and Experimental Therapeutics, 326*, 306–314.

Pin, J. P., & Prézeau, L. (2007). Allosteric modulators of $GABA_B$ receptors: Mechanism of action and therapeutic perspective. *Current Neuropharmacology, 5*, 195–201.

Ramshini, E., Alaei, H., Reisi, P., Alaei, S., & Shahidani, S. (2013). The role of $GABA_B$ receptors in morphine self-administration. *International Journal of Preventive Medicine, 4*, 158–164.

Ranaldi, R., & Poeggel, K. (2002). Baclofen decreases methamphetamine self-administration in rats. *Neuroreport, 13*, 1107–1110.

Riahi, E., Mirzaii-Dizgah, I., Karimian, S. M., Sadeghipour, H. R., & Dehpour, A. R. (2009). Attenuation of morphine withdrawal signs by a $GABA_B$ receptor agonist in the locus coeruleus of rats. *Behavioural Brain Research, 196*, 11–14.

Riddle, E. L., Kokoshka, J. M., Wilkins, D. G., Hanson, G. R., & Fleckenstein, A. E. (2002). Tolerance to the neurotoxic effects of methamphetamine in young rats. *European Journal of Pharmacology, 435*, 181–185.

Roberts, D. C., Andrews, M. M., & Vickers, G. J. (1996). Baclofen attenuates the reinforcing effects of cocaine in rats. *Neuropsychopharmacology, 15*, 417–423.

Robinson, T. E., & Berridge, K. C. (2001). Incentive-sensitization and addiction. *Addiction, 96*, 103–114.

Rothman, R. B., & Baumann, M. H. (2003). Monoamine transporters and psychostimulant drugs. *European Journal of Pharmacology, 479*, 23–40.

Sagara, H., Kitamura, Y., Yae, T., Shibata, K., Suemaru, K., Sendo, T., et al. (2008). Nicotinic acetylcholine alpha4beta2 receptor regulates the motivational effect of intracranial self-stimulation behavior in the runway method. *Journal of Pharmacological Sciences, 108*, 455–461.

Sahraei, H., Amiri, Y. A., Haeri-Rohani, A., Sepehri, H., Salimi, S. H., Pourmotabbed, A., et al. (2005). Different effects of GABAergic receptors located in the ventral tegmental area on the expression of morphine-induced conditioned place preference in rat. *European Journal of Pharmacology, 524*, 95–101.

Sahraei, H., Etemadi, L., Rostami, P., Pourmotabbed, A., Zarrindast, M. R., Shams, J., et al. (2009). $GABA_B$ receptors within the ventral tegmental area are involved in the expression and acquisi-

tion of morphine-induced place preference in morphine-sensitized rats. *Pharmacology, Biochemistry and Behavior, 91*, 409–416.

Shoaib, M., Swanner, L. S., Beyer, C. E., Goldberg, S. R., & Schindler, C. W. (1998). The $GABA_B$ agonist baclofen modifies cocaine self-administration in rats. *Behavioral Pharmacology, 9*, 195–206.

Shoptaw, S., Yang, X., Rotheram-Fuller, E. J., Hsieh, Y. C., Kintaudi, P. C., Charuvastra, V. C., et al. (2003). Randomized placebo-controlled trial of baclofen for cocaine dependence: Preliminary effects for individuals with chronic patterns of cocaine use. *Journal of Clinical Psychiatry, 64*, 1440–1448.

Sitte, H. H., Huck, S., Reither, H., Boehm, S., Singer, E. A., & Pifl, C. (1998). Carrier-mediated release, transport rates, and charge transfer induced by amphetamine, tyramine, and dopamine in mammalian cells transfected with the human dopamine transporter. *Journal of Neurochemistry, 71*, 1289–1297.

Slattery, D. A., Markou, A., Froestl, W., & Cryan, J. F. (2005). The $GABA_B$ receptor-positive modulator GS39783 and the GABAB receptor agonist baclofen attenuate the reward-facilitating effects of cocaine: Intracranial self-stimulation studies in the rat. *Neuropsychopharmacology, 30*, 2065–2072.

Smith, M. A., Greene-Naples, J. L., Lyle, M. A., Iordanou, J. C., & Felder, J. N. (2009). The effects of repeated opioid administration on locomotor activity: I. Opposing actions of mu and kappa receptors. *Journal of Pharmacology of Experimental and Therapeutics, 330*, 468–475.

Smith, M. A., Yancey, D. L., Morgan, D., Liu, Y., Froestl, W., & Roberts, D. C. (2004). Effects of positive allosteric modulators of the $GABA_B$ receptor on cocaine self-administration in rats. *Psychopharmacology, 173*, 105–111.

Solecki, W., Krówka, T., Filip, M., & Przewłocki, R. (2005). Role of opioidergic mechanisms and GABA uptake inhibition in the heroin-induced discriminative stimulus effects in rats. *Pharmacological Reports, 57*, 744–754.

Spano, M. S., Fattore, L., Fratta, W., & Fadda, P. (2007). The $GABA_B$ receptor agonist baclofen prevents heroin-induced reinstatement of heroin-seeking behavior in rats. *Neuropharmacology, 52*, 1555–1562.

Steketee, J. D., & Kalivas, P. W. (2011). Drug wanting: Behavioral sensitization and relapse to drug-seeking behavior. *Pharmacological Reviews, 63*, 348–365.

Sulzer, D. (2011). How addictive drugs disrupt presynaptic dopamine neurotransmission. *Neuron, 69*, 628–649.

Sulzer, D., Chen, T. K., Lau, Y. Y., Kristensen, H., Rayport, S., & Ewing, A. (1995). Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. *Journal of Neuroscience, 15*, 4102–4108.

Suzuki, T., Nurrochmad, A., Ozaki, M., Khotib, J., Nakamura, A., Imai, S., et al. (2005). Effect of a selective GABA(B) receptor agonist baclofen on the mu-opioid receptor agonist-induced antinociceptive, emetic and rewarding effects. *Neuropharmacology, 49*, 1121–1131.

Tolu, S., Eddine, R., Marti, F., David, V., Graupner, M., Pons, S., et al. (2013). Co-activation of VTA DA and GABA neurons mediates nicotine reinforcement. *Molecular Psychiatry, 18*, 382–393.

Tsuji, M., Nakagawa, Y., Ishibashi, Y., Yoshii, T., Takashima, T., Shimada, M., et al. (1996). Activation of ventral tegmental $GABA_B$ receptors inhibits morphine-induced place preference in rats. *European Journal of Pharmacology, 313*, 169–173.

Tyacke, R. J., Lingford-Hughes, A., Reed, L. J., & Nutt, D. J. (2010). $GABA_B$ receptors in addiction and its treatment. *Advances in Pharmacology, 58*, 373–396.

Varani, A. P., Antonelli, M. C., & Balerio, G. N. (2013) Mecamylamine-precipitated nicotine withdrawal syndrome and its prevention with baclofen: An autoradiographic study of $\alpha4\beta2$ nicotinic acetylcholine receptors in mice. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *44*, 217–225.

Varani, A. P., Aso, E., Moutinho, L. M., Maldonado, R., & Balerio, G. N. (2014). Attenuation by baclofen of nicotine rewarding properties and nicotine withdrawal manifestations. *Psychopharmacology, 231*, 3031–3040.

Varani, A. P., Pedrón, V. T., Machado, L. M., Antonelli, M. C., Bettler, B., & Balerio, G. N. (2015). Lack of $GABA_B$ receptors modifies behavioural and biochemical alterations induced by precipitated nicotine withdrawal. *Neuropharmacology, 90*, 90–101.

Vlachou, S., Guery, S., Froestl, W., Banerjee, D., Benedict, J., Finn, M. G., et al. (2011). Repeated administration of the $GABA_B$ receptor positive modulator BHF177 decreased nicotine self-administration, and acute administration decreased cue-induced reinstatement of nicotine seeking in rats. *Psychopharmacology, 215*, 117–128.

Vlachou, S., & Markou, A. (2010). $GABA_B$ receptors in reward processes. *Advances in Pharmacology, 58*, 315–371.

Voigt, R. M., Herrold, A. A., & Napier, T. C. (2011a). Baclofen facilitates the extinction of methamphetamine-induced conditioned place preference in rats. *Behavioral Neuroscience, 125*, 261–267.

Voigt, R. M., Herrold, A. A., Riddle, J. L., & Napier, T. C. (2011b). Administration of GABA(B) receptor positive allosteric modulators inhibit the expression of previously established methamphetamine-induced conditioned place preference. *Behavioural Brain Research, 216*, 419–423.

Vos, T., Barber, R. M., Bell, B., Bertozzi-Villa, A., Biryukov, S., Bolliger, I., et al. (2015). Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: A systematic analysis for the Global Burden of Disease Study 2013. *Lancet, 386*, 743–800.

Willick, M. L., & Kokkinidis, L. (1995). The effects of ventral tegmental administration of $GABA_A$, $GABA_B$ and NMDA receptor agonists on medial forebrain bundle self-stimulation. *Behavioural Brain Research, 70*, 31–36.

Wirtshafter, D., & Sheppard, A. C. (2001). Localization of GABA(B) receptors in midbrain monoamine containing neurons in the rat. *Brain Research Bulletin, 56*, 1–5.

Wise, R. A. (1978). Catecholamine theories of reward: A critical review. *Brain Research, 152*, 215–247.

Wise, R. A. (1987). The role of reward pathways in the development of drug dependence. *Pharmacology and Therapeutics, 35*, 227–263.

Woo, S. H., Kim, H. S., Yun, J. S., Lee, M. K., Oh, K. W., Seong, Y. H., et al. (2001). Inhibition of baclofen on morphine-induced hyperactivity, reverse tolerance and postsynaptic dopamine receptor supersensitivity. *Pharmacological Research, 43*, 335–340.

Xi, Z. X., Spiller, K., & Gardner, E. L. (2009). Mechanism-based medication development for the treatment of nicotine dependence. *Acta Pharmacologica Sinica, 30*, 723–739.

Xi, Z. X., & Stein, E. A. (1999). Baclofen inhibits heroin self-administration behavior and mesolimbic dopamine release. *Journal of Pharmacol and Experimental Therapeutics, 290*, 1369–1374.

Xi, Z. X., & Stein, E. A. (2000). Increased mesolimbic GABA concentration blocks heroin self-administration in the rat. *Journal of Pharmacol and Experimental Therapeutics, 294*, 613–619.

Xi, Z. X., & Stein, E. A. (2002). GABAergic mechanisms of opiate reinforcement. *Alcohol and Alcoholism, 37*, 485–494.

Xia, Y., Driscoll, J. R., Wilbrecht, L., Margolis, E. B., Fields, H. L., & Hjelmstad, G. O. (2011). Nucleus accumbens medium spiny neurons target non-dopaminergic neurons in the ventral tegmental area. *Journal of Neuroscience, 31*, 7811–7816.

Yang, K., Hu, J., Lucero, L., Liu, Q., Zheng, C., Zhen, X., et al. (2009). Distinctive nicotinic acetylcholine receptor functional phenotypes of rat ventral tegmental area dopaminergic neurons. *Journal of Physiology, 587*, 345–361.

Yoon, S. S., Lee, B. H., Kim, H. S., Choi, K. H., Yun, J., Jang, E. Y., et al. (2007). Potential roles of GABA receptors in morphine self-administration in rats. *Neuroscience Letters, 428*, 33–37.

Zaniewska, M., Przegaliński, E., & Filip, M. (2009). Nicotine dependence—human and animal studies, current pharmacotherapies and future perspectives. *Pharmacological Reports, 61*, 957–965.

Zarrindast, M. R., Ghadimi, M., Ramezani-Tehrani, B., & Sahebgharani, M. (2006). Effect of GABA receptor agonists or antagonists on morphine-induced Straub tail in mice. *International Journal of Neuroscience, 116*, 963–973.

Chapter 15
Targeting the $GABA_B$ Receptor for the Treatment of Alcohol Use Disorder

Roberta Agabio, Kimberly A. Leite-Morris, Giovanni Addolorato, and Giancarlo Colombo

Abstract Accumulating lines of experimental and clinical evidence suggest that the prototypic, orthosteric GABA type B ($GABA_B$) receptor agonist, baclofen, may possess therapeutic potential for treatment of alcohol use disorder (AUD). At preclinical level, acute or repeated treatment with nonsedative doses of baclofen has repeatedly been reported to suppress several alcohol-motivated behaviors, including alcohol drinking, in rats and mice. At clinical level, administration of doses of baclofen ranging from low to extremely high doses has been found to suppress alcohol consumption, craving for alcohol, and alcohol withdrawal signs and symptoms in patients affected by AUD, confirming that baclofen may represent a promising option for treatment of AUD. A more recent avenue of research is represented by generalization of baclofen effects to the positive allosteric modulators of the $GABA_B$ receptor: preclinical data collected to date suggest that these compounds reproduce, with a much higher therapeutic index, several suppressing effects of baclofen on alcohol-motivated behaviors.

Keywords Alcohol use disorder • Alcoholism • Baclofen • Positive allosteric modulators of the $GABA_B$ receptor • GS39783 • Animal models of alcohol use disorder

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15.1 Introductory Notes on Alcohol Use Disorder

15.1.1 Notes on Pharmacology of Alcohol

Ethyl alcohol or ethanol (commonly called alcohol) is a small two-carbon molecule produced in nature and consumed by a high percentage of women and men throughout nearly all Western countries. Alcohol use has been documented since at least 10,000 BC, and in today's society, more than 90% of the general population has consumed alcoholic beverages at least once (Schuckit 2011; O'Brien 2011). Alcohol shares with all other substances of abuse the ability to activate the brain "reward" system, producing feelings of pleasure and leading to repeated consumption (Koob and Le Moal 2006; APA 2013). Alcohol moreover interacts with inhibitory and excitatory systems in the brain. Namely, it potentiates the inhibitory activity of the γ-aminobutyric acid (GABA) system, with prominent effects on GABA type A ($GABA_A$) receptors, and reduces the stimulant activity of the glutamatergic system, producing pronounced effects on *N*-methyl-D-aspartate (NMDA) receptors (Koob and Le Moal 2006; Tabakoff and Hoffman 2013). In addition, alcohol interacts with serotonergic, endogenous opioid, epinephrine, cannabinoid, adenosine, and acetylcholine receptor systems, as well as with stress-related systems (Koob and Le Moal 2006; Tabakoff and Hoffman 2013).

Alcohol is a depressant psychoactive substance that induces sedation and sleep (Schuckit 2011; O'Brien 2011). After an initial behavioral disinhibition (likely due to inhibition of the brain inhibitory systems), acute alcohol consumption induces signs and symptoms of intoxication, typical of brain depression (Schuckit 2011). The severity of intoxication is directly related to the amount of alcohol consumed, ranging from muscle relaxation, somnolence, impaired judgment, and aggressiveness to general anesthesia, coma, and death (Schuckit 2011).

When large amounts of alcohol are consumed over long periods of time, the brain adapts to the continuous and prolonged exposure to alcohol through changes in receptors and other proteins (Koob and Le Moal 2006; Schuckit 2014). This phenomenon, known as tolerance, is characterized by a reduced response to the same amount of alcohol, resulting in the need to increase alcohol consumption to achieve similar responses. After prolonged exposure to alcohol, abrupt cessation of drinking produces symptoms opposite to those induced by acute alcohol intoxication: irritability, anxiety, tremors, agitation, and—in severe cases—hallucinations, seizures, delirium tremens, and coma (Koob and Le Moal 2006; Schuckit 2014). These symptoms and signs constitute the alcohol withdrawal syndrome (AWS).

Excessive alcohol consumption is a leading cause of disease and disability worldwide (Lim et al. 2012). Repeated episodes of excessive drinking are associated with high risk of developing cardiovascular disease, cancer, liver cirrhosis, fetal alcohol syndrome, neuropsychological impairment, psychiatric comorbidity, suicides, accidents, domestic violence, economic costs, loss of productivity, and mortality (Rehm et al. 2009; Roerecke and Rehm 2013). When combining the harms a drug may produce in users together with those it produces in others, alcohol is by far the most harmful psychoactive substance, with heroin and crack ranking second and third, respectively (Nutt et al. 2010).

15.1.2 Definition of Alcohol Use Disorder

In predisposed individuals, the repeated consumption of excessive amounts of alcohol may result in a severe mental disorder, known as alcohol use disorder (AUD) (APA 2013). AUD is a chronic and potentially lethal disorder, characterized by bouts of excessive drinking and the inability to control alcohol consumption despite the awareness of its negative consequences (APA 2013). In the last edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), diagnoses of alcohol dependence and alcohol abuse have been replaced by that of AUD, joining their criteria in a single set (APA 2000, 2013). AUD diagnosis requires repetitive alcohol-related problems in at least 2 out of 11 areas of life, described by its criterion set, including "craving," defined as a strong, obsessive, and irresistible desire to consume alcohol (APA 2013). Lifetime prevalence of AUD is dramatically high, being equal to 29.1 % of US adult population, with higher values in men (36.0 %) than in women (22.7 %) (Grant et al. 2015).

15.1.3 Pharmacotherapy for Alcohol Use Disorder

Medical treatment is aimed at helping AUD patients to achieve abstinence or, at least, decrease alcohol consumption, and reduce the frequency and severity of relapse, improving psychological and social functioning (Schuckit 2009; Connor et al. 2015). Approximately half of all AUD individuals who reduce or discontinue their alcohol consumption develop AWS (Schuckit 2014). The phase of AUD medical treatment aimed at reducing the severity of AWS and preventing possible complications is known as "detoxification." The second phase of AUD medical treatment, known as "rehabilitation," is aimed at reducing the severity of craving and the risk of relapse, helping AUD individuals to maintain the motivation to abstain from alcohol and to develop an alcohol-free lifestyle. First line treatment options in this second phase usually comprise psychological and social interventions, including *Alcoholics Anonymous* and various counseling approaches (Schuckit 2009). However, these approaches are not always completely successful for all patients and pharmacotherapy is emerging as a valuable tool for AUD treatment (Zindel and Kranzler 2014).

Benzodiazepines are the drugs of choice in the treatment of AWS as they reduce the severity of AWS symptoms and signs and prevent the incidence of complications (seizures and delirium tremens) (Amato et al. 2011). However, use of benzodiazepines is associated with several side effects, such as risk of developing dependence, excess sedation, and memory deficits.

Medications approved to achieve abstinence or reduce alcohol consumption in the USA and/or in Europe include disulfiram, naltrexone, acamprosate, nalmefene, and γ-hydroxybutyric acid (GHB) (Haass-Koffler et al. 2014; Connor et al. 2015). Disulfiram is indicated in preventing relapse in abstinent patients as it produces aversive effects after drinking alcohol (Skinner et al. 2014). However, efficacy of this drug is controversial, and patients show a poor adherence to treatment (Skinner

et al. 2014). Naltrexone and nalmefene are indicated in reducing the risk of heavy drinking as they act by decreasing the rewarding effects of alcohol (Zindel and Kranzler 2014). However, their efficacy is of moderate magnitude (Rosner et al. 2010a; Palpacuer et al. 2015). Acamprosate is indicated in reducing the risk of drinking, helping patients to maintain abstinence (Rosner et al. 2010b), although its mechanism of action is not completely known and its efficacy appears to be rather moderate in magnitude (Rosner et al. 2010b; Kiefer and Mann 2010). GHB is indicated to reduce the risk of drinking, acting as a substitutive medication: it produces subjective effects perceived as similar to those of alcohol, bearing subsequently abuse potential (Chick and Nutt 2012). Taken together, rehabilitation medications apparently feature only a moderate success rate, with no treatment adequately suiting all patients (Haass-Koffler et al. 2014). Although acamprosate and naltrexone are the medications displaying the best evidence of efficacy, the number of AUD patients who will need to be treated to prevent one person from returning to drinking is 12 and 20 for acamprosate and naltrexone, respectively (Jonas et al. 2014; Day et al. 2015).

Due to the limited efficacy of the approved medical treatments, there is a dramatic need to identify new and more effective medications. The prototypic, orthosteric agonist of GABA type B ($GABA_B$) receptor, baclofen—a drug employed successfully for 40 years in the treatment of muscle rigidity (see Chap. 17 of this book)—is one of the most intriguing pharmacological agents currently under evaluation for AUD treatment (Yardley and Ray 2016). The following sections will summarize the results obtained in preclinical and clinical studies supporting the hypothesis that the activation of the $GABA_B$ receptors may have beneficial effects in AUD.

15.2 Preclinical Studies

15.2.1 Baclofen

Baclofen has been the target of multiple animal studies designed to assess the ability of the compound to affect a series of alcohol-motivated behaviors. The majority of these studies have employed validated experimental procedures known to model different aspects of the human AUD; the results obtained have demonstrated that acute or repeated administration of nonsedative doses of baclofen suppressed (a) alcohol seeking and consumption, (b) the reinforcing, motivational, rewarding, and stimulating properties of alcohol, and (c) AWS severity in rats and mice. Notably, as reported below, most of these data have been successfully translated to AUD patients.

Several studies have tested the effects of baclofen on voluntary alcohol drinking in rats and mice exposed to the conventional, home cage 2-bottle "alcohol vs. water" choice regimen. Under this experimental procedure, animals can choose between an alcohol solution and water and are free to consume both fluids. Rats and mice selectively bred for alcohol preference can consume amounts of alcohol high enough to produce measurable psychopharmacological effects, including locomotor stimulation,

anxiolysis, and sedation. Repeated treatment with baclofen over the initial period of exposure to alcohol completely prevented the acquisition of alcohol drinking behavior in selectively bred, Sardinian alcohol-preferring (sP) rats (Colombo et al. 2002), suggesting that the activation of the $GABA_B$ receptor blocked the discovery and experience of those psychopharmacological effects of alcohol that sustain alcohol drinking. Furthermore, baclofen-induced suppression of alcohol drinking was observed in rats, including alcohol-preferring sP and University of Chile *bebedoras*, and mice (Daoust et al. 1987; Colombo et al. 2000; Stromberg 2004; Quintanilla et al. 2008; Peters et al. 2013) with consolidated high levels of alcohol consumption (models of the "active drinking" phase in human AUD).

Acute treatment with baclofen also suppressed "alcohol deprivation effect" in sP rats (Colombo et al. 2003a; 2006). "Alcohol deprivation effect" is defined as the temporary increase in voluntary alcohol consumption—often doubling the average consumption—occurring after a period of forced abstinence, or deprivation, from alcohol; it constitutes a validated experimental model of human relapse into heavy drinking. In addition, acute treatment with baclofen markedly reduced cue-induced reinstatement of alcohol-seeking behavior in sP rats (Maccioni et al. 2008a), which further confirms the "*anti*-relapse" potential of baclofen.

Recent studies have also reported the capability of acutely administered baclofen to suppress alcohol consumption in mice exposed to specific experimental procedures in which alcohol drinking escalated to intoxicating amounts over brief periods of time (Moore and Boehm 2009; Tanchuck et al. 2011), mimicking the human condition of binge drinking.

Relevant evidence on the "*anti*-addiction" profile of baclofen is provided by numerous studies testing the effect of baclofen on operant, oral alcohol self-administration. Under these procedures, alcohol is made available via the completion of a behavioral response (usually, responding on a lever for a given number of times), to allow the reinforcing and motivational properties of alcohol to be assessed (in addition to its mere consumption). Operant procedures differ from the alcohol-drinking procedures in that alcohol is not "freely" available and a specific amount of "work" is required to access alcohol. In studies using the fixed ratio (FR) schedule of reinforcement (in which the response requirement—i.e., the "cost" of each alcohol presentation in terms of number of lever responses—is predetermined and kept fixed throughout the session), acute and repeated treatment with baclofen reduced lever responding for alcohol and amount of self-administered alcohol in selectively bred alcohol-preferring sP, Indiana P, and Alko Alcohol (AA) rats (Maccioni et al. 2005, 2012; Liang et al. 2006), unselected rats (Petry 1997; Anstrom et al. 2003; Janak and Gill 2003; Walker and Koob 2007; Dean et al. 2012) and alcohol-consuming mice (Besheer et al. 2004). These data clearly demonstrate the ability of baclofen to suppress the reinforcing properties of alcohol.

Baclofen administration also suppressed lever responding under operant procedures intended to measure the motivational properties of alcohol. Indeed, acutely administered baclofen (a) reduced breakpoint for alcohol in rats, including those of the alcohol-preferring P, sP, and AA lines, exposed to the progressive ratio (PR) schedule of reinforcement (in which the response requirement is progressively

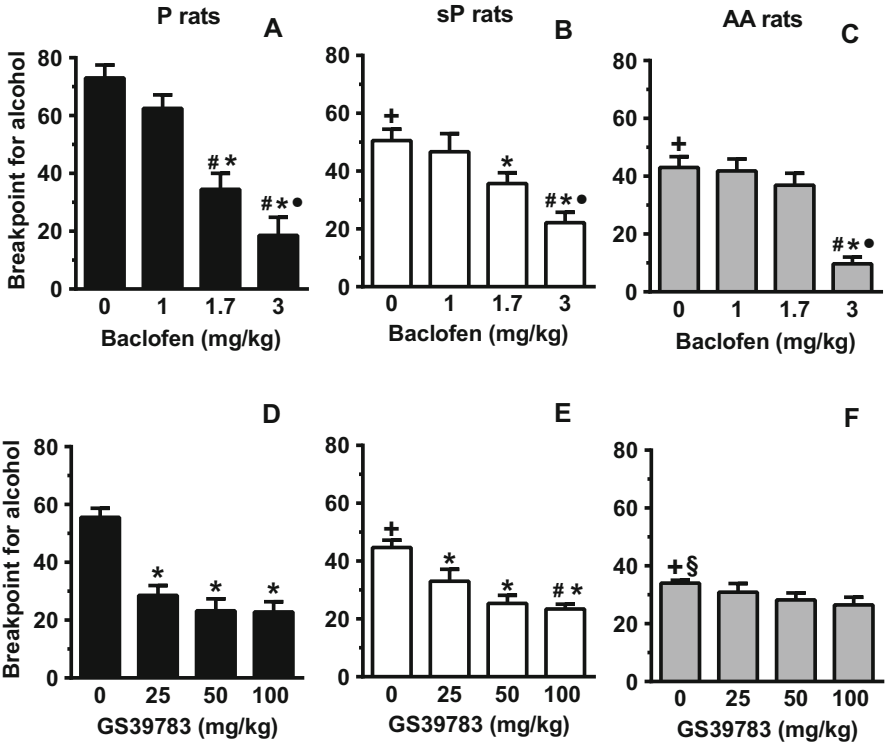

Fig. 15.1 Effect of treatment with the $GABA_B$ receptor agonist, baclofen (*panels* **a**–**c**), and the positive allosteric modulator of the $GABA_B$ receptor, GS39783 (*panels* **d**–**f**), on breakpoint for alcohol in selectively bred, Indiana alcohol-preferring (P) (*panels* **a**, **d**), Sardinian alcohol-preferring (sP) (*panels* **b**, **e**), and Alko Alcohol (AA) (*panels* **c**, **f**) rats. Rats were initially trained to lever respond for oral alcohol (15 % v/v, in water) [fixed ratio (FR) 4 (FR4)] and water (FR1) in daily 30-min sessions; once self-administration behavior had stabilized, rats were tested with baclofen (0, 1, 1.7, and 3 mg/kg; i.p.) or GS39783 (0, 25, 50, and 100 mg/kg; i.g.) under a progressive ratio (PR) schedule of reinforcement, in which the response requirement was increased progressively (namely, 4, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, etc.) over a 60-min session. Breakpoint was defined as the lowest response requirement not achieved by the rat. All four doses of baclofen or GS39783 were tested in each rat under a Latin-square design. Each bar is the mean ± SEM of $n = 12$ rats. +: $P < 0.05$ in comparison to vehicle-treated P rats (LSD test); §: $P < 0.05$ in comparison to vehicle-treated sP rats (LSD test); ★: $P < 0.05$ in comparison to vehicle-treated rats of the same line (LSD test); #: $P < 0.05$ in comparison to rats of the same line treated with 1 mg/kg baclofen or 25 mg/kg GS39783 (LSD test); ●: $P < 0.05$ in comparison to rats of the same line treated with 1.7 mg/kg baclofen (LSD test). Reproduced from Maccioni et al. (2012) with permission from John Wiley and Sons

increased, after each reinforcer, up to achievement of the breakpoint, defined as the lowest ratio not completed) (Walker and Koob 2007; Maccioni et al. 2008b, 2012) (Fig. 15.1, panels a–c) and (b) completely suppressed single-session extinction responding for alcohol in sP (Colombo et al. 2003b) and Long Evans (Leite-Morris et al. 2008) (Fig. 15.2, panel a) rats.

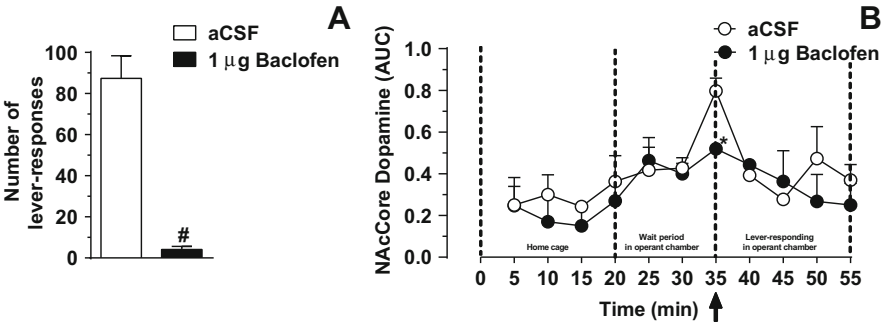

Fig. 15.2 Effects of intra-ventral tegmental area (VTA) microinjection of the $GABA_B$ receptor agonist, baclofen, on extinction responding for alcohol (*panel* **a**) and extracellular dopamine in the nucleus accumbens core (NAcCore) (*panel* **b**) during an extinction responding session in Long Evans rats. Rats were initially trained to lever respond for oral 2 % (v/v) alcohol/10 % (w/v) sucrose on fixed ratio (FR; increasing progressively from FR1 to FR4) and then transitioned to a continuous reinforcement schedule—progressively fading out sucrose from 10 to 0 % and increasing alcohol from 2 to 10 %—using an escalating response requirement (RR) RR4 to RR20, in daily 20-min sessions until stable lever responding. The reinforcement schedule of RR20 was performed over 2 consecutive weeks. Week 1 comprised 5 self-administration sessions (Monday to Friday) under RR20; Week 2 comprised 4 self-administration sessions (Monday to Thursday) under RR20 *plus* an extinction responding session (on Friday) with unlimited, non-reinforced lever responding. The extinction responding session lasted 20 min and was conducted to assess appetitive responding for alcohol. Concurrently, brain dialysate was collected at 5-min intervals and quantitatively analyzed using a high performance liquid chromatography system with electrochemical detection (Antec, Zoeterwoude, The Netherlands). Baclofen was dissolved in artificial cerebrospinal fluid (aCSF) and intra-VTA microinjected, at the doses of 0 and 1.0 µg, 30 min prior to placement into the operant chamber. Dialysate samples were collected from the NAcCore in 5-min intervals while the rat was in the home cage, during the wait period in the operant chamber (prior to the lever presentation), during lever responding and during a post-lever wait period (still into the operant chamber). Dialysate dopamine levels are presented as area under the curve (AUC). In *panel* **a**, each bar is the mean ± SEM of $n = 7$–8 rats; in *panel* **b**, each point is the mean ± SEM of $n = 3$ rats (due to technical complications, some rats did not undergo microdialysis sampling). In *panel* **b**, the *arrow* indicates lever insertion inside the operant chamber. #: $P < 0.0001$ in comparison to aCSF-treated rats (Student *t*-test); ★: $P < 0.05$ in comparison to aCSF-treated rats of the same time interval (last 5-min interval of the wait period prior the lever presentation)

Taken together, these results bear translational relevance as they have been collected using experimental procedures with demonstrated validity for human AUD. As further evidence of the translational value of these data, it may be worth noting that baclofen was more potent and effective in those rats (either selectively bred or made physically dependent on alcohol) seeking and consuming larger amounts of alcohol (Walker and Koob 2007; Maccioni et al. 2012). This view shares similarities with the clinical data suggesting higher efficacy of baclofen treatment in patients affected by severe forms of AUD (see below).

When investigated, the selectivity of the reducing effect of baclofen on alcohol drinking and self-administration was relatively limited, as treatment with baclofen tended to reduce consumption and self-administration of alternative, nondrug reinforcers (e.g., sucrose or saccharin solutions or regular food pellets) (Colombo et al.

2000, 2003b; Anstrom et al. 2003; Janak and Gill 2003; Maccioni et al. 2005, 2008b, 2012), suggestive of a more generalized suppression of reward-motivated behaviors and indicative of a relatively modest separation between the "desired" pharmacological effects and "unwanted," or adverse, effects. Notably, however, the enhancing effect of baclofen on alcohol-induced sedation was modest in alcohol-"experienced" mice (suggestive of the development of some degree of cross-tolerance between alcohol and baclofen) (Besheer et al. 2004) or even absent (Holstein et al. 2009).

In terms of the mechanism of action underlying baclofen effects on alcohol-related behaviors, it is likely that it is mediated by the activation of the $GABA_B$ receptors located in the ventral tegmental area (a key area of the brain "reward" system), both presynaptically (on the cell body of dopamine neurons) and postsynaptically (on the terminals of glutamatergic afferent neurons) (see Chap. 8 of this book); baclofen-induced activation of these receptors would result in the inhibition of alcohol-stimulated firing of dopaminergic neurons and dopamine release in the nucleus accumbens, and—in turn—of dopamine-controlled, alcohol-related responses and behaviors. Accordingly, baclofen microinjection into the ventral tegmental area suppressed (a) alcohol seeking in rats (Leite-Morris et al. 2008) (Fig. 15.2, panel a), (b) alcohol consumption in rats (Moore and Boehm 2009), (c) stimulation of dopamine release in the rat nucleus accumbens associated with anticipation of alcohol reinforcement (Leite-Morris et al. 2009) (Fig. 15.2, panel b), (d) alcohol-stimulated locomotor activity (the animal correlate of the stimulating and euphorigenic effects of alcohol) in mice (Boehm et al. 2002; Leite-Morris 2004), (e) alcohol-induced sensitization of locomotor activity (a phenomenon thought to play a central role in the development of drug addiction) in mice (Leite-Morris 2004), and (f) alcohol-induced place preference (the animal correlate of the rewarding effects of alcohol) in mice (Bechtholt and Cunningham 2005).

Treatment with baclofen was also reported to suppress several signs of AWS, including increase in alcohol self-administration, anxiety-related behaviors, tremors, and seizures, in rats made alcohol dependent by the forced, prolonged exposure to intoxicating amounts of alcohol (File et al. 1991; Colombo et al. 2000; Knapp et al. 2007; Walker and Koob 2007). These data have been effectively replicated in clinical studies (see below). In terms of the mechanism(s) of action, the suppressant effects of baclofen on AWS are likely due to a $GABA_B$ receptor-mediated counterbalance of the enhanced function of glutamate excitatory neurotransmission associated to AWS (Colombo et al. 2000; Knapp et al. 2007).

15.2.2 Positive Allosteric Modulators of the $GABA_B$ Receptor

Once the first positive allosteric modulators of the $GABA_B$ receptor ($GABA_B$ positive allosteric modulators) had been synthesized (see Chap. 3 of this book), interest grew regarding their ability to affect alcohol-related behaviors. The peculiar mode of action of $GABA_B$ positive allosteric modulators—i.e., augmentation of the affinity of the

$GABA_B$ receptor for GABA and agonists, synergistic potentiation of their effects, and lack of substantial intrinsic agonistic activity (see Chap. 18 of this book)—has led to hypothesize that $GABA_B$ positive allosteric modulators may represent a remarkable step forward in the use of $GABA_B$ receptor ligands as pharmacotherapies for AUD.

Preclinical data collected to date fully support this hypothesis. Virtually all studies demonstrate indeed that the suppressing effects of baclofen on alcohol-related behaviors can be reproduced by treatment with $GABA_B$ positive allosteric modulators. Specifically, repeated treatment with CGP7930, GS39783, and *rac*-BHFF delayed acquisition and reduced maintenance of alcohol drinking in sP rats exposed to the 2-bottle "alcohol vs. water" choice regimen (Orrù et al. 2005; Loi et al. 2013). Acute treatment with GS39783 and ADX71441 reduced excessive, or binge-like, drinking in sP rats (Colombo et al. 2015) and mice (Hwa et al. 2014; Linsenbardt and Boehm 2014). Treatment with CGP7930, GS39783, BHF177, and *rac*-BHFF reduced lever responding for alcohol in P, sP, and AA rats exposed to FR and PR schedules of reinforcement (Liang et al. 2006; Maccioni et al. 2007, 2008b, 2009, 2010, 2012) (Fig. 15.1, panels d–f). A recent study in sP rats found that the reducing effect of $GABA_B$ positive allosteric modulators on alcohol self-administration was maintained unaltered after repeated administration (Maccioni et al. 2015), suggesting the potential effectiveness of $GABA_B$ positive allosteric modulators under chronic treatment.

The combination of per se ineffective doses of GS39783 (or *rac*-BHFF) and baclofen markedly reduced alcohol self-administration in sP rats (Maccioni et al. 2015). These data corroborate that $GABA_B$ positive allosteric modulators are capable of facilitating in vivo the pharmacological activation of the $GABA_B$ receptor and possess relevant translational interest, as they suggest that low doses of a $GABA_B$ positive allosteric modulator would selectively potentiate the suppressing effect of baclofen on alcohol craving and consumption.

At variance with baclofen results, the reducing effect of $GABA_B$ positive allosteric modulators were highly selective for alcohol-related behaviors and occurred at doses far lower from those inducing motor-impairment and sedation; if transposed to humans, these data suggest that $GABA_B$ positive allosteric modulators possess remarkably higher therapeutic index and better side effect profile than baclofen.

15.3 Clinical Studies on Baclofen

15.3.1 Alcohol Withdrawal Syndrome

In agreement with the results of the preclinical studies, several case reports and randomized-controlled trials have suggested the ability of orally administered baclofen to reduce the severity of AWS in humans. However, the evidence obtained is still considered insufficient to recommend baclofen for the treatment of AWS (Liu and Wang 2015).

15.3.1.1 Case Reports

In an initial study, five patients with AWS received relatively low doses of baclofen [30 mg/day (10 mg three times a day, t.i.d.)] and severity of AWS was evaluated by means of the Clinical Institute Withdrawal Assessment for Alcohol-revised scale (CIWA-Ar), every hour for 4–8 h (Addolorato et al. 2002a). All patients displayed a rapid decrease in AWS severity and continued baclofen treatment for a 30-day period, maintaining abstinence without reporting any significant side effects (Addolorato et al. 2002a).

In a second study, a single patient with severe AWS, complicated by delirium tremens, received a higher oral dose of baclofen [75 mg/day (25 mg t.i.d.)] (Addolorato et al. 2003). Within 1 h of the first baclofen administration, AWS severity decreased dramatically. Following stabilization, the patient continued baclofen treatment at a lower dose (30 mg/day), maintaining abstinence without any relevant side effects for a 30-day period.

15.3.1.2 Comparative Studies

Two randomized open trials compared the effectiveness of baclofen with benzodiazepines in the treatment of AWS (Addolorato et al. 2006; Reddy et al. 2014). In the first study (Addolorato et al. 2006), 18 participants received baclofen (30 mg/day, for 10 days) and 19 diazepam (0.5–0.75 mg/kg/day for 6 days, tapering the dose by 25 % daily from day 7 to day 10); both medications produced a significant and similar decrease in CIWA-Ar scores. In the second study (Reddy et al. 2014), 30 participants received baclofen (30 mg/day progressively reduced to 10 mg/day) and 30 participants received chlordiazepoxide (75 mg/day progressively reduced to 25 mg/day); both medications significantly decreased CIWA-Ar scores, with baclofen resulting relatively less effective than chlordiazepoxide.

15.3.1.3 Randomized, Placebo-Controlled Studies

Only one randomized, double-blind trial has compared the effectiveness of baclofen (25 participants; 30 mg/day) with placebo (19 participants) (Lyon et al. 2011). Benzodiazepines were provided as rescue medication. Participants treated with baclofen required lower amounts of benzodiazepines than participants treated with placebo over the 72-h observation period.

15.3.2 Relapse Prevention and Suppression of Alcohol Craving and Consumption

Over the last few years and with a few exceptions, a large series of case reports, retrospective studies, open studies, and laboratory and randomized-controlled trials have reported that orally administered baclofen effectively reduced or

even suppressed craving for alcohol and alcohol consumption in AUD patients, particularly when baclofen was given at high doses. In the wake of these results, in March 2014 baclofen was approved in France for the treatment of AUD with a special temporary recommendation of use by the *Agence Nationale de Sécurité du Médicament et de Produits de Santé*.

15.3.2.1 Case Reports

A large number of case reports found that the administration of high doses of baclofen markedly reduced or completely suppressed alcohol consumption in AUD patients (Ameisen 2005; Agabio et al. 2007; Bucknam 2007; Dore et al. 2011; Pastor et al. 2012; Weibel et al. 2015). The first of these case reports described the original dose-finding study conducted by a French physician to treat his own AUD (Ameisen 2005). Namely, Dr. Olivier Ameisen started with 30 mg/day and increased the daily dose of baclofen up to 270 mg, achieving complete abstinence and suppression of craving for alcohol. Because of somnolence, Dr. Ameisen reduced the dose to 120 mg/day, adding 40 mg in stressful situations. This dose provided a complete and stable control over his alcohol craving without any particular side effect. Interestingly, Dr. Ameisen also suffered from comorbid anxiety, and the 120 mg/day baclofen dose completely suppressed anxiety. Another case report found that baclofen administration ameliorated anxiety in AUD subjects suggesting that the "*anti*-alcohol" effects of baclofen in patients affected by comorbid anxiety disorders may be due, at least in part, to a relief of anxiety symptoms (Dore et al. 2011).

15.3.2.2 Retrospective Studies

In France, following publication of the bestselling book entitled *The End of My Addiction* written by Dr. Ameisen (Ameisen 2008), a large number of AUD patients asked to be treated with baclofen (Enserink 2011). Between 2007 and 2013, approximately 200,000 subjects started AUD treatment with high doses of baclofen (Chaignot et al. 2015). Several retrospective studies conducted in France found that AUD patients treated with high doses of baclofen (average doses: 130–150 mg/day) achieved and maintained complete abstinence from alcohol or reduced their alcohol consumption to low risk drinking even after 12–24 months of treatment (de Beaurepaire 2012; Rigal et al. 2012). One study reported that some patients required extremely high doses of baclofen (>300 mg/day) to achieve and maintain abstinence (de Beaurepaire 2014). Another study conducted in India confirmed a relationship between baclofen dose and efficacy (Shukla et al. 2015). A French study found that AUD patients asking to be treated with baclofen consume larger amounts of alcohol and are approximately eight times more likely to be self-referred and treatment naive than other AUD patients who request medical treatment (Simioni et al. 2016). These findings suggest that the availability of baclofen as a treatment option may even induce previously untreated individuals with severe forms of AUD to seek medical treatment.

15.3.2.3 Open Studies

In two open studies, low doses of baclofen (30 mg/day) were administered to participants with AUD, for relatively short periods of time (4–12 weeks) (Addolorato et al. 2000; Flannery et al. 2004). These studies found that baclofen effectively reduced alcohol consumption, craving for alcohol, and anxiety; however, in one study, no patient achieved complete abstinence (Flannery et al. 2004). An additional 12-week open-label study confirmed the ability of low doses of baclofen (30 mg/day) to reduce alcohol craving and anxiety levels in 25 AUD participants; a reduction in stress-related hormones (e.g., cortisol, aldosterone) was also reported (Leggio et al. 2008a, b).

A more recent study found that administration of higher doses of baclofen (average dose: 145 mg/day) for 12 weeks helped the vast majority of patients (88 %) to achieve abstinence; many of them reported having effortlessly become indifferent to alcohol (Ameisen and de Beaurepaire 2010). Low-to-moderate doses of baclofen (30–60 mg/day) resulted as being effective and safe in the treatment of AUD patients affected by alcoholic liver disease, even after a 2-year treatment (Heydtmann et al. 2015; Yamini et al. 2014).

15.3.2.4 Laboratory Study

The efficacy of a low dose of baclofen was investigated in a randomized, placebo-controlled human laboratory study (Leggio et al. 2013). Fourteen AUD nontreatment-seeking patients received 30 mg/day baclofen or placebo for 7 days. On day 8, in a private bar-like atmosphere, participants were allowed to drink alcoholic beverages or receive alternative monetary reinforcers for not drinking: baclofen effectively reduced alcohol consumption in individuals with an endophenotype related with severe alcohol-related problems.

15.3.2.5 Randomized, Placebo-Controlled Studies

The first randomized, controlled trial was conducted in Italy (Addolorato et al. 2002b). In this study, 39 AUD patients were divided into two groups and received a low dose of baclofen (30 mg/day) or placebo for 4 weeks. Compared to placebo, baclofen treatment (a) reduced consumption of and craving for alcohol, (b) increased total alcohol abstinence, and (c) reduced anxiety.

Two clinical trials evaluated the effectiveness of low-to-moderate doses of baclofen (Addolorato et al. 2011; Morley et al. 2014). In each study, 42 AUD patients were divided into 3 groups and received low (30 mg/day) or moderate (60 mg/day) doses of baclofen or placebo for 12 weeks. In the first study (Addolorato et al. 2011), both doses effectively reduced alcohol consumption and increased abstinence, with 60 mg being more effective than 30 mg. In the second trial (Morley et al. 2014), no dose of baclofen was found to be effective in the entire sample of

AUD patients. However, both doses effectively increased abstinence in AUD patients affected by comorbid anxiety disorders. These findings are consistent with the hypothesis that craving for alcohol and anxiety may share common neural substrates, with $GABA_B$ receptors being involved (Cryan and Kaupmann 2005). Accordingly, the $GABA_B$ receptor may represent a target for treatment of AUD patients in which stress and anxiety contribute towards increasing alcohol consumption and/or risk of relapse.

In a subsequent study, 30 AUD heavy-smoker patients received a relatively high dose of baclofen [80 mg/day (20 mg, 4 times a day; q.i.d.)] or placebo for 12 weeks; baclofen increased the number of days of abstinence from alcohol and tobacco couse (Leggio et al. 2015a, b).

At variance with the consensus reached by the above studies, a trial conducted in the USA failed to demonstrate the efficacy of a low dose of baclofen (Garbutt et al. 2010). Two equal groups of 40 AUD participants received either placebo or 30 mg/day baclofen for 12 weeks: no difference in alcohol consumption, alcohol craving, and abstinence rate was observed (Garbutt et al. 2010). The high response to placebo, together with the relatively modest severity of AUD, was proposed as possible explanations for of the discrepancy with previous studies (Leggio et al. 2010).

Only one randomized controlled study has been conducted to date testing high doses of baclofen (Müller et al. 2015). In this trial, 56 AUD participants received individually titrated high doses of baclofen (30–270 mg/day) for 24 weeks (Müller et al. 2015). The treatment period included a 4-week escalation dose phase, a 12-week high-dose phase (when the maximum dose tolerated by each single patient was administered), and a 4-week tapering phase. The mean dose of baclofen administered during the high-dose phase was equal to 180 mg/day. Baclofen effectively increased alcohol abstinence and was well tolerated, producing no serious adverse events.

From a practical point of view, one of the main aspects of interest with baclofen is likely represented by the safety and efficacy of the drug in AUD patients affected by advanced liver disease (Addolorato et al. 2007). Alcohol is the most frequent cause of liver cirrhosis in the Western world. Persistent alcohol consumption has been associated with increased mortality in patients with liver cirrhosis; conversely, abstinence improves survival in patients with any stage of alcoholic liver disease. In these patients, medical and surgical treatments for alcoholic liver disease have limited success when drinking continues; the most effective management strategy consists in achieving total alcohol abstinence. However, AUD medications are often contraindicated and rarely administered to patients with liver disease because of concerns for their hepatic metabolism and possible drug-induced hepatic injury (Addolorato et al. 2016).

The safety and metabolism of baclofen (see below) suggested the feasibility of conducting a randomized, controlled trial in which placebo, or a low dose of baclofen (30 mg/day), was administered for 12 weeks to 84 AUD patients with liver cirrhosis (Addolorato et al. 2007). Baclofen treatment (a) increased the percentage of complete abstinence and (b) reduced the frequency of lapse and relapse to alcohol consumption in those patients not achieving complete abstinence.

Baclofen was manageable and no patient discontinued treatment because of side effects. Baclofen efficacy and safety were also observed in a subgroup of AUD patients with cirrhosis and HCV infection (Addolorato et al. 2007). These initial data have subsequently been confirmed by subsequent trials (Yamini et al. 2014; Heydtmann 2011; Barrault et al. 2015; Owens et al. 2015). Together, these different lines of evidence prompted inclusion of baclofen in European (European Association for the Study of Liver 2012) and US (Runyon 2013) guidelines for the management of alcoholic liver disease.

15.3.3 Safety Profile

The most common side effect of baclofen observed in AUD patients consists in a dose-dependent sedation: AUD patients treated with low doses of baclofen usually develop mild and transient sedation whereas those treated with higher doses may experience more severe episodes of sedation (e.g., Addolorato et al. 2000; Ameisen 2005). A randomized, placebo-controlled human laboratory study demonstrated the relatively safe profile of baclofen treatment even when AUD subjects continued to drink (Evans and Bisaga 2009). In this study, 18 heavy drinkers were given moderate-to-high doses of baclofen (40 and 80 mg), 2.5 h before the consumption of approximately 4 alcoholic or placebo beverages. Subsequently, participants performed different tasks to assess learning, memory, vigilance, and psychomotor ability. Both baclofen and alcohol increased sedation and impaired performance; however, the baclofen *plus* alcohol combination did not result in a further performance impairment. Not surprisingly, a prospective cohort study found that the risk of episodes of major sedation was directly related to both the baclofen dose and amount of alcohol consumed (Rolland et al. 2015).

As baclofen is predominantly eliminated by the kidneys, it is contraindicated in patients with impaired renal function (Reichmuth et al. 2015). Cases of intentional intoxication with baclofen (Dias et al. 2011; Franchitto et al. 2014; Pape et al. 2014) and baclofen withdrawal (Akosile and Klan 2015; Nasti and Brakoulias 2011; Rolland et al. 2014) have been described in AUD patients treated with high doses of baclofen. Other severe, although sporadic, side effects developed by AUD patients treated with high doses of baclofen (100–240 mg/day) include seizures (Rolland et al. 2012), tinnitus (Auffret et al. 2014), increase in triglyceride levels (Clarisse et al. 2013), manic symptoms (Geoffry et al. 2014), and central sleep apnea (Perogamvros et al. 2015).

To minimize the occurrence of baclofen side effects and withdrawal, it has been proposed that (a) renal function, comorbid psychiatric illness, and past history of suicide attempts should be investigated before prescribing any baclofen dose; (b) baclofen treatment should be initiated at a low dose, gradually titrating upward, and gradually discontinued, avoiding the abrupt discontinuation; and (c) baclofen treatment should be conducted under strict medical supervision.

15.4 Conclusions

Preclinical and clinical studies suggest that baclofen may constitute a useful pharmacological agent for AUD treatment. Indeed, the majority of results collected to date indicate that baclofen is effective and relatively safe in (a) AWS treatment, (b) reducing the severity of alcohol craving, and (c) suppressing alcohol consumption. This unique pharmacological profile apparently confers to baclofen the features of an ideal AUD medication, as baclofen may be used in both detoxification and rehabilitation phases without requiring any additional medication.

Preclinical data suggest that $GABA_B$ positive allosteric modulators may represent a step forward in a $GABA_B$ receptor-based pharmacology of AUD. $GABA_B$ positive allosteric modulators indeed reproduce baclofen-induced suppressant effects on several alcohol-related behaviors and display a much more favorable safety profile. Clinical studies are now needed to possibly generalize these data to AUD patients. As the majority of AUD patients still receive no treatment (Connor et al. 2015; Grant et al. 2015), the development of new, effective, safe, and manageable medications is expected to considerably increase the number of patients who seek and receive medical treatment.

References

Addolorato, G., Caputo, F., Capristo, E., Colombo, G., Gessa, G. L., & Gasbarrini, G. (2000). Ability of baclofen in reducing alcohol craving and intake: II—Preliminary clinical evidence. *Alcoholism: Clinical and Experimental Research, 24*, 67–71.

Addolorato, G., Caputo, F., Capristo, E., Domenicali, M., Bernardi, M., Janiri, L., et al. (2002a). Baclofen efficacy in reducing alcohol craving and intake: A preliminary double-blind randomized controlled study. *Alcohol and Alcoholism, 37*, 504–508.

Addolorato, G., Caputo, F., Capristo, E., Janiri, L., Bernardi, M., Agabio, R., et al. (2002b). Rapid suppression of alcohol withdrawal syndrome by baclofen. *American Journal of Medicine, 112*, 226–229.

Addolorato, G., Leggio, L., Abenavoli, L., DeLorenzi, G., Parente, A., Caputo, F., et al. (2003). Suppression of alcohol delirium tremens by baclofen administration: A case report. *Clinical Neuropharmacology, 26*, 258–262.

Addolorato, G., Leggio, L., Abenavoli, L., Agabio, R., Caputo, F., Capristo, E., et al. (2006). Baclofen in the treatment of alcohol withdrawal syndrome: A randomized comparative study versus diazepam. *American Journal of Medicine, 119*(3), 276.e13–276.e18.

Addolorato, G., Leggio, L., Ferrulli, A., Cardone, S., Vonghia, L., Mirijello, A., et al. (2007). Effectiveness and safety of baclofen for maintenance of alcohol abstinence in alcohol dependent patients with liver cirrhosis: Randomised, double-blind controlled study. *Lancet, 370*, 1915–1922.

Addolorato, G., Leggio, L., Ferrulli, A., Cardone, S., Bedogni, G., Caputo, F., et al. (2011). Dose-response effect of baclofen in reducing daily alcohol intake in alcohol dependence: Secondary analysis of a randomized, double-blind, placebo-controlled trial. *Alcohol and Alcoholism, 46*, 312–317.

Addolorato, G., Mirijello, A., Barrio, P., & Gual, A. (2016). Treatment of alcohol use disorders in patients with alcoholic liver disease. *Journal of Hepatology, 65*(3), 618–630.

Agabio, R., Marras, P., Addolorato, G., Carpiniello, B., & Gessa, G. L. (2007). Baclofen suppresses alcohol intake and craving for alcohol in a schizophrenic alcohol-dependent patient: A case report. *Journal of Clinical Psychopharmacology, 27*, 319–320.

Akosile, W., & Klan, M. (2015). Creating a new problem: The use of baclofen in the management of alcohol use disorder. *Drug and Alcohol Review*. doi:10.1111/dar.12306.

Amato, L., Minozzi, S., & Davoli, M. (2011). Efficacy and safety of pharmacological interventions for the treatment of the alcohol withdrawal syndrome. *Cochrane Database of Systematic Reviews, 6*, CD008537.

Ameisen, O. (2005). Complete and prolonged suppression of symptoms and consequences of alcohol-dependence using high-dose baclofen: A self-case report of a physician. *Alcohol and Alcoholism, 40*, 147–150.

Ameisen, O. (2008). *The end of my addiction*. New York: Farrar Straus Giroux.

Ameisen, O., & de Beaurepaire, R. (2010). Suppression de la dépendance à l'alcool par le baclofène à haute dose: un essai en ouvert. *Annales Médico-Psychologiques, 168*, 159–162.

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed., text revision). DSM-IV-TR. Washington, DC: American Psychiatric Association.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Arlington, VA: American Psychiatric Association.

Anstrom, K. K., Cromwell, H. C., Markowski, T., & Woodward, D. J. (2003). Effect of baclofen on alcohol and sucrose self-administration in rats. *Alcoholism: Clinical and Experimental Research, 27*, 900–908.

Auffret, M., Rolland, B., Deheul, S., Loche, V., Hennaux, C., Cottencin, O., et al. (2014). Severe tinnitus induced by off-label baclofen. *Annals of Pharmacotherapy, 48*, 656–659.

Barrault, C., Lison, H., Roudot-Thoraval, F., Garioud, A., Béhar, V., Rosa-Hézode, I., et al. (2015). One year effectiveness of baclofen treatment in 100 alcohol-dependent patients. *Journal of Hepatology, 62*, S758–S759.

Bechtholt, A. J., & Cunningham, C. L. (2005). Ethanol-induced conditioned place preference is expressed through a ventral tegmental area dependent mechanism. *Behavioral Neuroscience, 119*, 213–223.

Besheer, J., Lepoutre, V., & Hodge, C. W. (2004). $GABA_B$ receptor agonists reduce operant ethanol self-administration and enhance ethanol sedation in C57BL/6J mice. *Psychopharmacology, 74*, 358–366.

Boehm, S. L., Piercy, M. M., Bergstrom, H. C., Phillips, T. J., et al. (2002). Ventral tegmental area region governs $GABA_B$ receptor modulation of ethanol-stimulated activity in mice. *Neuroscience, 115*, 185–200.

Bucknam, W. (2007). Suppression of symptoms of alcohol dependence and craving using high-dose baclofen. *Alcohol and Alcoholism, 42*, 158–160.

Chaignot, C., Weill, A., Ricordeau, P., & Alla, F. (2015). Use in France of baclofen for alcohol dependence from 2007 to 2013: Cohort study based on the databases SNIIRAM and PMSI. *Thérapie, 70*, 443–453.

Chick, J., & Nutt, D. J. (2012). Substitution therapy for alcoholism: Time for a reappraisal? *Journal of Psychopharmacology, 26*, 205–212.

Clarisse, H., Imbert, B., Belzeaux, R., Jaquet, I., Lancon, C., & Simon, N. (2013). Baclofen and risperidone association increases dramatically triglycerides level. *Alcohol and Alcoholism, 48*, 515–516.

Colombo, G., Agabio, R., Carai, M. A. M., Lobina, C., Pani, M., Reali, R., et al. (2000). Ability of baclofen in reducing alcohol intake and withdrawal severity: I—Preclinical evidence. *Alcoholism: Clinical and Experimental Research, 24*, 58–66.

Colombo, G., Lobina, C., Maccioni, P., Carai, M. A., Lorrai, I., Zaru, A., et al. (2015). Anxiety-like behaviors at the end of the nocturnal period in sP rats with a "history" of unpredictable, limited access to alcohol. *Alcohol, 49*, 707–712.

Colombo, G., Serra, S., Brunetti, G., Atzori, G., Pani, M., Vacca, G., et al. (2002). The $GABA_B$ receptor agonists baclofen and CGP 44532 prevent acquisition of alcohol drinking behaviour in alcohol-preferring rats. *Alcohol and Alcoholism, 37*, 499–503.

Colombo, G., Serra, S., Brunetti, G., Vacca, G., Carai, M. A., & Gessa, G. L. (2003a). Suppression by baclofen of alcohol deprivation effect in Sardinian alcohol-preferring (sP) rats. *Drug and Alcohol Dependence, 70*, 105–108.

Colombo, G., Serra, S., Vacca, G., Carai, M. A., & Gessa, G. L. (2006). Baclofen-induced suppression of alcohol deprivation effect in Sardinian alcohol-preferring (sP) rats exposed to different alcohol concentrations. *European Journal of Pharmacology, 550*, 123–126.

Colombo, G., Vacca, G., Serra, S., Brunetti, G., Carai, M. A., & Gessa, G. L. (2003b). Baclofen suppresses motivation to consume alcohol in rats. *Psychopharmacology, 167*, 221–224.

Connor, J. P., Haber, P. S., & Hall, W. D. (2015). Alcohol use disorders. *Lancet, 387*, 988–998.

Cryan, J. F., & Kaupmann, K. (2005). Don't worry 'B' happy!: A role for GABA(B) receptors in anxiety and depression. *Trends in Pharmacological Sciences, 26*, 36–43.

Daoust, M., Saligaut, C., Lhuintre, J. P., Moore, N., Flipo, J. L., & Boismare, F. (1987). GABA transmission, but not benzodiazepine receptor stimulation, modulates ethanol intake by rats. *Alcohol, 4*, 469–472.

Day, E., Copello, A., & Hull, M. (2015). Assessment and management of alcohol use disorders. *BMJ, 350*, h715.

de Beaurepaire, R. (2012). Suppression of alcohol dependence using baclofen: A 2-year observational study of 100 patients. *Frontiers in Psychiatry, 3*, 103.

de Beaurepaire, R. (2014). The use of very high-doses of baclofen for the treatment of alcohol-dependence: A case series. *Frontiers in Psychiatry, 5*, 143.

Dean, R. L., Eyerman, D., Todtenkopf, M. S., Turncliff, R. Z., Bidlack, J. M., & Deaver, D. R. (2012). Effects of oral loperamide on efficacy of naltrexone, baclofen and AM-251 in blocking ethanol self-administration in rats. *Pharmacology, Biochemistry, and Behavior, 100*, 530–537.

Dias, L. S., Vivek, G., Manthappa, M., & Acharya, R. V. (2011). Role of hemodialysis in baclofen overdose with normal renal function. *Indian Journal of Pharmacology, 43*, 722–723.

Dore, G. M., Lo, K., Juckes, L., Bezyan, S., & Latt, N. (2011). Clinical experience with baclofen in the management of alcohol-dependent patients with psychiatric comorbidity: A selected case series. *Alcohol and Alcoholism, 46*, 714–720.

Enserink, M. (2011). Addiction research. Anonymous alcoholic bankrolls trial of controversial therapy. *Science, 332*, 653.

European Association for the Study of Liver. (2012). EASL clinical practical guidelines: Management of alcoholic liver disease. *Journal of Hepatology, 57*, 399–420.

Evans, S. M., & Bisaga, A. (2009). Acute interaction of baclofen in combination with alcohol in heavy social drinkers. *Alcoholism: Clinical and Experimental Research, 33*, 19–30.

File, S. E., Zharkovsky, A., & Gulati, K. (1991). Effects of baclofen and nitrendipine on ethanol withdrawal responses in the rat. *Neuropharmacology, 30*, 183–190.

Flannery, B. A., Garbutt, J. C., Cody, M. W., Renn, W., Grace, K., Osborne, M., et al. (2004). Baclofen for alcohol dependence: A preliminary open-label study. *Alcoholism: Clinical and Experimental Research, 28*, 1517–1523.

Franchitto, N., Pelissier, F., Lauque, D., Simon, N., & Lançon, C. (2014). Self-intoxication with baclofen in alcohol-dependent patients with coexisting psychiatric illness: An emergency department case series. *Alcohol and Alcoholism, 49*, 79–83.

Garbutt, J. C., Kampov-Polevoy, A. B., Gallop, R., Kalka-Juhl, L., & Flannery, B. A. (2010). Efficacy and safety of baclofen for alcohol dependence: A randomized, double-blind, placebo-controlled trial. *Alcoholism: Clinical and Experimental Research, 34*, 1849–1857.

Geoffroy, P. A., Auffret, M., Deheul, S., Bordet, R., Cottencin, O., & Rolland, B. (2014). Baclofen-induced manic symptoms: Case report and systematic review. *Psychosomatics, 55*, 326–332.

Grant, B. F., Goldstein, R. B., Saha, T. D., Chou, S. P., Jung, J., Zhang, H., et al. (2015). Epidemiology of DSM-5 alcohol use disorder: Results from the National Epidemiologic Survey on alcohol and related conditions III. *JAMA Psychiatry, 72*, 757–766.

Haass-Koffler, C. L., Leggio, L., & Kenna, G. A. (2014). Pharmacological approaches to reducing craving in patients with alcohol use disorders. *CNS Drugs, 28*, 343–360.

Heydtmann, M. (2011). Baclofen effect related to liver damage. *Alcoholism: Clinical and Experimental Research, 35*, 848.

Heydtmann, M., Macdonald, B., Lewsey, J., Masson, N., Cunningham, L., Irnazarow, A., et al. (2015). Tailored dose baclofen in patients with alcoholic liver disease: A case series with 2-year follow-up of hospitalization. *Addiction Research and Theory, 23*, 1–8.

Holstein, S. E., Dobbs, L., & Phillips, T. J. (2009). Attenuation of the stimulant response to ethanol is associated with enhanced ataxia for a $GABA_A$, but not a $GABA_B$, receptor agonist. *Alcoholism: Clinical and Experimental Research, 33*, 108–120.

Hwa, L. S., Kalinichev, M., Haddouk, H., Poli, S., & Miczek, K. A. (2014). Reduction of excessive alcohol drinking by a novel $GABA_B$ receptor positive allosteric modulator ADX71441 in mice. *Psychopharmacology, 231*, 333–343.

Janak, P. H., & Gill, T. M. (2003). Comparison of the effects of allopregnanolone with direct GABAergic agonists on ethanol self-administration with and without concurrently available sucrose. *Alcohol, 30*, 1–7.

Jonas, D. E., Amick, H. R., Feltner, C., Bobashev, G., Thomas, K., Wines, R., et al. (2014). Pharmacotherapy for adults with alcohol use disorders in outpatient settings: A systematic review and meta-analysis. *Journal of the American Medical Association, 311*, 1889–1900.

Kiefer, F., & Mann, K. (2010). Acamprosate: How, where, and for whom does it work? Mechanism of action, treatment targets, and individualized therapy. *Current Pharmaceutical Design, 16*, 2098–2102.

Knapp, D. J., Overstreet, D. H., & Breese, G. R. (2007). Baclofen blocks expression and sensitization of anxiety-like behavior in an animal model of repeated stress and ethanol withdrawal. *Alcoholism: Clinical and Experimental Research, 31*, 582–595.

Koob, G. F., & Le Moal, M. (2006). Alcohol. In G. F. Koob & M. Le Moal (Eds.), *Neurobiology of addiction* (pp. 173–241). Oxford: Academic.

Leggio, L., Ferrulli, A., Cardone, S., Malandrino, N., Mirijello, A., D'Angelo, C., et al. (2008a). Relationship between the hypothalamic-pituitary-thyroid axis and alcohol craving in alcohol dependent patients. A longitudinal study. *Alcoholism: Clinical and Experimental Research, 32*, 2047–2053.

Leggio, L., Ferrulli, A., Cardone, S., Miceli, A., Kenna, G. A., & Gasbarrini, G. (2008b). Renin and aldosterone but not the natriuretic peptide correlate with obsessive craving in medium-term abstinent alcohol-dependent patients: A longitudinal study. *Alcohol, 42*, 375–381.

Leggio, L., Garbutt, J. C., & Addolorato, G. (2010). Effectiveness and safety of baclofen in the treatment of alcohol dependent patients. *CNS & Neurological Disorders: Drug Targets, 9*, 33–44.

Leggio, L., Zywiak, W. H., Edwards, S. M., Tidey, J. W., Swift, R. M., & Kenna, G. A. (2015a). A preliminary double-blind, placebo-controlled randomized study of baclofen effects in alcoholic smokers. *Psychopharmacology, 232*, 233–243.

Leggio, L., Zywiak, W. H., Edwards, S. M., Tidey, J. W., Swift, R. M., & Kenna, G. A. (2015b). Erratum to: A preliminary double-blind, placebo-controlled randomized study of baclofen effects in alcoholic smokers. *Psychopharmacology, 232*, 1667.

Leggio, L., Zywiak, W. H., McGeary, J. E., Edwards, S., Fricchione, S. R., Shoaff, J. R., et al. (2013). A human laboratory pilot study with baclofen in alcoholic individuals. *Pharmacology, Biochemistry, and Behavior, 103*, 784–791.

Leite-Morris, K. A. (2004). The effects of ventral tegmental baclofen on ethanol-induced motor stimulation and sensitization in DBA/2 mice. *Alcoholism: Clinical and Experimental Research, 28*, 182A.

Leite-Morris, K. A., Kerestes, H. B., & Colombo, G. (2009). Intra-ventral tegmental area injection of the GABA B receptor positive allosteric modulator GS39783 inhibits ethanol seeking behavior in rats. *Alcoholism: Clinical and Experimental Research, 33*, 226A.

Leite-Morris, K. A., Misch, E. S., & Czachowski, C. L. (2008). Intra-VTA activation of GABA(B) receptors modulates accumbal dopamine during ethanol seeking behavior. *Alcoholism: Clinical and Experimental Research, 32*, 276A.

Liang, J. H., Chen, F., Krstew, E., Cowen, M. S., Carroll, F. Y., Crawford, D., et al. (2006). The $GABA_B$ receptor allosteric modulator CGP7930, like baclofen, reduces operant self-administration of ethanol in alcohol-preferring rats. *Neuropharmacology, 50*, 632–639.

Lim, S. S., Vos, T., Flaxman, A. D., Danaei, G., Shibuya, K., Adair-Rohani, H., et al. (2012). A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: A systematic analysis for the Global Burden of Disease Study 2010. *Lancet, 380*, 2224–2260.

Linsenbardt, D. N., & Boehm, S. L. (2014). Alterations in the rate of binge ethanol consumption: Implications for preclinical studies in mice. *Addiction Biology, 19*, 812–825.

Liu, J., & Wang, L. N. (2015). Baclofen for alcohol withdrawal. *Cochrane Database of Systematic Reviews, 4*, CD008502.

Loi, B., Maccioni, P., Lobina, C., Carai, M. A. M., Gessa, G. L., Thomas, A. W., et al. (2013). Reduction of alcohol intake by the positive allosteric modulator of the $GABA_B$ receptor, rac-BHFF, in alcohol-preferring rats. *Alcohol, 47*, 69–73.

Lyon, J. E., Khan, R. A., Gessert, C. E., Larson, P. M., & Renier, C. M. (2011). Treating alcohol withdrawal with oral baclofen: A randomized, double-blind, placebo-controlled trial. *Journal of Hospital Medicine, 6*, 474–479.

Maccioni, P., Bienkowski, P., Carai, M. A. M., Gessa, G. L., & Colombo, G. (2008a). Baclofen attenuates cue-induced reinstatement of alcohol-seeking behavior in Sardinian alcohol-preferring (sP) rats. *Drug and Alcohol Dependence, 95*, 284–287.

Maccioni, P., Carai, M. A. M., Kaupmann, K., Guery, S., Froestl, W., Leite-Morris, K. A., et al. (2009). Reduction of alcohol's reinforcing and motivational properties by the positive allosteric modulator of the $GABA_B$ receptor, BHF177, in alcohol-preferring rats. *Alcoholism: Clinical and Experimental Research, 33*, 1749–1756.

Maccioni, P., Fantini, N., Froestl, W., Carai, M. A., Gessa, G. L., & Colombo, G. (2008b). Specific reduction of alcohol's motivational properties by the positive allosteric modulator of the $GABA_B$ receptor, GS39783—Comparison with the effect of the $GABA_B$ receptor direct agonist, baclofen. *Alcoholism: Clinical and Experimental Research, 32*, 1558–1564.

Maccioni, P., Pes, D., Orrù, A., Froestl, W., Gessa, G. L., Carai, M. A. M., et al. (2007). Reducing effect of the positive allosteric modulator of the GABA receptor, GS39783, on alcohol self-administration in alcohol-preferring rats. *Psychopharmacology, 193*, 171–178.

Maccioni, P., Serra, S., Vacca, G., Orrù, A., Pes, D., Agabio, R., et al. (2005). Baclofen-induced reduction of alcohol reinforcement in alcohol-preferring rats. *Alcohol, 36*, 161–168.

Maccioni, P., Thomas, A. W., Carai, M. A. M., Gessa, G. L., Malherbe, P., & Colombo, G. (2010). The positive allosteric modulator of the $GABA_B$ receptor, rac-BHFF, suppresses alcohol self-administration. *Drug and Alcohol Dependence, 109*, 96–103.

Maccioni, P., Vargiolu, D., Thomas, A. W., Malherbe, P., Mugnaini, C., Corelli, F., et al. (2015). Inhibition of alcohol self-administration by positive allosteric modulators of the GABA receptor in rats: Lack of tolerance and potentiation of baclofen. *Psychopharmacology, 232*, 1831–1841.

Maccioni, P., Zaru, A., Loi, B., Lobina, C., Carai, M. A. M., Gessa, G. L., et al. (2012). Comparison of the effect of the $GABA_B$ receptor agonist, baclofen, and the positive allosteric modulator of the $GABAN_B$ receptor, GS39783, on alcohol self-administration in three different lines of alcoholpreferring rats. *Alcoholism: Clinical and Experimental Research, 36*, 1748–1766.

Moore, E. M., & Boehm, S. L. (2009). Site-specific microinjection of baclofen into the anterior ventral tegmental area reduces binge-like ethanol intake in male C57BL/6J mice. *Behavioral Neuroscience, 123*, 555–563.

Morley, K. C., Baillie, A., Leung, S., Addolorato, G., Leggio, L., & Haber, P. S. (2014). Baclofen for the treatment of alcohol dependence and possible role of comorbid anxiety. *Alcohol and Alcoholism, 49*, 654–660.

Müller, C. A., Geisel, O., Pelz, P., Higl, V., Krüger, J., Stickel, A., et al. (2015). High-dose baclofen for the treatment of alcohol dependence (BACLAD study): A randomized, placebo-controlled trial. *European Neuropsychopharmacology, 25*, 1167–1177.

Nasti, J. J., & Brakoulias, V. (2011). Chronic baclofen abuse and withdrawal delirium. *The Australian and New Zealand Journal of Psychiatry, 45*, 86–87.

Nutt, D. J., King, L. A., Phillips, L. D., & Independent Scientific Committee on Drugs. (2010). Drug harms in the UK: A multicriteria decision analysis. *Lancet, 376*, 1558–1565.

O'Brien, C. P. (2011). Drug addiction. In L. L. Brunton, B. A. Chabner, & B. C. Knollmann (Eds.), *Goodman & Gilman's the pharmacological basis of therapeutics* (12th ed., pp. 650–668). New York: McGraw-Hill.

Orrù, A., Lai, P., Lobina, C., Maccioni, P., Piras, P., Scanu, L., et al. (2005). Reducing effect of the positive allosteric modulators of the $GABA_B$ receptor, CGP7930 and GS39783, on alcohol intake in alcohol-preferring rats. *European Journal of Pharmacology, 525*, 105–111.

Owens, L., Rose, A., Thompson, A., Pirmohamed, M., Gilmore, I., & Richardson, P. (2015). Baclofen: Maintenance of abstinence in alcohol dependent patients attending liver clinic. *Journal of Hepatology, 62*, S767.

Palpacuer, C., Laviolle, B., Boussageon, R., Reymann, J. M., Bellissant, E., & Naudet, F. (2015). Risks and benefits of nalmefene in the treatment of adult alcohol dependence: A systematic literature review and meta-analysis of published and unpublished double-blind randomized controlled trials. *PLoS Medicine, 12*, e1001924.

Pape, E., Roman, E., Scala-Bertola, J., Thivilier, C., Javot, L., Saint-Marcoux, F., et al. (2014). Death of an alcohol-dependent patient following intentional drug intoxication: Implication of baclofen? *European Addiction Research, 20*, 300–304.

Pastor, A., Jones, D. M., & Currie, J. (2012). High-dose baclofen for treatment-resistant alcohol dependence. *Journal of Clinical Psychopharmacology, 32*, 266–268.

Perogamvros, L., Pépin, J. L., Thorens, G., Mégevand, P., Claudel, E., Espa, F., et al. (2015). Baclofen-associated onset of central sleep apnea in alcohol use disorder: A case report. *Respiration, 90*, 507–511.

Peters, S., Slattery, D. A., Flor, P. J., Neumann, I. D., & Reber, S. O. (2013). Differential effects of baclofen and oxytocin on the increased ethanol consumption following chronic psychosocial stress in mice. *Addiction Biology, 18*, 66–77.

Petry, N. M. (1997). Benzodiazepine-GABA modulation of concurrent ethanol and sucrose reinforcement in the rat. *Experimental and Clinical Psychopharmacology, 5*, 183–194.

Quintanilla, M. E., Perez, E., & Tampier, L. (2008). Baclofen reduces ethanol intake in high-alcohol-drinking University of Chile bibulous rats. *Addiction Biology, 13*, 326–336.

Reddy, V. K., Girish, K., Lakshmi, P., Vijendra, R., Kumar, A., & Harsha, R. (2014). Cost-effectiveness analysis of baclofen and chlordiazepoxide in uncomplicated alcohol-withdrawal syndrome. *Indian Journal of Pharmacology, 46*, 372–377.

Rehm, J., Mathers, C., Popova, S., Thavorncharoensap, M., Teerawattananon, Y., & Patra, J. (2009). Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. *Lancet, 373*, 2223–2233.

Reichmuth, P., Blanc, A. L., & Tagan, D. (2015). Unintentional baclofen intoxication in the management of alcohol use disorder. *BMJ Case Reports.* doi:10.1136/bcr-2015-212187.

Rigal, L., Alexandre-Dubroeucq, C., de Beaurepaire, R., Le Jeunne, C., & Jaury, P. (2012). Abstinence and 'low-risk' consumption 1 year after the initiation of high-dose baclofen: A retrospective study among 'high-risk' drinkers. *Alcohol and Alcoholism, 47*, 439–442.

Roerecke, M., & Rehm, J. (2013). Alcohol use disorders and mortality: A systematic review and meta-analysis. *Addiction, 108*, 1562–1578.

Rolland, B., Deheul, S., Danel, T., Bordet, R., & Cottencin, O. (2012). A case of de novo seizures following a probable interaction of high-dose baclofen with alcohol. *Alcohol and Alcoholism, 47*, 577–580.

Rolland, B., Jaillette, E., Carton, L., Bence, C., Deheul, S., Saulnier, F., et al. (2014). Assessing alcohol versus baclofen withdrawal syndrome in patients treated with baclofen for alcohol use disorder. *Journal of Clinical Psychopharmacology, 34*, 153–156.

Rolland, B., Labreuche, J., Duhamel, A., Deheul, S., Gautier, S., Auffret, M., et al. (2015). Baclofen for alcohol dependence: Relationships between baclofen and alcohol dosing and the occurrence of major sedation. *European Neuropsychopharmacology, 25*, 1631–1636.

Rosner, S., Hackl-Herrwerth, A., Leucht, S., Vecchi, S., Srisurapanont, M., & Soyka, M. (2010a). Opioid antagonists for alcohol dependence. *Cochrane Database of Systematic Reviews, 12*, CD001867.

Rosner, S., Hackl-Herrwerth, A., Leucht, S., Lehert, P., Vecchi, S., & Soyka, M. (2010b). Acamprosate for alcohol dependence. *Cochrane Database of Systematic Reviews, 9*, CD004332.

Runyon, B. A. (2013). Introduction to the revised American Association for the Study of Liver Diseases Practice Guideline management of adult patients with ascites due to cirrhosis 2012. *Hepatology, 57*, 1651–1653.

Schuckit, M. A. (2009). Alcohol-use disorders. *Lancet, 373*, 492–501.

Schuckit, M. A. (2011). Ethanol and methanol. In L. L. Brunton, B. A. Chabner, & B. C. Knollmann (Eds.), *Goodman & Gilman's the pharmacological basis of therapeutics* (12th ed., pp. 628–647). New York: McGraw-Hill.

Schuckit, M. A. (2014). Recognition and management of withdrawal delirium (delirium tremens). *New England Journal of Medicine, 371*, 2109–2113.

Shukla, L., Shukla, T., Bokka, S., Kandasamy, A., Benegal, V., Murthy, P., et al. (2015). Correlates of baclofen effectiveness in alcohol dependence. *Indian Journal of Psychological Medicine, 37*, 370–373.

Simioni, N., Preda, C., Deken, V., Bence, C., Cottencin, O., & Rolland, B. (2016). Characteristics of patients with alcohol dependence seeking baclofen treatment in France: A two-centre comparative cohort study. *Alcohol and Alcoholism, 51*(6), 664–669.

Skinner, M. D., Lahmek, P., Pham, H., & Aubin, H. J. (2014). Disulfiram efficacy in the treatment of alcohol dependence: A meta-analysis. *PLoS One, 9*, e87366.

Stromberg, M. F. (2004). The effect of baclofen alone and in combination with naltrexone on ethanol consumption in the rat. *Pharmacology, Biochemistry and Behavior, 78*, 743–750.

Tabakoff, B., & Hoffman, P. L. (2013). The neurobiology of alcohol consumption and alcoholism: An integrative history. *Pharmacology, Biochemistry and Behavior, 113*, 20–37.

Tanchuck, M. A., Yoneyama, N., Ford, M. M., Fretwell, A. M., & Finn, D. A. (2011). Assessment of GABA-B, metabotropic glutamate, and opioid receptor involvement in an animal model of binge drinking. *Alcohol, 45*, 33–44.

Walker, B. M., & Koob, G. F. (2007). The γ-aminobutyric acid-B receptor agonist baclofen attenuates responding for ethanol in ethanol-dependent rats. *Alcoholism: Clinical and Experimental Research, 31*, 11–18.

Weibel, S., Lalanne, L., Riegert, M., & Bertschy, G. (2015). Efficacy of high-dose baclofen for alcohol use disorder and comorbid bulimia: A case report. *Journal of Dual Diagnosis, 11*, 203–204.

Yamini, D., Lee, S. H., Avanesyan, A., Walter, M., & Runyon, B. (2014). Utilization of baclofen in maintenance of alcohol abstinence in patients with alcohol dependence and alcoholic hepatitis with or without cirrhosis. *Alcohol and Alcoholism, 49*, 453–456.

Yardley, M. M., & Ray, L. A. (2016). Medications development for the treatment of alcohol use disorder: Insights into the predictive value of animal and human laboratory models. *Addiction Biology*.

Zindel, L. R., & Kranzler, H. R. (2014). Pharmacotherapy of alcohol use disorders: seventy-five years of progress. *Journal of Studies on Alcohol and Drugs. Supplement, 17*, 79–88.

Chapter 16
Targeting the $GABA_B$ Receptors for the Treatment of Gastroesophageal Reflux Disease and Chronic Cough

Anders Lehmann, L. Ashley Blackshaw, and Brendan J. Canning

Abstract Transient lower esophageal sphincter relaxation (TLESR) is the dominating motor event behind gastroesophageal reflux (GER) and therefore an important factor in gastroesophageal reflux disease (GERD). TLESR is a reflex dependent on the vagus and as such, it shares many similarities with the cough reflex. Moreover, GER is a known cause of chronic cough in some patients, and agents inhibiting both TLESR and cough may be useful antitussives. There is no effective therapy for GERD patients whose symptoms are not fully resolved by proton pump inhibitor (PPI) therapy and marketed antitussives have poor effects in most patients suffering from chronic cough. The observations that GABA type B ($GABA_B$) receptor agonists reduce TLESR and inhibit cough regardless of stimulus open new inroads to the treatment of PPI-resistant GERD as well as GER-related cough. The present review focuses on these aspects and discusses the findings of $GABA_B$ receptor agonists on TLESR, GER, GERD, and cough in both the preclinical setting, healthy individuals and patients. Special attention is devoted to the role of the vagus in mediating these pharmacological effects.

Keywords Gastroesophageal reflux • Chronic cough • Vagus • $GABA_B$ • Baclofen • Lesogaberan • Transient lower esophageal sphincter relaxation

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16.1 Introduction

Over the years, the quest for new drugs acting on the GABA type B ($GABA_B$) receptor has intensified as the number of potential indications has grown. Despite all efforts, baclofen remains the only marketed $GABA_B$ receptor ligand with a narrow indication, spasticity (see Chap. 17 of this book). One major obstacle is to identify new compounds, whether they be agonists, antagonists, allosteric activators/inhibitors, or any other modality. The advent of high-throughput screening and other approaches to discover new, pharmacologically active chemical classes have only slightly changed this picture for $GABA_B$ receptor ligands. Nevertheless, a number of structurally diverse molecules have been identified that bind to sites other than the agonist-binding site and render the receptor more sensitive to endogenous GABA; the positive allosteric modulators. Since the activity of a positive allosteric modulator depends on endogenous tone at the receptor, the therapeutic effects may be particularly difficult to predict. One $GABA_B$ receptor-positive allosteric modulator, ADX71441, is approaching clinical phase with a wide number of potential indications (http://www.addextherapeutics.com/). The large number of suggested indications may actually be interpreted as a sign of uncertainty rather than a broad range of opportunities.

The ever growing list of potential indications for $GABA_B$ receptor agonists/positive allosteric modulators is not matched by clinical confirmation. The poor translatability of many preclinical disease models to the corresponding human disease (particularly in the central nervous system (CNS) field), combined with the challenging task to discover new, patentable agonists with a benefit/risk profile superior to baclofen, renders the field an uncertain investment object for pharmaceutical companies. The majority of suggestions on novel indications for $GABA_B$ receptor agonists is based on preclinical findings, and it is as surprising as it is disappointing that there seems to be so little enthusiasm for extension of preclinical findings to proof-of-principle studies in patients using baclofen as a tool compound. In this context, it is a bit perplexing to note that one disease for which there is exceptionally strong evidence for a therapeutic role for $GABA_B$ receptor agonists, gastroesophageal reflux disease (GERD), usually is referred to en passant (Kalinichev et al. 2014) if at all (Bowery 2006) in papers on the possible therapeutic utility of $GABA_B$ receptor stimulation. The number and quality of clinical trials on baclofen in GERD has even justified meta-analysis (Li et al. 2014). Here, we will review the case for $GABA_B$ receptor agonism in GERD based on preclinical as well as randomized, placebo-controlled double-blind clinical studies. A parallel case will be elaborated on, namely the effects of $GABA_B$ receptor agonists on cough. The rationale for this is threefold. First, cough and the chief motor event underlying gastroesophageal reflux (GER), transient lower esophageal sphincter relaxation (TLESR), share several features such as being critically dependent on the vagus nerve. Second, $GABA_B$ receptor agonists may act on the vagus to inhibit cough (Bolser et al. 1994; Canning et al. 2012) and reflux (Page and Blackshaw 1999). Third, there is a cross-talk between cough and GER (Kollarik and Brozmanova 2009), and in many patients suffering from chronic cough, GER is suspected to be the primary cause (Lv and Qiu 2015).

16.2 Neural Control of GER and Cough

16.2.1 Gastroesophageal Reflux

The important elements in the GER barrier are the esophageal body and its musculature, the lower esophageal sphincter (LES), the crural diaphragm, and the stomach (Mittal et al. 1995). It is frequently misconstrued that the cause of GER is an incompetent LES, which is driven to contract insufficiently by myogenic, neural, and/or humoral influences. This would suggest that reflux may occur freely across the open sphincter, but this is not the case. Even in quite severe GERD, reflux is episodic. The reason for this is that the LES is rarely, if ever, totally incompetent. There are myogenic mechanisms exclusive to sphincters that maintain them in a state of tonic contraction, so they are, at rest, constricted. This closed aperture must then be overcome by force behind the gastric contents for reflux to occur but this still accounts for a minority of reflux episodes.

The major cause of GER is via TLESR (Mittal et al. 1995), which is the result of a complex motor program involving skeletal and smooth muscle contraction and relaxation under the influence of several neural pathways. As such it can be compared with many other episodic events such as coughing and vomiting.

1. At the center of a TLESR is abrupt relaxation of the circular smooth muscle of the LES, which occurs in the absence of any peristaltic contraction of the esophageal body, which distinguishes it from a swallow-induced relaxation. TLESRs are mediated via a powerful activation of vagal motorneurons which in turn activate neurons of the esophageal and LES myenteric plexus. Spinal sympathetic influences would appear to be minimal (Staunton et al. 2000). The local myenteric neurons release inhibitory transmitters such as nitric oxide (NO) onto LES circular smooth muscle causing it to relax. This is therefore a process of active relaxation, which is not seen in skeletal muscle (Mittal et al. 1995).
2. Simultaneously, there is thought to be a removal of vagal drive to excitatory myenteric neurons which normally release acetylcholine onto the smooth muscle keeping it contracted.
3. Retrograde flow will not occur unless there is also inhibition of the crural diaphragm (Martin et al. 1992), which is controlled separately to the rest of the (costal) diaphragm, and forms two “legs” that wrap externally around the LES. Like the costal diaphragm it receives cervical spinal input from the phrenic nerve, but via a separate population of motorneurons. It also receives input from the vagus (Young et al. 2010). Being skeletal muscle, it relies entirely on cholinergic nicotinic receptor excitation to contract, so it is removal of this input that allows the crural diaphragm to relax. Thus the gastroesophageal junction, like the anal sphincter, has external skeletal and internal visceral muscle components. It is only the coordinated relaxation of the two that allows reflux to occur. This is a normal physiological mechanism for belching and vomiting in many species.

4. Simultaneous with the other three events is a shortening of the esophageal longitudinal smooth muscle, which pulls the LES up partly into the thoracic cavity. It is likely that the vagal command to relax the LES is shared with the esophageal body so there is a coordinated movement. This is controversial, however, with some reports suggesting the shortening precedes the other events and is therefore the trigger for the whole program (Leslie et al. 2012). Esophageal shortening serves to straighten the path for refluxed gas during belching and allow it to reach the upper esophageal sphincter prior to belching, and thus to be quickly sensed and expelled by straining. An unfortunate corollary of this is that esophageal shortening in GERD allows acidic fluid also to spread rapidly throughout the esophagus where it may cause pain and damage if prolonged and/or frequent.
5. After the contact of refluxed material with the esophageal mucosa, and presumably distension of the esophageal smooth muscle, swallowing or secondary peristalsis is triggered which rapidly clears remaining esophageal contents back into the stomach.

The motor program of TLESR involves classical somatic and autonomic neural pathways that utilize transmitter and receptor mechanisms that are ubiquitous in systems of neural control in the gastrointestinal tract and elsewhere. As such there is little that can be done to selectively reduce GER through these means without side effects. Attempts have been made to bolster the LES pharmacologically, and indeed this effect is seen after dosing with of $GABA_B$ receptor agonists. Anti-reflux surgery is aimed to do just that, by wrapping a part of the proximal stomach around the gastroesophageal junction to strengthen it (Lundell 2010). What is more important is the mechanism of triggering of reflux, which is where drugs like $GABA_B$ receptor agonists have much more potent and therapeutic effects. The process of TLESR triggering is most probably a very complex one; however, the fundamental element is distension of the proximal stomach—in particular the cardia region just distal to the LES (Franzi et al. 1990). Here, gas accumulates and it therefore makes sense as a location for initiating a venting mechanism (Little et al. 1989). The vagus nerve is a crucial component, since cooling blockade of the vagus abolishes TLESR completely (Martin et al. 1986). Complex neural interactions under the influence of central nervous arousal networks are also required, since general anesthesia blocks TLESR (Cox et al. 1988). These facts have given rise to a model summarized in Fig. 16.1, in which build-up of proximal gastric distension activates vagal afferent endings in the smooth muscle layers, which convey a mounting signal to the vagal nuclei in the brain stem. At a point of increasing input from these mechanoreceptors, a coordinated output is activated paroxysmally to the LES, crural diaphragm, and esophageal body. This output is terminated either by a signal from upper esophageal muscle mechanoreceptors that retrograde flow has occurred, or by a self-limiting auto-inhibitory circuit in the hindbrain.

Unlike the motor program, the trigger mechanism for TLESR is a rich source of possible pharmacotherapeutic targets with minimal overlap onto other systems. The most attractive cellular target is the gastric vagal mechanoreceptor that detects

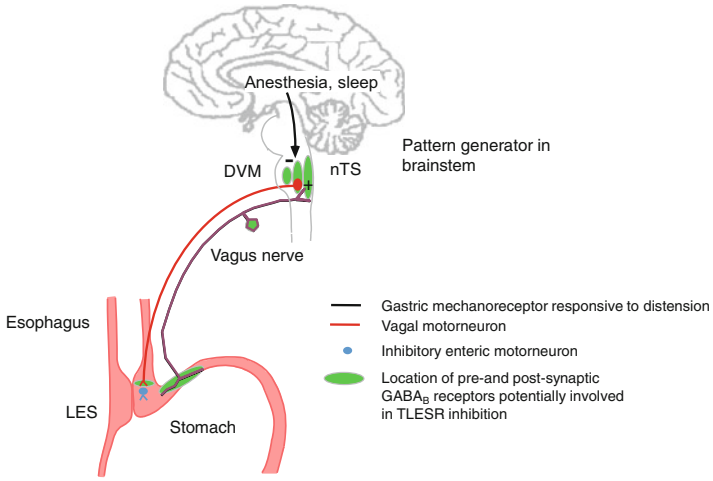

Fig. 16.1 Neural pathways of TLESR, with sites of $GABA_B$ receptors potentially involved. *DVM* dorsal vagal nucleus, *nTS* nucleus tractus solitarius

tension in the cardia region upon distension with fluid or gas. The second most attractive target is the central program generator in the brain stem that takes the input from these mechanoreceptors and decides at what point to initiate the motor program. As it happens, the same receptors may affect triggering at both locations, which may be a bonus. However, since much of the central program generator for TLESR may be behind the blood–brain barrier, CNS side effects are a potential disadvantage of drugs acting at this point.

Gastric mechanoreceptors providing input to the central program generator for TLESR are most probably intraganglionic laminar endings (IGLEs). They envelop myenteric ganglia, placing them in an ideal position to monitor tension generated by distension by intraluminal contents (Zagorodnyuk et al. 2001). They are potently inhibited by $GABA_B$ receptor activation in ferrets (Page and Blackshaw 1999) but not in guinea-pigs (Zagorodnyuk et al. 2002). Inhibition is via the classical coupling mechanisms of $GABA_B$ receptor, involving N-type calcium channels and inward rectifier potassium channels (Page et al. 2006). There are several other inhibitory G-protein-coupled receptors (GPCRs) on these vagal afferent endings that may have similar effects on triggering of GER (Page and Blackshaw 2009), the most notable of which are metabotropic glutamate receptors, which may work in a similar way to $GABA_B$ receptor (Page et al. 2005). There are also excitatory GPCRs that may enhance the sensitivity of gastric vagal afferents, such as metabotropic glutamate receptor 5 and cholecystokinin receptor 1, so that blockade of them may reduce GER (Slattery et al. 2006; Frisby et al. 2005; Jensen et al. 2005; Boulant et al. 1994; Blackshaw and Grundy 1990; Hirsch et al. 2002).

16.2.2 Cough

The airways and lungs are innervated by both vagal and spinal afferent and efferent projections, with a predominance of vagal innervation. In health, these nerves function in large part to preserve, protect, and optimize lung capacity for gas exchange by regulating respiratory rate, tidal volumes, airway caliber, mucus secretion, mucociliary clearance, and through protective reflexes such as cough. But in many acute and chronic conditions and in patients with GERD, airway neural control can become dysregulated and contribute to the pathophysiology and symptoms of disease.

16.2.2.1 Efferent Innervation of the Airways

The efferent innervation of the airways is comprised of both sympathetic and parasympathetic projections. Sympathetic nerves innervate the airways vasculature in all species (both the pulmonary circulation and the bronchial vasculature) but there are wide variations of sympathetic nerve-dependent regulation of airway smooth muscle amongst species. Parasympathetic nerves innervate airway smooth muscle in all species, and also project the airways vasculature and mucus glands.

Multiple neurotransmitters in addition to acetylcholine and norepinephrine have been localized to airway autonomic nerves (Kummer et al. 1992; Dey et al. 1996). These include peptide transmitters (vasoactive intestinal peptide (VIP and related peptides), and neuropeptide Y (NPY)) and the gaseous transmitters (NO and carbon monoxide). There is direct evidence for cotransmission of NPY with norepinephrine and NO with VIP, but there is also clear evidence for subtypes of postganglionic sympathetic and parasympathetic nerves innervating the airways. Notably, anatomically and physiologically distinct cholinergic and noncholinergic parasympathetic nerves innervate airway smooth muscle (Dey et al. 1996; Lama et al. 1988; Canning and Undem 1993; Fischer et al. 1998). Reflexes differentially regulate these distinct parasympathetic pathways projecting to the lung (Inoue et al. 1989; Mazzone and Canning 2002).

Preganglionic sympathetic nerves emanate bilaterally from thoracic segments of the spinal cord, while preganglionic parasympathetic neurons have their cell bodies in brainstem nuclei, with a preponderance of perikarya in or adjacent to nucleus ambiguus, and a minor, poorly defined population localized to the dorsal motor nucleus of the vagus nerve (Haxhiu et al. 2005; Oh et al. 2006).

A potential link between the airways and the esophagus has been described in studies of the noncholinergic parasympathetic nerves innervating the airways. In guinea pigs, the cell bodies of these VIP/NO nerves have been localized to the myenteric plexus of the esophagus (Fischer et al. 1998). A comparable projection from the esophagus has been described in mice (Balentova et al. 2013), and a subpopulation of neurons with similar neurochemistry are thought to play an important role in regulating esophageal motility in multiple species, including humans (Worl and Neuhuber 2005). These esophageal neurons are activated upon airway irritation

and initiate relaxations of airway smooth muscle (Mazzone and Canning 2002). These noncholinergic parasympathetic nerves also project to mucus glands and the bronchial vasculature, but their reflex regulation at these specific targets has not been described. The inhibitory neurons may also be regulated through peripheral reflexes following activation of neurokinin-containing capsaicin-sensitive C-fibers (Canning and Undem 1994; Canning et al. 1998). An extensive plexus of substance P-containing nerve terminals has been described in the esophageal myenteric plexus (Canning et al. 1998; Wank and Neuhuber 2001). To the extent these nerves have arborizations reaching the esophageal mucosa, which may be activated by luminal acid or perhaps carbon dioxide, has not been directly documented (Page et al. 2002; Akiba et al. 2008; Yu et al. 2014; Dusenkova et al. 2014).

16.2.2.2 Afferent Innervation of the Airways

Both vagal and spinal afferent nerves innervate the airways and lungs. Given that virtually all airway and lung reflexes are abolished by vagotomy, the preponderance of reflex regulation in the airways and lungs can be ascribed to vagal afferent nerves.

Vagal afferent nerve subtypes have been characterized in multiple mammalian species based on their sensitivity to physical and chemical stimuli, responses to sustained lung inflations, neurochemistry, axonal conduction velocity, central and peripheral terminations, gene expression, ganglionic origin, and the reflexes evoked upon their activation. These subtypes can be broadly differentiated into mechanoreceptors and chemically sensitive nociceptors, primarily C-fibers (Coleridge and Coleridge 1984; Ho et al. 2001; Canning et al. 2014).

Amongst mechanically sensitive vagal bronchopulmonary afferents, all of which are myelinated, subtypes have been identified by their responses to sustained lung inflations, the reflexes evoked and by their central terminations (Kubin et al. 2006). Slowly adapting receptors (SARs) are in many respects comparable to the IGLEs described in the esophagus, being exquisitely sensitive to distension/stretch, and producing action potentials in a nonadapting pattern with sustained lung inflations. SARs terminate peripherally in the lung parenchyma (Yu et al. 2003), and centrally in rostral and lateral subnuclei of nucleus of the solitary tract (nTS). Rapidly adapting receptors (RARs) are comparably sensitive to lung inflation, but rapidly adapt during static lung inflations. RARs are thus perhaps best described as dynamic receptors, sensing the rate and volume of lung inflations (Pack and DeLaney 1983). RARs are thought to terminate peripherally in the conducting intrapulmonary airways, and centrally in the commissural subnucleus of nTS (Kubin et al. 2006; Sant'Ambrogio and Widdicombe 2001). In general, RARs elicit reflexes that often oppose the reflexes evoked by SAR activation (Canning and Chou 2009).

A physiologically distinct subtype of mechanoreceptors has been described in the extrapulmonary airways, with the most extensive descriptions of these afferents in studies performed on the guinea pig trachea (Canning et al. 2004). These small, myelinated afferents are largely insensitive to changes in luminal pressures but exquisitely sensitive to punctate mechanical touch. They are insensitive to capsaicin, but

they are acid sensitive, presumably through the expression of acid sensing ion channels. Studies combining neuronal tracing, physiological recordings, and in vivo reflexes identified the essential role for these afferents in the initiation of cough. Aside from protons, these "cough receptors" are largely unresponsive to chemical stimuli applied to the airways mucosa. Their central terminations and the pharmacology of synaptic transmission have also been described (Mazzone et al. 2009; Canning and Mori 2010, 2011).

Unmyelinated C-fibers also innervate the airways and lungs (Coleridge and Coleridge 1984; Ho et al. 2001). C-fibers are only modestly sensitive to lung distensions and punctate mechanical stimuli, but they are robustly activated by autacoids associated with inflammation and other irritants, including capsaicin, ozone, cigarette smoke, and protons. C-fiber subtypes have been described, and can be differentiated by their ganglionic origin, neurochemistry, termination sites in the airways, and responsiveness to autacoids such as serotonin and adenosine (Undem et al. 2004; Nassenstein et al. 2010; Kwong et al. 2008). These subtypes may have opposing effects on respiratory rate and cough (Canning and Chou 2009; Chou et al. 2008).

16.2.2.3 Airway Defensive Reflexes

Airway defensive reflexes that contribute to the symptoms of respiratory disease and to the extrapulmonary manifestations of diseases such as GERD include reflex bronchospasm, mucus secretion, and cough.

Reflex bronchospasm has been described in several species including humans and results from an increased activation of parasympathetic cholinergic nerves. Multiple stimuli in the airways and in extrapulmonary structures have been shown to evoke reflex bronchospasm. In the airways, stimulants of C-fibers and RARs will initiate reflex bronchospasm (Coleridge and Coleridge 1984). These reflexes are opposed within the airways by reflex activation of noncholinergic parasympathetic nerves, sympathetic nerves and/or circulating catecholamines, and by inhibitory effects initiated through SAR activation (Inoue et al. 1989; Mazzone and Canning 2002; Oh et al. 2006). Extrapulmonary stimuli evoking reflex bronchospasm include esophageal acid stimulation and nasal irritation. Pathophysiological stimuli resulting in reflex bronchospasm include allergen challenge, pulmonary embolism, and GER. Reflex regulation of airway mucus secretion has also been described (Phipps and Richardson 1976; Schultz et al. 1985; Yu et al. 1989).

Cough has been studied extensively in humans and in other mammalian species possessing a cough reflex. Stimuli evoking cough include mechanical irritation of the airways mucosa, airway surface liquid acidification, and irritant inhalation, including capsaicin, cigarette smoke, bradykinin, and acidic solutions. The afferent nerves initiating cough upon stimulation include C-fibers and the acid-sensitive Aδ fibers innervating the extrapulmonary airways (Canning et al. 2014).

16.3 Pathophysiology of GERD and Chronic Cough

16.3.1 Gastroesophageal Reflux Disease

GERD is a multifactorial disease with a host of underlying mechanisms. As the name implies, GER is the cause of symptoms, the hallmark of which is heartburn. Many patients present with inflammation of the lower esophagus but even more show no evidence of macroscopic inflammation (Galmiche and des Varannes 2001). The symptomatology fails to differentiate between these categories, and the severity of esophagitis is not reflected by the symptoms (Galmiche and des Varannes 2001).

For a long time, gastric acid was considered the only factor responsible for heartburn. This view has been modified over the years, and in some affected individuals, efficient blockade of gastric acid secretion with proton pump inhibitors (PPIs) fails to afford complete symptomatic relief. However, esophagitis appears to be strictly dependent on reflux of acidic gastric juice. Studies combining impedance measurement to follow fluid and gas reflux, pHmetry, and manometry have illustrated the complexity of the disease (Woodland and Sifrim 2010). Recent work suggests that weakly acidic fluid and gas reflux sometimes is accompanied by heartburn (Conchillo and Smout 2009). However, there are patients with typical GERD symptoms not correlating with reflux episodes, whether acidic or not. This is known as "functional heartburn" (Zerbib et al. 2012) and this patient category most likely contaminated phase II studies on $GABA_B$ receptor agonist drug candidates aimed at reducing TLESRs (Kahrilas and Boeckxstaens 2012).

The concept of TLESR and its importance in GERD was developed in the 1980s (Dent et al. 1980, 1988; Dodds et al. 1982). Most studies have found that the incidence of TLESR is similar in healthy subjects and GERD patients. However, the probability of reflux to occur during a TLESR is higher in patients (Dent et al. 1988), so inhibition of TLESR is a rational therapeutic target.

16.3.2 Cough

Chronic cough is defined clinically as a persistent cough lasting 8 weeks or more and is amongst the most common presenting symptoms amongst patients seeking medical advice. Excluding conditions related to smoking, multiple pulmonary and extrapulmonary diseases result in chronic cough. Several severe and life-threatening illnesses (lung cancer, idiopathic pulmonary fibrosis) can first present clinically as a persistent cough. After smoking history and imaging rule out more serious causes, a triad of chronic conditions—asthma, rhinosinusitis, and GERD—are thought to account for the majority of chronic cough in patients at their initial presentation in primary care clinics. Treatment for these patients is in most instances empiric, with patient histories guiding diagnosis, and

confirmation provided by subsequent clinical testing or a course of disease-specific therapy (e.g. topical steroids for rhinosinusitis, inhaled corticosteroids, and/or bronchodilators for asthma, histamine H2 blockers, PPIs, and lifestyle modifications in patients suspected of having reflux disease). Positive clinical outcomes following empiric medical treatment is often used to confirm diagnoses made based solely on clinical histories. Few prospective studies have been reported in the literature, but there is some suggestion that an empiric approach targeting these three conditions alone or in combination diminishes cough frequency and severity in the majority of patients (McGarvey et al. 1998; Irwin et al. 2006). The role of GER in chronic cough is further discussed below.

16.4 The Role of $GABA_B$ receptor Agonists in the Treatment of GERD and Chronic Cough

16.4.1 GERD

16.4.1.1 Discovery of the $GABA_B$ receptor as a Target for GERD

Very few targets related to TLESR were known when AstraZeneca (Astra Hässle at the time) in close collaboration with Drs. Blackshaw, Dent and their colleagues at the Royal Adelaide Hospital launched a project on this topic in the mid-1990s. Due to the complex nature of TLESRs, an in vivo model was deemed as the most relevant screening model. To this end, combined manometric/pH metric studies were done in dogs and ferrets. This model consistently showed inhibitory effects of different $GABA_B$ receptor agonists (Blackshaw et al. 1999; Lehmann et al. 1999). The $GABA_B$ receptor was one of many possible targets considered at an early stage. Incidentally, several years later, it could be concluded that this field actually is quite target-poor so the evaluation of baclofen early on was perhaps a stroke of luck.

Once the receptor had been validated as a target, several actions were undertaken to discover new agonists lacking the CNS side effects of baclofen. High-throughput screening campaigns were launched and while they helped to identify new $GABA_B$ receptor-positive allosteric modulators, no novel agonists were found. The structure–activity relationship for agonists is very narrow, and to use baclofen as a starting point for optimization seemed doomed to fail. However, it was realized that 3-aminopropylphosphinates represented an unexplored group of agonists so there was a scope both in terms of medicinal chemistry, pharmacology, and patentability. Positive in vivo findings using the only known 3-aminopropylphosphinic acid at the time, the unsubstituted compound, subsequently led to the discovery of lesogaberan [(*R*)-(3-amino-2-fluoropropyl)phosphinic acid] (Alstermark et al. 2008).

16.4.2 Clinical Validation of Efficacy of $GABA_B$ Receptor Agonists in GERD

16.4.2.1 Effects on TLESR and Reflux

Following the observations that $GABA_B$ receptor stimulation inhibits TLESR and reflux in dogs and ferrets, baclofen was used to verify that humans responded similarly. In the first study in healthy volunteers, 40 mg of baclofen as a single dose was shown to reduce TLESR by some 40 % (Lidums et al. 2000). The dose was chosen from literature data and was expected to be effective and safe. The maximal inhibition noted in dogs and ferrets is close to 100 %, and when taking baclofen blood levels into account, there is an overlap between human and animal findings (Boeckxstaens et al. 2011a). Nevertheless, these results have repeatedly been misinterpreted with claims that humans are less responsive than dogs. There is no reason to believe that higher doses of baclofen would not yield more pronounced inhibition of TLESR in humans but investigators have been uninclined to up the dose due to the side-effect profile. There is one reproducible qualitative difference between the effects of baclofen and other $GABA_B$ receptor agonists in dogs and humans. The basal LES pressure is unaffected in the former but elevated in the latter. From a therapeutic point of view, this disparity is advantageous. The effects of baclofen on TLESR and acidic reflux were replicated in GERD patients but the effect was possibly somewhat weaker (Zhang et al. 2002). These clinical findings were uniformly reproduced in several other laboratories (Cange et al. 2002; Vela et al. 2003; Ciccaglione and Marzio 2003; Omari et al. 2006; Kawai et al. 2004; Koek et al. 2003; Grossi et al. 2008; Cossentino et al. 2012; Orr et al. 2012; Scarpellini et al. 2015; Curcic et al. 2014; Beaumont and Boeckxstaens 2009) suggestive of a robust response. They were also extended to show that baclofen inhibits duodenogastroesophageal reflux (Koek et al. 2003) and nocturnal reflux (Orr et al. 2012) as well as reflux in children with GERD (Omari et al. 2006) and in children with GERD related to neurological impairment (Kawai et al. 2004).

Arbaclofen placarbil is a prodrug of *R*-baclofen with improved absorption from the gut. The compound has not been evaluated with regard to effects on TLESR but it reduced total and acid reflux in GERD patients after a meal at the highest dose tested, 60 mg (Gerson et al. 2010).

Two substituted 3-aminopropylphosphinic acids, lesogaberan (formerly known as AZD3355) and AZD9343, predictably suppress TLESRs and reflux. AZD9343 was only studied in healthy volunteers (Curcic et al. 2014) but as it was not found to be superior to lesogaberan, its further development was discontinued. In stationary measurements, lesogaberan inhibits TLESRs (by 36 % at 0.8 mg/kg as a single dose) and acid reflux in healthy individuals, and elevates postprandial basal LES pressure (Boeckxstaens et al. 2010a). Similar results were obtained with lesogaberan at a comparable dose in a 24-h ambulatory pH metric/manometric study in partially PPI-resistant patients (Boeckxstaens et al. 2010b). These observations were extended to show that there is a dose-dependency in the dose range 30–240 mg b.i.d. (Miner et al. 2014).

16.4.2.2 Effects on Symptoms

Esophageal acid (pH < 4) exposure time has a relatively good predictive value in terms of esophagitis. However, the relationship is less obvious when it comes to symptoms but it is still the best predictor to date. PPIs represent the best therapeutic option to resolve GERD symptoms but in a significant minority of patients, the reduction in symptoms is insufficient (Hussain et al. 2015). These so-called "PPI partial responders" have therefore been nominated the target population for $GABA_B$ receptor agonist treatment (Shaheen et al. 2013). Since acid reflux is greatly reduced in GERD patients on PPIs, it has been proposed that weakly acidic or non-acidic reflux accounts for the symptoms. However, the predictive value of these measures is highly uncertain, leaving no option but to assess the effects of $GABA_B$ receptor stimulation in this patient population.

One of the first reports on the effects of baclofen on GERD symptoms was a double-blind, placebo-controlled 4-week study on GERD patients without or with mild esophagitis (Ciccaglione and Marzio 2003). As expected, dosing with baclofen (10 mg four times daily) pronouncedly reduced acid reflux and esophageal acid exposure. More surprisingly, given the small size of the study, all five symptoms recorded were markedly ameliorated by baclofen in the absence of any placebo effect. The symptomatic relief provided by baclofen was partially replicated in another small study on patients with duodenogastroesophageal reflux (Koek et al. 2003). Baclofen (20 mg three times daily) added on top of omeprazole (20 mg twice daily) significantly attenuated heartburn, choking, and odynophagia but not the other 11 symptoms recorded. Also, baclofen (40 mg single dose) suppressed symptoms related both to acid and non-acid reflux in an acute setting (Vela et al. 2003). Some GERD patients suffer from night-time reflux and symptoms, and baclofen has been shown to reduce both reflux and symptoms which improves sleep (Orr et al. 2012). The inhibitory effects of baclofen on reflux in children are paralleled by reports on symptomatic relief both in neurologically healthy (Vadlamudi et al. 2013) and impaired (Kawai et al. 2004) children.

Others have failed to confirm the effect of baclofen on symptoms. In a study on 37 GERD patients, 40 mg baclofen (single dose) neither reduced postprandial heartburn nor regurgitation (van Herwaarden et al. 2002) despite inhibitory effects on acid reflux. While doubling the standard dose of 40 mg esomeprazole afforded additional inhibition of reflux and symptoms in hard-to-treat GERD patients, those who still were symptomatic did not respond to 60 mg baclofen (3 × 20 mg daily) added on top of esomeprazole (Bajbouj et al. 2009). Neither reflux nor symptoms were affected but it has to be pointed out that this cohort consisted only of seven patients. The importance of the sensitivity of GERD symptoms to placebo was demonstrated by Cossentino et al. (2012) who found that while baclofen reduced both reflux parameters and symptoms, placebo suppressed symptoms equally effectively in the absence of any effects on reflux. In a phase IIa study on 252 subjects, lesogaberan was shown to reduce symptoms in GERD patients not responding sufficiently to PPIs (Boeckxstaens et al. 2011b). However, while the effect was statistically significant, it was modest (8 % responders in the placebo group vs. 18 % in the lesogaberan

group). A dose-ranging phase IIb study was done in 580 patients (Shaheen et al. 2013). Unfortunately, the maximal effect was similar to that observed in the phase IIa study (a finding consistent with the biphasic dose–response curve for inhibition of TLESRs in dogs) (Lehmann et al. 2009), and since it was below the prespecified lowest clinically meaningful effect, the development of lesogaberan was discontinued at that point. In retrospect, the inclusion of patients based predominantly on heartburn may have contributed significantly to the failure. A large proportion of those may have suffered from functional heartburn which is unrelated to GER. Regurgitation, on the other hand, is an unequivocal sign of reflux, and it is possible that the outcome would have been different if only patients with regurgitation as the predominant symptom would have been studied. This supposition is not only based on findings that lesogaberan inhibits TLESRs in GERD patients partially resistant to PPIs but also that the compound reduces acid, weakly acidic, and mixed gas/liquid reflux (Miner et al. 2014).

16.4.2.3 Translational Aspects

Lesogaberan has a side-effect profile superior to baclofen. In the preclinical setting, this was evident when the effect on TLESRs was compared with the potential to cause hypothermia, a central side-effect (Lehmann et al. 2009). Mechanistic investigations on the unusual profile of lesogaberan showed that it is not only a potent $GABA_B$ receptor agonist but also a substrate for GABA carriers (Lehmann et al. 2009). It is thought that uptake into neural cells of the small fraction of lesogaberan that crosses the blood–brain barrier limits access to central $GABA_B$ receptors accountable for the major side-effects of baclofen. Subsequent studies in healthy volunteers (Boeckxstaens et al. 2010a) and GERD patients (Boeckxstaens et al. 2010b) indicated excellent translation in this regard, and the incidence of central adverse effects was very low for lesogaberan. In addition, the inhibitory effects of different $GABA_B$ receptor agonists on TLESRs in dogs and ferrets were validated in a number of human studies on both healthy volunteers and GERD patients (Lidums et al. 2000; Zhang et al. 2002; Boeckxstaens et al. 2010a, b; van Herwaarden et al. 2002). Another key observation was that the inhibitory effects of lesogaberan on acid reflux measured with ambulatory pHmetry in dogs (Branden et al. 2010) were confirmed in GERD patients (Miner et al. 2014). These are examples of translational research that clearly justified progress into phase II studies. Two observations made in humans were not revealed in preclinical experiments. One was the increase in LES pressure only seen in humans, and the other a side-effect that cannot be detected in animals, paresthesia. This sensory phenomenon is most likely related to peripheral $GABA_B$ receptor activation and can be mitigated by adjusting the exposure profile to lesogaberan (Rydholm et al. 2016).

To date, no drug candidate inhibiting TLESRs has been advanced into phase III studies. For instance, the development of arbaclofen placarbil was discontinued after phase IIb following the heels of lesogaberan, and the negative allosteric mGluR5 inhibitor ADX10059 was stopped even earlier. However, this should not

be construed to conclude that the concept of TLESR inhibition as a therapy for GERD is flawed. As mentioned above, the group of GERD patients partially resistant to GERD appears more heterogeneous than originally believed (Kahrilas and Boeckxstaens 2012). There may still be a case for future $GABA_B$ receptor agonists in the treatment of GERD but for such endeavors to be successful, careful characterization and selection of patients is key (Kahrilas and Boeckxstaens 2012). In addition, none of the reflux inhibitors evaluated provide much more than 50 % inhibition of TLESR at the highest doses testable, and it may be that a more pronounced reduction is required to achieve clinically meaningful symptomatic relief.

16.4.3 Chronic Cough

16.4.3.1 Preclinical Studies

Both GABA type A ($GABA_A$) receptors and $GABA_B$ receptor have been localized to the airways and lungs and their capacity to regulate epithelial cell and airway smooth muscle cell function has been documented (Mizuta et al. 2008a, 2011, 2008b; Yabumoto et al. 2008). GABA synthesized by neuroendocrine cells and other airway epithelial cells may be the endogenous agonist for these receptors (Schnorbusch et al. 2013; Gallos et al. 2009, 2013; Danielsson et al. 2016). This endogenous GABAergic system has been implicated in mucus hypersecretion in asthma (Hirota et al. 2010; Xiang et al. 2007). GABA receptors may also regulate airway parasympathetic ganglia neurons (Itabashi et al. 1992; Allen and Burnstock 1990). GABA receptor activation has been shown to inhibit cholinergic nerve activity (Chapman et al. 1991; Tamaoki et al. 1987). GABA receptor agonists, including $GABA_B$ receptor-selective agonists, reverse bronchospasm and attenuate airways hyperresponsiveness in animals and in human subjects (Grimm et al. 1997; Gleason et al. 2009; Gentilini et al. 1995; Dicpinigaitis et al. 1994; Tohda et al. 1998). There are also GABAergic central circuits regulating cholinergic outflow to the airways, and cough (Canning and Mori 2010; Moore et al. 2004).

The effects of $GABA_B$ receptor activation on airway neural control have been described in multiple studies. In humans, $GABA_B$ receptor activation using baclofen reduces airways reactivity and reduces airways obstruction in patients with spinal cord injury (Grimm et al. 1997; Dicpinigaitis et al. 1994). It is likely that these effects of baclofen result from an inhibition of airway cholinergic tone (Chapman et al. 1991; Tamaoki et al. 1987). $GABA_B$ receptor activation also prevents neuropeptide release from the peripheral terminals of C-fibers in guinea pigs and rats (Belvisi et al. 1989; Ray et al. 1991). This prevents axonal reflexes in the airways of these species, but the physiological relevance of the axon reflex in humans is thought to be minimal. Whether or not axon reflexes are relevant to human disease, the studies of the axon reflex in rats and guinea pigs establishes the expression and functionality of $GABA_B$ receptor on the peripheral terminals of C-fibers, and this may be relevant to therapy targeting airway sensory nerves.

Perhaps the most extensive work on $GABA_B$ receptor actions in the airways has been performed describing the antitussive effects of baclofen and other $GABA_B$ receptor agonists. Studies performed in guinea pigs, cats, and rabbits described the antitussive effects of $GABA_B$ receptor agonists (Bolser et al. 1994; Canning et al. 2012; Bolser et al. 1993; Mutolo et al. 2008; Smith et al. 2012). Studies in patients confirmed the ability of baclofen to prevent cough in humans. These clinical observations included cough induced by capsaicin inhalation (Dicpinigaitis and Dobkin 1997; Dicpinigaitis et al. 1998), cough associated with angiotensin-converting enzyme inhibitor therapy (Dicpinigaitis 1996), coughing accompanying spinal cord injury (Dicpinigaitis et al. 2000), and more recently in patients presenting with cough and GERD (Xu et al. 2012, 2013) (see below).

Both the efficacy and tolerability of baclofen as an antitussive are limited by side effects thought to result from its central penetrance and activation of $GABA_B$ receptor in the brain. These effects include respiratory depression. As mentioned, there is direct physiological and pharmacological evidence for $GABA_B$ receptor expression by neuropeptide containing C-fibers, which have been implicated in cough (Belvisi et al. 1989; Ray et al. 1991). These observations led to the prediction that $GABA_B$ receptor activation at the peripheral terminals of C-fibers may provide cough suppression without the undesirable side effects of centrally penetrant $GABA_B$ receptor agonists. In support of this hypothesis, studies performed using the peripherally restricted agonists 3-aminopropylphosphinic acid and lesogaberan have documented profound antitussive effects of these drugs without coincident respiratory depression (Bolser et al. 1994; Canning et al. 2012). Although a preliminary report of the actions of lesogaberan in capsaicin-induced cough in healthy volunteers was not encouraging (Badri et al., 2014), the extensive work with baclofen and other $GABA_B$ receptor agonists in patients and in animals provides a strong rationale for continued analyses of the actions of these drugs in cough and in other respiratory reflexes that contribute to the symptoms and pathophysiological presentation of acute and chronic respiratory diseases.

One limitation of attempting to translate preclinical studies of cough to patients is the assumptions necessarily made regarding the sensory nerve subtypes regulating cough in humans, and more specifically, the sensory nerve subtypes responsible for cough hypersensitivity and thus the excessive coughing associated with acute and chronic diseases of the airways and GERD. As mentioned, in guinea pigs both C-fibers and the mechanically sensitive cough receptors are both capable of initiating cough upon activation (Canning et al. 2004; Mazzone et al. 2009). Structures in the mucosa of biopsies taken from chronic cough patients would seem to support the notion of multiple sensory nerve subtypes in the regulation of cough (West et al. 2015). Studies performed in guinea pigs and rats confirm the expression of $GABA_B$ receptor on the peripheral terminals of C-fibers (Belvisi et al. 1989; Ray et al. 1991), but comparable studies of the peripheral terminals of the cough receptors have not been performed. We have, however, evaluated the ability of baclofen to suppress cough when applied topically to the airways from which cough is evoked in anesthetized guinea pigs to the effects of baclofen

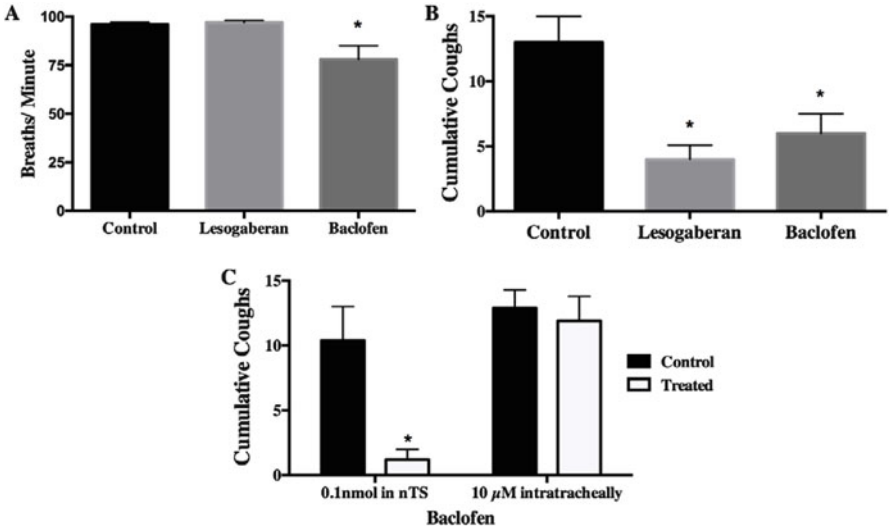

Fig. 16.2 $GABA_B$ receptor agonists modulate respiratory rate and cough via central and peripheral sites of action. (**a**) Respiratory rate in awake guinea pigs following intraperitoneal administration of vehicle, lesogaberan (10 mg/kg ip) and baclofen (3 mg/kg ip). Baclofen markedly reduced respiratory rate and induced overt signs of sedation while the peripherally acting lesogaberan was without effect on breathing or wakefulness. (**b**) Both baclofen (3 mg/kg) and lesogaberan (10 mg/kg) reduced coughing evoked by citric acid (0.01–0.3 M) inhalation, which in conscious guinea pigs is thought to be heavily dependent upon C-fiber activation (data modified from Canning et al. 2012). (**c**) Citric acid (0.001–2 M applied topically to the tracheal mucosa in 100 μL aliquots)-evoked coughing in anesthetized guinea pigs is mediated by capsaicin-insensitive myelinated Aδ fibers (Canning et al. 2004; Mazzone et al. 2009). Microinjecting baclofen bilaterally in nTS locations of the cough receptor terminals (Canning and Mori 2010) nearly abolished citric acid-evoked coughing while having no effect on respiratory rate, which averaged 53 ± 4 and 50 ± 5 in control and baclofen-treated animals, respectively. A tenfold higher dose of baclofen (1 nmol bilaterally) abolished coughing but also markedly decreased respiratory rate (29 ± 4 breaths/min). In contrast to its effects following nTS microinjection, baclofen applied topically to the tracheal mucosa at a concentration (10 μM) that prevents neuropeptide release from C-fiber terminals (Belvisi et al. 1989; Ray et al. 1991), was without effect on tracheal citric acid evoked coughing

microinjected at the central terminations of the cough receptors (Fig. 16.2). We observed that baclofen was without effect when applied topically to the tracheal mucosa, but profoundly inhibited cough upon nTS microinjection. These results would thus suggest that cough receptor-dependent cough would require a CNS penetrant $GABA_B$ receptor agonist for suppression.

16.4.3.2 $GABA_B$ Receptor Agonism in GER-Related Chronic Cough

The importance of GER as a cause of chronic cough has been extensively debated (Pauwels et al. 2009; Kahrilas et al. 2014). The challenge to interpreting this issue has been separating evidence based on the experience of primary care providers

from the results arising from cough specialty clinics. In these latter settings, patients have typically had a chronic, troublesome cough not for weeks but for years, have tried multiple medical approaches without success and have already had all obvious etiologies investigated, ruled out or aggressively treated and yet they are still coughing. In this latter group of patients, reflux medications have typically proven ineffective (Patterson et al. 2004; Shaheen et al. 2011; Faruqi et al. 2011). Without strong supportive data, some researchers still maintain that GER is important in many such patients with chronic cough, but implicate non-acid GER and not the GER effectively treated by PPIs and H2 blockers (Pearson et al. 2011). However, direct evidence for a role of nonacid reflux in cough is lacking. Outcomes in chronic cough patients following surgical treatment of reflux (fundoplication) may be no better than in patients receiving no intervention at all (Birring 2011). Chronic cough patients also display no obvious signs of aspiration or microaspiration as assessed by measurements of pepsin in sputum (Decalmer et al. 2012).

Despite the frequent failures of reflux-specific therapies in patients with chronic cough, there are several clinical observations that do support the hypothesis that reflux may in fact be permissive to cough and cough hypersensitivity. Simultaneous measurements of cough sounds and reflux events (using impedance catheters) revealed frequent associations between these reflexes (Smith et al. 2010). Motility studies in chronic cough patients also reveal disproportionate levels of pharyngeal, laryngeal, and esophageal motility irregularities in coughers when compared to control patients (Kastelik et al. 2003; Phua et al. 2005; Almansa et al. 2015; Kanemitsu et al. 2016). Although limited studies have been completed, patients carefully selected for evidence of a reflux-induced chronic cough respond well to anti-reflux therapy, including proton pump inhibitors, $GABA_B$ agonists, pro-motility agents, liquid alginate, and fundoplication (Xu et al. 2013; Olafsdottir 1995; Ziora et al. 2005; McGlashan et al. 2009; Qiu et al. 2011; Kahrilas et al. 2013). In patients with evidence for reflux, PPIs may also reduce sensitivity to tussive challenges (Benini et al. 2000).

The mechanisms by which reflux might induce cough (Fig. 16.3) are not firmly established. The simplest explanation would be that reflux is aspirated and induces cough following activation of airway sensory nerves that induce coughing upon activation. But as mentioned, there is little evidence for frequent aspiration events in chronic cough patients, and esophageal pH and impedance studies suggest that proximal reflux events are rare. The alternative hypothesis is that refluxate in susceptible individuals activates sensory nerves in the esophageal mucosa to increase sensitivity to tussive stimuli. This has been described in patients and in animals, and such synergistic interactions between afferent nerve subtypes and cough have been described (Canning et al. 2014; Ebihara et al. 2007; Javorkova et al. 2008). Both of the mechanisms outlined above seem operative in the rare genetic disorder autosomal dominant hereditary sensory neuropathy (Spring et al. 2005).

Since $GABA_B$ receptor agonists inhibit reflux irrespective of acidity as well as different types of vagal pulmonary afferents they may be expected to be particularly effective in GER-induced cough. Unfortunately, this notion has not yet been clearly supported by published studies. In a large phase IIb study, lesogaberan was

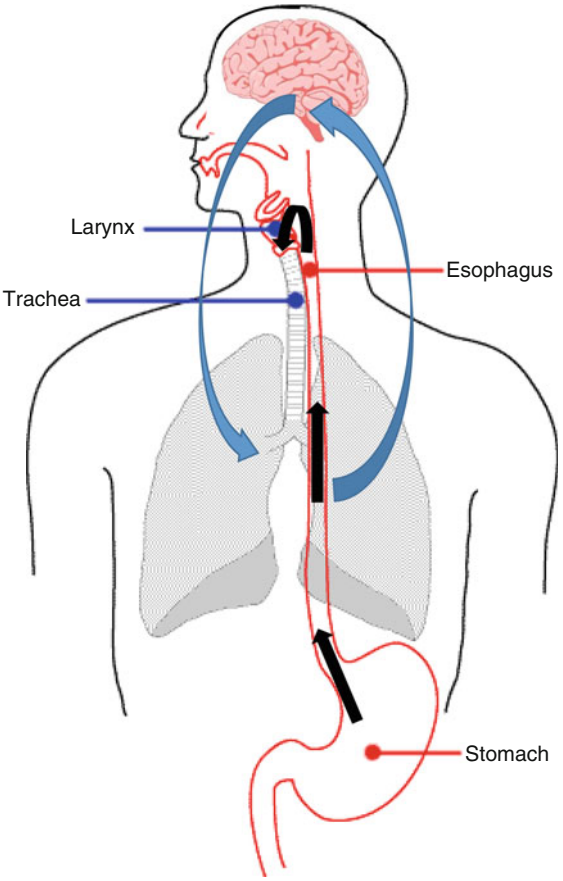

Fig 16.3 GER may trigger cough by two mechanisms. Firstly, reflux reaching the larynx (*black arrows*) may be inspired which excites acid-sensitive pulmonary afferents. Secondly, acid may activate esophageal vagal afferents which trigger the cough motor program via the nTS (*blue arrows*)

given to patients with a partial PPI response. While there was a statistically significant effect of lesogaberan on symptoms such as heartburn and regurgitation, no such effect was seen on hoarseness, cough, and difficulties to swallow (Shaheen et al. 2013). However, since these symptoms were lumped together, a lack of an effect on cough is uncertain.

Using a step-up therapeutic regimen, Xu et al. found that baclofen on top of esomeprazole and ranitidine reduced cough in patients with suspected GER-related cough (Xu et al. 2016). A smaller study from the same group demonstrated that baclofen (20 mg three times daily) combined with esomeprazole produced an antitussive action in GER-related cough patients and elevated the capsaicin tussigenic threshold (Xu et al. 2013). Three GER-related cough patients who gained no benefit from esomeprazole and the prokinetic domperidon reported resolution of symptoms after baclofen therapy (Xu et al. 2012). However, all three studies suffered from the shortcoming that they were not placebo-controlled. To conclude, more studies are clearly warranted to determine if $GABA_B$ agonists have a role to play in GER-related cough.

16.5 Conclusions

With the exception of spasticity, GERD is undoubtedly the indication for which the therapeutic effects of $GABA_B$ receptor agonism have been most convincingly documented. However, even if baclofen has been added to the compassionate use pharmacological toolbox for both adult (Tsoukali and Sifrim 2010) and pediatric (Vadlamudi et al. 2013) hard-to-treat GERD patients, no $GABA_B$ receptor agonist has been approved for GERD. It seems clear that centrally acting compounds similar to baclofen will not be broadly successful in the treatment of GERD and it is questionable if peripherally restricted ligands such as lesogaberan can reduce reflux sufficiently efficaciously. Despite this, there is still some cause for optimism. First, more careful characterization and selection of GERD patients may provide an opportunity for personalized medicine approaches using peripherally restricted $GABA_B$ receptor agonists. Also, whether or not positive allosteric modulators binding to the $GABA_B$ receptor can prevent TLESRs and reflux remains an open question. As the understanding of GER-related chronic cough grows, these patients may eventually prove to be the optimal target population for $GABA_B$ receptor agonist therapy since there may be a double effect of $GABA_B$ receptor agonists (inhibition of microaspiration and reduction of airway vagal afferent sensitivity). Whether this assumption is correct can only be addressed by well-designed, placebo-controlled randomized clinical trials.

References

Akiba, Y., Mizumori, M., Kuo, M., Ham, M., Guth, P. H., Engel, E., et al. (2008). CO_2 chemosensing in rat oesophagus. *Gut, 57*(12), 1654–1664. doi:10.1136/gut.2007.144378.

Allen, T. G., & Burnstock, G. (1990). GABAA receptor-mediated increase in membrane chloride conductance in rat paratracheal neurones. *British Journal of Pharmacology, 100*(2), 261–268.

Almansa, C., Smith, J. A., Morris, J., Crowell, M. D., Valdramidou, D., Lee, A. S., et al. (2015). Weak peristalsis with large breaks in chronic cough: Association with poor esophageal clearance. *Neurogastroenterology and Motility, 27*(3), 431–442. doi:10.1111/nmo.12513.

Alstermark, C., Amin, K., Dinn, S. R., Elebring, T., Fjellstrom, O., Fitzpatrick, K., et al. (2008). Synthesis and pharmacological evaluation of novel gamma-aminobutyric acid type B (GABAB) receptor agonists as gastroesophageal reflux inhibitors. *Journal of Medicinal Chemistry, 51*(14), 4315–4320. doi:10.1021/jm701425k.

Bajbouj, M., Becker, V., Phillip, V., Wilhelm, D., Schmid, R. M., & Meining, A. (2009). High-dose esomeprazole for treatment of symptomatic refractory gastroesophageal reflux disease—A prospective pH-metry/impedance-controlled study. *Digestion, 80*(2), 112–118. doi:10.1159/000221146.

Balentova, S., Conwell, S., & Myers, A. C. (2013). Neurotransmitters in parasympathetic ganglionic neurons and nerves in mouse lower airway smooth muscle. *Respiratory Physiology & Neurobiology, 189*(1), 195–202. doi:10.1016/j.resp.2013.07.006.

Beaumont, H., & Boeckxstaens, G. E. (2009). Does the presence of a hiatal hernia affect the efficacy of the reflux inhibitor baclofen during add-on therapy? *American Journal of Gastroenterology, 104*(7), 1764–1771. doi:10.1038/ajg.2009.247.

Belvisi, M. G., Ichinose, M., & Barnes, P. J. (1989). Modulation of non-adrenergic, non-cholinergic neural bronchoconstriction in guinea-pig airways via GABAB-receptors. *British Journal of Pharmacology, 97*(4), 1225–1231.

Benini, L., Ferrari, M., Sembenini, C., Olivieri, M., Micciolo, R., Zuccali, V., et al. (2000). Cough threshold in reflux oesophagitis: Influence of acid and of laryngeal and oesophageal damage. *Gut, 46*(6), 762–767.

Birring, S. S. (2011). Controversies in the evaluation and management of chronic cough. *American Journal of Respiratory and Critical Care Medicine, 183*(6), 708–715. doi:10.1164/rccm.201007-1017CI.

Blackshaw, L. A., & Grundy, D. (1990). Effects of cholecystokinin (CCK-8) on two classes of gastroduodenal vagal afferent fibre. *Journal of the Autonomic Nervous System, 31*(3), 191–201.

Blackshaw, L. A., Staunton, E., Lehmann, A., & Dent, J. (1999). Inhibition of transient LES relaxations and reflux in ferrets by GABA receptor agonists. *American Journal of Physiology, 277* (4 Pt 1), G867–G874.

Boeckxstaens, G. E., Beaumont, H., Hatlebakk, J. G., Silberg, D. G., Bjorck, K., Karlsson, M., et al. (2011). A novel reflux inhibitor lesogaberan (AZD3355) as add-on treatment in patients with GORD with persistent reflux symptoms despite proton pump inhibitor therapy: A randomised placebo-controlled trial. *Gut, 60*(9), 1182–1188. doi:10.1136/gut.2010.235630.

Boeckxstaens, G. E., Beaumont, H., Mertens, V., Denison, H., Ruth, M., Adler, J., et al. (2010). Effects of lesogaberan on reflux and lower esophageal sphincter function in patients with gastroesophageal reflux disease. *Gastroenterology, 139*(2), 409–417. doi:10.1053/j.gastro.2010.04.051.

Boeckxstaens, G. E., Denison, H., Jensen, J. M., Lehmann, A., & Ruth, M. (2011). Translational gastrointestinal pharmacology in the 21st century: 'The lesogaberan story'. *Current Opinion in Pharmacology, 11*(6), 630–633. doi:10.1016/j.coph.2011.10.011.

Boeckxstaens, G. E., Rydholm, H., Lei, A., Adler, J., & Ruth, M. (2010). Effect of lesogaberan, a novel GABA(B)-receptor agonist, on transient lower oesophageal sphincter relaxations in male subjects. *Alimentary Pharmacology & Therapeutics, 31*(11), 1208–1217. doi:10.1111/j.1365-2036.2010.04283.x.

Bolser, D. C., Aziz, S. M., DeGennaro, F. C., Kreutner, W., Egan, R. W., Siegel, M. I., et al. (1993). Antitussive effects of GABAB agonists in the cat and guinea-pig. *British Journal of Pharmacology, 110*(1), 491–495.

Bolser, D. C., DeGennaro, F. C., O'Reilly, S., Chapman, R. W., Kreutner, W., Egan, R. W., et al. (1994). Peripheral and central sites of action of GABA-B agonists to inhibit the cough reflex in the cat and guinea pig. *British Journal of Pharmacology, 113*(4), 1344–1348.

Boulant, J., Fioramonti, J., Dapoigny, M., Bommelaer, G., & Bueno, L. (1994). Cholecystokinin and nitric oxide in transient lower esophageal sphincter relaxation to gastric distention in dogs. *Gastroenterology, 107*(4), 1059–1066.

Bowery, N. G. (2006). GABAB receptor: A site of therapeutic benefit. *Current Opinion in Pharmacology, 6*(1), 37–43. doi:10.1016/j.coph.2005.10.002.

Branden, L., Fredriksson, A., Harring, E., Jensen, J., & Lehmann, A. (2010). The novel, peripherally restricted GABAB receptor agonist lesogaberan (AZD3355) inhibits acid reflux and reduces esophageal acid exposure as measured with 24-h pHmetry in dogs. *European Journal of Pharmacology, 634*(1-3), 138–141. doi:10.1016/j.ejphar.2010.02.015.

Cange, L., Johnsson, E., Rydholm, H., Lehmann, A., Finizia, C., Lundell, L., et al. (2002). Baclofen-mediated gastro-oesophageal acid reflux control in patients with established reflux disease. *Alimentary Pharmacology & Therapeutics, 16*(5), 869–873.

Canning, B. J., Chang, A. B., Bolser, D. C., Smith, J. A., Mazzone, S. B., & McGarvey, L. (2014). Anatomy and neurophysiology of cough: Chest guideline and expert panel report. *Chest, 146*(6), 1633–1648. doi:10.1378/chest.14-1481.

Canning, B. J., & Chou, Y. L. (2009). Cough sensors. I. Physiological and pharmacological properties of the afferent nerves regulating cough. *Handbook of Experimental Pharmacology* (187), 23–47. doi:10.1007/978-3-540-79842-2_2.

Canning, B. J., Fischer, A., & Undem, B. J. (1998). Pharmacological analysis of the tachykinin receptors that mediate activation of nonadrenergic, noncholinergic relaxant nerves that innervate guinea pig trachealis. *Journal of Pharmacology and Experimental Therapeutics, 284*(1), 370–377.

Canning, B. J., Mazzone, S. B., Meeker, S. N., Mori, N., Reynolds, S. M., & Undem, B. J. (2004). Identification of the tracheal and laryngeal afferent neurones mediating cough in anaesthetized guinea-pigs. *Journal of Physiology, 557*(Pt 2), 543–558. doi:10.1113/jphysiol.2003.057885.

Canning, B. J., & Mori, N. (2010). An essential component to brainstem cough gating identified in anesthetized guinea pigs. *FASEB Journal, 24*(10), 3916–3926. doi:10.1096/fj.09-151068.

Canning, B. J., & Mori, N. (2011). Encoding of the cough reflex in anesthetized guinea pigs. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology, 300*(2), R369–R377. doi:10.1152/ajpregu.00044.2010.

Canning, B. J., Mori, N., & Lehmann, A. (2012). Antitussive effects of the peripherally restricted GABAB receptor agonist lesogaberan in guinea pigs: Comparison to baclofen and other GABAB receptor-selective agonists. *Cough, 8*(1), 7. doi:10.1186/1745-9974-8-7.

Canning, B. J., & Undem, B. J. (1993). Evidence that distinct neural pathways mediate parasympathetic contractions and relaxations of guinea-pig trachealis. *Journal of Physiology, 471*, 25–40.

Canning, B. J., & Undem, B. J. (1994). Evidence that antidromically stimulated vagal afferents activate inhibitory neurones innervating guinea-pig trachealis. *Journal of Physiology, 480*(Pt 3), 613–625.

Chapman, R. W., Danko, G., Rizzo, C., Egan, R. W., Mauser, P. J., & Kreutner, W. (1991). Prejunctional GABA-B inhibition of cholinergic, neurally-mediated airway contractions in guinea-pigs. *Pulmonary Pharmacology, 4*(4), 218–224.

Chou, Y. L., Scarupa, M. D., Mori, N., & Canning, B. J. (2008). Differential effects of airway afferent nerve subtypes on cough and respiration in anesthetized guinea pigs. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology, 295*(5), R1572–R1584. doi:10.1152/ajpregu.90382.2008.

Ciccaglione, A. F., & Marzio, L. (2003). Effect of acute and chronic administration of the GABA B agonist baclofen on 24 hour pH metry and symptoms in control subjects and in patients with gastro-oesophageal reflux disease. *Gut, 52*(4), 464–470.

Coleridge, J. C., & Coleridge, H. M. (1984). Afferent vagal C fibre innervation of the lungs and airways and its functional significance. *Reviews of Physiology, Biochemistry and Pharmacology, 99*, 1–110.

Conchillo, J. M., & Smout, A. J. (2009). Review article: Intra-oesophageal impedance monitoring for the assessment of bolus transit and gastro-oesophageal reflux. *Alimentary Pharmacology & Therapeutics, 29*(1), 3–14. doi:10.1111/j.1365-2036.2008.03863.x.

Cossentino, M. J., Mann, K., Armbruster, S. P., Lake, J. M., Maydonovitch, C., & Wong, R. K. (2012). Randomised clinical trial: The effect of baclofen in patients with gastro-oesophageal reflux—A randomised prospective study. *Alimentary Pharmacology & Therapeutics, 35*(9), 1036–1044. doi:10.1111/j.1365-2036.2012.05068.x.

Cox, M. R., Martin, C. J., Dent, J., & Westmore, M. (1988). Effect of general anaesthesia on transient lower oesophageal sphincter relaxations in the dog. *Australian and New Zealand Journal of Surgery, 58*(10), 825–830.

Curcic, J., Schwizer, A., Kaufman, E., Forras-Kaufman, Z., Banerjee, S., Pal, A., et al. (2014). Effects of baclofen on the functional anatomy of the oesophago-gastric junction and proximal stomach in healthy volunteers and patients with GERD assessed by magnetic resonance imaging and high-resolution manometry: A randomised controlled double-blind study. *Alimentary Pharmacology & Therapeutics, 40*(10), 1230–1240. doi:10.1111/apt.12956.

Danielsson, J., Zaidi, S., Kim, B., Funayama, H., Yim, P. D., Xu, D., et al. (2016). Airway epithelial cell release of GABA is regulated by protein kinase A. *Lung, 194*(3), 401–408. doi:10.1007/s00408-016-9867-2.

Decalmer, S., Stovold, R., Houghton, L. A., Pearson, J., Ward, C., Kelsall, A., et al. (2012). Chronic cough: Relationship between microaspiration, gastroesophageal reflux, and cough frequency. *Chest, 142*(4), 958–964. doi:10.1378/chest.12-0044.

Dent, J., Dodds, W. J., Friedman, R. H., Sekiguchi, T., Hogan, W. J., Arndorfer, R. C., et al. (1980). Mechanism of gastroesophageal reflux in recumbent asymptomatic human subjects. *Journal of Clinical Investigation, 65*(2), 256–267. doi:10.1172/jci109667.

Dent, J., Holloway, R. H., Toouli, J., & Dodds, W. J. (1988). Mechanisms of lower oesophageal sphincter incompetence in patients with symptomatic gastrooesophageal reflux. *Gut, 29*(8), 1020–1028.

Dey, R. D., Altemus, J. B., Rodd, A., Mayer, B., Said, S. I., & Coburn, R. F. (1996). Neurochemical characterization of intrinsic neurons in ferret tracheal plexus. *American Journal of Respiratory Cell and Molecular Biology, 14*(3), 207–216. doi:10.1165/ajrcmb.14.3.8845170.

Dicpinigaitis, P. V. (1996). Use of baclofen to suppress cough induced by angiotensin-converting enzyme inhibitors. *Annals of Pharmacotherapy, 30*(11), 1242–1245.

Dicpinigaitis, P. V., & Dobkin, J. B. (1997). Antitussive effect of the GABA-agonist baclofen. *Chest, 111*(4), 996–999.

Dicpinigaitis, P. V., Dobkin, J. B., Rauf, K., & Aldrich, T. K. (1998). Inhibition of capsaicin-induced cough by the gamma-aminobutyric acid agonist baclofen. *Journal of Clinical Pharmacology, 38*(4), 364–367.

Dicpinigaitis, P. V., Grimm, D. R., & Lesser, M. (2000). Baclofen-induced cough suppression in cervical spinal cord injury. *Archives of Physical Medicine and Rehabilitation, 81*(7), 921–923. doi:10.1053/apmr.2000.5612.

Dicpinigaitis, P. V., Spungen, A. M., Bauman, W. A., Absgarten, A., & Almenoff, P. L. (1994). Inhibition of bronchial hyperresponsiveness by the GABA-agonist baclofen. *Chest, 106*(3), 758–761.

Dodds, W. J., Dent, J., Hogan, W. J., Helm, J. F., Hauser, R., Patel, G. K., et al. (1982). Mechanisms of gastroesophageal reflux in patients with reflux esophagitis. *New England Journal of Medicine, 307*(25), 1547–1552. doi:10.1056/nejm198212163072503.

Dusenkova, S., Ru, F., Surdenikova, L., Nassenstein, C., Hatok, J., Dusenka, R., et al. (2014). The expression profile of acid-sensing ion channel (ASIC) subunits ASIC1a, ASIC1b, ASIC2a, ASIC2b, and ASIC3 in the esophageal vagal afferent nerve subtypes. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 307*(9), G922–G930. doi:10.1152/ajpgi.00129.2014.

Ebihara, S., Ebihara, T., Yamasaki, M., Asada, M., Yamanda, S., Niu, K., et al. (2007). Contribution of gastric acid in elderly nursing home patients with cough reflex hypersensitivity. *Journal of American Geriatrics Society, 55*(10), 1686–1688. doi:10.1111/j.1532-5415.2007.01324.x.

Faruqi, S., Molyneux, I. D., Fathi, H., Wright, C., Thompson, R., & Morice, A. H. (2011). Chronic cough and esomeprazole: A double-blind placebo-controlled parallel study. *Respirology, 16*(7), 1150–1156. doi:10.1111/j.1440-1843.2011.02014.x.

Fischer, A., Canning, B. J., Undem, B. J., & Kummer, W. (1998). Evidence for an esophageal origin of VIP-IR and NO synthase-IR nerves innervating the guinea pig trachealis: A retrograde neuronal tracing and immunohistochemical analysis. *Journal of Comparative Neurology, 394*(3), 326–334.

Franzi, S. J., Martin, C. J., Cox, M. R., & Dent, J. (1990). Response of canine lower esophageal sphincter to gastric distension. *American Journal of Physiology, 259*(3 Pt 1), G380–G385.

Frisby, C. L., Mattsson, J. P., Jensen, J. M., Lehmann, A., Dent, J., & Blackshaw, L. A. (2005). Inhibition of transient lower esophageal sphincter relaxation and gastroesophageal reflux by metabotropic glutamate receptor ligands. *Gastroenterology, 129*(3), 995–1004. doi:10.1053/j.gastro.2005.06.069.

Gallos, G., Gleason, N. R., Virag, L., Zhang, Y., Mizuta, K., Whittington, R. A., et al. (2009). Endogenous gamma-aminobutyric acid modulates tonic guinea pig airway tone and propofol-induced airway smooth muscle relaxation. *Anesthesiology, 110*(4), 748–758.

Gallos, G., Townsend, E., Yim, P., Virag, L., Zhang, Y., Xu, D., et al. (2013). Airway epithelium is a predominant source of endogenous airway GABA and contributes to relaxation of airway smooth muscle tone. *American Journal of Physiology. Lung Cellular and Molecular Physiology, 304*(3), L191–L197. doi:10.1152/ajplung.00274.2012.

Galmiche, J. P., & des Varannes, S. B. (2001). Endoscopy-negative reflux disease. *Current Gastroenterology Reports, 3*(3), 206–214.

Gentilini, G., Franchi-Micheli, S., Mugnai, S., Bindi, D., & Zilletti, L. (1995). GABA-mediated inhibition of the anaphylactic response in the guinea-pig trachea. *British Journal of Pharmacology, 115*(3), 389–394.

Gerson, L. B., Huff, F. J., Hila, A., Hirota, W. K., Reilley, S., Agrawal, A., et al. (2010). Arbaclofen placarbil decreases postprandial reflux in patients with gastroesophageal reflux disease. *American Journal of Gastroenterology, 105*(6), 1266–1275. doi:10.1038/ajg.2009.718.

Gleason, N. R., Gallos, G., Zhang, Y., & Emala, C. W. (2009). The GABAA agonist muscimol attenuates induced airway constriction in guinea pigs in vivo. *Journal of Applied Physiology (1985), 106*(4), 1257–1263. doi:10.1152/japplphysiol.91314.2008.

Grimm, D. R., DeLuca, R. V., Lesser, M., Bauman, W. A., & Almenoff, P. L. (1997). Effects of GABA-B agonist baclofen on bronchial hyperreactivity to inhaled histamine in subjects with cervical spinal cord injury. *Lung, 175*(5), 333–341.

Grossi, L., Spezzaferro, M., Sacco, L. F., & Marzio, L. (2008). Effect of baclofen on oesophageal motility and transient lower oesophageal sphincter relaxations in GORD patients: A 48-h manometric study. *Neurogastroenterology and Motility, 20*(7), 760–766. doi:10.1111/j.1365-2982.2008.01115.x.

Guo, G. F., Cai, Y. C., Zhang, B., Xu, R. H., Qiu, H. J., Xia, L. P., et al. (2011). Overexpression of SGLT1 and EGFR in colorectal cancer showing a correlation with the prognosis. *Medical Oncology, 28*(Suppl 1), S197–S203. doi:10.1007/s12032-010-9696-8.

Haxhiu, M. A., Kc, P., Moore, C. T., Acquah, S. S., Wilson, C. G., Zaidi, S. I., et al. (2005). Brain stem excitatory and inhibitory signaling pathways regulating bronchoconstrictive responses. *Journal of Applied Physiology (1985), 98*(6), 1961–1982. doi:10.1152/japplphysiol.01340.2004.

Hirota, J. A., Budelsky, A., Smith, D., Lipsky, B., Ellis, R., Xiang, Y. Y., et al. (2010). The role of interleukin-4Ralpha in the induction of glutamic acid decarboxylase in airway epithelium following acute house dust mite exposure. *Clinical and Experimental Allergy, 40*(5), 820–830. doi:10.1111/j.1365-2222.2010.03458.x.

Hirsch, D. P., Mathus-Vliegen, E. M., Holloway, R. H., Fakhry, N., D'Amato, M., & Boeckxstaens, G. E. (2002). Role of CCK(A) receptors in postprandial lower esophageal sphincter function in morbidly obese subjects. *Digestive Diseases and Sciences, 47*(11), 2531–2537.

Ho, C. Y., Gu, Q., Lin, Y. S., & Lee, L. Y. (2001). Sensitivity of vagal afferent endings to chemical irritants in the rat lung. *Respiration Physiology, 127*(2-3), 113–124.

Hussain, Z. H., Henderson, E. E., Maradey-Romerao, C., George, N., Fass, R., & Lacy, B. E. (2015). The proton pump inhibitor non-responder: A clinical conundrum. *Clinical and Translational Gastroenterology, 6*, e106. doi:10.1038/ctg.2015.32.

Inoue, H., Ichinose, M., Miura, M., Katsumata, U., & Takishima, T. (1989). Sensory receptors and reflex pathways of nonadrenergic inhibitory nervous system in feline airways. *American Review of Respiratory Disease, 139*(5), 1175–1178. doi:10.1164/ajrccm/139.5.1175.

Irwin, R. S., Baumann, M. H., Bolser, D. C., Boulet, L. P., Braman, S. S., Brightling, C. E., et al. (2006). Diagnosis and management of cough executive summary: ACCP evidence-based clinical practice guidelines. *Chest, 129*(1 Suppl), 1s–23s. doi:10.1378/chest.129.1_suppl.1S.

Itabashi, S., Aibara, K., Sasaki, H., & Akaike, N. (1992). gamma-Aminobutyric acid-induced response in rat dissociated paratracheal ganglion cells. *Journal of Neurophysiology, 67*(5), 1367–1374.

Javorkova, N., Varechova, S., Pecova, R., Tatar, M., Balaz, D., Demeter, M., et al. (2008). Acidification of the oesophagus acutely increases the cough sensitivity in patients with gastro-oesophageal reflux and chronic cough. *Neurogastroenterology and Motility, 20*(2), 119–124. doi:10.1111/j.1365-2982.2007.01020.x.

Jensen, J., Lehmann, A., Uvebrant, A., Carlsson, A., Jerndal, G., Nilsson, K., et al. (2005). Transient lower esophageal sphincter relaxations in dogs are inhibited by a metabotropic glutamate receptor 5 antagonist. *European Journal of Pharmacology, 519*(1-2), 154–157. doi:10.1016/j.ejphar.2005.07.007.

Kahrilas, P. J., & Boeckxstaens, G. (2012). Failure of reflux inhibitors in clinical trials: Bad drugs or wrong patients? *Gut, 61*(10), 1501–1509. doi:10.1136/gutjnl-2011-301898.

Kahrilas, P. J., Howden, C. W., Hughes, N., & Molloy-Bland, M. (2013). Response of chronic cough to acid-suppressive therapy in patients with gastroesophageal reflux disease. *Chest, 143*(3), 605–612. doi:10.1378/chest.12-1788.

Kahrilas, P. J., Smith, J. A., & Dicpinigaitis, P. V. (2014). A causal relationship between cough and gastroesophageal reflux disease (GERD) has been established: A pro/con debate. *Lung, 192*(1), 39–46. doi:10.1007/s00408-013-9528-7.

Kalinichev, M., Donovan-Rodriguez, T., Girard, F., Riguet, E., Rouillier, M., Bournique, B., et al. (2014). Evaluation of peripheral versus central effects of GABA(B) receptor activation using a novel, positive allosteric modulator of the GABA(B) receptor ADX71943, a pharmacological tool compound with a fully peripheral activity profile. *British Journal of Pharmacology, 171*(21), 4941–4954. doi:10.1111/bph.12812.

Kanemitsu, Y., Niimi, A., Matsumoto, H., Iwata, T., Ito, I., Oguma, T., et al. (2016). Gastroesophageal dysmotility is associated with the impairment of cough-specific quality of life in patients with cough variant asthma. *Allergology International, 65*(3), 320–326. doi:10.1016/j.alit.2016.02.014.

Kastelik, J. A., Redington, A. E., Aziz, I., Buckton, G. K., Smith, C. M., Dakkak, M., et al. (2003). Abnormal oesophageal motility in patients with chronic cough. *Thorax, 58*(8), 699–702.

Kawai, M., Kawahara, H., Hirayama, S., Yoshimura, N., & Ida, S. (2004). Effect of baclofen on emesis and 24-hour esophageal pH in neurologically impaired children with gastroesophageal reflux disease. *Journal of Pediatrics Gastroenterology and Nutrition, 38*(3), 317–323.

Koek, G. H., Sifrim, D., Lerut, T., Janssens, J., & Tack, J. (2003). Effect of the GABA(B) agonist baclofen in patients with symptoms and duodeno-gastro-oesophageal reflux refractory to proton pump inhibitors. *Gut, 52*(10), 1397–1402.

Kollarik, M., & Brozmanova, M. (2009). Cough and gastroesophageal reflux: Insights from animal models. *Pulmonary Pharmacology & Therapeutics, 22*(2), 130–134. doi:10.1016/j.pupt.2008.12.017.

Kubin, L., Alheid, G. F., Zuperku, E. J., & McCrimmon, D. R. (2006). Central pathways of pulmonary and lower airway vagal afferents. *Journal of Applied Physiology (1985), 101*(2), 618–627. doi:10.1152/japplphysiol.00252.2006.

Kummer, W., Fischer, A., Kurkowski, R., & Heym, C. (1992). The sensory and sympathetic innervation of guinea-pig lung and trachea as studied by retrograde neuronal tracing and double-labelling immunohistochemistry. *Neuroscience, 49*(3), 715–737.

Kwong, K., Kollarik, M., Nassenstein, C., Ru, F., & Undem, B. J. (2008). P2X2 receptors differentiate placodal vs. neural crest C-fiber phenotypes innervating guinea pig lungs and esophagus. *American Journal of Physiology. Lung Cellular and Molecular Physiology, 295*(5), L858–L865. doi:10.1152/ajplung.90360.2008.

Lama, A., Delpierre, S., & Jammes, Y. (1988). The effects of electrical stimulation of myelinated and non-myelinated vagal motor fibres on airway tone in the rabbit and the cat. *Respiration Physiology, 74*(3), 265–274.

Lehmann, A., Antonsson, M., Bremner-Danielsen, M., Flardh, M., Hansson-Branden, L., & Karrberg, L. (1999). Activation of the GABA(B) receptor inhibits transient lower esophageal sphincter relaxations in dogs. *Gastroenterology, 117*(5), 1147–1154.

Lehmann, A., Antonsson, M., Holmberg, A. A., Blackshaw, L. A., Branden, L., Brauner-Osborne, H., et al. (2009). (R)-(3-amino-2-fluoropropyl) phosphinic acid (AZD3355), a novel GABAB receptor agonist, inhibits transient lower esophageal sphincter relaxation through a peripheral mode of action. *Journal of Pharmacology and Experimental Therapeutics, 331*(2), 504–512. doi:10.1124/jpet.109.153593.

Leslie, E., Bhargava, V., & Mittal, R. K. (2012). A novel pattern of longitudinal muscle contraction with subthreshold pharyngeal stimulus: A possible mechanism of lower esophageal sphincter relaxation. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 302*(5), G542–G547. doi:10.1152/ajpgi.00349.2011.

Li, S., Shi, S., Chen, F., & Lin, J. (2014). The effects of baclofen for the treatment of gastroesophageal reflux disease: A meta-analysis of randomized controlled trials. *Gastroenterology Research and Practice, 2014*, 307805. doi:10.1155/2014/307805.

Lidums, I., Lehmann, A., Checklin, H., Dent, J., & Holloway, R. H. (2000). Control of transient lower esophageal sphincter relaxations and reflux by the GABA(B) agonist baclofen in normal subjects. *Gastroenterology, 118*(1), 7–13.

Little, A. F., Cox, M. R., Martin, C. J., Dent, J., Franzi, S. J., & Lavelle, R. (1989). Influence of posture on transient lower oesophageal sphincter relaxation and gastro-oesophageal reflux in the dog. *Journal of Gastroenterology and Hepatology, 4*(1), 49–54.

Lundell, L. (2010). Surgical therapy of gastro-oesophageal reflux disease. *Best Practice & Research Clinical Gastroenterology, 24*(6), 947–959. doi:10.1016/j.bpg.2010.09.006.

Lv, H. J., & Qiu, Z. M. (2015). Refractory chronic cough due to gastroesophageal reflux: Definition, mechanism and management. *World Journal of Methodology, 5*(3), 149–156. doi:10.5662/wjm.v5.i3.149.

Martin, C. J., Dodds, W. J., Liem, H. H., Dantas, R. O., Layman, R. D., & Dent, J. (1992). Diaphragmatic contribution to gastroesophageal competence and reflux in dogs. *American Journal of Physiology, 263*(4 Pt 1), G551–G557.

Martin, C. J., Patrikios, J., & Dent, J. (1986). Abolition of gas reflux and transient lower esophageal sphincter relaxation by vagal blockade in the dog. *Gastroenterology, 91*(4), 890–896.

Mazzone, S. B., & Canning, B. J. (2002). Evidence for differential reflex regulation of cholinergic and noncholinergic parasympathetic nerves innervating the airways. *American Journal of Respiratory and Critical Care Medicine, 165*(8), 1076–1083. doi:10.1164/ajrccm.165.8.2001121270c.

Mazzone, S. B., Reynolds, S. M., Mori, N., Kollarik, M., Farmer, D. G., Myers, A. C., et al. (2009). Selective expression of a sodium pump isozyme by cough receptors and evidence for its essential role in regulating cough. *Journal of Neuroscience, 29*(43), 13662–13671. doi:10.1523/jneurosci.4354-08.2009.

McGarvey, L. P., Heaney, L. G., Lawson, J. T., Johnston, B. T., Scally, C. M., Ennis, M., et al. (1998). Evaluation and outcome of patients with chronic non-productive cough using a comprehensive diagnostic protocol. *Thorax, 53*(9), 738–743.

McGlashan, J. A., Johnstone, L. M., Sykes, J., Strugala, V., & Dettmar, P. W. (2009). The value of a liquid alginate suspension (Gaviscon Advance) in the management of laryngopharyngeal reflux. *European Archives of Oto-Rhino-Laryngology, 266*(2), 243–251. doi:10.1007/s00405-008-0708-7.

Miner, P. B., Jr., Silberg, D. G., Ruth, M., Miller, F., & Pandolfino, J. (2014). Dose-dependent effects of lesogaberan on reflux measures in patients with refractory gastroesophageal reflux disease: A randomized, placebo-controlled study. *BMC Gastroenterology, 14*, 188. doi:10.1186/1471-230x-14-188.

Mittal, R. K., Holloway, R. H., Penagini, R., Blackshaw, L. A., & Dent, J. (1995). Transient lower esophageal sphincter relaxation. *Gastroenterology, 109*(2), 601–610.

Mizuta, K., Mizuta, F., Xu, D., Masaki, E., Panettieri, R. A., Jr., & Emala, C. W. (2011). Gi-coupled gamma-aminobutyric acid-B receptors cross-regulate phospholipase C and calcium in airway smooth muscle. *American Journal of Respiratory Cell and Molecular Biology, 45*(6), 1232–1238. doi:10.1165/rcmb.2011-0088OC.

Mizuta, K., Osawa, Y., Mizuta, F., Xu, D., & Emala, C. W. (2008). Functional expression of GABAB receptors in airway epithelium. *American Journal of Respiratory Cell and Molecular Biology, 39*(3), 296–304. doi:10.1165/rcmb.2007-0414OC.

Mizuta, K., Xu, D., Pan, Y., Comas, G., Sonett, J. R., Zhang, Y., et al. (2008). GABAA receptors are expressed and facilitate relaxation in airway smooth muscle. *American Journal of Physiology. Lung Cellular and Molecular Physiology, 294*(6), L1206–L1216. doi:10.1152/ajplung.00287.2007.

Moore, C. T., Wilson, C. G., Mayer, C. A., Acquah, S. S., Massari, V. J., & Haxhiu, M. A. (2004). A GABAergic inhibitory microcircuit controlling cholinergic outflow to the airways. *Journal of Applied Physiology (1985), 96*(1), 260–270. doi:10.1152/japplphysiol.00523.2003.

Mutolo, D., Bongianni, F., Cinelli, E., Fontana, G. A., & Pantaleo, T. (2008). Modulation of the cough reflex by antitussive agents within the caudal aspect of the nucleus tractus solitarii in the rabbit. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology, 295*(1), R243–R251. doi:10.1152/ajpregu.00184.2008.

Nassenstein, C., Taylor-Clark, T. E., Myers, A. C., Ru, F., Nandigama, R., Bettner, W., et al. (2010). Phenotypic distinctions between neural crest and placodal derived vagal C-fibres in mouse lungs. *Journal of Physiology, 588*(Pt 23), 4769–4783. doi:10.1113/jphysiol.2010.195339.

Oh, E. J., Mazzone, S. B., Canning, B. J., & Weinreich, D. (2006). Reflex regulation of airway sympathetic nerves in guinea-pigs. *Journal of Physiology, 573*(Pt 2), 549–564. doi:10.1113/jphysiol.2005.104661.

Olafsdottir, E. (1995). Gastro-oesophageal reflux and chronic respiratory disease in infants and children: Treatment with cisapride. *Scandinavian Journal of Gastroenterology. Supplement, 211*, 32–34.

Omari, T. I., Benninga, M. A., Sansom, L., Butler, R. N., Dent, J., & Davidson, G. P. (2006). Effect of baclofen on esophagogastric motility and gastroesophageal reflux in children with gastroesophageal reflux disease: A randomized controlled trial. *Journal of Pediatrics, 149*(4), 468–474. doi:10.1016/j.jpeds.2006.05.029.

Orr, W. C., Goodrich, S., Wright, S., Shepherd, K., & Mellow, M. (2012). The effect of baclofen on nocturnal gastroesophageal reflux and measures of sleep quality: A randomized, cross-over trial. *Neurogastroenterology and Motility, 24*(6), 553–559. doi:10.1111/j.1365-2982.2012.01900.x. e253.

Pack, A. I., & DeLaney, R. G. (1983). Response of pulmonary rapidly adapting receptors during lung inflation. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology, 55*(3), 955–963.

Page, A. J., & Blackshaw, L. A. (1999). GABA(B) receptors inhibit mechanosensitivity of primary afferent endings. *Journal of Neuroscience, 19*(19), 8597–8602.

Page, A. J., & Blackshaw, L. A. (2009). Roles of gastro-oesophageal afferents in the mechanisms and symptoms of reflux disease. *Handbook of Experimental Pharmacology* (194), 227–257. doi:10.1007/978-3-540-79090-7_7.

Page, A. J., Martin, C. M., & Blackshaw, L. A. (2002). Vagal mechanoreceptors and chemoreceptors in mouse stomach and esophagus. *Journal of Neurophysiology, 87*(4), 2095–2103. doi:10.1152/jn.00785.2001.

Page, A. J., O'Donnell, T. A., & Blackshaw, L. A. (2006). Inhibition of mechanosensitivity in visceral primary afferents by GABAB receptors involves calcium and potassium channels. *Neuroscience, 137*(2), 627–636. doi:10.1016/j.neuroscience.2005.09.016.

Page, A. J., Young, R. L., Martin, C. M., Umaerus, M., O'Donnell, T. A., Cooper, N. J., et al. (2005). Metabotropic glutamate receptors inhibit mechanosensitivity in vagal sensory neurons. *Gastroenterology, 128*(2), 402–410.

Patterson, R. N., Johnston, B. T., MacMahon, J., Heaney, L. G., & McGarvey, L. P. (2004). Oesophageal pH monitoring is of limited value in the diagnosis of "reflux-cough". *European Respiratory Journal, 24*(5), 724–727. doi:10.1183/09031936.04.00007404.

Pauwels, A., Blondeau, K., Dupont, L., & Sifrim, D. (2009). Cough and gastroesophageal reflux: From the gastroenterologist end. *Pulmonary Pharmacology & Therapeutics, 22*(2), 135–138. doi:10.1016/j.pupt.2008.11.007.

Pearson, J. P., Parikh, S., Orlando, R. C., Johnston, N., Allen, J., Tinling, S. P., et al. (2011). Review article: Reflux and its consequences—The laryngeal, pulmonary and oesophageal manifestations. Conference held in conjunction with the 9th International Symposium on Human Pepsin (ISHP) Kingston-upon-Hull, UK, 21–23 April 2010. *Alimentary Pharmacology & Therapeutics, 33 Suppl 1*, 1–71. doi:10.1111/j.1365-2036.2011.04581.x.

Phipps, R. J., & Richardson, P. S. (1976). The effects of irritation at various levels of the airway upon tracheal mucus secretion in the cat. *Journal of Physiology, 261*(3), 563–581.

Phua, S. Y., McGarvey, L. P., Ngu, M. C., & Ing, A. J. (2005). Patients with gastro-oesophageal reflux disease and cough have impaired laryngopharyngeal mechanosensitivity. *Thorax, 60*(6), 488–491. doi:10.1136/thx.2004.033894.

Ray, N. J., Jones, A. J., & Keen, P. (1991). GABAB receptor modulation of the release of substance P from capsaicin-sensitive neurones in the rat trachea in vitro. *British Journal of Pharmacology, 102*(4), 801–804.

Rydholm, H., von Corswant, C., Denison, H., Jensen, J. M., Lehmann, A., Ruth, M., et al. (2016). Reducing adverse effects during drug development: The example of lesogaberan and paresthesia. *Clinical Therapeutics, 38*(4), 946–960. doi:10.1016/j.clinthera.2016.02.012.

Sant'Ambrogio, G., & Widdicombe, J. (2001). Reflexes from airway rapidly adapting receptors. *Respiration Physiology, 125*(1–2), 33–45.

Scarpellini, E., Boecxstaens, V., Broers, C., Vos, R., Pauwels, A., & Tack, J. (2015). Effect of baclofen on gastric acid pocket in subjects with gastroesophageal reflux disease symptoms. *Diseases of the Esophagus*. doi:10.1111/dote.12443.

Schnorbusch, K., Lembrechts, R., Pintelon, I., Timmermans, J. P., Brouns, I., & Adriaensen, D. (2013). GABAergic signaling in the pulmonary neuroepithelial body microenvironment: Functional imaging in GAD67-GFP mice. *Histochemistry and Cell Biology, 140*(5), 549–566. doi:10.1007/s00418-013-1093-x.

Schultz, H. D., Roberts, A. M., Bratcher, C., Coleridge, H. M., Coleridge, J. C., & Davis, B. (1985). Pulmonary C-fibers reflexly increase secretion by tracheal submucosal glands in dogs. *Journal of Applied Physiology (1985), 58*(3), 907–910.

Shaheen, N. J., Crockett, S. D., Bright, S. D., Madanick, R. D., Buckmire, R., Couch, M., et al. (2011). Randomised clinical trial: High-dose acid suppression for chronic cough—A double-blind, placebo-controlled study. *Alimentary Pharmacology & Therapeutics, 33*(2), 225–234. doi:10.1111/j.1365-2036.2010.04511.x.

Shaheen, N. J., Denison, H., Bjorck, K., Karlsson, M., & Silberg, D. G. (2013). Efficacy and safety of lesogaberan in gastro-oesophageal reflux disease: A randomised controlled trial. *Gut, 62*(9), 1248–1255. doi:10.1136/gutjnl-2012-302737.

Slattery, J. A., Page, A. J., Dorian, C. L., Brierley, S. M., & Blackshaw, L. A. (2006). Potentiation of mouse vagal afferent mechanosensitivity by ionotropic and metabotropic glutamate receptors. *Journal of Physiology, 577*(Pt 1), 295–306. doi:10.1113/jphysiol.2006.117762.

Smith, J. A., Decalmer, S., Kelsall, A., McGuinness, K., Jones, H., Galloway, S., et al. (2010). Acoustic cough-reflux associations in chronic cough: Potential triggers and mechanisms. *Gastroenterology, 139*(3), 754–762. doi:10.1053/j.gastro.2010.06.050.

Smith, J. A., Hilton, E. C., Saulsberry, L., & Canning, B. J. (2012). Antitussive effects of memantine in guinea pigs. *Chest, 141*(4), 996–1002. doi:10.1378/chest.11-0554.

Spring, P. J., Kok, C., Nicholson, G. A., Ing, A. J., Spies, J. M., Bassett, M. L., et al. (2005). Autosomal dominant hereditary sensory neuropathy with chronic cough and gastro-oesophageal reflux: Clinical features in two families linked to chromosome 3p22-p24. *Brain, 128*(Pt 12), 2797–2810. doi:10.1093/brain/awh653.

Staunton, E., Smid, S. D., Dent, J., & Blackshaw, L. A. (2000). Triggering of transient les relaxations in ferrets: Role of sympathetic pathways and effects of baclofen. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 279*(1), G157–G162.

Tamaoki, J., Graf, P. D., & Nadel, J. A. (1987). Effect of gamma-aminobutyric acid on neurally mediated contraction of guinea pig trachealis smooth muscle. *Journal of Pharmacology and Experimental Therapeutics, 243*(1), 86–90.

Tohda, Y., Ohkawa, K., Kubo, H., Muraki, M., Fukuoka, M., & Nakajima, S. (1998). Role of GABA receptors in the bronchial response: Studies in sensitized guinea-pigs. *Clinical and Experimental Allergy, 28*(6), 772–777.

Tsoukali, E., & Sifrim, D. (2010). The role of weakly acidic reflux in proton pump inhibitor failure, has dust settled? *Journal of Neurogastroenterology and Motility, 16*(3), 258–264. doi:10.5056/jnm.2010.16.3.258.

Undem, B. J., Chuaychoo, B., Lee, M. G., Weinreich, D., Myers, A. C., & Kollarik, M. (2004). Subtypes of vagal afferent C-fibres in guinea-pig lungs. *Journal of Physiology, 556*(Pt 3), 905–917. doi:10.1113/jphysiol.2003.060079.

Vadlamudi, N. B., Hitch, M. C., Dimmitt, R. A., & Thame, K. A. (2013). Baclofen for the treatment of pediatric GERD. *Journal of Pediatrics Gastroenterology and Nutrition, 57*(6), 808–812. doi:10.1097/MPG.0b013e3182a2747b.

van Herwaarden, M. A., Samsom, M., Rydholm, H., & Smout, A. J. (2002). The effect of baclofen on gastro-oesophageal reflux, lower oesophageal sphincter function and reflux symptoms in patients with reflux disease. *Alimentary Pharmacology & Therapeutics, 16*(9), 1655–1662.

Vela, M. F., Tutuian, R., Katz, P. O., & Castell, D. O. (2003). Baclofen decreases acid and non-acid post-prandial gastro-oesophageal reflux measured by combined multichannel intraluminal impedance and pH. *Alimentary Pharmacology & Therapeutics, 17*(2), 243–251.

Wank, M., & Neuhuber, W. L. (2001). Local differences in vagal afferent innervation of the rat esophagus are reflected by neurochemical differences at the level of the sensory ganglia and by different brainstem projections. *Journal of Comparative Neurology, 435*(1), 41–59.

West, P. W., Canning, B. J., Merlo-Pich, E., Woodcock, A. A., & Smith, J. A. (2015). Morphologic characterization of nerves in whole-mount airway biopsies. *American Journal of Respiratory and Critical Care Medicine, 192*(1), 30–39. doi:10.1164/rccm.201412-2293OC.

Woodland, P., & Sifrim, D. (2010). The refluxate: The impact of its magnitude, composition and distribution. *Best Practice & Research Clinical Gastroenterology, 24*(6), 861–871. doi:10.1016/j.bpg.2010.09.002.

Worl, J., & Neuhuber, W. L. (2005). Enteric co-innervation of motor endplates in the esophagus: State of the art ten years after. *Histochemistry and Cell Biology, 123*(2), 117–130. doi:10.1007/s00418-005-0764-7.

Xiang, Y. Y., Wang, S., Liu, M., Hirota, J. A., Li, J., Ju, W., et al. (2007). A GABAergic system in airway epithelium is essential for mucus overproduction in asthma. *Nature Medicine, 13*(7), 862–867. doi:10.1038/nm1604.

Xu, X., Chen, Q., Liang, S., Lu, H., & Qiu, Z. (2012). Successful resolution of refractory chronic cough induced by gastroesophageal reflux with treatment of baclofen. *Cough, 8*(1), 8. doi:10.1186/1745-9974-8-8.

Xu, X., Lv, H., Yu, L., Chen, Q., Liang, S., & Qiu, Z. (2016). A stepwise protocol for the treatment of refractory gastroesophageal reflux-induced chronic cough. *Journal of Thoracic Disease, 8*(1), 178–185. doi:10.3978/j.issn.2072-1439.2016.01.50.

Xu, X. H., Yang, Z. M., Chen, Q., Yu, L., Liang, S. W., Lv, H. J., et al. (2013). Therapeutic efficacy of baclofen in refractory gastroesophageal reflux-induced chronic cough. *World Journal of Gastroenterology, 19*(27), 4386–4392. doi:10.3748/wjg.v19.i27.4386.

Yabumoto, Y., Watanabe, M., Ito, Y., Maemura, K., Otsuki, Y., Nakamura, Y., et al. (2008). Expression of GABAergic system in pulmonary neuroendocrine cells and airway epithelial cells in GAD67-GFP knock-in mice. *Medical Molecular Morphology, 41*(1), 20–27. doi:10.1007/s00795-007-0391-6.

Young, R. L., Page, A. J., Cooper, N. J., Frisby, C. L., & Blackshaw, L. A. (2010). Sensory and motor innervation of the crural diaphragm by the vagus nerves. *Gastroenterology, 138*(3), 1091–1101.e1091–e1095. doi:10.1053/j.gastro.2009.08.053.

Yu, X., Hu, Y., & Yu, S. (2014). Effects of acid on vagal nociceptive afferent subtypes in guinea pig esophagus. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 307*(4), G471–G478. doi:10.1152/ajpgi.00156.2014.

Yu, J., Schultz, H. D., Goodman, J., Coleridge, J. C., Coleridge, H. M., & Davis, B. (1989). Pulmonary rapidly adapting receptors reflexly increase airway secretion in dogs. *Journal of Applied Physiology (1985), 67*(2), 682–687.

Yu, J., Wang, Y. F., & Zhang, J. W. (2003). Structure of slowly adapting pulmonary stretch receptors in the lung periphery. *Journal of Applied Physiology (1985), 95*(1), 385–393. doi:10.1152/japplphysiol.00137.2003.

Zagorodnyuk, V. P., Chen, B. N., & Brookes, S. J. (2001). Intraganglionic laminar endings are mechano-transduction sites of vagal tension receptors in the guinea-pig stomach. *Journal of Physiology, 534*(Pt 1), 255–268.

Zagorodnyuk, V. P., D'Antona, G., Brookes, S. J., & Costa, M. (2002). Functional GABAB receptors are present in guinea pig nodose ganglion cell bodies but not in peripheral mechanosensitive endings. *Autonomic Neuroscience, 102*(1-2), 20–29.

Zerbib, F., Bruley des Varannes, S., Simon, M., & Galmiche, J. P. (2012). Functional heartburn: Definition and management strategies. *Current Gastroenterology Reports, 14*(3), 181–188. doi:10.1007/s11894-012-0255-7.

Zhang, Q., Lehmann, A., Rigda, R., Dent, J., & Holloway, R. H. (2002). Control of transient lower oesophageal sphincter relaxations and reflux by the GABA(B) agonist baclofen in patients with gastro-oesophageal reflux disease. *Gut, 50*(1), 19–24.

Ziora, D., Jarosz, W., Dzielicki, J., Ciekalski, J., Krzywiecki, A., Dworniczak, S., et al. (2005). Citric acid cough threshold in patients with gastroesophageal reflux disease rises after laparoscopic fundoplication. *Chest, 128*(4), 2458–2464. doi:10.1378/chest.128.4.2458.

Chapter 17
Baclofen: Therapeutic Use and Potential of the Prototypic $GABA_B$ Receptor Agonist

Norman G. Bowery

Abstract Baclofen (β-chlorophenyl GABA) is a stereospecific agonist at GABA type B ($GABA_B$) receptors and is inactive at $GABA_A$ receptors. It has therefore been employed as a marker for $GABA_B$ sites. Its selectivity for this receptor provides a unique pharmacology which is covered in this chapter. Numerous effects have been reported but currently only its anti-spasticity and analgesic actions are utilized in clinical medicine. It is the preferred treatment in spasticity of different origins. Its potential for use in other conditions, for example, in reducing drug addiction, in the treatment of gastroesophageal reflux disease, chronic cough and hiccup is very strong. However, as it is a directly acting receptor agonist its action is likely to be diminished by desensitization. Moreover, its access to the CNS is limited requiring the administration of high doses. This increases the chance of unwanted side effects. To overcome this in spasticity, the administration of lower doses into the intrathecal space through an indwelling cannula has had a major influence on the acceptance of the drug by patients. In other conditions, an alternative approach is required and the possible solution may be the use of positive allosteric modulators of the $GABA_B$ receptor. This could reduce receptor desensitization and improve access to the site of action.

Keywords Baclofen • Spasticity • Analgesia • Epilepsies • Antidepressant • Cognition • Addiction • Hiccup • Cough • Gastroesophageal reflux disease

β-Chlorophenyl GABA (Baclofen) was first introduced into clinical medicine in 1972 after evidence was obtained showing it had a muscle relaxant effect in an animal model (Bein 1972). It was designed to be a GABA-mimetic, which could cross the blood–brain barrier through increased lipophilicity (Keberle and Faigle 1968). Whilst it is able to gain access to the brain after peripheral administration, this is not due to passive diffusion but instead it appears to enter via a neutral amino acid transporter (van Bree et al. 1988, 1991). Although baclofen was designed to mimic the

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action of γ-aminobutyric acid (GABA), there was no evidence, when it was first used clinically, that it acted in this manner. Numerous in vitro studies failed to show any activity at the GABA receptor (Curtis et al. 1974; Davies and Watkins 1974). Only some 10–5 years later did evidence emerge that supported an interaction with a GABA receptor that is not associated with chloride ion channels. It was this selective effect at a metabotropic GABA receptor that provided, in part, the basis for the existence of a novel receptor, which we designated GABA type B '$GABA_B$' receptor (Hill and Bowery 1981).

Activation of this receptor in brain tissue can produce neuronal hyperpolarization or a decrease in evoked neurotransmitter release. The former effect is mediated by K^+ conductance predominantly via postsynaptic receptors (Luscher et al. 1997) whilst the latter effect is due to an action on presynaptic terminals mediated via a decrease in membrane Ca^{2+} conductance (Dunlap 1981; Doze et al. 1995; Isaacson 1998; Wu and Saggau 1995). Both of these mechanisms are coupled to G-proteins that are members of the pertussis toxin-sensitive family $G_{io}/G_{o\alpha}$ (Odagaki and Koyama 2001; Odagaki et al. 2000) although some presynaptically mediated events, associated with reduced Ca^{2+} conductance, appear to be insensitive to pertussis toxin (Harrison et al. 1990).

Multiple types of K^+ channels seem to be associated postsynaptic $GABA_B$ receptors (Wagner and Deakin 1993) whilst the predominant calcium channel linked to $GABA_B$ sites appears to be the 'N' type although 'P' and 'Q' type channels have also been implicated (Santos et al. 1995; Lambert and Wilson 1996; Barral et al. 2000). $GABA_B$ sites are also associated with adenylyl cyclase, which is normally inhibited by receptor activation (Xu and Wojcik 1986) although when the enzyme is activated by a G_s-coupled receptor agonist such as isoprenaline, $GABA_B$ receptor activation increases the formation of cyclic adenosine monophosphate (cAMP) above the level produced by isoprenaline alone (Karbon et al. 1984; Hill 1985).

Throughout these studies baclofen (and GABA) has been used as the $GABA_B$ receptor agonist.

17.1 Actions of Baclofen

The actions of baclofen in mammals are not confined to the brain which is hardly surprising given that $GABA_B$ receptors have been shown to be present in many peripheral tissues (Giotti et al. 1990; Erdo and Bowery 1986). The receptors are not even confined to nervous tissue (Erdo and Bowery 1986), but the majority of receptor activation stems from an action on neural systems. $GABA_B$ receptors are widely distributed throughout the mammalian brain although there are regional variations. Whilst there are high receptor densities in the interpeduncular nucleus, dorsal horn of the spinal cord, the thalamic nuclei, cerebellar molecular layer and cerebral cortex, the densities in other brain regions are much lower (Bowery et al. 1987; Chu et al. 1990). Unfortunately, the receptor density does not necessarily reflect the physiological importance of the receptor in all brain regions. For example, in the hippocampus the

Table 17.1 Site of action and potential therapeutic application of baclofen and $GABA_B$ receptor activation

Effect	Site of action	Clinical
Muscle relaxation	Spinal cord reflexes	Spasticity
Antinociception	Spinal cord sensory fibres	Analgesia
	Thalamus	
Antitussive	Cough centre in medulla	Cough suppression
Suppression of drug addiction	Mesolimbic system	Drug abuse
Oesophageal sphincter relaxation	Intestine	Gastroesophageal reflux disease
Smooth muscle relaxation	Lung	Asthma
Food intake modified	Higher centres	Enhance feeding
Insulin/glucagon release	Pancreas	Diabetes
Adenylyl cyclase inhibition	Pancreas	Adenocarcinoma
Reduction in fat intake	Higher centres	Binge eating
Enhancement of neutrophil chemotaxis	Leucocytes	Inflammation
Suppression of pain behaviour	Dorsal periaqueductal grey	Anxiety/panic disorder

overall receptor density, as assessed by receptor autoradiography, appears to relatively low although there is variation within the structure. Many studies have been conducted using hippocampal tissue, and this structure provided the initial evidence for a physiological role of the $GABA_B$ receptor (Dutar and Nicoll 1988; Nicoll 2004).

Table 17.1 summarizes the effects of baclofen throughout the mammalian system with the sites of action implicated in each case. We can consider each locus in turn examining what effect(s) has been observed and how this may relate to any clinical potential.

17.2 Spasticity

Numerous studies have been performed on the action of baclofen in the spinal cord. In initial studies using GABA in vivo in anaesthetized animals, it was shown that it depresses all types of spinal neurons and could inhibit monosynaptic and polysynaptic reflex activity (Curtis and Watkins 1965). In a preliminary report using baclofen as a GABA derivative, Birkmayer et al. (1967) were able to show control of spasticity after spinal cord lesions. Subsequent clinical studies supported this finding in a placebo-controlled trial in 6 patients (Jones et al. 1970) and in a double-blind trial in 23 patients (Hudgson and Weightman 1971). Baclofen rapidly became the drug of choice in spasticity in patients with hemi- and tetraplegia (Brogden et al. 1974), in multiple sclerosis (Giesser 1985; Brar et al. 1991; Smith et al. 1991) in cerebral palsy (Buonaguro et al. 2005; Scheinberg et al. 2006; Krach 2009; Navarrete-Opazo et al. 2016) in stiff-man syndrome (Whelan 1980; Silbert et al. 1995; Stayer et al. 1997) tetanus (Mueller et al.

Table 17.2 Possible unwanted side effects of baclofen after oral administration

Nausea
Drowsiness
Dizziness
Hypotension
Seizures
Muscle weakness
Hallucinations
Mental disturbance

1987; Demaziere et al. 1991; Engrand et al. 1999; Santos et al. 2004) and after stroke (O'Brien et al. 1996). The underlying effect of baclofen in these conditions is a reduction in spinal reflex mechanisms, but how this arose was not established. No evidence for an action at GABA receptors was obtained. Although baclofen had a beneficial effect in spasticity, it has many unwanted side effects (Table 17.2) primarily because it had to be administered orally in large doses due to poor penetration into the brain. In 1984, Penn and Kroin (1984, 1985) made an important discovery that baclofen could be administered intrathecally using an indwelling mini-pump. This enabled much smaller doses to be given as Leisen et al. (2003) demonstrated; an intrathecal dose of 600 μg baclofen produced a local plasma level of 5–20 ng/g whereas 100 mg baclofen given orally produced a level of <3 ng/g in cerebrospinal fluid (CSF). Numerous studies have been made since the Penn and Kroin (1984) original report and in all cases positive effects have emerged following intrathecal application (Ochs 1993; Becker et al. 1997; Gianino et al. 1998; Rawlins 1998; Sasaki and Ogiwara 2016; Heetla et al. 2016; Bonouvrie et al. 2016; Okazaki et al. 2016; Al-Kadalry et al. 2015; Naito 2014; Khurana and Gang 2014; Mathur et al. 2014).

17.3 Analgesia

Baclofen has been shown to be anti-nociceptive in animal models of acute pain. For example, in the rat tail flick and hot plate tests at doses lower than required to produce muscle relaxation (Wilson and Yaksh 1978; Aley and Kulkarni 1991). Visceral pain-related responses to colorectal distension in rats are also inhibited by baclofen (Brusberg et al. 2009). An analgesic effect of baclofen in clinical medicine has been noted in cluster headache, migraine and trigeminal neuralgia (Hering-Hanit and Gadoth 2000; Hering-Hanit 1999; Fromm et al. 1980, 1984; Fromm and Terrence 1987). However, following its systemic administration rapid tolerance to the effects of baclofen occurs, and this limits its use as an analgesic.

The sites of action of baclofen responsible for analgesia are within the spinal cord and thalamus. Systemic, intrathecal or injection into discrete regions of the thalamus can produce analgesia in acute pain models (Cutting and Jordan 1975; Sawynok and LaBella 1982; Vaught et al. 1985). Whilst spinal transection reduces the anti-nociceptive effect

of baclofen (Proudfit and Levy 1978), there is evidence for a distinct contribution from a reduction in primary afferent transmitter release within the spinal cord. $GABA_B$ receptor activation in spinal cord slices prevents the release of Substance P, glutamate and calcitonin gene-related peptide (CGRP) evoked by electrical stimulation of the dorsal roots (Malcangio and Bowery 1993, 1996; Teoh et al. 1996). All three compounds are believed to contribute to the transmission of nociceptive impulses in the spinal cord. If a $GABA_B$ receptor antagonist is administered to the isolated spinal cord preparation, there is no increase in transmitter release. However, if chronic inflammation is produced by in vivo administration of Freunds adjuvant this produces an increase of around 25% in the concentration of GABA in the dorsal horn (Castro-Lopes et al. 1992). This facilitates the $GABA_B$ receptor control of Substance P release. When a $GABA_B$ receptor antagonist is applied to the isolated cord prepared from adjuvant-treated rat, the evoked release of Substance P is dramatically increased (Malcangio and Bowery 1994). Furthermore, if the GABA antagonist is administered to the intact adjuvant-treated rat, there is a striking increase in nociception. This contrasts with the lack of effect of the antagonist in naïve animals. This would suggest that during chronic inflammation there is an increase in $GABA_B$ innervation to primary afferent terminals that acts as a pathological anti-nociceptive control to oppose the enhanced sensory input that occurs.

Administration of baclofen into the thalamic ventrobasal complex contralateral to the inflamed joint in monoarthritic rats can attenuate allodynia in the ankle-bend test (Potes et al. 2006) supporting a higher centre role in nociception. However, evidence for a spinal role is also strong. $GABA_B$ receptor expression is required for inhibition of N-type (Cav 2.2) calcium channels by α-conotoxins in rat models of neuropathic pain. Conotoxins are anti-nociceptive. Knockdown of $GABA_B$ receptors in rat-isolated dorsal root ganglia using RNA interference produced a significant reduction in the inhibition of N-type calcium channels produced by baclofen. This would indicate that $GABA_B$ receptor activation must occur to allow the modulation of N-type calcium channels and consequent analgesia by α-conotoxins (Cuny et al. 2012; Huynh et al. 2015)

Further support for the involvement of $GABA_B$ receptors in pain mechanisms comes from studies with 'knock-out' mice (Schuler et al. 2001) in which the subunits $GABA_{B1}$ or $GABA_{B2}$ are not formed. In either case, functional $GABA_B$ receptors are not produced and hyperalgesia occurs indicating that heteromeric receptors are required to maintain nociceptive thresholds.

As indicated earlier, the potential for baclofen as a clinical analgesic is limited by rapid tolerance and the adverse effects which can develop after systemic administration (Table 17.2). The introduction of positive allosteric modulators of the $GABA_B$ receptor has provided an alternative approach to reduce both tolerance and unwanted side effects.

Many examples of $GABA_B$ positive allosteric modulators have been described (see Chap. 18 of this volume) and tested in vivo in a variety of animal models for anti-nociceptive activity. For example, ADX 71943 reduced the pain-associated behaviours in the acetic acid writhing and formalin tests in mice and the $GABA_B$ antagonist, CGP63360, blocked this effect. The compound was inactive in the marble burying and elevated plus maze tests for anxiolytic or anxiogenic activity (Kalinichev et al. 2014). Baclofen produces anti-nociceptive effects in animal models of visceral pain including mechanically

Table 17.3 Comparison of effects of baclofen and positive allosteric modulators of $GABA_B$ receptors

Effect	Baclofen	Positive allosteric modulator
Tolerance	Yes[a]	No[b]
Anti-nociception	Yes[c]	Yes[d]
Body temperature	Decrease[e]	No effect[f]
Gastroesophageal disease	Reduction[g]	?
Systemic blood pressure	Decrease (in hypertension)[h]	?
Sedation	Yes[i]	No[f]
Myorelaxation (locomotor activity↓)	Yes[j]	No[f]
Cognition	Decrease[k]	?
Food intake	Increase[l]	Increase[l]
	Decrease[m]	Decrease[m]
Anticonvulsant	Yes[n]	Yes[o]
Overactive bladder	Reduces[p]	Reduces[q]
Anxiety	Anxiogenic/variable[r]	Anxiolytic[f]
Drug addiction (nicotine, cocaine, opiates, alcohol)	Decrease[s]	Decrease[b]

Table 17.3 References (numbers after each effect refer to reference list below):
[a]Gjoni T, Urwyler S (2008) Neuropharmacology 55: 1293–1299
[b]Ong J, Kerr DI (2005) CNS Drug Rev 11: 317–334
[c]Brusberg M, Raynefjord A, Martinsson R et al. (2009) Neuropharmacology 56: 362–367
[d]Kalinichev M, Donovan-Rodriguez T, Girard F et al. (2014) Br J Pharmacol 171: 4941–4954
[e]Queva C, Bremner-Danielsen M, Edlund A et al. (2003) Br J Pharmacol 140: 315–322
[f]Cryan JF, Kelly PH, Chaperon F et al. (2004) J Pharmacol Exp Ther 310: 952–963
[g]Miner PB jr, Silberg DG, Ruth M et al. (2014) Gastroentorology 14: 188
[h]Li DP, Pan HL (2010) Adv Pharmacol 58: 257–286
[i]From A, Heltberg A (1975) Acta Neurol Scand 51: 158–166
[j]Stefanski RI, Plaznik A, Palejko W, Kostowski WJ (1990) J Neural Transm Park Dis Dement Sect 2(3): 179–191
[k]Stackman RJ, Walsh TJ (1994) Behav Neural Biol 61: 181–185
[l]Ebenezer IS (2014) Eur J Pharmacol 690: 115–118
[m]Perdona E, Costantini VJ, Tessari M et al. (2011) Neuropharmacology 61: 957–966
[n]Brown JW, Moeller A, Schmidt M et al. (2016) Neuropharmacology 101: 358–367
[o]Mares P, Ticha K, Mikulecki A (2013) Epilepsy Behav 28: 113–120
[p]Frost F, Nanninga J, Penn R et al. (1989). Am J Phys Med Rehabil 68: 112–115
[q]Kalinichev M, Palea S, Haddouk H, et al. (2014) Br J Pharmacol 171: 995–1006
[r]Cryan JF, Slattery DA (2010) Adv Pharmacol 58: 427–451
[s]Viachou S, Markov A (2010) Adv Pharmacol 58: 315–371

induced visceral pain. CGP7930, a positive allosteric modulator (Adams and Lawrence 2007), is also anti-nociceptive in such a model (Brusberg et al. 2009) and rac-BHFF, another positive allosteric modulator, enhances baclofen-mediated anti-nociception in neuropathic mice (Zemoura et al. 2016). This would suggest that positive allosteric modulators may provide a better approach to analgesia having less unwanted side effects. Positive allosteric modulators appear to have an improved profile compared to baclofen or other direct acting agonists (Table 17.3).

17.4 Epilepsy: Convulsant Seizures

GABA receptors have long been associated with the control of epileptic seizures. Deficits in GABA type A ($GABA_A$) receptor function within the central nervous system (CNS) provide an underlying mechanism for the production of seizures (Gale 1992; Olsen et al. 1992) and positive allosteric modulation of $GABA_A$ receptors with benzodiazepines can reduce seizure activity (Upton and Blackburn 1997). However, $GABA_B$ receptors have also been implicated (Gamardella et al. 2003; Pacey et al. 2009, 2011; Vienne et al. 2010). 'Knock-out' mice lacking functional $GABA_B$ receptors exhibit both spontaneous and audiogenic-induced seizures (Prosser et al. 2001; Schuler et al. 2001; Vienne et al. 2010), and baclofen is known to produce anticonvulsant effects in the DBA/2J mouse audiogenic seizure test (Meldrum and Horton 1980; Brown et al. 2016). However, baclofen appears to augment some seizures whilst inhibiting others. For example, it has been shown to increase the incidence of seizures evoked by pentylenetetrazole without increasing seizures due to local injections of excitatory amino acids (Snodgrass 1992).

In temporal lobe epilepsy, impairment of $GABA_B$ receptor function has been noted in cerebrocortical slices obtained from patients undergoing surgery for drug-resistant epilepsy (Teichgraber et al. 2009) suggesting that $GABA_B$ receptor activation could be important in seizure suppression.

17.5 Epilepsy: Absence Seizures

The characteristic electroencephalography (EEG) activity of a 3-Hz spike and wave, which is manifest during typical absence seizures, appears to stem from discharges in the thalamic nuclei. But this is probably not the site of origin of these discharges. Meeren et al. (2005) have demonstrated unequivocally in a genetic rat model of absence epilepsy (Wistar Albino Glaxo/Rij-rat, WAG/Rij) that the origin is outside the thalamus in the perioral region of the somatosensory cortex. These discharges spread across the cortex and initiate a corticothalamic cascade.

Inaba et al.(2009) examined the effects induced by baclofen (0.1–10 μM) on the inhibitory events recorded in vitro from neocortical slices obtained from these epileptic WAG/Rij (>180 day-old) and from age-matched, non-epileptic control (NEC) rats. They found that higher doses of baclofen were required to depress pharmacologically isolated, stimulus-induced inhibitory postsynaptic potentials (IPSPs) generated in WAG/Rij neurons as compared to those in NEC neurones. These authors suggest that this indicates a decreased function of presynaptic $GABA_B$ receptors in the WAG/Rij rat neocortex.

In another rat model of absence seizures (genetic absence epilepsy rat of Strasbourg, GAERS), when baclofen is injected into the ventrobasal thalamus or reticular nucleus this exacerbates the seizures (Manning et al. 2003). Conversely, if a $GABA_B$ antagonist is injected into the same brain regions this suppresses the seizures. These studies in a rat

model may implicate the $GABA_B$ receptor in the generation or modulation of absence seizures but certainly indicates that baclofen might exacerbate seizures clinically and, therefore, would be contraindicated in patients with absence epilepsy.

Another model, this time in mice, DBA/2J, which are prone to audiogenic seizures (see above) also provides an in vivo system for studying spontaneous absence seizures. Baclofen, administered i.p. (0.5–10 mg/kg), dose-dependently increased the number of spike and wave discharge episodes in this model. This increase was also reversed by the $GABA_B$ antagonist, SCH50911 (50 mg/kg i.p.) (Bortolato et al. 2010).

Atypical absence epilepsy is another distinct form of absence in which the seizures differ markedly from those in typical absence seizures notably in the EEG pattern. A model of this disorder can be produced in rats treated with the cholesterol synthesis inhibitor, AY-9944 (Cortez et al. 2001). In this animal model, the spike and wave discharges are significantly prolonged by baclofen and abolished by the $GABA_B$ antagonist, CGP 35348 (Cortez et al. 2001). Over expression of the $GABA_B$ receptor 1a subtype in transgenic mice also appears to produce atypical absence seizures which are exacerbated by baclofen (Wu et al. 2007).

Taken together these data support a role for $GABA_B$ receptors in the aetiology of both typical and atypical absence epilepsy.

17.6 Affective Disorders

Lloyd et al. (1985) and Lloyd (1989) were the first to report that a variety of antidepressant drugs, e.g. fluoxetine, citalopram and amitriptyline given chronically (6–18 days) in rats, produces a significant upregulation in $GABA_B$ binding sites in the frontal cortex. By contrast neuroleptics, psychostimulants and anxiolytics had no effect (Lloyd 1989). From the data produced by Lloyd (1989) there would appear to be a close connection between $GABA_B$ receptor mechanisms and cerebral beta-adrenoceptors. Repeated administration of baclofen to rats produces a down-regulation in cerebral adrenoceptors in a manner similar to chronic treatment with nomifensine or imipramine (Suzdak and Gianutsos 1986). Although these observations were disputed (Cross and Horton 1987, 1988), there is now good evidence that $GABA_B$ mechanisms are associated with depression. Baclofen administered to rats undergoing the forced swim test and the learned helplessness test, which are animal models for determining the action of antidepressant drugs, attenuates the action of recognized antidepressant drugs such as mianserin and desipramine (Nagakawa et al. 1996; see Chap. 12 of this volume). However, baclofen given alone had no direct effect in these models whereas $GABA_B$ receptor antagonists administered to mice in the forced swim test produced an antidepressant-like effect (Mombereau et al. 2004). Knock-out mice lacking $GABA_{B1}$ or $GABA_{B2}$ receptor subunits exhibit antidepressant behaviour and anxiety (Mombereau et al. 2004). So we might conclude that a reduction in $GABA_B$

receptor function produces antidepressant-like behaviour whilst an increase in receptor activation produces a depressant effect. But baclofen does not fit into this category; it merely appears to attenuate the effects of antidepressant drugs.

Very little is known about the potential clinical significance of these observations, as there is a paucity of clinical data. However, Post et al. (1991) found in 3 out of 5 patients with depression that baclofen exacerbated their symptoms.

In a study by Keegan et al. (1983), a patient with pre-existing bipolar affective disorder developed increased depression whilst on baclofen. This led the authors to conclude that baclofen should be used with caution in patients with neuropsychiatric problems.

17.7 Cognitive Behaviour

Baclofen and other $GABA_B$ receptor agonists suppress cognitive behaviour in animals (Carletti et al. 1993; Stackman and Walsh 1994; De Sousa et al. 1994; Pitsikas et al. 2003), and this effect is reversed by $GABA_B$ receptor antagonism (Pitsikas et al. 2003; Liu et al. 2014). Baclofen has also been shown to contribute to different varieties of amnesia in the human brain (Zerman et al. 2016). By contrast, impairment of recognition memory induced in mice by methamphetamine (1 mg/day for 7 days) was ameliorated by baclofen but not by the $GABA_A$ receptor agonist, gaboxadol (Arai et al. 2009). Similarly, in a study performed in Macaca mulatta primates in which cocaine (0.2–0.6 mg/kg iv) induced a cognitive decline in a delayed match to sample task, baclofen, co-administered with cocaine, reversed the task performance back to nondrug (saline iv) control levels (Porrino et al. 2013). Baclofen has also been shown to ameliorate spatial memory impairment induced by chronic cerebral hypoperfusion in rats (Luo et al. 2016). These effects of baclofen seem contrary to previous reports but still implicate $GABA_B$ mechanisms in cognitive function.

Improvement in cognitive behaviour by $GABA_B$ receptor antagonists is well established in a variety of animal models such as in an active and passive avoidance tests in rats and mice (Getova and Bowery 1998; Getova and Dimitrova 2007) and age-related learning in Fischer rats (Lasarge et al. 2009). Intrahippocampal administration of the $GABA_B$ antagonist, 2-hydroxy saclofen (20 μM) markedly reversed the scopolamine-induced impairment in behavioural long-term potentiation (LTP) and maze performance in rats indicating that blockade of the $GABA_B$ receptor displays a facilitatory role in the induction of behavioural LTP and a maze learning task (Liu et al. 2014).

Pentylenetetrazole-kindling-provoked amnesia in rats is also prevented by $GABA_B$ antagonists such as CGP36742 (Genkova-Papazova et al. 2000)

The potential for $GABA_B$ receptor antagonists as cognition enhancers is covered in Chap. 19 of this volume, but there is already evidence of limited clinical effects with the antagonist SGS742 [also known as CGP36742] (Froestl et al. 2004).

17.8 Drug Addiction

A major goal in clinical therapeutics is the successful treatment of drug dependence, and many targets are under consideration. This topic is covered in Chaps. 14 and 15 of this volume, but it seems appropriate to consider baclofen here as it was the first $GABA_B$-related compound to be shown to reduce the reinforcing effects of cocaine in rats (Roberts and Andrews 1997). However, it soon became clear that the addictive behaviour in animals associated with morphine-like substances, nicotine and alcohol could also be attenuated by baclofen and other $GABA_B$ agonists (Xi and Stein 1999; Corrigall et al. 2000; Addolorato et al. 2000, 2002; Cousins et al. 2002; Lorrai et al. 2016). Clinical evidence has been obtained for baclofen reducing alcohol craving in alcoholics (Imbert et al. 2015; Rolland et al. 2015; Chaignot et al. 2015; Lesouef et al. 2014) and reducing the craving for nicotine evidenced by a reduction in the number of cigarettes smoked per day (Colombo et al. 2004; Franklin et al. 2009). Whilst the attenuation of craving for cocaine by baclofen in animals is well established (e.g. Ling et al. 1998; Shoptaw et al. 2003; Haney et al. 2006), the effects in the clinic are not so clear. In a multi-site double-blind study, treatment of severe cocaine-dependent patients with a single dose of baclofen (60 mg/day) for 8 weeks did not show any significant difference from addicts treated with placebo. It was suggested that a higher dose should be tested (Kahn et al. 2009).

One possible area for clinical efficacy is in the use of baclofen for opiate withdrawal (Akhondzadeh et al. 2000), but overall more evidence is required to substantiate the clinical use of baclofen in drug addiction, in part, because of the unwanted side effects of the compound. The advent of positive allosteric modulators of $GABA_B$ sites may provide the answer as preliminary reports seem to suggest (Agabio and Colombo 2014, 2015; Maccioni et al. 2015) and see Chaps. 14, 15, and 18 of this volume.

17.9 Gastroesophageal Reflux Disease

Gastroesophageal reflux disease (GERD) is a disease that affects about 1 in 5 of the population in the western world (Dent et al. 2005) and manifests as heartburn and regurgitation in patients but can give rise to oesophagitis (Wiklund 2004; Vakil et al. 2006). Baclofen can relieve these symptoms, and this was first noted as an unexpected observation in patients treated for spasticity (e.g. Cange et al. (2002). Whilst antacid treatment can help in many cases, persistent symptoms often occur. The $GABA_B$ receptor in the intestine appears to provide a target for inhibiting GERD. Baclofen and other $GABA_B$ receptor agonists inhibit transient lower oesophageal sphincter relaxation, which occurs after a meal and this inhibition appears to be the basis for its beneficial action (Lehmann et al. 1999; see also Chap. 16 of this book)). However, the side effects of baclofen, which result from its access to the brain, would indicate the need for an agent with restricted access. Lehmann

et al. (2009) have described the agonist lesogaberan that has good selectivity for the $GABA_B$ receptor over the $GABA_A$ site and higher affinity than baclofen for $GABA_B$ receptors. This has led to its introduction into clinical trials for GERD (Boeckxstaens et al. 2009) but Astrazeneca have since discontinued these trials although Miner et al. reported a successful randomized placebo-controlled study in 2014.

17.10 Other Actions of Baclofen

Many effects produced by baclofen have been reported since its clinical introduction in 1972. A number of these may be of importance when unwanted side effects are avoided, for example, if and when positive allosteric modulators are pursued clinically.

Baclofen is an antitussive agent and has been shown to be effective in the treatment of chronic refractory cough after systemic administration (Dicpinigaitis and Dobkin 1997; Dicpinigaitis and Rauf 1998; Xu et al. 2013; Chung 2015; see also Chap. 16 of this book). Another interesting action is the suppression of chronic hiccup. In patients with hiccup, over a prolonged period of years baclofen has been shown to stop the hiccup in at least 50 % of them. The mechanism underlying this effect is unclear but may stem from rectifying gastroesophageal abnormalities (Guelaud et al. 1995; Twycross 2003; Turkyilmaz and Eroglu 2008; Steger et al. 2015; Sharma 2015; Zhang et al. 2014; Thompson et al. 2014; Baumann et al. 2014).

Inhibition of vagally and histamine-induced bronchoconstriction by baclofen was first shown by Chapman and colleagues who reported that $GABA_B$ receptor activation in guinea pigs could oppose the bronchoconstriction (for review, see Chapman et al. 1993) Initial suggestions were that baclofen or an analogue might be useful in the clinical treatment of asthma. Although the action of baclofen against histamine was pursued clinically in patients with cervical spinal cord injury, nothing more has emerged with reference to asthma (Grimm et al. 1997). The underlying mechanism for this effect may well be the suppression of Substance P release from nerve terminal in the bronchi.

A major problem in patients with spinal cord lesions is unstable bladder function. Baclofen produces a marked improvement in such cases, which is a beneficial side effect of treatment for spasticity (Taylor and Bates 1979; Nanninga et al. 1989).

17.11 Conclusions

After more than 40 years since its introduction to clinical medicine, baclofen remains the drug of choice in the treatment of spastic disorders. It is by no means the perfect drug producing many unwanted side effects, and therefore improvements are needed. The introduction of positive allosteric modulators of the $GABA_B$ receptor may provide the answer, but their clinical use has yet to emerge. But baclofen has been an extremely

useful compound providing a tool to define the $GABA_B$ site. It was introduced as a muscle relaxant in man before its mechanism of action had been obtained. Only subsequently was its site of action defined (see Chap. 1 of this volume) and since then a multitude of effects, wanted and unwanted, have been reported. The synthesis of analogues has produced more selective agonists such as lesogaberan (Lehmann et al. 2009), which does not cross the blood–brain barrier so well and thus limits its actions to the periphery. However, one important introduction for the application of baclofen to the brain was its administration via the intrathecal route. This meant that much less drug is required which had an enormous influence on the incidence of unwanted side effects. One wonders from where the next improvement will stem.

References

Adams, C. L., & Lawrence, A. J. (2007). CGP7930: A positive allosteric modulator of the $GABA_B$ receptor. *CNS Drug Reviews, 13*, 308–316.

Addolorato, G., Caputo, F., Capristo, E., Colombo, G., Gessa, G. L., & Gasbarrini, G. (2000). Ability of baclofen in reducing alcohol craving and intake: II—Preliminary clinical evidence. *Alcoholism, Clinical and Experimental Research, 24*, 67–71.

Addolorato, G., Caputo, F., Capristo, E., Domenicali, M., Bernardi, M., Janiri, L., et al. (2002). Baclofen efficacy in reducing alcohol craving and intake: A preliminary double-blind randomized controlled study. *Alcohol and Alcoholism, 37*, 504–508.

Agabio, R., & Colombo, G. (2014). $GABA_B$ receptor ligands for the treatment of alcohol use disorder: Preclinical and clinical evidence. *Frontiers in Neuroscience, 8*, 140.

Agabio, R., & Colombo, G. (2015). GABAB receptor as therapeutic target for drug addiction: From baclofen to allosteric modulators. *Psychiatria Polska, 49*, 215–223.

Akhondzadeh, S., Ahmadi-Abhari, S. A., Assadi, S. M., Shabestari, O. L., Kashani, A. R., & Farzanehgan, Z. M. (2000). Double-blind randomized controlled trial of baclofen vs clonidine in the treatment of opiates withdrawal. *Journal of Clinical Pharmacy and Therapeutics, 25*, 347–353.

Aley, K. O., & Kulkarni, S. K. (1991). Baclofen analgesia in mice: A $GABA_B$-mediated response. *Methods and Findings in Experimental and Clinical Pharmacology, 13*, 681–686.

Al-Kadalry, A. T., Wicky, G., Nicolo, D., & Vuadens, P. (2015). Influence of intrathecal baclofen on the level of consciousness and mental functions after extremely severe traumatic brain injury: Brief report. *Brain Injury, 29*, 527–532.

Arai, S., Takuma, K., Mizoguchi, H., Ibi, D., Nagai, T., Kamei, H., et al. (2009). $GABA_B$ receptor agonist baclofen improves methamphetamine-induced cognitive deficit in mice. *European Journal of Pharmacology, 602*, 101–104.

Barral, J., Toro, S., Galarraga, E., & Bargas, J. (2000). GABAergic presynaptic inhibition of rat neostriatal afferents is mediated by Q-type Ca^{2+} channels. *Neuroscience Letters, 283*, 33–36.

Baumann, A., Weicker, T., Alb, I., & Audibert, G. (2014). Baclofen for the treatment of hiccup related to brainstem compression. *Annales Françaises d'Anesthèsie et de Rèanimation, 33*, 27–28.

Becker, R., Alberti, O., & Bauer, B. L. (1997). Continuous intrathecal baclofen infusion in severe spasticity after traumatic or hypoxic brain injury. *Journal of Neurology, 244*, 160–166.

Bein, H. J. (1972). Pharmacological differentiation of muscle relaxants. In W. Birkmayer (Ed.), *Spasticity—A topical survey* (pp. 76–82). Vienna: Huber.

Birkmayer, W., Danielczyk, W., & Weller, G. (1967). On the objectivization of the myotonolitic effect of an aminobutyric acid derivative (Ciba 34647-Ba). *Wiener Medizinische Wochenschrift (1946), 117*, 7–9.

Boeckxstaens, G., Denison, H., Ruth, M., Adler, J., Silberg, D., & Sifrim, D. (2009). Effect of AZD3355, a novel $GABA_B$ agonist, on reflux and lower esophageal sphincter function in patients with GERD with symptoms despite proton pump inhibitor treatment. *Gastroenterology, 136*(Suppl. 1), M1861.

Bonouvrie, L., Becher, J., Soudant, D., Buizer, A., van Ouwerkerk, W., Vles, G., et al. (2016). The effect of intrathecal baclofen treatment on activities of daily life in children and young adults with cerebral palsy and progressive neurological disorders. *European Journal of Paediatric Neurology, 20*(4), 538–544. doi:10.1016/ejpn.2016.02.013.

Bortolato, M., Frau, R., Orrù, M., Fà, M., Dessì, C., Puligheddu, M., et al. (2010). $GABA_B$ receptor activation exacerbates spontaneous spike-and-wave discharges in DBA/2J mice. *Seizure, 19*, 226–231.

Bowery, N. G., Price, G. P., & Hudson, A. L. (1987). $GABA_A$ and $GABA_B$ receptor site distribution in rat central nervous system. *Neuroscience, 20*, 365–385.

Brar, S. P., Smith, M. B., Nelson, I. M., Franklin, G. M., & Cobble, N. D. (1991). Evaluation of treatment protocols on minimal to moderate spasticity in multiple sclerosis. *Archives of Physical Medicine and Rehabilitation, 72*, 186–189.

Brogden, R. N., Speight, T. M., & Avery, G. S. (1974). Baclofen: A preliminary report of its pharmacological properties and therapeutic efficacy in spasticity. *Drugs, 8*, 1–14.

Brown, J. W., Moeller, A., Schmidt, M., Turner, S. C., Nimmrich, V., Ma, J., et al. (2016). Anticonvulsant effects of structurally diverse $GABA_B$ positive allosteric modulators in the DBA/2J audiogenic seizure test: Comparison to baclofen and utility as a pharmacodynamics screening model. *Neuropharmacology, 101*, 358–369.

Brusberg, M., Ravenefjord, A., Martinsson, R., Larsson, H., Martinez, V., & Lindström, E. (2009). The GABA(B) receptor agonist, baclofen, and the positive allosteric modulator, CGP7930, inhibit visceral pain-related responses to colorectal distension in rats. *Neuropharmacology, 56*, 362–367.

Buonaguro, V., Scelsa, B., Curci, D., Monforte, S., Iuorno, T., & Motta, F. (2005). Epilepsy and intrathecal baclofen therapy in children with cerebral palsy. *Pediatric Neurology, 33*, 110–113.

Cange, L., Johnston, E., Rydholm, H., Lehmann, A., Finizia, C., Lundell, L., et al. (2002). Baclofen-mediated gastro-oesophageal acid reflux control in patients with established reflux disease. *Alimentary Pharmacology and Therapeutics, 16*, 869–873.

Carletti, R., Libri, V., & Bowery, N. G. (1993). The $GABA_B$antagonist CGP36742 enhances spatial learning performance and antagonizes baclofen-induced amnesia in mice. *British Journal of Clinical Pharmacology, 109*, 74.

Castro-Lopes, J. M., Tavares, I., Tölle, T. R., Coito, A., & Coimbra, A. (1992). Increase in GABAergic cells and GABA levels in the spinal cord in unilateral inflammation of the hindpaw of the rat. *European Journal of Neuroscience, 4*, 296–301.

Chaignot, C., Weill, A., Ricordeau, P., & Alla, F. (2015). Use in France of baclofen for alcohol dependence from 2007 to 2013: Cohort study based on the databases SNTTRAM and PMSTL. *Thérapie, 70*, 443–453.

Chapman, R. W., Hey, J. A., Rizzo, C. A., & Bolser, D. C. (1993). $GABA_B$ receptors in the lung. *Trends in Pharmacological Sciences, 14*, 26–29.

Chu, D. C. M., Albin, R. L., Young, A. B., & Penney, J. B. (1990). Distribution and kinetics of $GABA_B$ binding sites in rat central nervous system: A quantitative autoradiography study. *Neuroscience, 34*, 341–357.

Chung, K. F. (2015). NMDA and GABA receptors as potential targets in cough hypersensitivity syndrome. *Current Opinion in Pharmacology, 22*, 29–36.

Colombo, G., Addolorato, G., Agabio, R., Carai, M. A. M., Pibiri, F., Serra, S., et al. (2004). Role of $GABA_B$ receptor in alcohol dependence: Reducing effect of baclofen on alcohol intake and alcohol motivational properties in rats and amelioration of alcohol withdrawal syndrome and alcohol craving in human alcoholics. *Neurotoxicity Research, 6*, 403–414.

Corrigall, W. A., Coen, K. M., Adamson, K. L., Chow, B. L., & Zhang, J. (2000). Response of nicotine self-administration in the rat to manipulations of mu-opioid and gamma-aminobutyric acid receptors in the ventral tegmental area. *Psychopharmacology, 149*, 107–114.

Cortez, M. A., McKerlie, C., & Snead, O. C. (2001). A model of atypical absence seizures: EEG, pharmacology, and developmental characterization. *Neurology, 56*, 341–349.

Cousins, M. S., Roberts, D. C., & de Wit, H. (2002). $GABA_B$ receptor agonists for the treatment of drug addiction: A review of recent findings. *Drug and Alcohol Dependence, 65*, 209–220.

Cross, J. A., & Horton, R. W. (1987). Are increases in $GABA_B$ receptors consistent findings following chronic antidepressant administration? *European Journal of Pharmacology, 141*, 159–162.

Cross, J. A., & Horton, R. W. (1988). Effects of chronic oral administration of the antidepressants desmethylimipramine and zimelidine on rat cortical $GABA_B$ binding sites: A comparison with $5HT_2$ binding site changes. *British Journal of Clinical Pharmacology, 93*, 331–336.

Cuny, H., de Faoite, A., Huynh, T. G., Yasuda, T., Berecki, G., & Adams, D. J. (2012). γ-Aminobutyric acid type B (GABAB) receptor expression is needed for inhibition of N-type (Cav2.2) calcium channels by analgesic α-conotoxins. *Journal of Biological Chemistry, 287*, 23948–23957.

Curtis, D. R., Game, C. J. A., Johnston, G. A. R., & McCulloch, R. M. (1974). Central effects of β-(p-chlorophenyl)-γ-aminobutyric acid. *Brain Research, 70*, 493–499.

Curtis, D. R., & Watkins, J. C. (1965). The pharmacology of amino acids related to gamma-aminobutyric acid. *Pharmacological Reviews, 17*, 347–391.

Cutting, D. A., & Jordan, C. C. (1975). Alternative approaches to analgesia: Baclofen as a model compound. *British Journal of Clinical Pharmacology, 54*, 171–179.

Davies, J., & Watkins, J. C. (1974). The action of β-phenyl-GABA derivatives on neurons of the cat cerebral cortex. *Brain Research, 70*, 501–505.

De Sousa, N. J., Beninger, R., Jhamandas, K., & Boegman, R. J. (1994). Stimulation of $GABA_B$ receptors in the basal forebrain selectivity impairs working memory of rats in the double Y-maze. *Brain Research, 641*, 29–38.

Demaziere, J., Saissy, J. M., Vitris, M., Seck, M., Marcoux, L., & Ndiaye, M. (1991). Intermittent intrathecal baclofen for severe tetanus. *Lancet, 337*(8738), 427.

Dent, J., El-Serag, H. B., Wallander, M. A., & Johansson, S. (2005). Epidemiology of gastroesophageal reflux disease: A systemic review. *Gut, 54*, 710–717.

Dicpinigaitis, P. V., & Dobkin, J. B. (1997). Antitussive effect of the GABA-agonist baclofen. *Chest, 111*, 996–999.

Dicpinigaitis, P. V., & Rauf, K. (1998). Treatment of chronic refractory cough with baclofen. *Respiration, 65*, 86–88.

Doze, V. A., Cohen, G. A., & Madison, D. V. (1995). Calcium channel involvement in $GABA_B$ receptor-mediated inhibition of GABA release in area CA1 of the rat hippocampus. *Journal of Neurophysiology, 74*, 43–53.

Dunlap, K. (1981). Two types of γ-aminobutyric acid receptor on embryonic sensory neurons. *British Journal of Pharmacology, 74*, 579–585.

Dutar, P., & Nicoll, R. A. (1988). A physiological role for $GABA_B$ receptors in the central nervous system. *Nature, 332*, 156–158.

Engrand, N., Guerot, E., Rouamba, A., & Vilain, G. (1999). The efficacy of intrathecal baclofen in severe tetanus. *Anesthesiology, 90*, 1773–1776.

Erdo, S. L., & Bowery, N. G. (Eds.). (1986). *GABAergic mechanisms in mammalian periphery* (pp. 1–364). New York: Raven.

Franklin, T. R., Harper, D., Kampman, K., Kildea-McCrea, S., Jens, W., Lynch, K. G., et al. (2009). The $GABA_B$ agonist baclofen reduces cigarette consumption in a preliminary double-blind placebo-controlled smoking reduction study. *Drug and Alcohol Dependence, 103*, 30–36.

Froestl, W., Gallagher, M., Jenkins, H., Madrid, A., Melcher, T., Teichman, S., et al. (2004). SGS742: The first $GABA_B$receptor antagonist in clinical trials. *Biochemical Pharmacology, 68*, 1479–1487.

Fromm, G. H., & Terrence, C. F. (1987). Comparison of L-baclofen and racemic baclofen in trigeminal neuralgia. *Neurology, 37*, 1725–1728.

Fromm, G. H., Terrence, C. F., Chatta, A. S., et al. (1980). Baclofen in trigeminal neuralgia: Its effect on the spinal trigeminal nucleus: A pilot study. *Archives of Neurology, 15*, 240–244.

Fromm, G. H., Terrence, C. F., Chatta, A. S., & Glass, J. D. (1984). Baclofen in the treatment of trigeminal neuralgia: Double-blind study and long-term follow-up. *Annals of Neurology, 15*, 240–244.

Gale, K. (1992). GABA and epilepsy: Basic concepts from preclinical research. *Epilepsia, 33*(Suppl. 5), S3–S12.

Gamardella, A., Manna, I., Labate, A., Chifari, R., La Russa, A., Serra, P., et al. (2003). GABA(B) receptor 1 polymorphism (G1465A) is associated with temporal lobe epilepsy. *Neurology, 60*, 560–563.

Genkova-Papazova, M. G., Perkova, B., Shishkova, N., & Lazarova-Bakarova, M. (2000). The $GABA_B$ antagonist CGP36742 prevents PTZ-kindling-provoked amnesia in rats. *European Neuropsychopharmacology, 10*, 273–278.

Getova, D. P., & Bowery, N. G. (1998). The modulatory effects of high affinity $GABA_B$ receptor antagonists in an active avoidance learning paradigm in rats. *Psychopharmacology, 137*, 369–373.

Getova, D. P., & Dimitrova, D. D. (2007). Effects of $GABA_B$ receptor antagonists CGP63360, CGP76290A and CGP76291A on learning and memory processes in rodents. *Central European Journal of Medicine, 2*, 280–293.

Gianino, J. M., York, M. M., Paice, J. A., & Shott, S. (1998). Quality of life: Effect of reduced spasticity from intrathecal baclofen. *Journal of Neuroscience Nursing, 30*, 47–54.

Giesser, B. (1985). Multiple sclerosis. Current concepts in management. *Drugs, 29*, 88–95.

Giotti, A., Bartolini, A., Failli, P., Gentilini, G., Malcangio, M., & Zilletti, L. (1990). Review of peripheral GABAB effects. In N. G. Bowery, H. Bittiger, & H.-R. Olpe (Eds.), *GABAB receptors in mammalian function* (pp. 101–123). Chichester: Wiley.

Grimm, D. R., DeLuca, R. V., Lesser, M., Bauman, W. A., & Almenoff, P. L. (1997). Effects of $GABA_B$ agonist baclofen on bronchial hyperreactivity to inhaled histamine in subjects with cervical spinal cord injury. *Lung, 175*, 333–341.

Guelaud, C., Similowski, T., Bizec, J., Cabane, J., Whitelaw, W. A., & Derenne, J. P. (1995). Baclofen therapy for chronic hiccup. *European Respiratory Journal, 8*, 235–237.

Haney, M., Hart, C. L., & Foltin, R. W. (2006). Effects of baclofen on cocaine self-administration: Opioid- and nonopioid-dependent volunteers. *Neuropsychopharmacology, 31*, 1814–1821.

Harrison, N. L., Lambert, N. A., & Lovinger, D. M. (1990). Presynaptic GABAB receptors on rat hippocampal neurons. In N. G. Bowery, H. Bittiger, & H.-R. Olpe (Eds.), *GABAB receptors in mammalian function* (pp. 208–221). Chichester: Wiley.

Heetla, H. W., Proost, J. H., Molmans, B. H., Staal, M. J., & van Laar, T. (2016). A pharmacokinetic-pharmacodynamic model for intrathecal baclofen in patients with severe spasticity. *British Journal of Clinical Pharmacology, 81*, 101–112.

Hering-Hanit, R. (1999). Baclofen for the prevention of migraine. *Cephalalgia, 19*, 589–591.

Hering-Hanit, R., & Gadoth, N. (2000). Baclofen in cluster headache. *Headache, 40*, 48–51.

Hill, D. R. (1985). $GABA_B$ receptor modulation of adenylate cyclase activity in rat brain slices. *British Journal of Clinical Pharmacology, 84*, 249–257.

Hill, D. R., & Bowery, N. G. (1981). ^{3}H-Baclofen and ^{3}H-GABA bind to bicuculline insensitive $GABA_B$ sites in rat brain. *Nature, 290*, 149–152.

Hudgson, P., & Weightman, D. (1971). Baclofen in the treatment of spasticity. *British Medical Journal, 15*, 7.

Huynh, T. G., Cuny, H., Siesinger, P. A., & Adams, D. J. (2015). Novel mechanism of voltage-gated N-type (Cav2.2) calcium channel inhibition revealed through α-conotoxin Vc1.1 activation of the GABA(B) receptor. *Molecular Pharmaceutics, 87*, 240–250.

Imbert, B., Alvarez, J. C., & Simon, N. (2015). Anticraving effect of baclofen in alcohol-dependent patients. *Alcoholism, Clinical and Experimental Research, 39*, 1602–1608.

Inaba, Y., D'Antuono, M., Bertazzoni, G., Biagini, G., & Avoli, M. (2009). Diminished presynaptic GABA(B) receptor function in the neocortex of a genetic model of absence epilepsy. *Neurosignals, 17*, 121–131.

Isaacson, J. S. (1998). $GABA_B$ receptor-mediated modulation of presynaptic currents and excitatory transmission at a fast central synapse. *Journal of Neurophysiology, 80*, 1571–1576.

Jones, R. F., Burke, D., Marosszeky, J. E., & Gillies, J. D. (1970). A new agent for the control of spasticity. *Journal of Neurology, Neurosurgery & Psychiatry, 33*, 464–468.

Kahn, R., Biswas, K., Childress, A. R., Shoptaw, S., Fudala, P. J., Gorgon, L., et al. (2009). Multicenter trial of baclofen for abstinence initiation in severe cocaine-dependent individuals. *Drug Alcohol Dependence, 103*, 59–64.

Kalinichev, M., Donovan-Rodriguez, T., Girard, F., Riguet, E., Rouillier, M., Bournique, B., et al. (2014). Evaluation of peripheral versus central effects of GABA(B) receptor activation using a novel, positive allosteric modulator of the GABA(B) receptor ADX71943, a pharmacological tool compound with a fully peripheral activity profile. *British Journal of Clinical Pharmacology, 171*, 4941–4954.

Karbon, E. W., Duman, R. S., & Enna, S. J. (1984). $GABA_B$ receptors and norepinephrine-stimulated cAMP production in rat brain cortex. *Brain Research, 306*, 327–332.

Keberle, H., & Faigle, J. W. (1968). Synthesis and structure-activity relationships of the γ-aminobutyric acid derivatives. In W. Birkmayer (Ed.), *Spasticity—A topical survey* (pp. 90–93). Vienna: Huber.

Keegan, D. L., Richardson, J. S., & Kirby, A. R. (1983). A possible neurochemical basis for the neuropsychiatric aspects of baclofen therapy. *International Journal of Neuroscience, 20*, 249–254.

Khurana, S. R., & Gang, D. S. (2014). Spasticity and the use of intrathecal baclofen in patients with spinal cord injury. *Physical Medicine and Rehabilitation Clinics of North America, 25*, 655–669.

Krach, L. E. (2009). Intrathecal baclofen use in adults with cerebral palsy. *Developmental Medicine and Child Neurology, 51*(Suppl. 4), 106–112.

Lambert, N. A., & Wilson, W. A. (1996). High-threshold Ca^{2+} currents in rat hippocampal interneurons and their selective inhibition by activation of $GABA_B$ receptors. *Journal of Physiology, 492*, 115–127.

Lasarge, C. L., Banuelos, C., Mayse, J. D., & Bizon, J. L. (2009). Blockade of GABA(B) receptors completely reverses age-related learning impairment. *Neuroscience, 164*, 941–947.

Lehmann, A., Antonsson, M., Bremner-Danielson, M., Flärdh, M., Hansson-Brändén, L., & Kärrberg, L. (1999). Activation of the $GABA_B$ receptor inhibits transient lower esophageal sphincter relaxations in dogs. *Gastroenterology, 117*, 1147–1154.

Lehmann, A., Antonsson, M., Holmberg, A., Blackshaw, L. A., Brändén, L., Bräuner-Osborne, H., et al. (2009). (R)-(3-amino-2-fluoropropyl) phosphinic acid (AZD3355), a novel GABAB receptor agonist, inhibits transient lower esophageal sphincter relaxation through a peripheral mode of action. *Journal of Pharmacol and Experimental Therapeutics, 331*, 504–512.

Leisen, C., Langguth, P., Herbert, B., Dressler, C., Koggel, A., & Spahn-Langguth, H. (2003). Lipophilicities of baclofen ester prodrug correlate with affinities to the ATP-dependent efflux pump P-glycoprotein: Relevance for their permeation across the blood-brain barrier? *Pharmaceutical Research, 20*, 772–778.

Lesouef, N., Bellet, F., Mourier, G., & Beyens, M. N. (2014). Efficacy of baclofen on abstinence and craving in alcohol-dependent patients: A meta-analysis of randomized controlled trials. *Thérapie, 69*, 427–435.

Ling, W., Shoptaw, S., & Majewska, D. (1998). Baclofen as a cocaine anti-craving medication: A preliminary clinical study. *Neuropsychopharmacology, 18*, 403–404.

Liu, Q. Y., Wang, C. Y., Cai, Z. L., Xu, S. T., Liu, W. X., Xiao, P., et al. (2014). Effects of intrahippocampal GABAB receptor antagonist treatment on the behavioral long-term potentiation and Y-maze learning performance. *Neurobiology of Learning and Memory, 114*, 26–31.

Lloyd, K. G. (1989). GABA and depression. In G. Nistico & N. G. Bowery (Eds.), *GABA basic research and clinical applications* (pp. 301–343). Pythagora: Rome.

Lloyd, K. G., Thuret, F., & Pilc, A. (1985). Upregulation of γ-aminobutyric acid (GABA)B binding sites in rat frontal cortex: A common action of repeated administration of different classes of antidepressants and electroshock. *Journal of Pharmacol and Experimental Therapeutics, 235*, 191–199.

Lorrai, I., Maccioni, P., Gessa, G. L., & Colombo, G. (2016). R (+)-Baclofen but not S (−) baclofen alters alcohol self administration in alcohol preferring rats. *Frontiers in Psychiatry, 7*, 68. doi:10.3389/fpsyt.2016.04.028.

Luo, P., Chen, C., Lu, Y., Fu, T., Lu, Q., Xu, X., et al. (2016). Baclofen ameliorates spatial working memory impairments induced by chronic cerebral hypoperfusion via up-regulation of HCN2 expression in the PPC in rats. *Behavioural Brain Research, 308*, 6–13.

Luscher, C., Jan, I. Y., Stoffel, M., Malenka, R. C., & Nicoll, R. A. (1997). G-protein inwardly rectifying K^+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron, 19*, 687–695.

Maccioni, P., Vargiolu, D., Thomas, A. W., Malherbe, P., Mugnaini, C., Corelli, F., et al. (2015). Inhibition of alcohol self-administration by positive allosteric modulators of the $GABA_B$ receptor in rats: Lack of tolerance and potentiation of baclofen. *Psychopharmacology, 232*, 1831–1841.

Malcangio, M., & Bowery, N. G. (1993). Gamma-aminobutyric $acid_B$ but not gamma-aminobutyric $acid_A$ receptor activation inhibits electrically evoked substance P-like immunoreactivity release from rat spinal cord in vitro. *Journal of Pharmacol and Experimental Therapeutics, 266*, 1490–1496.

Malcangio, M., & Bowery, N. G. (1994). Spinal cord SP release and hyperalgesia in monoarthritic rats: Involvement of the $GABA_B$ receptor system. *British Journal of Clinical Pharmacology, 113*, 1561–1566.

Malcangio, M., & Bowery, N. G. (1996). Calcitonin gene-related peptide content, basal outflow and electrically evoked release from monoarthritic rat spinal cord in vitro. *Pain, 66*, 351–358.

Manning, J. P., Richards, D. A., & Bowery, N. G. (2003). Pharmacology of absence epilepsy. *Trends in Pharmacological Sciences, 17*, 457–462.

Mathur, S. N., Chu, S. K., McCormick, Z., Chang Chien, G. C., & Marciniak, C. M. (2014). Long-term intrathecal baclofen: Outcomes after more than 10 years of treatment. *PM & R: The Journal of Injury, Function, and Rehabilitation, 6*, 506–513.

Meeren, H., van Luitelaar, G., Lopes da Silva, F., & Coenen, A. (2005). Evolving concepts on the pathophysiology of absence seizures. *Archives of Neurology, 62*, 371–376.

Meldrum, B., & Horton, R. (1980). Effects of the bicyclic GABA agonist, THIP, on myoclonic and seizure responses in mice and baboons with reflex epilepsy. *European Journal of Pharmacology, 61*, 231–237.

Miner, P. B., Jr., Silberg, D. G., Ruth, M., Miller, F., & Pandolfino, J. (2014). Dose-dependent effects of lesogaberan on reflux measures in patients with refractory gastroesophageal reflux disease: A randomized, placebo-controlled study. *BMC Gastroenterol, 14*, 188.

Mombereau, C., Kaupmann, K., Froestl, W., Sansig, G., van der Putten, H., & Cryan, J. F. (2004). Genetic and pharmacological evidence of a role for $GABA_B$ receptors in the modulation of anxiety- and antidepressant-like behavior. *Neuropsychopharmacology, 29*, 1050–1062.

Mueller, H., Borner, U., Zierski, J., & Hempelmann, G. (1987). Intrathecal baclofen for treatment of tetanus-induced spasticity. *Anesthesiology, 66*, 76–79.

Nagakawa, Y., Ishima, T., Ishibashi, Y., Tsuji, M., & Takashima, T. (1996). Involvement of GABAB receptor systems in experimental depression: Baclofen but not bicuculline exacerbates helplessness in rats. *Brain Research, 741*, 240–245.

Naito, Y. (2014). Intrathecal baclofen therapy and management of severe spasticity. *Brain and Nerve, 66*, 1049–1055.

Nanninga, J. B., Frost, F., & Penn, R. (1989). Effect of intrathecal baclofen on bladder and sphincter function. *Journal of Urology, 142*, 101–105.

Navarrete-Opazo, A. A., Gonzalez, W., & Nahuelhual, P. (2016). Effectiveness of oral baclofen in the treatment of spasticity in children and adolescents with cerebral palsy. *Archives of Physical Medicine and Rehabilitation, 97*, 604–618.

Nicoll, R. A. (2004). My close encounter with $GABA_B$ receptors. *Biochemical Pharmacology, 68*, 1667–1674.

O'Brien, C. F., Seeberger, I. C., & Smith, D. B. (1996). Spasticity after stroke: Epidemiology and optimal treatment. *Drugs & Aging, 9*, 332–340.

Ochs, G. A. (1993). Intrathecal baclofen. *Baillière's Clinical Neurology, 2*, 73–86.
Odagaki, Y., & Koyama, T. (2001). Identification of G alpha subtype(s) involved in gamma-aminobutyric acid (B) receptor-mediated high affinity guanosine triphosphate activity in rat cerebral cortical membranes. *Neuroscience Letters, 297*, 137–141.
Odagaki, Y., Nishi, N., & Koyama, T. (2000). Functional coupling of GABA(B) receptors with G proteins that are sensitive to N-ethylmaleimide treatment, suramin and benzalkonium chloride in rat cerebral cortical membranes. *Journal of Neural Transmission, 107*, 1101–1116.
Okazaki, T., Saito, Y., Ueda, R., Sughara, S., Tamasaki, A., Nishimura, Y., Ohno, K., et al. (2016). Effect of intrathecal baclofen on delayed-onset paroxysmal dystonia due to compression injury resulting from congenital and progressive spinal bone deformaties in chondrodysplasia punctata. *Pediatric Neurology, 56*, 80–85.
Olsen, R. W., Bureau, M., Houser, C. R., Delgado-Escueta, A. V., Richards, J. G., & Möhler, H. (1992). GABA/Benzodiazepine receptors in human focal epilepsy. *Epilepsy Research. Supplement, 8*, 383–391.
Pacey, L. K., Heximer, S. P., & Hampson, D. R. (2009). Increased GABA(B) receptor-mediated signaling reduces the susceptibility of fragile X knockout mice to audiogenic seizures. *Molecular Pharmacology, 76*, 18–24.
Pacey, L. K., Tharmlingam, S., & Hampson, D. R. (2011). Subchronic administration and combination metabotropic glutamate and GABAB receptor drug therapy in fragile X syndrome. *Journal of Pharmacol and Experimental Therapeutics, 338*, 897–905.
Penn, R. D., & Kroin, J. S. (1984). Intrathecal baclofen alleviates spinal cord spasticity. *Lancet, 323*(8385), 1078.
Penn, R. D., & Kroin, J. S. (1985). Continuous intrathecal baclofen for severe spasticity. *Lancet, 326*(8447), 125–127.
Pitsikas, N., Rigamonti, A. E., Cella, S. G., & Muller, E. E. (2003). The GABAB receptor and recognition memory: Possible modulation of its behavioral effects by the nitrergic system. *Neurosience, 118*, 1121–1127.
Porrino, L. J., Hampson, R. E., Opris, I., & Deadwyler, S. A. (2013). Acute cocaine induced deficits in cognitive performance in rhesus macaque monkeys treated with baclofen. *Psychopharmacology, 225*, 105–114.
Post, R. M., Ketter, T. A., Joffe, R. T., & Kramlinger, K. L. (1991). Lack of beneficial effects of l-baclofen in affective disorder. *International Clinical Psychopharmacology, 6*, 197–207.
Potes, C. S., Neto, F. L., & Castro-Lopes, J. M. (2006). Administration of baclofen, a gamma-aminobutyric acid type B agonist in the thalamic ventrobasal complex, attenuates allodynia in monoarthritic rats subjected to the ankle-bend test. *Journal of Neuroscience Research, 83*, 515–523.
Prosser, H. M., Gill, C. H., Hirst, W. D., Grau, E., Robbins, M., Calver, A., et al. (2001). Epileptogenesis and enhanced prepulse inhibition in GABA(B1)-deficient mice. *Molecular and Cellular Neurosciences, 17*(6), 1059–1070.
Proudfit, H. K., & Levy, R. A. (1978). Delimitation of neuronal substrates necessary for the analgesic actions of baclofen and morphine. *European Journal of Pharmacology, 47*, 159–166.
Rawlins, P. (1998). Patient management of cerebral origin spasticity with intrathecal baclofen. *Journal of Neuroscience Nursing, 30*, 32–35, 40–46.
Roberts, D. C. S., & Andrews, M. M. (1997). Baclofen suppression of cocaine self-administration: Demonstration using a discrete trials procedure. *Psychopharmacology, 131*, 271–277.
Rolland, B., Labreuche, J., Duhammel, A., Deheul, S., Gautier, S., Auffret, M., et al. (2015). Baclofen for alcohol dependence: Relationships between baclofen and alcohol dosing and the occurrence of major sedation. *European Neuropsychopharmacology, 25*, 1631–1636.
Santos, A. E., Carvalho, C. M., Macedo, T. A., & Carvalho, A. P. (1995). Regulation of intracellular [Ca^{2+}] GABA release by presynaptic $GABA_B$ receptors in rat cerebrocortical synaptosomes. *Neurochemistry International, 27*, 397–406.
Santos, M. L., Mota-Miranda, A., Alves-Pereira, G. A., Correia, J., & Marçal, N. (2004). Intrathecal baclofen for the treatment of tetanus. *Clinical Infectious Diseases, 38*, 321–328.

Sasaki, N., & Ogiwara, M. (2016). Intrathecal baclofen therapy in a child with severe scoliosis. Report of 2 cases. *Neuromodulation, 19*(6), 664–666. doi:10.1111/ner.12449.

Sawynok, J., & LaBella, F. S. (1982). On the involvement of GABA in the analgesia produced by baclofen, muscimol and morphine. *Neuropharmacology, 21*, 397–404.

Scheinberg, A., Hall, K., Lam, L. T., & O'Flaherty, S. (2006). Oral baclofen in children with cerebral palsy: A double-blind cross-over pilot study. *Journal of Paediatrics and Child Health, 42*, 715–720.

Schuler, V., Luscher, C., Blanchet, C., Klix, N., Sansig, G., Klebs, K., et al. (2001). Epilepsy, hyperalgesia, impaired memory, and loss of pre- and post-synaptic $GABA_B$ responses in mice lacking $GABA_{B1}$. *Neuron, 31*, 47–58.

Sharma, R. C. (2015). Successful treatment of idiopathic intractable hiccup with baclofen and supportive treatment: A case report. *Journal of Neuropsychiatry and Clinical Neurosciences, 27*, e62–e63.

Shoptaw, S., Yang, X., Rotheram-Fuller, E. J., Hsieh, Y. C., Kintaudi, P. C., Charuvastra, V. C., et al. (2003). Randomized placebo-controlled trial of baclofen for cocaine dependence: Preliminary effects for individuals with chronic patterns of cocaine use. *Journal of Clinical Psychiatry, 64*, 1440–1448.

Silbert, P. L., Matsumoto, J. Y., McManis, P. G., Stolp-Smith, K. A., Elliott, B. A., & McEvoy, K. M. (1995). Intrathecal baclofen therapy in stiff-man syndrome: A double-blind, placebo-controlled trial. *Neurology, 45*, 1893–1897.

Smith, C. R., La Rocca, N. G., Giesser, B. S., & Scheinberg, L. C. (1991). High-dose oral baclofen: Experience in patients with multiple sclerosis. *Neurology, 41*, 1829–1831.

Snodgrass, S. R. (1992). GABA and epilepsy: Their complex relationship and the evolution of our understanding. *Journal of Child Neurology, 7*, 77–86.

Stackman, R. J., & Walsh, T. J. (1994). Baclofen reduces dose related working memory impairments after intraseptal injection. *Behavioral and Neural Biology, 61*, 181–185.

Stayer, C., Tronnier, V., Dressnandt, J., Mauch, E., Marquardt, G., Rieke, K., et al. (1997). Intrathecal baclofen therapy for stiff-man syndrome and progressive encephalomyeopathy with rigidity and myoclonus. *Neurology, 49*, 1591–1597.

Steger, M., Schneemann, M., & Fox, M. (2015). Systemic review: The pathogenesis and pharmacological treatment of hiccups. *Alimentary Pharmacology & Therapeutics, 42*, 1037–1050.

Suzdak, P. D., & Gianutsos, G. (1986). Effect of chronic imipramine or baclofen on $GABA_B$ binding and cyclic AMPproduction in cerebral cortex. *European Journal of Pharmacology, 131*, 129–133.

Taylor, M., & Bates, C. P. (1979). A double-blind crossover trial of baclofen- a new treatment for the unstable bladder syndrome. *British Journal of Urology, 51*, 504–505.

Teichgraber, L. A., Lehmann, T. N., Meencke, H. J., Weiss, T., Nitsch, R., & Deisz, R. A. (2009). Impaired function of $GABA_B$ receptors in tissues from pharmacoresistant epilepsy patients. *Epilepsia, 50*, 1697–1716.

Teoh, H., Malcangio, M., & Bowery, N. G. (1996). The effects of novel $GABA_B$ antagonists on the release of amino acids from spinal cord of the rat. *British Journal of Clinical Pharmacology, 118*, 1153–1160.

Thompson, A. N., Ehret Leal, J., & Brzezinski, W. A. (2014). Olanzapine and baclofen for the treatment of intractable hiccups. *Pharmacotherapy, 34*, e4–e8.

Turkyilmaz, A., & Eroglu, A. (2008). Use of baclofen in the treatment of esophageal stent-related hiccups. *Annals of Thoracic Surgery, 85*, 328–330.

Twycross, R. (2003). Baclofen for hiccups. *American Journal of Hospice & Palliative Care, 20*, 262.

Upton, N., & Blackburn, T. (1997). Pharmacology of mammalian $GABA_A$ receptors. In S. J. Enna & N. G. Bowery (Eds.), *The GABA receptors* (pp. 83–120). Totowa, NJ: Humana.

Vakil, N., van Zanten, S. V., Kahrilas, P., Dent, J., Jones, R., & Global Consensus Group. (2006). The Montreal definition and classification gastroesophageal reflux disease: A global evidence-based consensus. *American Journal of Gastroenterology, 101*, 1900–1920.

Van Bree, J. B., Audus, K. I., & Borchardt, R. T. (1988). Carrier-mediated transport of baclofen across monolayers of bovine brain endothelial cells in primary culture. *Pharmaceutical Research, 5*, 369–371.

Van Bree, J. B., Heijligers-Feijen, C. D., De Boer, A. G., Danhof, M., & Breimer, D. D. (1991). Stereoselective transport of baclofen across the blood-brain barrier in rats as determined by the unit impulse response methodology. *Pharmaceutical Research, 8*, 259–262.

Vaught, J. L., Pelley, K., Costa, L. G., Setler, P., & Enna, S. J. (1985). A comparison of the antinociceptive responses to the GABA-receptor agonists THIP and baclofen. *Neuropharmacology, 24*, 211–216.

Vienne, J., Bettler, B., Franken, P., & Tafti, M. (2010). Differential effects of GABAB receptor subtypes, {gamma}-hydroxybutyric Acid, and Baclofen on EEG activity and sleep regulation. *Journal of Neuroscience, 30*, 14194–14204.

Wagner, P. G., & Deakin, M. S. (1993). $GABA_B$ receptors are coupled to a barium-sensitive outward rectifying potassium conductance in premotor respiratory neurons. *Journal of Neurophysiology, 69*, 286–289.

Whelan, J. L. (1980). Baclofen in treatment of the "stiff-man" syndrome. *Archives of Neurology, 37*, 600–601.

Wiklund, I. (2004). Review of the quality of life and burden of illness in gastroesophageal reflux disease. *Digestive Diseases, 22*, 108–114.

Wilson, P. R., & Yaksh, T. L. (1978). Baclofen is anti-nociceptive in the intrathecal space of animals. *European Journal of Pharmacology, 51*, 323–330.

Wu, Y., Chan, K. F., Eubanks, J. H., Guin Ting Wong, C., Cortez, M. A., Shen, L., et al. (2007). Transgenic mice over-expressing GABA(B)R1a receptors acquire an atypical absence epilepsy-like phenotype. *Neurobiology of Disease, 26*, 439–451.

Wu, L. G., & Saggau, P. (1995). $GABA_B$ receptor-mediated presynaptic inhibition in guinea-pig hippocampus is caused by reduction of presynaptic Ca^{2+}influx. *Journal of Physiology, 485*, 649–657.

Xi, Z. X., & Stein, E. A. (1999). Baclofen inhibits heroin self-administration behavior and mesolimbic dopamine release. *Journal of Pharmacol and Experimental Therapeutics, 290*, 1369–1374.

Xu, J., & Wojcik, W. J. (1986). Gamma aminobutyric acid B receptor-mediated inhibition of adenylate cyclase in cultured cerebellar granule cells: Blockade by islet-activating protein. *Journal of Pharmacol and Experimental Therapeutics, 239*, 568–573.

Xu, X. H., Yang, Z. M., Chen, Q., Yu, L., Liang, S. W., Lv, H. J., et al. (2013). Therapeutic efficacy of baclofen in refractory gastroesophageal reflux-induced chronic cough. *World Journal of Gastroenterology, 19*, 4386–4392.

Zemoura, K., Ralvenius, W. T., Malherbe, P., & Benke, D. (2016). The positive allosteric $GABA_B$ receptor modulator rac-BHFF enhances baclofen-mediated analgesia in neuropathic rats. *Neuropharmacology, 108*, 172–178.

Zerman, A., Hoefeijzers, S., Milton, F., Dewar, M., Carr, M., & Streatfield, C. (2016). The $GABA_B$ receptor agonist baclofen contributes to three distinct varieties of amnesia in the human brain—A detailed case report. *Cortex, 74*, 9–19.

Zhang, C., Zhang, R., Zhang, S., Xu, M., & Zhang, S. (2014). Baclofen for stroke patients with persistent hiccups: A randomized, double-blind, placebo-controlled trial. *Trials, 15*, 295.

Chapter 18
Allosteric Modulators: The New Generation of $GABA_B$ Receptor Ligands

Stephan Urwyler

Abstract Allosteric modulators are molecules that interact with a site on a receptor which is distinct from the orthosteric recognition site for the endogenous ligand. By modifying the receptor conformation, they change the affinity and/or efficacy of agonists, but often have no intrinsic activity on their own. Because of this use-dependent mechanism, they are expected to have a much better side-effect profile than agonist drugs. The first positive GABA type B ($GABA_B$) receptor modulators, CGP7930 and GS39783, have been described more than 10 years ago. They were discovered in a high-throughput screen using GTP(γ)^{35}S assays, in which they enhanced both the affinity and the maximal effect of γ-aminobutyric acid (GABA), without having any agonist activity of their own. This positive modulation was subsequently confirmed in a number of different radioligand binding, biochemical and electrophysiological assay systems. The recombinant expression of engineered receptor constructs allowed to locate the site of action of these positive modulators to the seven-transmembrane domain of the $GABA_{B2}$ subunit, through which they could to some extent directly activate the receptor in sufficiently sensitive assay systems. These early findings have fostered the search for other molecules acting in a similar way, and a number of positive $GABA_B$ receptor modulators, and also the first negative modulators, have been described in recent years. In vivo microdialysis experiments have demonstrated at the biochemical level that the mechanism of positive allosteric $GABA_B$ receptor modulation also applies in living animals. Behavioural experiments have confirmed that positive $GABA_B$ receptor modulators have a better side-effect profile than the therapeutically used agonist drug baclofen. Numerous studies have shown that these compounds show promising activity in animal models for anxiety, drug and alcohol abuse, pain, gastrointestinal indications and possibly more.

Keywords $GABA_B$ receptor • Allosteric modulators • Affinity cooperativity • Activation cooperativity • Radioligand binding • GTPγ^{35}S binding • Intracellular Ca^{2+} signalling • Electrophysiology • In vivo effects • Clinical indications

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18.1 Introduction: Principles of Allosteric Modulation

Allosteric receptor modulation is an appealing and proven principle in drug targeting (Bowery 2006; Conn et al. 2009; Changeux 2013). The concept of allosterism was introduced for the first time 50 years ago in a classic paper by Monod et al. (1965). Allosteric modulators are molecules that act at a site on a receptor (or enzyme) which is distinct from the "orthosteric" recognition site for the endogenous ligand (or substrate). Numerous recent articles (e.g. Hall 2000; Christopoulos and Kenakin 2002; Urwyler 2011; Christopoulos 2014) address the principles and theoretical aspects of allosteric drug action. By inducing conformational changes in the receptor protein, positive or negative modulators enhance or decrease, respectively, the affinity and/or the efficacy of orthosteric ligands (the constants γ and δ in the model by Hall 2000, Fig. 18.1). Although they often have no intrinsic effects on their own, it is basically possible that they also act as agonists or inverse agonists to various degrees through the allosteric site (intrinsic efficacy constant β in Fig. 18.1). Moreover, neutral allosteric ligands are "silent" ($\beta = 1$, $\gamma = 1$ and $\delta = 1$), but they block the effects of allosteric modulators or agonists by competitive displacement from the allosteric site. The cooperativity constants γ (binding) and δ (activation/efficacy) in Fig. 18.1 are determined by the conformational changes induced by given chemical structures of the orthosteric and allosteric ligands; therefore, their interactions are "probe dependent". Because the thermodynamic stability of the ternary complex between

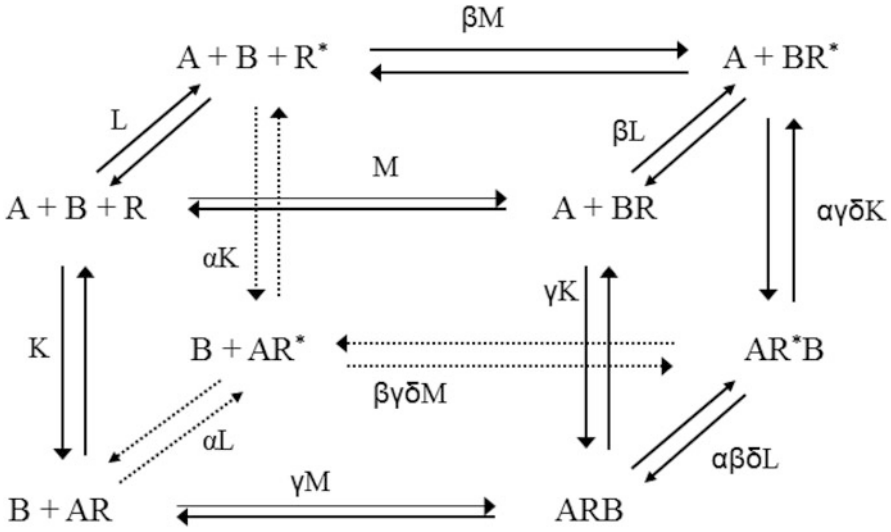

Fig. 18.1 The allosteric two-state model. *R* inactive state of the receptor, *R** active state of the receptor, *A* orthosteric ligand, *B* allosteric ligand, *K* binding constant of A, *L* receptor isomerization constant, *M* binding constant of B, α intrinsic efficacy of A, β intrinsic efficacy of B, γ binding cooperativity between A and B, δ activation cooperativity between A and B. Slightly modified after Hall (2000). Reproduced from reference Urwyler 2011, with permission

the receptor and its two ligands is independent of the way on which it is formed, cooperativity between allosteric and orthosteric ligands is reciprocal.

Benzodiazepines are an early generation of clinically successful drugs acting as allosteric receptor modulators. These sedative/anxiolytic agents enhance the function of the inhibitory ionotropic (chloride channel) GABA type A ($GABA_A$) receptor, basically without stimulating it by themselves. While these positive allosteric modulators are clinically useful drugs, compounds acting directly at the γ-aminobutyric acid (GABA) recognition site (such as the agonist muscimol or the antagonist bicuculline) have prohibitive side-effects.

A more recent case of a clinically successful positive modulator drug is the "calcimimetic" agent cinacalcet, which enhances the sensitivity of the G protein-coupled calcium sensing receptor for its natural ligand, the Ca^{2+} ion (reviewed in Urwyler 2011). This example nicely illustrates the potential of drugs acting as allosteric modulators. The calcium sensing receptor is a key regulator of calcium homeostasis; it acts by monitoring extracellular calcium concentrations in blood plasma and various organs and by triggering tissue responses that restore these to normal when needed. There are several diseases in which malfunction of the calcium sensing receptor is critically involved. An important example is secondary hyperparathyroidism resulting from chronic renal failure, in which a downregulation of the parathyroid calcium sensing receptor and a concomitant change of the calcium set point towards higher concentrations are observed. This situation can be corrected with the positive allosteric modulator cinacalcet, which increases receptor affinity and thereby shifts the calcium response curve towards lower concentrations. On the other hand, orthosteric agonist drugs (difficult to find for a site recognizing the calcium ion!), which would persistently activate the receptor, would not be useful in targeting a receptor whose function is to monitor changes in calcium concentrations.

Allosteric modulators undoubtedly have numerous advantages over orthosteric ligands as therapeutically active molecules. Firstly, whereas an agonist drug will basically stimulate each of its receptors which it encounters, a positive allosteric modulator will typically only enhance the receptor activation by its endogenous ligand (for example, when and where a neurotransmitter is released). Thus, compared to an agonist, it acts much more in concert with the temporal and spatial organization of physiological receptor activation. For this reason, a positive allosteric modulator can be expected to have a much lower side-effect potential compared to agonists. In addition, if a positive allosteric modulator were to be administered concomitantly with a drug acting as an agonist, the dose of the latter could be substantially reduced, thereby minimizing putative off-target side-effects. Secondly, whereas persistent receptor activation by agonists often leads to receptor desensitization (and thereby tolerance development), positive allosteric modulators, again because of their use-dependent mechanism, may well have a lower potential for inducing receptor desensitization. Thirdly, whereas the recognition sites for the endogenous ligands usually remained well conserved between different receptor subtypes during evolution, allosteric binding sites in general appear more variable. For example, while it has proven to be virtually impossible to find subtype-selective orthosteric agonists for a given group of metabotropic glutamate receptors, highly selective allosteric modulators for particular subtypes have been described (for

review see Urwyler 2011). As a last point, in those cases where it is inherently difficult to conceive organic molecules mimicking the endogenous ligand, allosteric modulators may offer an elegant solution to the problem. The calcium-sensing receptor discussed above is a nice example for such a situation.

The agonist molecule baclofen, so far the only marketed drug targeting the GABA type B ($GABA_B$) receptor, is used since decades for the treatment of muscle spasticity in patients suffering from multiple sclerosis or spinal cord injury (see Chap. 17 of this book for a comprehensive review on baclofen pharmacology). Moreover, more recently several "off-label" clinical indications for baclofen have emerged, such as, e.g. gastroesophageal reflux disease (GERD) (see Chap. 16 of this book) or alcohol use disorder (see Chap. 15 of this book). However, in uses other than the treatment of muscle spasticity, the strong muscle relaxant properties of baclofen are an unwanted side-effect. Baclofen also has other important shortcomings, such as a short duration of action, a narrow therapeutic window and rapid development of tolerance to some of its effects (Vacher and Bettler 2003, see also Chap. 17 of this book). For these reasons, in the light of their advantages mentioned above, positive allosteric $GABA_B$ receptor modulators seem to have therapeutic potential possibly avoiding the shortcomings of baclofen. Although information on such molecules is only available from preclinical models so far, optimism for therapeutic efficacy in man is justified on the basis of the success of cinacalcet, since the calcium sensing receptor belongs to the same G protein-coupled receptor (GPCR) family C as the $GABA_B$ receptor. Cinacalcet may thus well have paved the way for $GABA_B$ receptor modulators.

18.2 Molecular and Cellular Pharmacology of Allosteric $GABA_B$ Receptor Modulators

The first positive allosteric $GABA_B$ receptor modulators, CGP7930 and GS39783 (Fig. 18.2), were discovered and described at the beginning of this century (Urwyler et al. 2001, 2003). Since then, high-throughput screening and synthetic chemistry efforts have produced more such compounds, and allosteric $GABA_B$ receptor modulators have been the subject of extensive pharmacological characterization in vitro

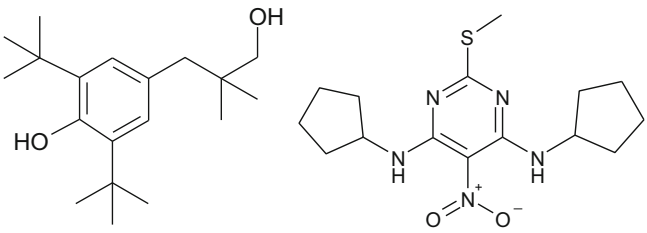

Fig. 18.2 Chemical structures of the prototypical positive allosteric $GABA_B$ receptor modulators CGP7930 (*left*) and GS39783 (*right*). See Chap. 3 of this book for the structures of other compounds acting in a similar way

and in vivo. Several reviews have addressed this topic in recent years (e.g. Pin and Prézeau 2007; Urwyler 2011; Brown et al. 2015, see also Froestl 2010 for a patent survey). The chemistry of allosteric $GABA_B$ receptor modulators is covered by C. Mugnaini and F. Corelli in Chap. 3 of this book; it also contains the structures of modulators other than CGP7930 and GS39783.

CGP7930 and GS39783, and a number of analogues thereof, were discovered in a high-throughput screening program based on a GTPγ^{35}S binding assay using membranes from a Chinese hamster ovary (CHO) cell line expressing the two $GABA_B$ receptor subunits, and subsequently characterized in detail in vitro (Urwyler et al. 2001, 2003). CGP7930 and GS39783 at low micromolar concentrations enhanced GTPγ^{35}S binding stimulated by GABA or baclofen, without having any effect by themselves. The two positive modulators had no effect on GABA-stimulated GTPγ^{35}S binding in the presence of the competitive antagonist CGP56999A, confirming that their activity is dependent on concomitant stimulation of the orthosteric receptor site. Very similar results were obtained on native $GABA_B$ receptors in rat brain membranes (Urwyler et al. 2001, 2003) or in human post-mortem brain tissue (Olianas et al. 2005). Unexpectedly, CGP7930 and GS39783 not only enhanced the potency of GABA in the GTPγ^{35}S assay (by a five- to tenfold), but also its maximal effect (by a 1.5- to 2-fold) (Fig. 18.3). This was a rather novel type of allosteric modulatory effect at the time, but is accounted for by recent theoretical receptor models (Hall 2000; Christopoulos and Kenakin 2002, see also Urwyler 2011).

Radioligand binding assays are a powerful tool to assess the binding cooperativity (affinity modulation) between two molecules interacting with distinct sites on the same receptor protein. The rate constants of association and dissociation of orthosteric ligands are sensitive to changes in receptor confor-

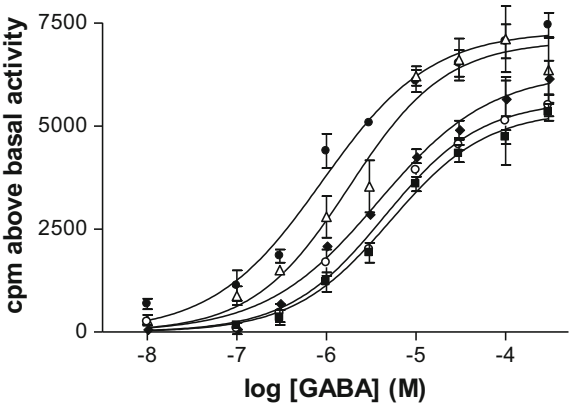

Fig. 18.3 Effects of CGP7930 in membranes from a recombinant Chinese hamster ovary (CHO) cell line expressing the $GABA_B$ receptor. In a GTPγ[^{35}S] stimulation assay, CGP7930 enhances at the same time the potency and the maximal efficacy of GABA as an agonist at the $GABA_B$ receptor. GABA concentration–response curves were measured in the absence (*filled square*) and in the presence of 1 μM (*open circle*), 3 μM (*filled diamond*), 10 μM (*triangle*) and 30 μM (*filled circle*) CGP7930. Reproduced from reference Urwyler et al. (2001), with permission

mation induced by an allosteric ligand. In our experiments, 30 μM GS39783 unexpectedly reduced the rate of association of the agonist radioligand [^{3}H]3-aminopropylphosphinic acid ([^{3}H]APPA) to native $GABA_B$ receptors, but this effect was overcompensated by an even greater reduction in the rate of dissociation, resulting in a net increase of ligand affinity (Urwyler et al. 2003). GPCRs exist in a high agonist affinity (G protein-bound) and a low agonist affinity (uncoupled from the G protein) state. The agonist radioligand [^{3}H]APPA labels only the high affinity state of the $GABA_B$ receptor. When saturation experiments were performed with this ligand using native $GABA_B$ receptors in rat brain membranes, 30 μM CGP7930 increased the agonist affinity by about a 2.5- to 3-fold, and also marginally but not significantly its maximal binding capacity (Urwyler et al. 2001). On the other hand, antagonist radioligands such as [^{3}H] CGP62349 label both receptor states. Therefore, agonist displacement curves are biphasic, comprising a high- and a low-affinity component. CGP7930 and GS39783 (30 μM) enhanced the affinities for agonists of both components (Fig. 18.4), indicating that both the G protein-coupled and -uncoupled receptor forms are amenable to allosteric modulation. Moreover, the relative proportion of the high affinity component was increased, suggesting that the two positive modulators also enhance the interaction between receptor and G protein (Urwyler et al. 2003, 2004). This interpretation is in line with the slight increase in maximal agonist binding capacity observed earlier, the meaning of which was overlooked at the time.

A complex situation was encountered when radioligand binding experiments were performed with recombinant $GABA_B$ receptor preparations. In membranes from CHO cells expressing the $GABA_{B1}$ subunit only, GABA displaced the antagonist radioligand [^{3}H]CGP62349 with low affinity, which was not affected by CGP7930. On the other hand, in membranes from cells expressing both $GABA_B$ receptor subunits, biphasic displacement curves were observed with GABA. The low-affinity

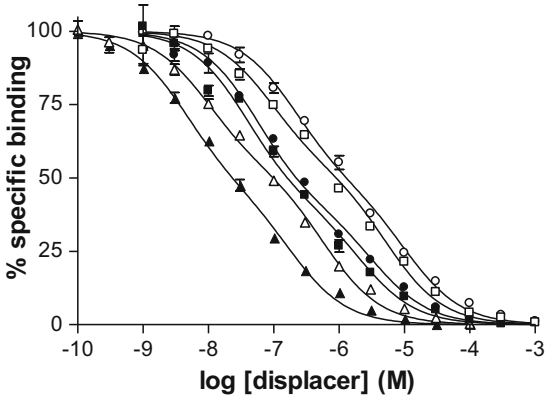

Fig. 18.4 Displacement of the competitive antagonist radioligand [^{3}H]CGP62349 from native $GABA_B$ receptors in rat cortical membranes by GABA (*squares*), L-baclofen (*circles*) and APPA (*triangles*) in the absence (*open symbols*) and in the presence (*filled symbols*) of 30 μM GS39783. Reproduced from Urwyler et al. (2003), with permission

component comprised the major part (about 70 %) of the total binding, had a similar IC_{50} as in the $GABA_{B1}$ monomer, was not influenced by CGP7930 and was therefore attributed to the strongly overexpressed $GABA_{B1}$ subunit in these cells. However, the remaining part corresponding to dimeric $GABA_B$ receptors displayed a much higher affinity for GABA, which was increased by a 2.5-fold in the presence of 30 μM CGP7930. The binding assay was not sufficiently sensitive to further resolve this part into G protein-coupled and -uncoupled components. These results demonstrate that the $GABA_{B2}$ subunit is essential for the modulatory action of CGP7930 and suggest that it harbours the modulator binding site (Urwyler et al. 2001).

Allosteric modulation by GS39783 is not observed with the *Drosophila melanogaster* $GABA_B$ receptor (Dupuis et al. 2006). However, co-expression of the *Drosophila* $GABA_{B1}$ subunit together with rat $GABA_{B2}$ gave functional receptors positively modulated by GS39783 in GTPγ^{35}S binding assays. Further data obtained with chimeric *Drosophila melanogaster*/rat $GABA_{B2}$ subunit constructs and point mutations demonstrated a critical role of the $GABA_{B2}$ transmembrane region for positive modulation. Of particular interest was the finding that in a construct containing two key amino acid substitutions in transmembrane domain VI, GS39783 directly activated the rat $GABA_{B2}$ subunit even in the absence of $GABA_{B1}$, in contrast to wild-type $GABA_{B2}$ (Dupuis et al. 2006). Binet et al. (2004), using a sensitive phosphoinositol turnover assay, have found that CGP7930 activated the wild-type $GABA_B$ receptor on its own as a partial agonist with low efficacy. Moreover, it could also directly activate $GABA_{B2}$ expressed alone, as well as a truncated construct in which $GABA_{B2}$ was deprived of its extracellular domain (Binet et al. 2004). Taken together, these results demonstrate that CGP7930 and GS39783 not only act as positive allosteric $GABA_B$ receptor modulators, but they are also the first compounds shown to be able to directly activate the $GABA_{B2}$ receptor subunit, via its heptahelical domain. Such "allosteric agonism" is accounted for (intrinsic efficacy constant β in Fig. 18.1) and allowed by the theoretical model proposed by Hall (2000, see also Urwyler 2011 for more explanations).

The model shown in Fig. 18.1 is basically applicable to all kinds of orthosteric ligands, independently of their intrinsic properties. This means that partial or full agonists, inverse agonists, as well as competitive antagonists, should be equally amenable to modulation by allosteric drugs. In fact, in radioligand binding assays the affinities of a number of competitive $GABA_B$ receptor antagonists were found to be decreased in the presence of 30 μM CGP7930 or GS39783 (Urwyler et al. 2005). In a GTPγ^{35}S assay, the maximal response of the partial agonist CGP47656 was increased by approximately fourfold by the two modulators, compared to only 1.5- to 2-fold increases found with GABA. Interestingly, in the same study (Urwyler et al. 2005) it was found that the two compounds CGP35348 and 2-hydroxy-saclofen, previously believed to be neutral or "silent" competitive antagonists, did not stimulate GTPγ^{35}S binding on their own, but became partial agonists in the presence of 30 μM CGP7930 or GS39783. Apparently the modulators amplified hidden, marginal agonistic effects of these two compounds. Partial agonistic properties of CGP35348 and 2-hydroxy-saclofen were also seen in seemingly more sensitive cyclic AMP (cAMP) measurements and were further enhanced by 10 μM CGP7930 and GS39783 (Urwyler et al. 2005).

Matsushita et al. (2010) have looked at the mechanisms of $GABA_B$ receptor activation in a novel way. They fused each $GABA_B$ receptor subunit with either Cerulean or enhanced yellow fluorescent protein at intracellular loops and measured changes in fluorescence resonance energy transfer (FRET) after agonist application. FRET decreases were observed between $GABA_{B1a}$ loop 2 and $GABA_{B2}$ loops 1 or 2. These FRET decreases were more pronounced when 3 or 10 μM GABA were applied together with CGP7930 (100 μM); the allosteric modulator alone had no effect. In contrast, intrasubunit constructs labelled with Cerulean at the C terminus and enhanced yellow fluorescent protein at the intracellular loop 1 of either subunit did not reveal any FRET change upon application of GABA, with or without CGP7930. The authors proposed a model according to which $GABA_B$ receptor activation would result in the widening of a cleft between the two receptor subunits, leaving the configuration of the transmembrane domains of each subunit unchanged. CGP7930 would act by binding in the cleft, thereby further widening it. However, this model seems difficult to reconcile with the view that CGP7930 acts through the $GABA_{B2}$ subunit in the transmembrane domain as discussed above.

Whereas radioligand binding and GTPγ^{35}S stimulation assays are mostly performed on membrane preparations, the two prototypical positive $GABA_B$ receptor modulators were also tested in a number of biochemical and electrophysiological experiments in cellular or intact tissue preparations. CGP7930 (10 μM) and GS39783 (10 μM) enhanced the potency of GABA to inhibit adenylyl cyclase activity in a recombinant $GABA_B$ receptor expressing CHO cell line (Urwyler et al. 2005). This experimental system was highly sensitive, apparently due to a substantial degree of receptor reserve, the potency of GABA being considerably higher than in GTPγ^{35}S assays. This high sensitivity again allowed to detect a low degree of partial agonistic activity of CGP7930 and GS39783 on their own, via the allosteric site.

Masharina et al. (2012) have developed an elegant $GABA_B$ receptor-based biosensor to determine concentrations of GABA and synthetic $GABA_B$ receptor ligands on the surface of living cells, using a FRET readout. In their hands, 30 μM CGP7930 increased the receptor affinity for GABA, and thereby the sensitivity of the biosensor, by a threefold.

In *Xenopus laevis* oocytes which were injected with mRNA for the two $GABA_B$ receptor subunits and for inwardly rectifying (Kir3) potassium channels, the application of 0.3 μM GABA elicited potassium currents which were enhanced in the presence of 30 μM CGP7930 or 3 μM GS39783 (Urwyler et al. 2001, 2003). In the case of CGP7930, this effect was observed with both $GABA_B$ receptor isoforms $GABA_{B(1a/2)}$ and $GABA_{B(1b/2)}$. No potassium currents were produced by either of the modulators alone. In human embryonic kidney (HEK293) cells transiently co-transfected with $GABA_B$ receptors and a chimeric G-protein coupling them to the phospholipase C pathway, the application of GABA elicited a transient increase in intracellular cytosolic calcium concentration. Both modulators enhanced that signal with EC_{50}-values in the low micromolar range, depending on the GABA concentrations used, with lower EC_{50}s at higher GABA concentrations. This is a good example of the reciprocity of allosteric modulation mentioned in the Introduction. In the

case of GS39783, this effect was again observed with both receptor isoforms, and the modulator not only increased the potency, but also the maximal efficacy of GABA. No intrinsic agonist activity was observed with either CGP7930 or GS39783 in this assay system (Urwyler et al. 2001, 2003). GABAergic effects on intracellular calcium were also observed in a neuronal network preparation. *R*(−)-baclofen reduces the frequency of synchronized intracellular calcium oscillations in mouse or rat cortical neurons in primary culture. Both CGP7930 (0.3 μM) and GS39783 (1 and 3 μM) further reduced the calcium oscillation frequency in the presence of baclofen, but not on their own (Urwyler et al. 2001; Gjoni and Urwyler 2008). In a hippocampal slice preparation, two consecutive stimulations of afferent pathways result in inhibition of the second population response as a consequence of the activity of GABAergic interneurons. Activation of presynaptic $GABA_B$ receptors will inhibit GABA release from these interneurons and thereby reverse paired pulse inhibition. This is what was observed with 10 μM GS39783 applied alone, like with baclofen (Urwyler et al. 2003). The effects of both compounds were counteracted by the competitive antagonist CGP55845A, indicating that the reversal of paired pulse inhibition by GS39783 was due to a potentiation of the activity of endogenous GABA rather than a direct activation of presynaptic $GABA_B$ receptors. On a similar line, Chen et al. (2006) have found that CGP7930 enhances the inhibitory effects of baclofen on synaptic inhibition in the CA1 area of the hippocampus. Also, CGP7930 enhanced the inhibitory effect of baclofen on dopamine neuron activity in rat midbrain slices (Chen et al. 2005).

$GABA_B$ receptors couple to multiple intracellular pathways, either directly or via "crosstalk" with other G protein-coupled receptors. Onali et al. (2003) have studied the allosteric effects of CGP7930 on native $GABA_B$ receptors in membrane preparations from different rat brain regions. In membranes from olfactory bulb and frontal cortex, $GABA_B$ receptor activation enhances basal and corticotropin-releasing hormone-stimulated adenylyl cyclase activity via the β/γ-subunits of $G_{i/o}$-proteins. In both cases, CGP7930 at concentrations in the range from 10 to 100 μM enhanced the stimulatory effects on cAMP formation produced by baclofen or GABA, without having any agonistic effect on its own. It increased both the potencies and maximal effects of the two agonists. On the other hand, when inhibition of forskolin- or Ca^{2+}/calmodulin-stimulated adenylyl cyclase (in frontal cortex or striatum and cerebellum, respectively) was measured, CGP7930 increased the potency of baclofen or GABA with little or no effect on the maximal inhibition. These results indicate that signalling via both the α- and β/γ-subunits of the $G_{i/o}$-proteins coupled to $GABA_B$ receptors is enhanced by CGP7930.

Mannoury la Cour et al. (2008) used an immunocapture scintillation proximity assay to investigate the coupling of $GABA_B$ receptors to different G-protein subtypes. GABA and (R)-baclofen stimulated GTPγ^{35}S binding to $G\alpha_o$ and $G\alpha_{i1/3}$, but not to $G\alpha_q$ and $G\alpha_{s/olf}$ in membranes from rat cortex, hippocampus and cerebellum. CGP7930 and GS39783 did not stimulate GTPγ^{35}S binding on their own, but they enhanced agonist potencies and maximal efficacy for the stimulation of GTPγ^{35}S binding to $G\alpha_o$, but not to $G\alpha_{i1/3}$, in all three brain regions. On the other hand, in a recombinant human embryonic kidney (HEK) cell line expressing $GABA_B$ receptors,

the modulators enhanced the potency, but not the maximal efficacy, of GABA to stimulate GTPγ^{35}S binding to $G\alpha_{i1/3}$. This G-protein subtype might have been a limiting factor in both native and recombinant assay systems.

In cultured cerebellar granule neurons, GABA and baclofen induce ERK1/2 phosphorylation (Tu et al. 2007). This effect was also found with 50 μM CGP7930 applied on its own, this assay system thus giving another example of allosteric agonism by this compound. Again using cerebellar granule neurons, research from the same group (Tu et al. 2010) later demonstrated interesting neuroprotective effects of CGP7930. Using potassium deprivation to induce apoptosis in these neurons, they observed that baclofen (30 μM) and CGP7930 (3–30 μM) on its own significantly decreased the number of apoptotic neurons. This neuroprotection seemed to follow a complex pathway involving $GABA_B$/insulin-like growth factor 1 receptor transactivation ("crosstalk") and ultimately leading to inhibition of caspase-3 activity (Tu et al. 2010).

Receptor desensitization is a mechanism often involved in the development of tolerance upon chronic drug treatment. Rapid development of tolerance is indeed one of the major problems in the clinical use of baclofen, at least for some indications (Vacher and Bettler 2003). Because of their use-dependent mechanism of action, positive allosteric modulators can be expected to induce less receptor desensitization than orthosteric agonists. We tested this hypothesis by measuring different functional responses after continuous exposure of native or recombinant $GABA_B$ receptors on the one hand to desensitizing agonist concentrations and, on the other hand, to a combination of a low agonist concentration and GS39783 that activated the receptor to the same extent (Gjoni and Urwyler 2008). In our recombinant $GABA_B$ receptor expressing CHO cell line, we observed a decrease of the potency of GABA to inhibit adenylyl cyclase activity after pre-exposure to a saturating concentration (100 μM) of GABA. On the other hand, no such desensitization was seen after pre-exposure to a low GABA concentration (0.3 μM) in combination with 10 μM GS39783. We made similar observations in primary neuronal cultures for baclofen-induced inhibition of spontaneous Ca^{2+} oscillations (Gjoni and Urwyler 2008). Thus it seems that the change in receptor conformation induced by GS39783 would only enhance functional $GABA_B$ receptor-mediated responses, but not its desensitization pathway—a finding reminiscent of the phenomenon of "agonist-directed trafficking". However, because of the probe-dependence of allosteric effects mentioned above, the outcome in such experiments might be different with an allosteric modulator other than GS39783.

In this context, it is also of interest to note that cAMP measurements in a recombinant cell line revealed that GS39783 became an allosteric agonist at desensitized $GABA_B$ receptors in which the activation mechanisms of the receptor have apparently undergone fundamental changes (Gjoni and Urwyler 2009).

The prototypical positive allosteric $GABA_B$ receptor modulators CGP7930 and GS39783 have of course fostered the search for more compounds with such properties. The extracellular Ca^{2+} sensing receptor is allosterically modulated by amino acids and arylalkylamines. For this reason, Kerr et al. (2002) and Kerr and Ong (2003) have examined the effects of such compounds on $GABA_B$ receptor-mediated

responses. They concluded that several amino acids, dipeptides and arylalkylamines such as fendiline are allosteric modulators because they enhanced baclofen-induced field potentials in rat neocortical slices, without having an effect on their own. However, in our radioligand binding, GTPγ^{35}S stimulation and intracellular Ca^{2+} signalling assays, these compounds were completely inactive (Urwyler et al. 2004). It therefore appears that the observations made in brain tissue slices are due to "downstream" effects, rather than allosteric mechanisms. Later on, Kerr et al. (2006, 2007) have made a series of derivatives of CGP7930, none of which, however, surpassed the lead compound in terms of potency and efficacy.

In an effort to obtain allosteric $GABA_B$ receptor modulators devoid of the genotoxic potential of GS39783, Guery et al. (2007) made a number of derivatives thereof lacking the nitro group. The most active molecule from their series is BHF177, with a potency (EC_{50} = 1.7 μM) and cooperative effects in the GTPγ^{35}S assay similar to those of the lead compound.

The compound rac-BHFF (Malherbe et al. 2008) acted in a way similar to CGP7930, but with higher potency (EC_{50} = 234 nM) at recombinant $GABA_B$ receptors. In GTPγ^{35}S and intracellular calcium mobilization assays, it enhanced both the potency and maximal effects of GABA. However, unlike CGP7930, it stimulated GTPγ^{35}S binding also in the absence of GABA (allosteric agonism). (+)-BHFF was more potent than (−)-BHFF, the first described case of enantioselectivity at the allosteric $GABA_B$ receptor binding site. In the FRET-biosensor setup by Masharina et al. (2012), rac-BHFF at 10 μM increased the affinity of GABA by a ninefold and was thus more potent than CGP7930. Because rac-BHFF is quickly hydrolysed in vivo, Malherbe et al. (2008) also made its more stable lactam analogue BHFI, which has similar activity as a positive allosteric $GABA_B$ receptor modulator. The more recently described positive modulator ADX71943 (Kalinichev et al. 2014a) also has good, submicromolar potency in intracellular calcium mobilization and GTPγ^{35}S binding assays.

Another positive allosteric modulator which was found in a high-throughput screening campaign is the compound CMPPE (Perdonà et al. 2011). In GTPγ^{35}S assays in a recombinant cell line expressing the human $GABA_B$ receptor or in rat cortical membranes, it enhanced the stimulation produced by a low concentration of GABA in a way seemingly similar to that of CGP7930 and GS39783. However, unlike the two prototypical modulators, CMPPE displayed a high degree of allosteric agonism, stimulating GTPγ^{35}S binding on its own to a maximal level approximately the same as that produced by GABA. It thus seems difficult to distinguish to which degree the GABA-enhancing effects of CMPPE were truly of cooperative nature or rather due to additivity of agonism through the orthosteric and allosteric sites. On the other hand, when CMPPE (1 μM) was tested on currents through inwardly rectifying potassium channels in rat hippocampal neurons, it was found, like GS39783, to enhance currents produced by baclofen but did not evoke any current when applied alone up to 10 μM.

Interestingly, the two compounds COR627 and COR628 were identified by a virtual screening protocol using a pharmacophore model based on previously described positive allosteric $GABA_B$ receptor modulators (Castelli et al. 2012). In

rat cortical membranes, both compounds enhanced GABA- or baclofen-stimulated GTPγ^{35}S binding at low micromolar concentrations, without any agonist effect when given alone. However, unlike the observations made with CGP7930 and GS39783, the allosteric effect of these compounds consisted almost solely in binding cooperativity, i.e. an increase in the potency of GABA. The maximal efficacy of GABA was only little (with COR627) or not at all (with COR628) increased by these two compounds. In radioligand binding experiments (displacement of an antagonist radioligand by GABA in rat cortical membranes), both compounds enhanced the affinities of GABA for both high- and low-affinity sites. Unlike CGP7930 and GS39783, the two COR compounds did not increase the relative proportion of the high agonist affinity component, suggesting that they do not facilitate the coupling between the receptor and its G-protein. COR627 and COR628 then served as a starting point for the synthesis of a whole series of 2-(acylamino) thiophene derivatives (Mugnaini et al. 2013). These molecules had a profile in vitro similar to that of the two COR compounds. Although their potency in vitro was lower compared to the reference compound GS39783, a few among them showed good effectiveness in vivo.

Until recently, no negative allosteric $GABA_B$ receptor modulators have been known. From a synthetic program based on the scaffold of CGP7930, Chen et al. (2014) obtained three compounds which were found to inhibit GABA-induced inositol phosphate 3 (IP3) production in HEK cells overexpressing $GABA_B$ receptors together with a G_q-protein. They did so by decreasing the maximal effect of GABA without changing its EC_{50}, thus acting as non-competitive antagonists. For one of the compounds it was shown in a radioligand binding assay that it did not bind to the orthosteric receptor site, thus confirming that it acted as an allosteric modulator with negative efficacy cooperativity. Furthermore, these compounds not only inhibited baclofen-induced ERK1/2 phosphorylation in HEK293 cells overexpressing $GABA_B$ receptors, but they also blocked the stimulation of this same pathway by CGP7930, which had been previously shown to be an allosteric agonist in this assay system (see above, Tu et al. 2007). Most likely the compounds inhibited the effect of baclofen by negative modulation, whereas the stimulation by CGP7930 may well have been blocked by a competitive mechanism at the allosteric site, in the light of the structural similarity between the positive and negative modulators. It would be interesting to see how these negative modulators behave in membrane-based assays (GTPγ^{35}S and radioligand binding), in particular whether their lack of binding cooperativity could be confirmed.

Taken together, screening and synthetic chemistry efforts have yielded so far a number of interesting allosteric $GABA_B$ receptor modulators displaying binding and/or efficacy cooperativity toward orthosteric agonists in different ways, and with different intrinsic agonist activity. The two prototypical modulators CGP7930 and GS39783, along with COR627 and COR628, seem to be among those exerting the lowest degree of allosteric agonism and might therefore be considered the allosteric $GABA_B$ receptor modulators acting mostly through binding and/or activation cooperativity.

18.3 Effects of Positive Allosteric $GABA_B$ Receptor Modulators In Vivo

To address the question of whether allosteric $GABA_B$ receptor modulators act in the same way in a living organism, we have carried out an in vivo microdialysis study measuring cyclic AMP formation in the brain of freely moving rats (Gjoni et al. 2006). Orally applied GS39783 dose dependently inhibited cAMP formation in rat striatum only in conjunction with a threshold concentration of locally administered baclofen (Fig.18.5). On its own, GS39783 at the doses tested was inactive, suggesting a sub-threshold endogenous GABA-tone. The inhibition of forskolin-stimulated cAMP production by GS39783 and baclofen was reversed by a competitive $GABA_B$ receptor antagonist. Thus, just as the early in vitro GTPγ^{35}S experiments, this study at the biochemical-mechanistic level in vivo demonstrated that the effects of GS39783 are dependent on the concomitant activation of the orthosteric agonist site.

The agonist baclofen has long been the almost only available tool compound to investigate the role of $GABA_B$ receptors in behavioural processes. However, baclofen induces sedation, hypothermia and muscle relaxation, which may seriously interfere with behavioural observations in animals. The effectiveness of

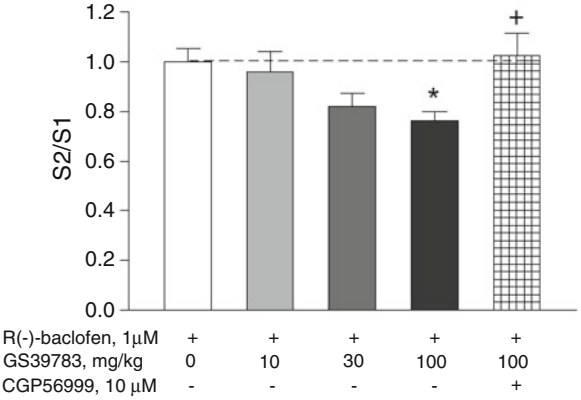

Fig. 18.5 GS39783 enhances the inhibition of cAMP formation by the $GABA_B$ agonist baclofen in vivo. cAMP concentrations were measured in rat brain striatum by in vivo microdialysis. Adenylyl cyclase was stimulated by two consecutive administrations of a water-soluble forskolin analogue through the dialysis probe. Drugs were administered before the second stimulation, and drug effects were calculated as the ratio between the areas under the two cAMP peaks (*S2*/*S1*). Baclofen was administered through the dialysis probe at a threshold concentration (1 μM), which by itself did not evoke a detectable inhibition of cAMP formation (*S2*/*S1* = 1). However, when the positive allosteric modulator GS39783 was administered orally in addition to 1 μM baclofen, a dose-dependent and significant (*asterisk*) inhibition of cAMP production was observed. This inhibition was significantly (+) reverted by coapplication of the competitive antagonist CGP56999A, indicating the dependence of the effects of GS39783 on the presence of an orthosteric agonist. GS39783 alone had no effect (not shown). Reproduced from reference Gjoni et al. (2006), with permission

CGP7930 in vivo has in fact been demonstrated by its ability to enhance the sedative/hypnotic effects (loss of righting reflex) of threshold doses of the $GABA_B$ receptor agonists baclofen and γ-hydroxybutyrate (GHB) in DBA mice (Carai et al. 2004). Conversely, at the doses tested CGP7930 did not induce any loss of righting reflex when administered alone. Similar results were obtained with COR627 and COR628 (Castelli et al. 2012). Koek et al. (2010) have also observed that CGP7930 and rac-BHFF enhanced baclofen- and GHB-induced loss of righting reflex, but not hypothermia. These results have fostered the interest in testing allosteric modulators, which are potentially devoid of the unwanted effects of $GABA_B$ receptor agonists such as baclofen, in behavioural paradigms. Numerous such studies have been performed in recent years; many of them are covered in separate chapters in this book and are therefore only briefly mentioned here, mainly highlighting the impact of some mechanistic aspects on the in vivo situation.

In pigeons discriminating baclofen from saline, CGP7930 and rac-BHFF enhanced the discriminative stimulus effects of baclofen (but not of GHB), but produced only a partial baclofen-appropriate responding by themselves (Koek et al. 2012). In the latter regard, rac-BHFF was more effective than CGP7930, a finding possibly related to the fact that rac-BHFF has a higher intrinsic agonist efficacy at the $GABA_B$ receptor. In pigeons which were, conversely, trained to discriminate rac-BHFF from its vehicle, the discriminative stimulus produced by rac-BHFF was not mimicked by baclofen and not antagonized by the competitive antagonist CGP35348 (Koek et al. 2013). On the other hand, it was attenuated by CGP7930, suggesting that rac-BHFF produces its effects by directly activating the $GABA_{B2}$ receptor subunit. For more details on $GABA_B$ receptor-mediated mechanisms in drug discrimination paradigms, see Chap. 9 of this book.

Cryan et al. (2004) studied the effects of GS39783 in various rodent models for anxiety and depression. Treatment with GS39783 produced anxiolytic-like activity in the light-dark box and elevated zero maze tests, but no effect of GS39783 was found in the forced swim test for antidepressant activity. Importantly, the positive modulator did not show any of the side-effects known to be associated with the use of baclofen or benzodiazepines. A similar profile was later reported for CGP7930, which showed anxiolytic-like activity in different mouse models without inducing motor impairment or hypothermia (Jacobson and Cryan 2008). The role of the $GABA_B$ receptor and its pharmacology in psychiatric indications is covered in detail in Chap. 12 of this book.

$GABA_B$ receptors seem to play an important role in drug addiction (Filip and Frankowska 2008). Baclofen reduces the consumption of different drugs of abuse in laboratory animals and seemingly also in humans, an effect most likely due to the fact that it inhibits nicotine-, cocaine- and opiate-induced release of dopamine in the nucleus accumbens, which is believed to mediate the rewarding effects of drugs of abuse. Xi et al. (2003) and Amantea et al. (2004) have reported that on repeated administration of cocaine or nicotine, respectively, to rats, $GABA_B$ receptor density in the nucleus accumbens was not altered. However, the level of G-protein coupling to the receptor, assessed by the stimulation of $GTP\gamma^{35}S$ binding by baclofen, was reduced. It is exactly in this type of situation that positive allosteric modulators are expected to be of benefit, because at least some precisely act by enhancing the

efficiency of the coupling of the $GABA_B$ receptor to its G-proteins. Lhuillier et al. (2007) have aligned behavioural effects of short- and long-term cocaine administration with several biochemical correlates. GS39783 counteracted the increase in both locomotion and in striatal expression of the immediate early gene c-fos produced by acutely administered cocaine. After long-term cocaine treatment, GS39783 somewhat attenuated behavioural sensitization and at the same time reduced the upregulation of cAMP-response-element-binding protein (CREB) and dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) and the accumulation of the transcription factor ΔFosB in the nucleus accumbens and/or dorsal striatum. These findings strongly suggest that the positive $GABA_B$ receptor modulator prevents long-term adaptive changes in dopaminergic signalling pathways induced by chronic cocaine intake. In a similar study, it was shown that GS39783 blocked the rewarding effects of nicotine in rats; at the same time, GS39783 inhibited nicotine-induced accumulation of ΔFosB in the nucleus accumbens of these animals (Mombereau et al. 2007). Numerous behavioural studies have been performed in recent years to investigate the role of $GABA_B$ receptors in alcohol and drug use disorder. They have been reviewed recently by Filip et al. (2015) and are covered in detail in Chaps. 14 and 15 of this book. The compound ADX71441 is the first positive allosteric $GABA_B$ receptor modulator to be tested clinically, after it has been found to reduce voluntary alcohol consumption in two animal models of binge-like drinking and of long-term excessive drinking, respectively (Hwa et al. 2014).

Systemic CGP7930, like baclofen, has analgesic properties in a mechanically evoked visceral pain model (Brusberg et al. 2009). Both drugs reduced colorectal distension-induced visceromotor and cardiovascular responses in conscious rats. $GABA_B$ receptors might therefore be a promising target for the treatment of painful gastrointestinal disorders, such as irritable bowel syndrome. Peripheral $GABA_B$ receptors are localized on nerve endings innervating different regions of the gastrointestinal tract. Allosteric modulators devoid of blood–brain barrier permeability would selectively target these peripheral receptors. One such compound might be ADX71943, which also showed analgesic effects in peripheral pain models (acetic acid-induced writhing, formalin tests), but was suggested to have a fully peripheral activity profile based on its lack of anxiolytic-like activity (marble burying, elevated plus maze tests) despite reaching high concentrations in plasma (Kalinichev et al. 2014a). Also, ADX71943 had no effect on body temperature, muscle relaxation (rotarod test) and spontaneous locomotor activity. Another clinical indication calling for a drug acting only peripherally is overactive bladder syndrome, in animal models for which ADX71441 proved to be effective (Kalinichev et al. 2014b).

18.4 Outlook: Therapeutic Perspectives

Allosteric $GABA_B$ receptor modulators have come a long way since their first description in 2001. High-throughput screening and synthetic chemistry efforts have yielded a variety of structural classes of compounds acting through this

mechanism. Most of these have served as research tools, while only few compounds have reached a development stage close to clinical trials. The optimization of such molecules in terms of potency, selectivity, in vivo efficacy and desired brain permeability (depending on peripheral or central site of action, respectively) still remains challenging for pharmacologists and medicinal chemists. On the other hand, based on extensive testing in a large number of animal models in vivo, it appears that allosteric $GABA_B$ receptor modulators hold their promise of a better side-effect profile compared to the agonist baclofen, because of their use-dependent mechanism.

The clinical indications for which a therapy with allosteric $GABA_B$ receptor modulators might be successful include anxiety, epilepsy, pain, drug abuse, gastrointestinal disorders, overactive bladder syndrome and possibly more. The $GABA_B$ receptor agonist baclofen has been in clinical use as an antispastic agent for decades. However, its strong muscle-relaxant property precludes its therapeutic application in other indications, in which it would be an unwanted side-effect. On the other hand, the fact that allosteric modulators are devoid of this side-effect in animal experiments means that spasticity will likely not be a potential indication for such compounds. It thus seems that $GABA_B$ receptor agonists and positive modulators might well cover complementary sets of clinical indications. The first results from clinical trials should become available in a not too distant future.

References

Amantea, D., Tessari, M., & Bowery, N. G. (2004). Reduced G-protein coupling to the $GABA_B$ receptor in the nucleus accumbens and the medial prefrontal cortex of the rat after chronic treatment with nicotine. *Neuroscience Letters, 355*, 161–164.

Binet, V., Brajon, C., Le Corre, L., Acher, F., Pin, J. P., & Prézeau, L. (2004). The heptahelical domain of $GABA_{B2}$ is activated directly by CGP7930, a positive allosteric modulator of the $GABA_B$ receptor. *Journal of Biological Chemistry, 279*, 29085–29091.

Bowery, N. G. (Ed.). (2006). *Allosteric receptor modulation in drug targeting*. London: Taylor & Francis.

Brown, K. M., Roy, K. K., Hockerman, G. H., Doerksen, R. J., & Colby, D. A. (2015). Activation of the γ-aminobutyric acid type B ($GABA_B$) receptor by agonists and positive allosteric modulators. *Journal of Medicinal Chemistry, 58*, 6336–6347.

Brusberg, M., Ravnefjord, A., Martinsson, R., Larsson, H., Martinez, V., & Lindström, E. (2009). The $GABA_B$ receptor agonist, baclofen, and the positive allosteric modulator, CGP7930, inhibit visceral pain-related responses to colorectal distension in rats. *Neuropharmacology, 56*, 362–367.

Carai, M. A. M., Colombo, G., Froestl, W., & Gessa, G. L. (2004). In vivo effectiveness of CGP7930, a positive allosteric modulator of the $GABA_B$ receptor. *European Journal of Pharmacology, 504*, 213–216.

Castelli, M. P., Casu, A., Casti, P., Lobina, C., Carai, M. A. M., Colombo, G., et al. (2012). Characterization of COR627 and COR628, two novel positive allosteric modulators of the $GABA_B$ receptor. *Journal of Pharmacology and Experimental Therapeutics, 340*, 529–538.

Changeux, J. P. (2013). The concept of allosteric interaction and its consequences for the chemistry of the brain. *Journal of Biological Chemistry, 288*, 26969–26986.

Chen, L. H., Sun, B., Zhang, Y., Xu, T. J., Xia, Z. X., Liu, J. F., et al. (2014). Discovery of a negative allosteric modulator of $GABA_B$ receptors. *ACS Medicinal Chemistry Letters, 5*, 742–747.

Chen, Y., Menendez-Roche, N., & Sher, E. (2006). Differential modulation by the $GABA_B$ receptor allosteric potentiator 2,6-di-tert-butyl-4-(3-hydroxy-2,2dimethylpropyl)-phenol (CGP7930) of synaptic transmission in the rat hippocampal CA1 area. *Journal of Pharmacology and Experimental Therapeutics, 317*, 1170–1177.

Chen, Y., Phillips, K., Minton, G., & Sher, E. (2005). $GABA_B$ receptor modulators potentiate baclofen-induced depression of dopamine neuron activity in the rat ventral tegmental area. *British Journal of Pharmacology, 144*, 926–932.

Christopoulos, A. (2014). Advances in G protein-coupled receptor allostery: From function to structure. *Molecular Pharmacology, 86*, 463–478.

Christopoulos, A., & Kenakin, T. (2002). G protein-coupled receptor allosterism and complexing. *Pharmacological Reviews, 54*, 323–374.

Conn, P. J., Christopoulos, A., & Lindsley, C. W. (2009). Allosteric modulators of GPCRs: A novel approach for the treatment of CNS disorders. *Nature Reviews Drug Discovery, 8*, 41–54.

Cryan, J. F., Kelly, P. H., Chaperon, F., Gentsch, C., Mombereau, C., Lingenhoehl, K., et al. (2004). Behavioral characterization of the novel $GABA_B$ receptor-positive modulator GS39783 (N,N-dicyclopentyl-2methylsulfanyl-5-nitro-pyrimidine-4,6-diamine): Anxiolytic-like activity without side effects associated with baclofen or benzodiazepines. *Journal of Pharmacology and Experimental Therapeutics, 310*, 952–963.

Dupuis, D. S., Relkovic, D., Lhuillier, L., Mosbacher, J., & Kaupmann, K. (2006). Point mutations in the transmembrane region of $GABA_{B2}$ facilitate activation by the positive modulator N,N-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6diamine (GS39783) in the absence of the $GABA_{B1}$ subunit. *Molecular Pharmacology, 70*, 2027–2036.

Filip, M., & Frankowska, M. (2008). $GABA_B$ receptors in drug addiction. *Pharmacological Reports, 60*, 755–770.

Filip, M., Frankowska, M., Sadakierska-Chudy, A., Suder, A., Szumiec, L., Mierzejewski, P., et al. (2015). $GABA_B$ receptors as a therapeutic strategy in substance use disorders: Focus on positive allosteric modulators. *Neuropharmacology, 88*, 36–47.

Froestl, W. (2010). Novel $GABA_B$ receptor positive modulators: A patent survey. *Expert Opinion on Therapeutic Patents, 20*, 1007–1017.

Gjoni, T., Desrayaud, S., Imobersteg, S., & Urwyler, S. (2006). The positive allosteric modulator GS39783 enhances $GABA_B$ receptor-mediated inhibition of cyclic AMP formation in rat striatum *in vivo. Journal of Neurochemistry, 96*, 1416–1422.

Gjoni, T., & Urwyler, S. (2008). Receptor activation involving positive allosteric modulation, unlike full agonism, does not result in $GABA_B$ receptor desensitization. *Neuropharmacology, 55*, 1293–1299.

Gjoni, T., & Urwyler, S. (2009). Changes in the properties of allosteric and orthosteric $GABA_B$ receptor ligands after a continuous, desensitizing agonist pretreatment. *European Journal of Pharmacology, 603*, 37–41.

Guery, S., Floersheim, P., Kaupmann, K., & Froestl, W. (2007). Syntheses and optimization of new GS39783 analogues as positive allosteric modulators of $GABA_B$ receptors. *Bioorganic and Medicinal Chemistry Letters, 17*, 6206–6211.

Hall, D. A. (2000). Modeling the functional effects of allosteric modulators at pharmacological receptors: An extension of the two-state model of receptor activation. *Molecular Pharmacology, 58*, 1412–1423.

Hwa, L. S., Kalinichev, M., Haddouk, H., Poli, S., & Miczek, K. A. (2014). Reduction of excessive alcohol drinking by a novel $GABA_B$ receptor positive allosteric modulator ADX71441 in mice. *Psychopharmacology, 231*, 333–343.

Jacobson, L. H., & Cryan, J. F. (2008). Evaluation of the anxiolytic-like profile of the $GABA_B$ receptor positive modulator CGP7930 in rodents. *Neuropharmacology, 54*, 854–862.

Kalinichev, M., Donovan-Rodriguez, T., Girard, F., Riguet, E., Rouillier, M., Bournique, B., et al. (2014a). Evaluation of peripheral versus central effects of $GABA_B$ receptor activation using a novel, positive allosteric modulator of the $GABA_B$ receptor ADX71943, a pharmacological tool compound with a fully peripheral activity profile. *British Journal of Pharmacology, 171*, 4941–4954.

Kalinichev, M., Palea, S., Haddouk, H., Royer-Urios, I., Guilloteau, V., Lluel, P., et al. (2014b). ADX71441, a novel, potent and selective positive allosteric modulator of the $GABA_B$ receptor, shows efficacy in rodent models of overactive bladder. *British Journal of Pharmacology, 171*, 995–1006.

Kerr, D. I., Khalafy, J., Ong, J., Perkins, M. V., Prager, R. H., Puspawati, N. M., et al. (2006). Synthesis and biological activity of allosteric modulators of $GABA_B$ receptors, part 2. 3-(2,6-Bis-tert-butyl-4-hydroxyphenyl)propanols. *Australian Journal of Chemistry, 59*, 457–462.

Kerr, D. I., Khalafy, J., Ong, J., Prager, R. H., & Rimaz, M. (2007). Synthesis and biological activity of allosteric modulators of $GABA_B$ receptors part 3. 3-(2,6-Bis-iso-propyl-4-hydroxyphenyl) propanols. *Journal of the Brazilian Chemical Society, 18*, 721–727.

Kerr, D. I., & Ong, J. (2003). Potentiation of metabotropic $GABA_B$ receptors by L-aminoacids and dipeptides in rat neocortex. *European Journal of Pharmacology, 468*, 103–108.

Kerr, D. I., Ong, J., Puspawati, N. M., & Prager, R. H. (2002). Arylalkylamines are a novel class of positive allosteric modulators at $GABA_B$ receptors in rat neocortex. *European Journal of Pharmacology, 51*, 69–77.

Koek, W., Cheng, K., & Rice, K. C. (2013). Discriminative stimulus effects of the $GABA_B$ receptor-positive modulator rac-BHFF: Comparison with $GABA_B$ receptor agonists and drugs of abuse. *Journal of Pharmacology and Experimental Therapeutics, 344*, 553–560.

Koek, W., France, C. P., Cheng, K., & Rice, K. C. (2010). $GABA_B$ receptor-positive modulators: Enhancement of $GABA_B$ receptor agonist effects in vivo. *Journal of Pharmacology and Experimental Therapeutics, 335*, 163–171.

Koek, W., France, C. P., Cheng, K., & Rice, K. C. (2012). Effects of the $GABA_B$ receptor-positive modulators CGP7930 and rac-BHFF in baclofen- and γ-hydroxybutyrate-discriminating pigeons. *Journal of Pharmacology and Experimental Therapeutics, 341*, 369–376.

Lhuillier, L., Mombereau, C., Cryan, J. F., & Kaupmann, K. (2007). $GABA_B$ receptor positive modulation decreases selective molecular and behavioral effects of cocaine. *Neuropsychopharmacology, 32*, 388–398.

Malherbe, P., Masciadri, R., Norcross, R. D., Knoflach, F., Kratzeisen, C., Zenner, M. T., et al. (2008). Characterization of (R,S)-5,7-di-tert-butyl-3-hydroxy-3-trifluoromethyl-3H-benzofuran-2-one as a positive allosteric modulator of $GABA_B$ receptors. *British Journal of Pharmacology, 154*, 797–811.

Mannoury la Cour, C., Herbelles, C., Pasteau, V., de Nanteuil, G., & Millan, M. J. (2008). Influence of positive allosteric modulators on $GABA_B$ receptor coupling in rat brain: A scintillation proximity assay characterisation of G protein subtypes. *Journal of Neurochemistry, 105*, 308–323.

Masharina, A., Reymond, L., Maurel, D., Umezawa, K., & Johnsson, K. (2012). A fluorescent sensor for GABA and synthetic $GABA_B$ receptor ligands. *Journal of the American Chemical Society, 134*, 19026–19034.

Matsushita, S., Nakata, H., Kubo, Y., & Tateyama, M. (2010). Ligand-induced rearrangements of the $GABA_B$ receptor revealed by fluorescence resonance energy transfer. *Journal of Biological Chemistry, 285*, 10291–10299.

Mombereau, C., Lhuillier, L., Kaupmann, K., & Cryan, J. F. (2007). $GABA_B$ receptor positive modulation-induced blockade of the rewarding properties of nicotine is associated with a reduction in nucleus accumbens ΔFosB accumulation. *Journal of Pharmacology and Experimental Therapeutics, 321*, 172–177.

Monod, J., Wyman, J., & Changeux, J. P. (1965). On the nature of allosteric transition. *Journal of Molecular Biology, 12*, 88–118.

Mugnaini, C., Pedani, V., Casu, A., Lobina, C., Casti, A., Maccioni, P., et al. (2013). Synthesis and pharmacological characterization of 2-(acylamino)-thiophene derivatives as metabolically stable, orally effective, positive allosteric modulators of the $GABA_B$ receptor. *Journal of Medicinal Chemistry, 56*, 3620–3635.

Olianas, M. C., Ambu, R., Garau, L., & Onali, P. (2005). Allosteric modulation of $GABA_B$ receptor function in human frontal cortex. *Neurochemistry International, 46*, 149–158.

Onali, P., Mascia, F. M., & Olianas, M. C. (2003). Positive regulation of $GABA_B$ receptors dually coupled to cyclic AMP by the allosteric agent CGP7930. *European Journal of Pharmacology, 471*, 77–84.

Perdonà, E., Costantini, V. J. A., Tessari, M., Martinelli, P., Carignani, C., Valerio, E., et al. (2011). *In vitro* and *in vivo* characterization of the novel $GABA_B$ receptor positive allosteric modulator, 2-{1-[2-(4-chlorophenyl)-5-methylpyrazolo[1,5-a]pyrimidin-7-yl]-2-piperidinyl}ethanol (CMPPE). *Neuropharmacology, 61*, 957–966.

Pin, J. P., & Prézeau, L. (2007). Allosteric modulators of $GABA_B$ receptors: Mechanism of action and therapeutic perspective. *Current Neuropharmacology, 5*, 195–201.

Tu, H., Rondard, P., Xu, C., Bertaso, F., Cao, F., Zhang, X., et al. (2007). Dominant role of $GABA_{B2}$ and $G_{\beta\gamma}$ for $GABA_B$ receptor-mediated-$ERK_{1/2}$/CREB pathway in cerebellar neurons. *Cellular Signalling, 19*, 1996–2002.

Tu, H., Xu, C., Zhang, W., Liu, Q., Rondard, P., Pin, J. P., et al. (2010). $GABA_B$ receptor activation protects neurons from apoptosis via IGF-1 receptor transactivation. *Journal of Neuroscience, 30*, 749–759.

Urwyler, S. (2011). Allosteric modulation of family C G-protein-coupled receptors: From molecular insights to therapeutic perspectives. *Pharmacological Reviews, 63*, 59–126.

Urwyler, S., Gjoni, T., Kaupmann, K., Pozza, M. F., & Mosbacher, J. (2004). Selected amino acids, dipeptides and arylalkylamine derivatives do not act as allosteric modulators at $GABA_B$ receptors. *European Journal of Pharmacology, 483*, 147–153.

Urwyler, S., Gjoni, T., Koljatić, J., & Dupuis, D. S. (2005). Mechanisms of allosteric modulation at $GABA_B$ receptors by CGP7930 and GS39783: Effects on affinities and efficacies of orthosteric ligands with distinct intrinsic properties. *Neuropharmacology, 48*, 343–353.

Urwyler, S., Mosbacher, J., Lingenhoehl, K., Heid, J., Hofstetter, K., Froestl, W., et al. (2001). Positive allosteric modulation of native and recombinant γ-aminobutyric $acid_B$ receptors by 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethylpropyl)-phenol (CGP7930) and its aldehyde analog CGP13501. *Molecular Pharmacology, 60*, 963–971.

Urwyler, S., Pozza, M. F., Lingenhoehl, K., Mosbacher, J., Lampert, C., Froestl, W., et al. (2003). N,N′Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) and structurally related compounds: Novel allosteric enhancers of γ-aminobutyric $acid_B$ receptor function. *Journal of Pharmacology and Experimental Therapeutics, 307*, 322–330.

Vacher, C. M., & Bettler, B. (2003). $GABA_B$ receptors as potential therapeutic targets. *Current Drug Targets: CNS & Neurological Disorders, 2*, 248–259.

Xi, Z. X., Ramamoorthy, S., Shen, H., Lake, R., Samuvel, D. J., & Kalivas, P. W. (2003). GABA transmission in the nucleus accumbens is altered after withdrawal from repeated cocaine. *Journal of Neuroscience, 23*, 3498–3505.

Chapter 19
$GABA_B$ Receptor Antagonists as Cognition Enhancers

Furhan Iqbal and Quratul Ane Gillani

Abstract Over two decades of preclinical research demonstrates that the $GABA_B$ receptor has an important role in cognition. In this chapter, we discuss the effects of a number of $GABA_B$ receptor antagonists (CGP 35348, CGP 55845, CGP 36742 (also known as SGS742), CGP 51176, CGP 62349, and CGP 71982) during normal brain functioning and under a variety of pathological conditions in which neuronal functioning was compromised. It was observed that $GABA_B$ receptor antagonists enhanced function, in various animal models, during different cognitive tasks employing spatial learning and memory, as well as during the passive and active avoidance test, indicating that there was large variation in structure and functioning of $GABA_B$ receptors. In addition, we discuss the $GABA_B$ receptor antagonist, SGS742, which has been studied in human clinical trials as a potential pharmacotherapy for mild cognitive impairment.

Keywords $GABA_B$ receptor • Antagonists • Learning • Memory • Clinical trials

19.1 Learning and Memory

Learning is in general a comparatively permanent change in performance caused by an experience (Kandel et al. 2000). Memory is debatably the most basic and important operation of the brain, referring to the knowledge stored in the brain and to the processes of acquiring, consolidating, and retrieving this knowledge (Martin et al. 2000).

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Theoretically and experimentally, memory can be divided into two major types: declarative and nondeclarative. Declarative memory deals with facts and events that can be brought into consciousness (like mental images), whereas nondeclarative memories are knowledge that cannot be intentionally brought into consciousness and, instead, are a collection of various motor skills, habits, and conditioning (Tranel and Damasio 2002). Specific brain regions are associated with various types of learning and memory. The medial temporal lobe, consisting of the hippocampus and adjacent brain regions, is important for forming and organizing memory and appears to play a part in converting memory from short to long term. Cortical areas are important for the long-term storage of knowledge about facts and events and are implicated in everyday situations (Carey et al. 2002). In addition, the amygdala constitutes our emotional memory, and the cerebellum is responsible for motor learning with accurate timing (Tranel and Damasio 2002).

19.2 $GABA_B$ Receptors and Cognition

The role of the GABA type B ($GABA_B$) receptors in cognition has been established for approximately two decades (Bowery et al. 2002). $GABA_B$ receptors can affect the regulation of memory-related neuronal plasticity. Vigot et al. (2006) conducted an object-recognition task in control (wild-type) and $GABA_B$(1a) or $GABA_B$(1b) isoforms knockout mice; they observed that wild-type and $GABA_B$(1b) knockout mice performed the task correctly, attending a novel object for a longer time than the familiar one. Conversely, mice with the $GABA_B$(1a) isoform knockout were unable to perform the object-recognition task and had impaired hippocampal-mediated long-term potentiation (LTP). The impairment in LTP induction in $GABA_B$(1a) knockout mice was due to a decrease in the proportion of silent synapses, which provide an ideal substrate for LTP (Malinow and Malenka 2002). These data have significantly added to our knowledge about the role of $GABA_B$ receptors in rodent LTP as it was the first report on $GABA_B$(1a) and $GABA_B$(1b) receptor subunits being capable of separate in vivo functions.

The entorhinal cortex (EC) densely expresses $GABA_B$ receptors in the principal cells like stellate neurons; however, the functions of $GABA_B$ receptors in this brain region are still not known (Mizukami et al. 2002). A structure like EC is essential for the consolidation and recall of memories (Steffenach et al. 2005). The EC forms the majority of the connections between the hippocampus and other cortical areas (Witter et al. 2000a). Superficial layers (II–III) of the EC, which give rise to dense projections to the hippocampus, include the sensory inputs. Axons of the stellate neurons in layer II of the EC form the perforant path that innervates the dentate gyrus and CA3. Pyramidal neurons in layer III form the temporoammonic pathway that synapses onto the distal dendrites of pyramidal neurons in CA1 and the subiculum (Witter et al. 2000a, b). This extensive networking shows that neurons in the deep layers of the EC (V–VI) depend on a large portion of hippocampal output projections back to the superficial layers of the EC and to other cortical areas

(van Haeften et al. 2003). Neuronal pathology and atrophy of the EC can result in the onset of Alzheimer's disease (Kotzbauer et al. 2001) and schizophrenia (Prasad et al. 2004) and induction and maintenance of temporal lobe epilepsy (Avoli et al. 2002). It has been reported that $GABA_B$ receptor activation leads to drastic inhibition of neuronal excitability in the superficial layers of the EC via activation of TREK-2, a type of two-pore domain potassium (K2P) channel (Deng et al. 2009). Mizukami et al. (2002) reported that a high density of $GABA_B$ receptors are expressed in the EC and activation of $GABA_B$ receptors remarkably depresses neuronal excitability in the EC, suggesting that learning and memory formation is controlled by activation of $GABA_B$ receptors. $GABA_B$ receptor-induced impairment of spatial learning is mediated by the downregulation of protein kinase A (PKA) activity (Huang and Yu 2008). The PKA target is likely the TREK-2 channel because the downregulation of TREK-2 by small interfering RNA (siRNA) prevents $GABA_B$ receptor-mediated impairment of spatial learning (Deng et al. 2009).

19.3 $GABA_B$ Receptor Antagonists and Cognition During Normal Brain Function in Rodents

$GABA_B$ receptors in both pre- and postsynaptic membranes are known to play a role in memory formation via inhibition and excitation of neurons (Mott and Lewis 1994). Behavioral work on the effect of $GABA_B$ receptor blockade has produced results ranging from memory facilitation to impairment (Brucato et al. 1996; Getova et al. 1996). Possible reasons for these inconsistencies include the use of tasks with unknown relationships to hippocampal processes and the failure to establish dose–response relationships between the drug and the behavior tested.

CGP 35348 is one of the $GABA_B$ receptor antagonists capable of crossing the blood–brain barrier (Olpe et al. 1993a, b). Farr et al. (2000) conducted a T maze foot shock avoidance test in 8–10 weeks old male CD-1 mice and documented that CGP 35348 was approximately 200 and 500 times more potent than the $GABA_B$ receptor antagonists OH-saclofen and phaclofen, respectively, in enhancing retention memory. $GABA_B$ receptor activation may regulate pyramidal cell activity in interneurons of the hippocampus. This increased activity on the pyramidal neurons impaired retention, while decreased receptor activity improved retention (Farr et al. 2000). Pitsikas et al. (2003) reported that pretreatment with CGP 35348 prevented performance deficits induced by treatment with the $GABA_B$ receptor agonist, baclofen, in adult Sprague Dawley male rats exposed to an object-recognition task.

It has been reported that inhibition of postsynaptic $GABA_B$ receptors can enhance the period of dendritic *N*-methyl-D-aspartate (NMDA) receptor-mediated currents that contribute to the induction of LTP, while inhibition of presynaptic $GABA_B$ receptors resulted in self-inhibition of γ-aminobutyric acid (GABA) release that does not facilitate the induction of LTP. Recently Gillani et al. (2014a) demonstrated that CGP 35348 treatment affected selected parameters of mouse (BALB/c strain) behavior with remarkable sex differences. Specifically, CGP 35348 treatment improved learn-

ing and memory in male mice during the retention phase of the Morris Water Maze task, while this effect was lacking in female mice. However, the exploratory behavior of CGP 35348-treated female mice was drastically affected: when exposed to an open field, CGP 35348-treated female mice displayed lower locomotor activity than vehicle-treated female rats; conversely, treatment with CGP 35348 did not alter locomotor activity in male mice (Gillani et al. 2014a). It is well established that sex differences in learning are associated with the neuroanatomical differences of males and females (Dalla and Shors 2009) and they are detectable at all behavioral levels of analysis. Even the trait that does not differ between genders may be subserved by different mechanisms in females and males (Cahill 2006).

The $GABA_B$ receptor antagonist CGP 36742 (also known as SGS742) has been demonstrated to be active at pre- and postsynaptic $GABA_B$ receptors, including autoreceptors regulating the release of GABA in the cerebral cortex (Olpe et al. 1993a; Froestl et al. 1995) and hippocampus (Pozza et al. 1999). CGP 36742 has been reported to facilitate memory in a social recognition test in rats (Mondadori et al. 1996a, b) and to improve cognitive performance in mice, rats, and rhesus monkeys (Mondadori et al. 1993; Froestl et al. 1995). In young rodents and non-human primates, CGP 36742 improved performance in a two-way active avoidance task and spatial reference memory in the eight-arm radial and Morris water mazes (Getova and Bowery 2001; Helm et al. 2005).

CGP 36742 is known to facilitate memory formation by regulating the release of somatostatin. In an initial report using superfused rat and human cerebrocortical synaptosomes, CGP 36742 antagonized baclofen-induced inhibition of somastotatin release and acted selectively on the pharmacologically distinct presynaptic $GABA_B$ receptor subtype that regulates the release of somatostatin (Bonanno et al. 1999). Somatostatin receptor activation was also found to decrease GABA release from hippocampal slices (Meyer et al. 1989). In addition, Nyitrai et al. (2003) reported that somatostatin function in the hippocampus of rats can be modulated through CGP 36742 in in vivo and in vitro treatment. Moreover, CGP 36742 may control the release of GABA and glutamate together with somatostatin. The following observations were reported. First, the blockade of CGP 36742-sensitive $GABA_B$ receptors altered somatostatin level and release: CGP 36742 increased the level of extracellular somatostatin in vivo in the hippocampus of anesthetized rats and increased the outflow of somatostatin from hippocampal nerve endings. Second, somatostatin decreased the basal release of GABA and enhanced the basal release of glutamate. Third, pre- and postsynaptic effects of CGP 36742 were altered in the presence of somatostatin: the enhancement of basal GABA release in response to CGP 36742 was inverted to a net decrease whereby basal glutamate release remained elevated; additionally, CGP 36742 increased somatostatin-activated K+ conductance in hippocampal slices. These data suggest pre- and postsynaptic ‘cross talks’ between coexisting $GABA_B$ and somatostatin receptors in the rat hippocampus (Fig. 19.1).

Getova and Bowery (1998) examined the effects of some newly synthesized (at that time) and more potent $GABA_B$ receptor antagonists—namely CGP 71982, CGP 62349, and CGP 55845—in an active avoidance test in rats. They observed

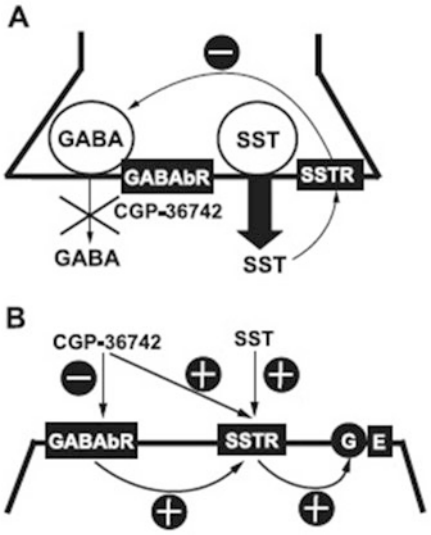

Fig. 19.1 A simplified model of the functional cross talk between coexisting $GABA_B$ and somatostatin receptors. (**a**) Presynaptic cross talk between somatostatin and GABA cotransmitters. (**b**) Postsynaptic interaction of $GABA_B$ and somatostatin receptors. Reproduced from Nyitrai et al. (2003) with permission from Elsevier

that CGP 71982 and CGP 55845, at all doses applied (0.01–1.0 mg/kg), had an improving effect on learning and memory retention. CGP 62349 was only active at the lowest dose tested (0.01 mg/kg).

19.4 $GABA_B$ Receptor Antagonists and Cognition in Neurologically Compromised Rodents

The studies reviewed above suggest that $GABA_B$ receptor antagonists are effective as spatial memory enhancers when administered to "normal," or healthy, rats and mice. However, very limited information is available regarding their potential role in various neurologically compromised rodent models. Below we discuss the reports regarding the effects of $GABA_B$ receptor antagonists affecting cognitive functions is animals after brain damage and/or during various neurological disorders, such as hypoxic ischemic encephalopathy (HIE), age-related decline and, notably, Alzheimer's disease.

In spite of the major advances in monitoring technology and knowledge of fetal and neonatal pathologies, perinatal asphyxia or, more appropriately, HIE remains a serious condition (Busl and Greer 2010). Specifically, severe perinatal HIE is a serious problem in full-term infants and more so in premature infants, with a high risk of long-term behavioral and neurological deficits (Iqbal et al. 2015). In most industrialised countries, the incidence of severe HIE is 2–4:1000 full term births, approaching 60 % in low birth weight premature newborns (Vannucci et al. 2001). Between 20 and 50 % of asphyxiated newborns with HIE die within the newborn period, and up to 25 % of the survivors exhibit permanent neuropsychological handicaps, including

mental retardation, cerebral palsy, epilepsy, or learning disabilities (Vannucci et al. 2001; Vannucci and Hagberg 2004).

Gillani et al. (2014b) applied hypoxia ischemia insult to mouse (BALB/c strain) pups on postnatal day 10 (corresponding to the brain development of 40-week gestational age in human fetus). During postnatal week 13, mice received intraperitoneal injections of either CGP 35348 or saline solution for 12 days. Female mice displayed poor exploratory and locomotory behavior following CGP 35348 treatment. While CGP 35348 improved spatial learning and memory in male mice, cognition remained unaffected in female mice, indicating that CGP 35348 has a potential to improve neuromuscular coordination and spatial learning in a sex-specific manner.

$GABA_B$ receptors on the pre- and postsynaptic membranes are different from one another in their structure and function (Bowery et al. 2002). It has been reported that CGP 55845 has higher affinity for receptors on pre- than postsynaptic membrane (Fukuda et al. 1993). Gillani et al. (2015) also reported the effects of CGP 55845 on mouse behavior following neonatal brain damage. Specifically, treatment with CGP 55845 had no effect on learning and memory formation at the Morris water maze test and brain infract size in both sexes following HIE. However, treatment with CGP 55845 impaired several parameters of locomotor and exploratory behavior in female mice following HIE (Gillani et al. 2015).

Impaired cognitive functions are established facts associated with the aging process. LaSarge et al. (2009) reported a complete reversal of olfactory discrimination learning deficits in cognitively impaired aged Fischer 344 rats by using CGP 55845.

Absence seizures in humans are behaviorally manifested as arrest and mild jerks mainly of facial muscles. Gamma-hydroxybutyrolactone (GHBL) administration is widely used as an experimental model of absence seizures in rats and mice. Getova and Bowery (2001) used GHBL to develop absence seizures [according to Snead (1992)] and treated rats and mice with CGP 55845, CGP 62349, or CGP 71982. They observed that CGP 55845A and CGP 62349 suppressed GHBL-induced absence seizures to a greater extent in mice than rats after 3 or 4 weeks of treatment, while all three tested $GABA_B$ receptor antagonists suppressed development of the absence seizures after 5 weeks of treatment in both mice and rats.

19.5 SGS742, the Only $GABA_B$ Receptor Antagonist Used in Clinical Trials

Despite that a number of studies have indicated the potential role of $GABA_B$ receptor antagonists in rats, mice, and monkeys, very few studies have tested this class of compounds in clinical trials. Froestl et al. (2004) reported that oral administration of SGS742 for 8 weeks significantly improved attention, in particular choice reaction time and visual information processing as well as working memory measured as pattern recognition speed, in 110 volunteers having mild cognitive impairment (MCI). No serious adverse events or drug-related effects on cardiovascular or

laboratory variables were reported. Mild to moderate nonspecific and non-dose-related adverse events, including headache, tiredness, sleepiness, and dizziness, were reported by some subjects. These results suggested that SGS742 may exert beneficial effects on various cognitive domains. However, Sabbagh (2009) failed to confirm those initial results, as they reported that treatment with SGS742 did not meet its efficacy end points in a larger ($N=280$) phase 2b monotherapy trial involving patients with mild to moderate Alzheimer's disease. Therefore, additional clinical studies exploring the use of SGS742 as a treatment for disorders associated with cognitive impairment are warranted.

19.6 Conclusions

In conclusion, we report that a variety of $GABA_B$ receptor antagonists influence cognition-related behaviors in rodents, under normal brain functioning as well as diverse neurological abnormalities. Specific $GABA_B$ receptor antagonists affect specific behavioral aspects indicating that $GABA_B$ receptors are diverse in structure and function of $GABA_B$ receptors and selective antagonists can potentially be used for the treatment of specific cognitive deficits in humans as well. It is evident that further studies are needed to characterize the $GABA_B$ receptor antagonists as a clinical pharmacotherapy.

References

Avoli, M., D'Antuono, M., Louvel, J., Köhling, R., Biagini, G., Pumain, R., et al. (2002). Network and pharmacological mechanisms leading to epileptiform synchronization in the limbic system in vitro. *Progress in Neurobiology, 68*, 167–207.

Bonanno, G., Carita, F., Cavazzani, P., Munari, C., & Raiteri, M. (1999). Selective block of rat and human neocortex GABAB receptors regulating somatostatin release by a GABAB antagonist endowed with cognition enhancing activity. *Neuropharmacology, 38*, 1789–1795.

Bowery, N. G., Bettler, B., Froestl, W., Gallagher, J. P., Marshall, F., Raiteri, M., et al. (2002). International Union of Pharmacology XXXIII. Mammalian gamma-aminobutyric acid (B) receptors: Structure and function. *Pharmacological Reviews, 54*, 247–264.

Brucato, F. H., Levin, E. D., Mott, D. D., Lewis, D. V., Wilson, W. A., & Swartzwelder, H. S. (1996). Hippocampal long-term potentiation and spatial learning in the rat: Effects of GABAB receptor blockade. *Neuroscience, 74*, 331–339.

Busl, K. M., & Greer, D. M. (2010). Hypoxic-ischemic brain injury: Pathophysiology, neuropathology and mechanisms. *NeuroRehabilitation, 26*, 5–13.

Cahill, L. (2006). Why sex matters for neuroscience. *Nature Reviews Neuroscience, 7*, 477–484.

Carey, J., Ariniello, L., & McComb, M. (2002). *Brain facts* (4th ed.). Washington, DC, USA: The Society for Neuroscience.

Dalla, C., & Shors, T. J. (2009). Sex differences in learning processes of classical and operant conditioning. *Physiology and Behavior, 97*, 229–238.

Deng, P. Y., Xiao, Z., Yang, C., Rojanathammanee, L., Grisanti, L., Watt, J., et al. (2009). GABAB receptor activation inhibits neuronal excitability and spatial learning in the entorhinal cortex by activating TREK-2 K+ channels. *Neuron, 63*(2), 230–243.

Farr, S. A., Flood, J. F., & Morley, J. E. (2000). The effect of cholinergic, GABAergic, serotonergic, and glutamatergic receptor modulation on posttrial memory processing in the hippocampus. *Neurobiology of Learning and Memory, 73*, 150–167.

Froestl, W., Gallagher, M., Jenkins, H., Madrid, A., Melcher, T., Teichman, S., et al. (2004). SGS742: The first GABAB receptor antagonist in clinical trials. *Biochemical Pharmacology, 68*, 1479–1487.

Froestl, W., Mickel, S. J., Von Sprecher, G., Diel, P. J., Hall, R. G., Maier, L., et al. (1995). Phosphinic acid analogues of GABA. 2. Selective, orally active GABAB antagonists. *Journal of Medicinal Chemistry, 38*, 3313–3331.

Fukuda, A., Mody, I., & Prince, D. A. (1993). Differential ontogenesis of presynaptic and postsynaptic $GABA_B$ inhibition in rat somatosensory cortex. *Journal of Neurophysiology, 70*, 448–452.

Getova, D., & Bowery, N. G. (1998). The modulatory effects of high affinity $GABA_B$ receptor antagonists in an active avoidance learning paradigm in rats. *Psychopharmacology, 137*, 369–373.

Getova, D., & Bowery, N. (2001). Effects of high-affinity $GABA_B$ receptor antagonists on active and passive avoidance responding in rodents with gamma-hydroxybutyrolactone-induced absence syndrome. *Psychopharmacology, 157*, 89–95.

Getova, D., Bowery, N. G., & Spassov, V. (1996). Effects of $GABA_B$ receptor antagonists on learning and memory retention in a rat model of absence epilepsy. *Pharmacology Reviews and Communications, 8*, 141–143.

Gillani, Q., Akbar, A., Ali, M., & Iqbal, F. (2015). Gender-specific effects of CGP 55845, $GABA_B$ receptor antagonist, on neuromuscular coordination, learning and memory formation in albino mouse following neonatal hypoxia–ischemia insult. *Neurological Science, 36*, 961–969.

Gillani, Q., Ali, M., & Iqbal, F. (2014). CGP 35348, $GABA_B$ receptor antagonist, has a potential to improve neuromuscular coordination and spatial learning in albino mouse following neonatal brain damage. *BioMed Research International, 2014,* 295215. http://dx.doi.org/10.1155/2014/295215.

Gillani, Q. A., Iqbal, S., Arfa, F., Khakwani, S., Akbar, A., Ullah, A., et al. (2014). Effect of GABAB receptor antagonist (CGP 35348) on learning and memory in albino mice. *Scientific World Journal, 2014*, 983651. doi:10.1155/2014/983651.

Helm, K. A., Haberman, R. P., Dean, S. L., Hoyt, E. C., Melcher, T., Lund, P. K., et al. (2005). GABAB receptor antagonist SGS742 improves spatial memory and reduces protein binding to the cAMP response element (CRE) in the hippocampus. *Neuropharmacology, 48*, 956–964.

Huang, D., & Yu, B. (2008). Recent advance and possible future in TREK-2: A two-pore potassium channel may involved in the process of NPP, brain ischemia and memory impairment. *Medical Hypothesis, 70*, 618–624.

Iqbal, S., Ali, M., & Iqbal, F. (2015). Long term creatine monohydrate supplementation, following neonatal hypoxic ischemic insult, improves neuromuscular coordination and spatial learning in male albino mouse. *Brain Research, 1603*, 76–83.

Kandel, E. R., Schwartz, J. H., & Jessell, T. M. (2000). *Principles of neural science* (4th ed.). New York: Elsevier.

Kotzbauer, P. T., Trojanowsk, J. Q., & Lee, V. M. (2001). Lewy body pathology in Alzheimer's disease. *Journal of Molecular Neuroscience, 17*, 225–232.

LaSarge, C. L., Bañuelos, C., Mayse, J. D., & Bizon, J. L. (2009). Blockade of GABA(B) receptors completely reverses age related learning impairment. *Neuroscience, 164*(3), 941–947.

Malinow, R., & Malenka, R. C. (2002). AMPA receptor trafficking and synaptic plasticity. *Annual Review of Neuroscience, 25*, 103–126.

Martin, S. J., Grimwood, P. D., & Morris, R. G. M. (2000). Synaptic plasticity and memory: An evaluation of the hypothesis. *Annual Review of Neuroscience, 23*, 649–711.

Meyer, D. K., Conzelmann, U., & Schultheiss, K. (1989). Effects of somatostatin-14 on the in vitro release of [3H]GABA from slices of rat caudatoputamen. *Neuroscience, 28*, 61–68.

Mizukami, K., Ishikawa, M., Hidaka, S., Iwakiri, M., Sasaki, M., & Iritani, S. (2002). Immunohistochemical localization of GABAB receptor in the entorhinal cortex and inferior temporal cortex of schizophrenic brain. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 26*, 393–396.

Mondadori, C., Jaekel, J., & Preiswerk, G. (1993). CGP 36742: The first orally active $GABA_B$ blocker improves the cognitive performance of mice, rats, and rhesus monkeys. *Behavioral and Neural Biology, 60*, 62–68.

Mondadori, C., Mobius, H.-J., & Borkowski, J. (1996). The $GABA_B$ receptor antagonist CGP 36742 and the nootropic oxiracetam facilitate the formation of long-term memory. *Behavioural Brain Research, 77*, 223–225.

Mondadori, C., Moebius, H.-J., & Zingg, M. (1996). CGP 36742, an orally active $GABA_B$ receptor antagonist, facilitates memory in a social recognition test in rats. *Behavioural Brain Research, 77*, 227–229.

Mott, D. D., & Lewis, D. V. (1994). The pharmacology and function of central $GABA_B$ receptors. *International Review of Neurobiology, 36*, 97–223.

Nyitrai, G., Kékesi, K. A., Emri, Z., Szárics, E., Juhász, G., & Kardos, J. (2003). GABAB receptor antagonist CGP-36742 enhances somatostatin release in the rat hippocampus in vivo and in vitro. *European Journal of Pharmacology, 478*, 111–119.

Olpe, H.-R., Steinmann, M. W., Ferrat, T., Pozza, M. F., Greiner, K., Brugger, F., et al. (1993). The actions of orally active GABAB receptor antagonists on GABAergic transmission in vivo and in vitro. *European Journal of Pharmacology, 233*, 179–186.

Olpe, H.-R., Wörner, W., & Ferrat, T. (1993). Stimulation parameters determine role of $GABA_B$ receptors in long-term potentiation. *Experientia, 49*, 542–546.

Pitsikas, N., Rigamonti, A. E., Cella, S. G., & Muller, E. E. (2003). The GABAB receptor and recognition memory: Possible modulation of its behavioral effects by the nitrergic system. *Neuroscience, 118*, 1121–1127.

Pozza, M. F., Manuel, N. A., Steinmann, M., Froestl, W., & Davies, C. H. (1999). Comparison of antagonist potencies at pre- and post-synaptic $GABA_B$ receptors at inhibitory synapses in the CA1 region of the rat hippocampus. *British Journal of Pharmacology, 127*, 211–219.

Prasad, K. M., Patel, A. R., Muddasani, S., Sweeney, J., & Keshavan, M. S. (2004). The entorhinal cortex in first-episode psychotic disorders: A structural magnetic resonance imaging study. *American Journal of Psychiatry, 161*, 1612–1619.

Sabbagh, M. N. (2009). Drug development for Alzheimer's disease: Where are we now and where are we headed? *The American Journal of Geriatric Pharmacotherapy, 67*, 167–185.

Snead, O. C., III. (1992). GABAB receptor mediated mechanisms in experimental absence seizures in rat. *Pharmacology Communications, 2*, 63–69.

Steffenach, H. A., Witter, M., Moser, M. B., & Moser, E. I. (2005). Spatial memory in the rat requires the dorsolateral band of the entorhinal cortex. *Neuron, 45*, 301–313.

Tranel, D., & Damasio, A. R. (2002). Neurobiological foundations of human memory. In A. D. Baddeley, M. D. Kopelman, & B. A. Wilson (Eds.), *The handbook of memory disorders* (2nd ed.). West Sussex, England: Wiley.

van Haeften, T., Baks-te-Bulte, L., Goede, P. H., Wouterlood, F. G., & Witter, M. P. (2003). Morphological and numerical analysis of synaptic interactions between neurons in deep and superficial layers of the entorhinal cortex of the rat. *Hippocampus, 13*, 943–952.

Vannucci, S. J., & Hagberg, H. (2004). Hypoxia-ischemia in the immature brain. *Journal of Experimental Biology, 207*, 3149–3154.

Vannucci, R. C., Towfighi, J., Brucklacher, R. M., & Vannucci, S. J. (2001). Effect of extreme hypercapnia on hypoxic-ischemic brain damage in the immature rat. *Pediatric Research, 49*, 799–803.

Vigot, R., Barbieri, S., Brauner-Osborne, H., Turecek, R., Shigemoto, R., Zhang, Y. P., et al. (2006). Differential compartmentalization and distinct functions of GABAB receptor variants. *Neuron, 50*(4), 589–601.

Witter, M. P., Naber, P. A., van Haeften, T., Machielsen, W. C., Rombouts, S. A., Barkhof, F., et al. (2000). Cortico-hippocampal communication by way of parallel parahippocampal-subicular pathways. *Hippocampus, 10*, 398–410.

Witter, M. P., Wouterlood, F. G., Naber, P. A., & Van Haeften, T. (2000b). Anatomical organization of the parahippocampal-hippocampal network. *Annals of the New York Academy of Science, 911*, 1–24.