The Receptors

Giancarlo Colombo *Editor*

$GABA_B$ Receptor

Second Edition

Humana Press

The Receptors

Editor-in-Chief

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The Receptors book Series, founded in the 1980's, is a broad-based and well-respected series on all aspects of receptor neurophysiology. The series presents published volumes that comprehensively review neural receptors for a specific hormone or neurotransmitter by invited leading specialists. Particular attention is paid to in-depth studies of receptors' role in health and neuropathological processes. Recent volumes in the series cover chemical, physical, modeling, biological, pharmacological, anatomical aspects and drug discovery regarding different receptors. All books in this series have, with a rigorous editing, a strong reference value and provide essential up-to-date resources for neuroscience researchers, lecturers, students and pharmaceutical research.

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Foreword

This volume is intended as an update, continuation, and completion of a similar book we published in 2016 for the same series "The Receptors" [1]. In the same way as the previous volume, this book is entirely focused on the $GABA_B$ receptor, a major modulator of neuronal activity in both central and peripheral nervous systems.

More specifically, this new book includes chapters on issues that were not fully covered in the first book as well as chapters providing an update on issues, already covered in the previous book, that are most relevant and timely. Accordingly, particular focus is placed on $GABA_B$ receptor-related issues exploited in recent years, such as use of the orthosteric $GABA_B$ receptor agonist, baclofen, as an effective pharmacotherapy for alcohol use disorder or the growing, convincing lines of experimental evidence on positive allosteric modulators of the $GABA_B$ receptor reproducing baclofen effects but overcoming its limitations; based on these lines of evidence, positive allosteric modulators of the $GABA_B$ receptor are nowadays seen as promising, improved medications for a series of diseases.

Additional features of this book are: (i) a wide spectrum of themes, ranging from medicinal chemistry, molecular and cellular biology, and physiology to preclinical and clinical pharmacology; (ii) an overall "lab-bench to bedside" translational approach; (iii) a roster of authors (to whom I am deeply grateful for their valuable contribution) chosen from among the most influential scientists in the field.

The previous book was dedicated to Wolfgang Froestl, the chemist who contributed to the synthesis of numerous $GABA_B$ receptor ligands (agonists, antagonists, and positive allosteric modulators) extremely useful for the research activity of hundreds of investigators. Conversely, this book is a commemoration to Norman G. Bowery, the "giant" to whom we owe the discovery of the $GABA_B$ receptor. Norman passed away a few months after contributing two chapters to our previous book [2, 3]. The last chapter of the present book is a touching tribute written by one of his closest collaborators to honor—on behalf of all us—Norman's extraordinary contribution and his legacy to the entire $GABA_B$ receptor research field.

Cagliari, Italy — Giancarlo Colombo

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About the Editor

Giancarlo Colombo is a researcher at the Neuroscience Institute, National Research Council of Italy, Cagliari, Italy.His research interest is primarily on neurobiology and pharmacology of alcohol use disorder, with focus on the role of $GABA_B$ receptor in the control of multiple alcohol-related behaviors and the development of $GABA_B$ receptor ligands as possible pharmacotherapies for alcohol use disorder.He has published approximately 240 papers in peer-reviewed journals and about 20 book chapters; he was also the editor of the 2016 volume on $GABA_B$ receptor.He serves as co-chief-editor of the journal *Alcohol and Alcoholism*.

Abbreviations

α2R	α2-Adrenergic receptor
µg	Microgram
µw	Microwaves
Δ^9-THC	Δ^9-Tetrahydrocannabinol
1,4-BD	1,4-Butanediol
3D	Three-dimensional
4-ACA	*Trans* 4-aminocrotonic acid
4-ATA	4-Aminotetrolic acid
5-HT	5-Hydroxytryptamine or serotonin
7TM	Heptahelical transmembrane
[^{3}H]CGP54626	*P*-([3,4-^{3}H]-Cyclohexylmethyl)-*P*-[(2*S*)-3-[[(1*S*)-1-(3,4-dichlorophenyl)ethyl]amino]-2-hydroxypropyl]phosphinic acid
AA	Alko alcohol
ACC	Anterior cingulate cortex
ACN	Acetonitrile
ADE	Alcohol deprivation effect
ADR	Adverse drug reaction
ADX71441	*N*-(5-(4-(4-Chloro-3-fluorobenzyl)-6-methoxy-3,5-dioxo-4,5-dihydro-1,2,4-triazin-2(3*H*)-yl)-2-fluorophenyl)acetamide
ADX71943	*N*-(5-(4-(4-cyano-3-methoxybenzyl)-6-methoxy-3,5-dioxo-4,5-dihydro-1,2,4-triazin-2(3*H*)-yl)-2-fluorophenyl)acetamide
ALD	Alcohol-associated liver disease
AM4113	5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide
AMPK	AMP-activated protein kinase
APP	Amyloid-β precursor protein
ASD	Autism spectrum disorder

ASP8062	4-[[6-(4,4-Dimethylcyclohexyl)-2-methylthieno [2,3-*d*]pyrimidin-4-yl]methyl] thiomorpholine 1,1-dioxide
ATP	Adenosine triphosphate
AUD	Alcohol use disorder
AWS	Alcohol withdrawal syndrome
BA	Basal amygdala
BADS	Barry Albright Dystonia Scale
BAM	Biased allosteric modulator
BBB	Blood-brain barrier
BED	Binge eating disorder
BHF177	*N*-[(1*R*,2*R*,4*S*)-Bicyclo[2.2.1]heptan-2-yl]-2-methyl-5-[4-(trifluoromethyl)phenyl]pyrimidin-4-amine
BHFF	5,7-Di-*tert*-butyl-3-hydroxy-3-trifluoromethyl-3*H*-benzofuran-2-one
BHFI	5,7-Di-*tert*-butyl-3-hydroxy-3-trifluoromethyl-1,3-dihydroindol-2-one
BRET	Bioluminescence resonance energy transfer
C5	Capsaicin concentration eliciting 5 or more coughs
CADD	Computer-aided drug design
CaMKII	Calcium calmodulin-dependent kinase II
cAMP	Cyclic adenosine monophosphate
CAPS	Clinician-Administered PTSD Scale
CaS	Calcium-sensing
Ca_V	Voltage-gated Ca^{2+} channel
CB_1	Cannabinoid type-1
CCP	Complement control protein
CEN	Central executive network
CFCS	Communication Function Classification System
CGP13501	3,5-*bis*-(1,1-Dimethylethyl)-4-hydroxy-α,α-dimethylbenzene-propanal
CGP27492	3-Aminopropylphosphinic acid
CGP35024 (SKF97541)	*P*-(3-Aminopropyl)-*P*-(methyl)phosphinic acid
CGP35348	*P*-(3-Aminopropyl)-*P*-(diethoxymethyl) phosphinic acid;
CGP36742	*P*-(3-Aminopropyl)-*P*-(*n*-butyl)phosphinic acid
CGP44532	*P*-[(*S*)-3-Amino-2-hydroxypropyl]-*P*-(methyl) phosphinic acid
CGP46381	*P*-(3-Aminopropyl)-*P*-(cyclohexylmethyl)phosphinic acid
CGP52432	*P*-[3-[[((3,4-Dichlorophenyl)methyl)amino]propyl]-*P*-(diethoxymethyl)phosphinic acid
CGP54626	*P*-(Cyclohexylmethyl)-*P*-[(2*S*)-3-[[(1*S*)-1-(3,4-dichlorophenyl)ethyl]amino]-2-hydroxypropyl] phosphinic acid

CGP7930	2,6-Di-*tert*-butyl-4-(3-hydroxy-2,2-dimethylpropyl) phenol
CGRP	Calcitonin gene-related peptide
CHO	Chinese hamster ovary
CHOP	C/EBP homologous protein
CIWA-Ar	Clinical Institute Withdrawal Assessment for Alcohol-revised scale
CLH304a	(*E*)-4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-oxobut-3-enoic acid
CLH391	(*E*)-4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-*N*-(2-(2-hydroxyethoxy)ethyl)-2-oxobut-3-enamide
CLH393	4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-*N*-(2-(2-hydroxyethoxy)ethyl)-2-oxobutanamide
ClogP	Calculated logarithm of the partition coefficient
C_{max}	Maximum plasma concentration
CMPPE	2-[1-[2-(4-Chlorophenyl)-5-methylpyrazolo[1,5-*a*] pyrimidin-7-yl]piperidin-2-yl]ethanol
CNS	Central nervous system
COPI	Coat protein complex I
COR627	Methyl2-[((adamantan-1-yl)carbonyl)amino]-4-ethyl-5-methylthiophene-3-carboxylate
COR628	Methyl 2-[((cyclohexyl)carbonyl)amino]-4-ethyl-5-methylthiophene-3-carboxylate
COR659	Methyl 2-[((4-chlorophenyl)carbonyl)amino]-4-ethyl-5-methylthiophene-3-carboxylate
COR758	4-Hydroxy-1-isobutyl-3,6-diisopropylquinolin-2(1*H*)-one
CP	Cerebral palsy
CPP	Conditioned place preference
Cryo-EM	Cryo-electron microscopy
CSF	Cerebrospinal fluid
CT	Computerized tomography
CUD	Cocaine use disorder
CV	Collective variable
CYP	Cytochrome P450
DAPI	4′,6-diamidino-2-phenylindole
DAT	Dopamine transporter
DCM	Dichloromethane
DH-VAL	Dihydrovaltrate, or [(1*S*,4a*S*,6*S*,7*R*,7a*S*)-6-Acetyloxy-1-(3-methylbutanoyloxy)spiro[4a,5,6,7a-tetrahydro-1*H*-cyclopenta[*c*]pyran-7,2′-oxirane]-4-yl]methyl 3-methylbutanoate
DID	Drinking in the dark
DIS	Dyskinesia Impairment Scale
DMF	*N*,*N*-Dimethylformamide

DMN	Default mode network
DMSO	Dimethyl sulfoxide
DOI	(±)1-(2.5-dimethoxy-4-iodophenyl)-2-aminopropane)
DRG	Dorsal root ganglia
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECL2	Extracellular loop 2
EDS	Excessive daytime sleepiness
EEG	Electroencephalography
eGFR	Estimated glomerular filtration rate
EKSRLL	Di-leucine internalization
EM	Electron microscopy
EPM	Elevated plus maze
EPSC	Excitatory postsynaptic current
ER	Endoplasmic reticulum (Chap. 1)
ER	Extinction responding (Chap. 12)
ERK	Extracellular-signal regulated kinase
ERK1/2	Extracellular signal-regulated kinase 1/2
EZM	Elevated zero maze
FA	Ferulic acid, or *trans*-3-(4-Hydroxy-3-methoxy)cinnamic acid
FDA	Food and Drug Administration
Fmr1	Fragile X Mental Retardation 1
fMRI	Functional magnetic resonance imaging
FMRP	Fragile X mental retardation protein
FPS	Fear-potentiated startle
FR	Fixed ratio
FRET	Fluorescence resonance energy transfer
FT218	Extended-release oral suspension of GHB
FXS	Fragile X syndrome
GABA	γ-Aminobutyric acid
$GABA_A$	GABA type-A
$GABA_B$	GABA type-B
$GABA_{B1}$	GABA type-B receptor subunit 1
$GABA_{B1a}$	$GABA_B$ receptor subunit 1a
$GABA_{B1b}$	$GABA_B$ receptor subunit 1b
$GABA_{B2}$	GABA type-B receptor subunit 2
$GABA_C$	GABA type-C
GADD153	Growth arrest and DNA damage-inducible gene 153
GAERS	Genetics Absence Epilepsy Rats from Strasburg
GAS	Goal Attainment Scaling
GBD	Global disease burden
GBL	γ-Butyrolactone
GDP	Guanosine 5′-diphosphate
GERD	Gastroesophageal reflux disease
GHB	γ-Hydroxybutyrate or γ-Hydroxybutyric acid

GINIP	Gα inhibitory interacting protein
GIRK	G protein-coupled inwardly rectifying potassium
GISP	G protein-coupled receptor interacting scaffolding protein
GMFCS	Gross Motor Function Classification System
GPCR	G protein-coupled receptor
GPRC6a	G protein-coupled receptor family C group 6 subtype a
GRADE	Grading of Recommendation, Assessment, Development and Evaluation
GRK	G protein-coupled receptor kinase
GS39783	*N*,*N*-Dicyclopentyl-2-methylsulfanyl-5-nitropyrimidine-4,6-diamine
GTPγ^{35}S	Guanosine 5-*O*-(3-[^{35}S]thio)triphosphate
HCV	Hepatitis C virus
HEK 293	Human embryonic kidney 293
HIV	Human immunodeficiency virus
HNC	Hyperpolarization activated cyclic nucleotide-gated channel
HS	Hippocampal sclerosis
HTS	High-throughput screening
i.c.v.	Intracerebroventricular
i.g.	Intragastric
i.p.	Intraperitoneal
IAA	Imidazole-4-acetic acid
ICD	International Classification of Diseases
ICL3	Intracellular loop 3
ICSS	Intracranial self-stimulation
ICU	Intensive care unit
IF	Interfering peptide
IPSP	Inhibitory postsynaptic potential
IRS-1	Insulin receptor substrate 1
ISL	Isoliquiritigenin or 2′,4,4′-Trihydroxychalcone
IT	Intrathecal
KCTD	K^+ channel tetramerization domain
K_i	Inhibitory constant
KK-92A	(4-(Cycloheptylamino)-5-(4-(trifluoromethyl)phenyl) pyrimidin-2-yl)methanol
KO	Knockout
LAH	Lithium aluminum hydride
LB	Lobe
LB1	N-terminal Lobe
LB2	C-terminal Lobe
LBDD	Ligand-based drug design
LDB	Light-dark box
LES	Light-enhanced startle

LiBERO	Ligand-guided receptor optimization
LIVS	Leucine/isoleucine/valine binding protein
LORR	Loss of righting reflex
LOS	Lower oesophageal sphincter
LTP	Long-term potentiation
mACh	M_2 muscarinic acetylcholine
MACS	Manual Ability Classification System
MAOI	Monoamine oxidase inhibitor
MB	Marble burying
MCAO	Middle cerebral artery occlusion
MD	Molecular dynamics
MDD	Major depressive disorder
MDMA	3,4-Methylenedioxy-*N*-methylamphetamin
mGlu	Metabotropic glutamate
MIB2	Mind bomb 2
MK-801 (Dizocilpine)	5-Methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene-5,10-imine
Msec7-1	Rat homolog of B2-1/cytohesin-1
MUPP1	Multi-PDZ domain protein1
NAc	Nucleus accumbens
NADPH	β-Nicotinamide adenine dinucleotide 2′-phosphate reduced
NAL	Neutral allosteric ligand
NAM	Negative allosteric modulator
NCS-382	6,7,8,9-tetrahydro-5-hydroxy-5H-benzo-cyclohept-6-ylideneacetic
NMDA	*N*-methyl-D-aspartate
NMR	Nuclear magnetic resonance
NMS	N-methyl scopolamine
NREM	Non-rapid-eye movement sleep
NSF	N-ethylmaleimide-sensitive fusion protein
NT157	(*E*)-3-(3-Bromo-4,5-dimethoxyphenyl)-*N*-(3,4,5-trimethoxybenzyl)prop-2-enethioamide
NTS	Nucleus tractus solitarius
ODM-106	3-(4-Bromobenzyl)-7-fluoro-1-methylquinazoline-2,4(1*H*,3*H*)-dione
OGD	Oxygen and glucose deprivation
ORM-27669	(*S*)-1-(5-Fluoro-2,3-dihydro-1*H*-inden-2-yl)-4-methyl-6,7,8,9-tetrahydro-[1,2,4]triazolo[4,3-*a*]quinazolin-5(4*H*)-one
OUD	Opioid use disorder
P	Indiana alcohol-preferring
PAM	Positive allosteric modulator
PCC	Posterior cingulate cortex
PD	Parkinson's disease
PFC	Prefrontal cortex

P-gp	P-glycoprotein
PI3K	Phosphoinositide 3-kinase
PKA	Protein kinase A
PNS	Peripheral nervous system
PP2A	Protein phosphatase 2A
PPI	Prepulse inhibition (Chap. 10)
PPI	Proton pump inhibitor (Chap. 5)
PR	Progressive ratio
PRAF2	Prenylated Rab acceptor 1 domain family member 2
PsUD	Polysubstance use disorder
PTSD	Post-traumatic stress disorder
PV	Parvalbumin
q4-6h	Every 4-6 hours
q8h	Every 8 hours
rac-BHFF	(*R*,*S*)-5,7-Di-*tert*-butyl-3-hydroxy-3-trifluoromethyl-3*H*-benzofuran-2-one
Rap1	Ras-associated protein 1
RCC	Refractory chronic cough
RCT	Randomized controlled trial
RE	Refractory epilepsy
REM	Rapid-eye movement sleep
RGS	Regulators of G protein signaling
RLM	Rat liver microsome
RR	Response requirement
rsFC	Resting-state functional connectivity
RSRR	Endoplasmatic reticulum retention
RTU	*Recommandations temporaires d'utilisation*
SAA	Succinic semialdehyde
SAP	Symptom-associated probability
SAR	Structure-activity relationship
SBDD	Structure-based drug design
SCH50911	2-[(2*S*)-5,5-Dimethylmorpholin-2-yl]acetic acid.
SCR	Short consensus repeats
SD	Sushi domain
Shrm4	Shroom 4
SIH	Stress-induced hyperthermia
SKF97541 (CGP35024)	*P*-(3-Aminopropyl)-*P*-(methyl)phosphinic acid
smFRET	Single-molecule Förster resonance energy transfer
SN	Salience network
SOM	Somatostatin
sP	Sardinian alcohol-preferring
SS	Saikosaponin
SSA	Saikosaponin A or (2*S*,3*R*,4*S*,5*S*,6*R*)-2-[(2*R*,3*R*,4*S*,5*S*,6*R*)-3,5-dihydroxy-2-[[(1*S*,2*S*,4*S*,5*R*,8*R*,9*R*,10*S*,13*S*,14*R*,17*S*,18*R*)-2-hydroxy-9-(hydroxymethyl)-4,5,9,13,20,20-

	hexamethyl-24-oxahexacyclo[15.5.2.$0^{1,18}$.$0^{4,17}$.$0^{5,14}$.$0^{8,13}$] tetracos-15-en-10-yl]oxy]-6-methyloxan-4-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol
SSD	Saikosaponin D or (2*S*,3*R*,4*S*,5*S*,6*R*)-2-[(2*R*,3*R*,4*S*,5*S*,6*R*)-3,5-dihydroxy-2-[[(1*S*,2*R*,4*S*,5*R*,8*R*,9*R*,10*S*,13*S*,14*R*,17*S*,18*R*)-2-hydroxy-9-(hydroxymethyl)-4,5,9,13,20,20-hexamethyl-24-oxahexacyclo [15.5.2.$0^{1,18}$.$0^{4,17}$.$0^{5,14}$.$0^{8,13}$]tetracos-15-en-10-yl]oxy]-6-methyloxan-4-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol
SSD114	*N*-Cyclohexyl-4-methoxy-6-(4-(trifluoromethyl)phenyl)pyrimidin-2-amine
SUD	Substance use disorder
SWS	Slow-wave sleep
t.i.d.	*Ter in die* (three times per day)
T1R	Taste receptor type-1
T3P	Propanephosphonic acid anhydride
TEA	Triethylamine
TLE	Temporal lobe epilepsy
TLOSR	Transient lower oesophageal sphincter relaxation
TM5	Transmembrane helix 5
TM6	Transmembrane helix 6
T_{max}	Time to maximum plasma concentration
TMD	Transmembrane domain
TPSA	Topological polar surface area
TREK	Two domain potassium channel
TRPV1	Transient receptor potential vanilloid 1
TRU	Temporary recommendation for use
USP14	Ubiquitin-specific protease 14
VAS	Visual analogue scale
VFT	Venus flytrap
VGCC	Voltage-gated calcium channel
VIP	Vasoactive intestinal polypeptide
VS	Virtual screening
VTA	Ventral tegmental area
WT	Wild type

Part I
Molecular Biology, Biochemistry, and Functioning of the $GABA_B$ Receptor

Chapter 1
$GABA_B$ Receptors: Molecular Organization, Function, and Alternative Drug Development by Targeting Protein-Protein Interactions

Dietmar Benke, Musadiq Ahmad Bhat, and Mohammad Hleihil

Abstract $GABA_B$ receptors couple via Gi/o proteins to multiple effector proteins to mediate prolonged inhibition in the nervous system. They are important elements in maintaining the excitation/inhibition balance by controlling neuronal excitation. $GABA_B$ receptors are expressed in virtually all neurons and are involved in the regulation of most brain functions. In neurological diseases associated with a disturbed excitation/inhibition balance, $GABA_B$ receptors are often de-regulated and represent promising drug targets for the development of novel therapeutic interventions. In this chapter, we briefly review the basic functions and the structural organization of the receptors. Then, we describe pathways that de-regulate $GABA_B$ receptors in neuropathic pain, cerebral ischemia, and psychostimulant-induced addiction and discuss efforts for alternative drug discovery by targeting disease-relevant protein-protein interactions.

Keywords $GABA_B$ receptor · Receptor structure · Receptor trafficking · Protein-protein interaction · Interfering peptides · Neuropathic pain · Cerebral ischemia · Addiction

1.1 $GABA_B$ Receptor Function

Regulation of neuronal activity requires tight control of the excitation/inhibition balance to ensure precise timing of synaptic input and generation of action potentials (Haider et al. 2006). The main inhibitory neurotransmitter in the central nervous system (CNS) is γ-aminobutyric acid (GABA), which activates two types of GABA-sensitive receptors: ionotropic type-A ($GABA_A$) and metabotropic type-B ($GABA_B$) receptors. The binding of GABA to $GABA_A$ receptors produces transient

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and fast inhibitory postsynaptic currents (<10 ms), driven mainly by chloride influx into the neuron (Sieghart and Savić 2018). The localization of $GABA_A$ receptors in the synapse is crucial to counterbalancing excitatory input. In addition to synaptic localization, $GABA_A$ receptors are also present extrasynaptically generating tonic inhibition (Glykys and Mody 2007; Chuang and Reddy 2018).

In contrast to $GABA_A$ receptors, $GABA_B$ receptors mediate prolonged inhibition (hundreds of ms) in the CNS by activating $G_{i/o}$-type G proteins, which in turn predominately activate G protein-coupled inwardly rectifying potassium channels (GIRK, also named $K_{ir}3$), inhibit voltage-gated calcium channels (VGCC), and adenylate cyclases (Padgett and Slesinger 2010) (Fig. 1.1). $GABA_B$ receptors are ubiquitously expressed in neurons throughout the CNS (Castelli and Gessa 2016) and most abundant in peri- and extrasynaptic sites, where they are mainly activated by spillover of GABA from intensely activated GABAergic synapses during burst firing, rhythmic network activity, or ambient GABA released from astrocytes (Scanziani 2000; Kohl and Paulsen 2010; Kilb and Kirischuk 2022).

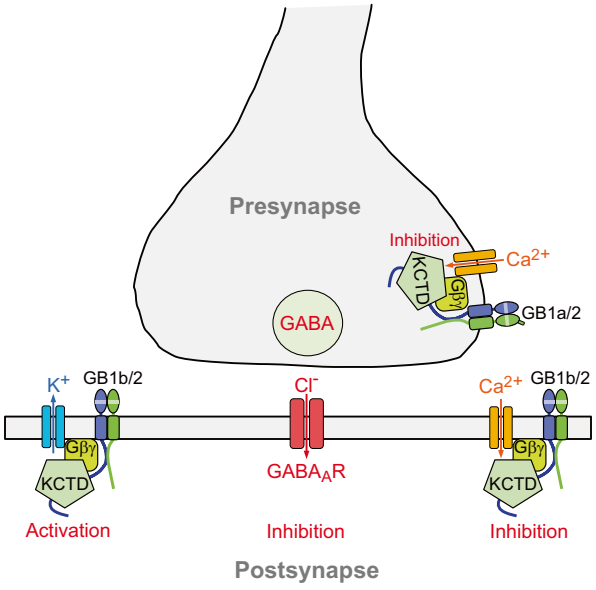

Fig. 1.1 Illustration of a GABAergic synapse containing $GABA_A$ receptors ($GABA_AR$) and $GABA_B$ receptors (GB1a/2 and GB1b/2) with their main effectors. $GABA_A$ receptors are located mainly underneath the presynaptic terminal, where they mediated fast inhibition (<10 ms) by opening an integral Cl^- channel. $GABA_B$ receptors are predominantly located at peri- and extrasynaptic sites in the postsynaptic membrane, where they mediate long-lasting inhibition (hundreds of ms) by activating K^+ channels (GIRK channels) and inhibiting Ca^{2+} channels (VGCC). $GABA_B$ receptors activate $G_{i/o}$ proteins. The activated $G\alpha_{i/o}$ subunit inhibits adenylate cyclases (not depicted), lowering cAMP levels, while the Gβγ dimer activates K^+ channels and inhibits Ca^{2+} channels

Several studies showed a functional crosstalk between $GABA_A$ and $GABA_B$ receptors in synaptic and extrasynaptic sites (Shrivastava et al. 2011; Kardos et al. 1994; Tao et al. 2013; Connelly et al. 2013). For example, in developing hypothalamic neurons, $GABA_B$ receptor activation by the selective agonist baclofen strongly inhibits synaptic $GABA_A$ receptor-mediated Ca^{2+} influx ($GABA_A$ receptors are excitatory in the early stages of development) (Obrietan and Van Den Pol 1998). Postsynaptic $GABA_B$ receptors have been shown to enhance the tonic activity of extrasynaptic $GABA_A$ receptors in different neuron populations by a mechanism involving adenylate cyclase and protein kinase A (PKA) (Tao et al. 2013; Connelly et al. 2013; Khatri et al. 2019). Conversely, $GABA_A$ receptors can also affect $GABA_B$ receptor activity. $GABA_A$ receptor-mediated chloride influx has been shown to alter the reversal potential of $GABA_B$ receptor/GIRK-mediated synaptic potentials in hippocampal pyramidal neurons, causing a reduction of $GABA_B$ receptor-mediated inhibitory postsynaptic potentials (IPSPs) (Lopantsev and Schwartzkroin 2001).

One important function of $GABA_B$ receptors is the modulation of neuronal network dynamics, which underlie cognitive functions including processing sensory input and memory processes (Kohl and Paulsen 2010; Villalobos 2024). $GABA_B$ receptors are activated by burst firing and rhythmic neuronal activity and thereby regulate slow and fast network activity and neuronal firing during network oscillations. During slow network activity, $GABA_B$ receptors are involved in termination of the Up state (Mann et al. 2009), and during fast network activity, $GABA_B$ receptors modulate the duration of stimulus-evoked gamma oscillations (Brown et al. 2007).

$GABA_B$ receptors modulate synaptic function in a cell-type specific manner. For instance, $GABA_B$ receptors modulate the release of GABA from distinct interneurons in the hippocampus with different efficiencies. The activation of presynaptic $GABA_B$ receptors inhibits GABA release from parvalbumin (PV) interneurons in the hippocampus by blocking P/N-type VGCC with significantly less efficiency than in non-PV interneurons. This relieves inhibition of hippocampal pyramidal neurons and increases their excitability to different levels (Liu et al. 2019). However, in somatostatin (SOM) interneurons of the hippocampus, postsynaptic dendritic $GABA_B$ receptors couple to L-type VGCC instead of GIRK channels. This reduces Ca^{2+} influx and inhibits long-term potentiation at excitatory input synapses onto SOM interneurons (Booker et al. 2018).

The functional output of $GABA_B$ receptor-mediated inhibition depends on the cellular context and can result in net excitatory output by disinhibiting excitatory target neurons (Fig. 1.2). For example, in the piriform cortex, activation of postsynaptic $GABA_B$ receptors hyperpolarizes layer 2 principal neurons, resulting in reduced excitability of these neurons. However, activation of presynaptic $GABA_B$ receptors located on the axons of GABAergic interneurons reduced the inhibitory input onto principal neurons, promoting their excitability by disinhibition (Gerrard et al. 2018).

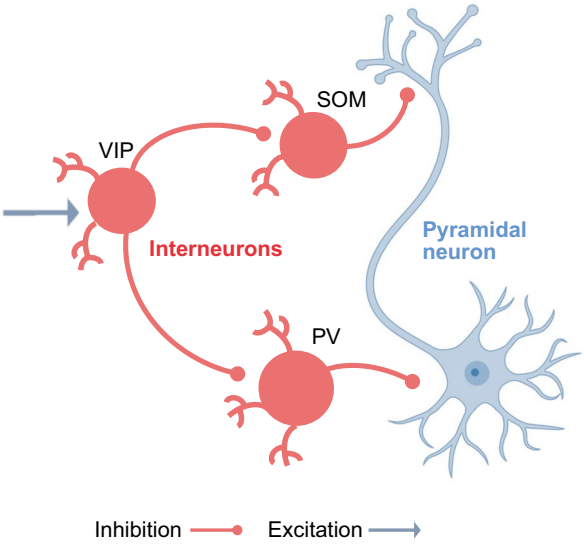

Fig. 1.2 Example depicting $GABA_B$ receptor-mediated disinhibition of excitatory pyramidal neurons in the cerebral cortex. Excitatory inputs from cortical or subcortical areas stimulate vasoactive intestinal polypeptide (VIP)-expressing interneurons, which in turn suppress two additional inhibitory neurons: parvalbumin (PV) and somatostatin (SOM)-expressing interneurons. PV-expressing interneurons specifically target the perisomatic area of pyramidal neurons, while SOM-expressing interneurons specifically target the basal and apical dendrites of pyramidal cells. This process removes inhibitory gating and disinhibits pyramidal neurons to enhance their excitability (Pi et al. 2013; Letzkus et al. 2015)

1.1.1 Function of Pre- and Postsynaptic $GABA_B$ Receptors

Functional $GABA_B$ receptors require the heterodimerization of the two subunits, $GABA_{B1}$ and $GABA_{B2}$. The $GABA_{B1}$ subunit exists in two major isoforms ($GABA_{B1a}$ and $GABA_{B1b}$), differing in their N-terminal domain by two sushi protein-protein interaction domains selectively present in $GABA_{B1a}$. Each of the subunits displays classical structural features of class C GPCRs, such as a large extracellular N-terminal domain containing the binding site for GABA, seven transmembrane domains, and a large intracellular C-terminal domain (for details, see Sect. 1.2). Receptors containing $GABA_{B1a}$ or $GABA_{B1b}$ isoforms ($GABA_{B1a/2}$ and $GABA_{B1b/2}$) are largely localized to distinct subcellular sites and exert different physiological functions. For example, the predominantly presynaptic localized $GABA_{B1a/2}$ subtype, but not the mainly postsynaptic localized $GABA_{B1b/2}$ subtype, is involved in hippocampal long-term potentiation (LTP) and memory formation. Ablation of $GABA_{B1a}$ impaired synaptic plasticity and hippocampus-dependent memory (Vigot et al. 2006; Jacobson et al. 2007). Furthermore, the absence of $GABA_{B1a}$ affected,

amongst others, locomotor activity (Jacobson et al. 2006a), sleep regulation (Vienne et al. 2010), and the acquisition of aversive taste memory (Jacobson et al. 2006b). On the other hand, the mainly postsynaptic $GABA_{B1b/2}$ subtype inhibited dendritic Ca^{2+} spikes (Pérez-Garci et al. 2006) and is involved in fear conditioning (Shaban et al. 2006).

1.1.1.1 Presynaptic $GABA_B$ Receptors

$GABA_{B1a}$-containing receptors are largely localized to presynaptic sites and are particularly abundant in glutamatergic neurons, where they suppress glutamate release via reducing VGCC activity (Vigot et al. 2006; Biermann et al. 2010). Depolarization of the plasma membrane activates VGCC, and the resulting increase in intracellular Ca^{2+} level triggers fusion of the synaptic vesicles with the plasma membrane and thereby neurotransmitter release. VGCC are composed of the pore-forming α1 subunits and several associated subunits (α1, α2δ, β1–4, γ), which modulate channel kinetics, stability, and trafficking (Proft and Weiss 2015). Based on their amino acid sequence, VGCCs are divided into Ca_v1 (also known as L-type), Ca_v2 (P/Q-type, N-type, and R-type), and Ca_v3 (T-type) channel families with distinct structural diversity and biophysical activity (Ertel et al. 2000; Catterall 2000). While N-, P/Q-, and R-type channels are primarily expressed in neurons, L-type and T-type channels are additionally expressed in numerous other cell types (Zamponi et al. 2015; Catterall 2011).

Presynaptic $GABA_B$ receptors mainly modulate the activity of N-type (Ca_v 2.2) and P/Q-type (Ca_v 2.1) calcium channels. The Gβγ subunits of $G_{i/o}$ proteins activated by $GABA_B$ receptors bind to VGCC and reduce the activation kinetics of the channels (Menon-Johansson et al. 1993; Mintz and Bean 1993; Takahashi et al. 1998). In addition to N- and P/Q-type VGCCs, in specific neuronal populations, presynaptic $GABA_B$ receptors also alter the activity of R- and T-type VGCCs (Bhandari et al. 2021; Moldavan et al. 2006; Huang et al. 2015). Although $GABA_B$ receptors generally inhibit VGCC, there are exceptions. For example, $GABA_B$ receptors in neurons of the medial habenula activate R-type VGCC to trigger the co-release of glutamate, acetylcholine, and neurokinin B, which reduced fear responses (Zhang et al. 2016).

In addition to the inhibition of presynaptic VGCC, $GABA_B$ receptors suppress neurotransmitter release via the regulation of adenylate cyclase during sustained neuronal activity. The Gα subunit of the activated $G_{i/o}$ protein inhibits the adenylate cyclase, reducing cyclic adenosine monophosphate (cAMP) levels in the presynaptic terminal. The shortage of cAMP impairs the Ca^{2+}-promoted recruitment of presynaptic vesicles to the plasma membrane by increasing the energy barrier for vesicle fusion (Sakaba and Neher 2003; Rost et al. 2011).

1.1.1.2 Postsynaptic $GABA_B$ Receptors

In postsynaptic sites in the soma and dendrites of neurons, activation of $GABA_B$ receptors opens GIRK channels via direct interaction with Gβγ. This increases the efflux of K^+, causing long-lasting membrane hyperpolarization which shunts depolarization from excitatory input (Lüscher and Slesinger 2010). GIRK channels are a family of four proteins (GIRK 1–4, also known as Kir3.1–3.4), which can exist as homomers (GIRK2, GIRK4) and heteromers (GIRK1/2, GIRK1/3, GIRK1/4, GIRK2/3, GIRK2/4). GIRK1 cannot form functional homomers but is present in most neuronal GIRK channels and conveys high sensitivity for activation through GPCRs. GIRK1, GIRK2, and GIRK3 are broadly distributed in the CNS and colocalize with $GABA_B$ receptors, while GIRK4 is mainly expressed in other tissues (Luján et al. 2014; Fernandez-Alacid et al. 2009; Lüscher and Slesinger 2010).

The type and composition of GIRK channels present in a given neuron can have a profound impact on $GABA_B$ receptor-mediated inhibition (Luján et al. 2014). A prominent example is the differential expression of GIRK channels in GABAergic and dopaminergic neurons of the ventral tegmental area (VTA). VTA GABAergic neurons express GIRK1–3 and display high sensitivity for activation by $GABA_B$ receptors. Dopaminergic neurons, on the other hand, express GIRK2/3 and are significantly less sensitive to $GABA_B$ receptor-mediated activation (Cruz et al. 2004).

In addition to GIRK channels, VGCC (N-, P/Q-, and L-type) are also expressed in dendritic membranes and spines, where they are activated by $GABA_B$ receptors (Chalifoux and Carter 2011; Booker et al. 2018). $GABA_B$ receptor-mediated inhibition of postsynaptic VGCC contributes to neuronal inhibition by decreasing dendritic Ca^{2+} spikes and inhibiting backpropagating action potentials (Pérez-Garci et al. 2006; Chalifoux and Carter 2011).

As mentioned above for presynaptic $GABA_B$ receptors, postsynaptic receptors also efficiently inhibit adenylate cyclases to convey various cAMP-mediated effects. Adenylate cyclases are a large family of mainly transmembrane proteins that generate cAMP from adenosine triphosphate (ATP) (Halls and Cooper 2017; Ostrom et al. 2022). $GABA_B$ receptors inhibit the activity of adenylate cyclases via the $Ga_{i/o}$ subunit, resulting in reduced cAMP levels.

The Ca^{2+} permeability of NMDA receptors is enhanced by phosphorylation of constitutive active cAMP-dependent PKA (Skeberdis et al. 2006). In basal dendritic spines, $GABA_B$ receptor-mediated reduction of cAMP levels inhibits the activity of PKA, which in turn reduces phosphorylation of NMDA receptors and thereby their Ca^{2+} permeability (Chalifoux and Carter 2010). This mechanism impacts NMDA receptor-mediated neuronal plasticity.

Although GIRK channels produce the major postsynaptic hyperpolarization upon activation of $GABA_B$ receptors, further channels are involved in altering the permeability of neuronal membranes to potassium. For example, by reducing adenylate cyclase activity, $GABA_B$ receptors activate TREK-2 (two domain potassium channels) in the entorhinal cortex by relieving tonic protein kinase A (PKA)-dependent inhibition from TREK-2 channels (Deng et al. 2009). This pathway inhibits neuronal activity and is involved in the depression of spatial learning.

1.2 Molecular Organization of $GABA_B$ Receptors

The minimum functional entity of $GABA_B$ receptors is composed of the two subunits $GABA_{B1}$ and $GABA_{B2}$, constituting a heterodimer (Fig. 1.3). Each subunit consists of an intracellular C-terminal domain and a typical heptahelical transmembrane (7TM) domain linked to a bi-lobed extracellular Venus Flytrap (VFT) domain via a linker region (Chun et al. 2012). The linker region is a short sequence lacking cysteine residues that are otherwise conserved among other members of class C GPCRs. The extracellular located VFT domain of the $GABA_{B1}$ subunit contains the orthosteric binding site (Kniazeff et al. 2002), whereas the transmembrane domain of the $GABA_{B2}$ subunit contains a binding site for allosteric modulators and is the main interaction site for $G_{i/o}$-type G proteins (Margeta-Mitrovic et al. 2001a; Binet et al. 2004; Galvez et al. 2001). $GABA_B$ receptors can form higher-order oligomers by weak and transient interaction between transmembrane domains of the $GABA_{B1}$ subunits (Xue et al. 2019; Calebiro et al. 2013; Maurel et al. 2008; Stewart et al. 2018). The receptor is believed to be in equilibrium between higher-order oligomers and heterodimers.

The heterodimeric assembly of the two subunits is stabilized by the interaction of coiled-coil domains present in their intracellularly located C-terminal domains. This interaction masks a di-leucine internalization (EKSRLL) and an endoplasmic reticulum (ER) retention (RSRR) signal in $GABA_{B1}$ and therefore allows the forward trafficking of functional heteromeric receptor to the plasma membrane (White et al. 1998; Margeta-Mitrovic et al. 2000; Pagano et al. 2001; Restituito et al. 2005).

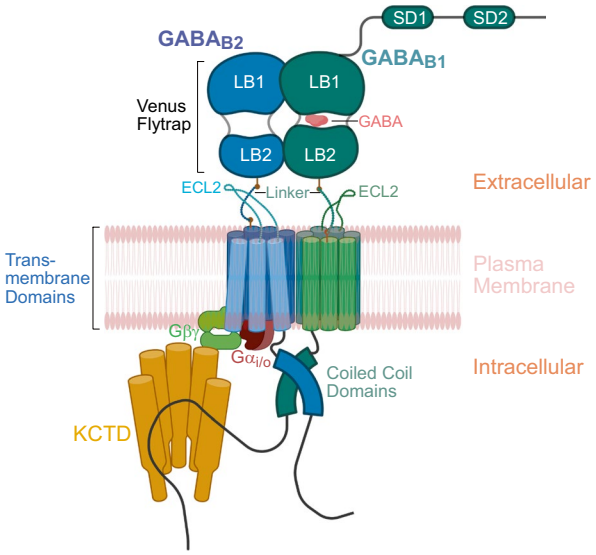

Fig. 1.3 Illustration of the structural organization of $GABA_B$ receptors, indicating the main domains such as the extracellular VFTs, heptahelical transmembrane domain, and intracellular coiled-coil domain. In addition, the association with the accessory KCTD proteins and $G_{i/o}$ proteins is depicted. ECL2: extracellular loop 2, LB1: Lobe 1, LB2: Lobe 2, SD1: sushi domain1, SD2: sushi domain2. SD1 and SD2 are only present in $GABA_{B1a}$ and absent in $GABA_{B1b}$

Multiple isoforms of the $GABA_{B1}$ subunit have been described, but the isoforms $GABA_{B1a}$ and $GABA_{B1b}$, both encoded by the same gene and generated by differential promoter usage (Steiger et al. 2004), appear to be constituents of the vast majority of $GABA_B$ receptors in the CNS. Structurally, these two isoforms differ only in the N-terminal domain by the presence of two sushi domain repeats (SD1 and SD2) in the $GABA_{B1a}$ subunit (Kaupmann et al. 1997). These sushi repeats play an important role as an intracellular sorting signal for preferential trafficking of $GABA_{B1a}$ into axons (Biermann et al. 2010) via a protein complex involving the amyloid-β precursor protein (APP) (Dinamarca et al. 2019). Interestingly, SD1 can bind to the secreted ectodomain of APP to inhibit neurotransmitter release (Rice et al. 2019).

1.2.1 The Extracellular Domain

The VFT domains of $GABA_{B1}$ and $GABA_{B2}$ subunits contain two distinct lobes, the N-terminal-oriented Lobe 1 (LB1) and the C-terminal-oriented Lobe 2 (LB2) (Geng et al. 2013). A grove at the LB1/LB2 interface constitutes the orthosteric binding site in the $GABA_{B1}$ subunit. The VFT domain of $GABA_{B2}$ is similarly structured but does not form a functional ligand binding site. Agonist binding is associated with a closed/active conformation, and antagonist binding is associated with an open/inactive conformation of the $GABA_{B1}$ VFT domain (Fig. 1.4). The LB1s of $GABA_{B1}$ and

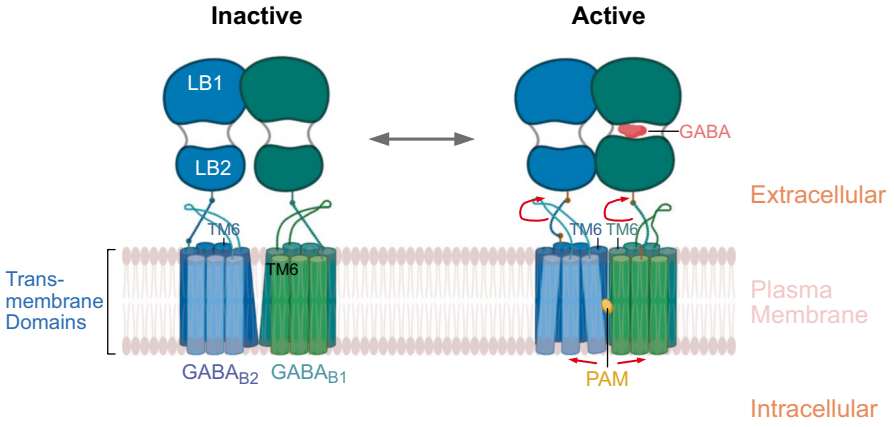

Fig. 1.4 Illustration of activation-related transitions in $GABA_B$ receptor domains from the inactive state to the active state. $GABA_{B1}$ and $GABA_{B2}$ VFTs adopt fully open conformations, and their LB2s are well separated in an inactive state. The ends of TM5 of both subunits are very close in an inactive state. In the active state, agonist binding closes the VFT of $GABA_{B1}$, bringing the LB2s into contact. These conformational changes in the VFTs propagate toward the TMDs, which results in the contact interface of TMD6 by mutual orientation. The binding of positive allosteric modulators (PAM) results in the stabilization of the active conformation and leads to orientation shifts of the intracellular ends of TMD3 and TMD5 and the stabilization of a cleft used for the engagement of G proteins. LB1: Lobe 1, LB2: Lobe 2, TM6: transmembrane domain 6, VFT: Venus fly trap domain

$GABA_{B2}$ interact with each other both in the active and inactive conformational states. The interaction involves hydrogen bonds, hydrophobic interactions, and a salt bridge (Geng et al. 2012). The hydrophobic interactions are mainly facilitated by three tyrosine residues and are conserved among all VFT domains of the $GABA_B$ receptor subunits (Geng et al. 2013). These tyrosine residues are important for agonist-dependent activation of the G protein (Geng et al. 2012; Rondard et al. 2008). Agonist binding induces conformation changes in the LB2 domains, resulting in the formation of a heterodimer interface stabilized by a series of hydrogen bonds (Geng et al. 2012). The residues associated with the ligand binding in the VFT domain of $GABA_{B1}$ are not conserved in the VFT domain of $GABA_{B2}$ (Geng et al. 2012). The sequence homology between the VFT domains of $GABA_{B1}$ and $GABA_{B2}$ is only 33% (Kniazeff et al. 2002). The $GABA_{B2}$ VFT domain remains in an open state despite the agonist-induced closed conformation of the $GABA_{B1}$ VFT domain (Geng et al. 2013). The $GABA_{B1}$ VFT domain is also functional in the absence of the $GABA_{B2}$ VFT domain but exhibits lower affinity to agonists (Liu et al. 2004; Nomura et al. 2008). The $GABA_{B2}$ VFT domain impacts receptor activation by promoting signal transduction and increasing agonist affinity (Nomura et al. 2008; Liu et al. 2004).

The VFT domains and the transmembrane domains in both subunits are connected via an approximately 20-residue linker region (Margeta-Mitrovic et al. 2001b; Rondard et al. 2011). Upon agonist binding, the distance of the two LB2 domains decreases from approximately 45 to 32 Å (Geng et al. 2013). The linker interacts with the extracellular loop 2 (ECL2) of the transmembrane domains through an anti-parallel β-sheet. This stabilizes the transmembrane domains of $GABA_{B1}$ and $GABA_{B2}$ in the active conformation (Shaye et al. 2020).

1.2.2 The Transmembrane Domain

One hallmark of the activation of class C GPCRs is the reorientation of transmembrane domains. Agonist binding induces conformation changes in the transmembrane domains of $GABA_B$ receptors, as shown by cross-linking experiments (Xue et al. 2019) and cryo-electron microscopy structure studies (Mao et al. 2020; Shaye et al. 2020). In the inactive state, the transmembrane domain (TMD) interface interactions are formed between $GABA_{B1}$-TMD3 with $GABA_{B2}$-TMD5 and $GABA_{B2}$-TMD3 with $GABA_{B1}$-TMD5. These interactions are facilitated through ionic interactions and stabilized by aromatic residues (Liu et al. 2004; Park et al. 2020; Xue et al. 2019). Upon agonist binding, the transmembrane domains rotate, leading to the formation of new interfaces consisting of TMD6 domains of $GABA_{B1}$ and $GABA_{B2}$ (Fig. 1.4) (Liu et al. 2004; Park et al. 2020; Rondard et al. 2008). It had been shown that the transmembrane domain of $GABA_{B2}$ mainly recruits the G proteins (Margeta-Mitrovic et al. 2001a; Binet et al. 2004; Galvez et al. 2001). However, there is evidence from recent cryo-electron microscopy data that $GABA_{B1}$ may also couple to G proteins (Mao et al. 2020). Due to steric hindrance, G proteins may bind to either of the subunits at a time, but $GABA_{B2}$ was favored as this was the most

frequent populated distribution found (Mao et al. 2020). In both the active and inactive conformations, the extracellular oriented half of the transmembrane domain of $GABA_{B1}$ and $GABA_{B2}$ is occupied by phospholipid, at a site corresponding to the orthosteric binding sites in most family A GPCRs (Mao et al. 2020; Papasergi-Scott et al. 2020; Park et al. 2020). These lipids are believed to be crucial for receptor activation and maintenance of its structural integrity (Papasergi-Scott et al. 2020; Park et al. 2020). However, it should be noted that Shaye et al. did not find lipids in the recently released structure (Shaye et al. 2020).

1.2.3 The Intracellular Domain

Both $GABA_{B1}$ and $GABA_{B2}$ contain an intracellular C-terminal coiled-coil domain, which is involved in formation of the heterodimer and controls the ER exit and forward trafficking of the heterodimeric receptor to the plasma membrane. A di-leucine internalization motif of $GABA_{B1}$ is buried at the heterodimer interface and directly interacts with $GABA_{B2}$ as part of the coiled-coil structure. Access to the ER retention signal RSRR in $GABA_{B1}$ appears to be blocked by steric hindrance upon heterodimerization with $GABA_{B2}$ (Burmakina et al. 2014; Gassmann et al. 2005).

It is now well established that the functional diversity of native $GABA_B$ receptors arises from the interactions of their intracellular domain with many associated proteins (Gassmann and Bettler 2012; Schwenk et al. 2010, 2016; Pin and Bettler 2016; Bettler and Fakler 2017). The most important group of proteins tightly associated with the $GABA_B$ receptor are the cytosolic K^+ channel tetramerization domain (KCTD)-containing proteins (Schwenk et al. 2010, 2016). KCTDs 8, 12, 12b, and 16 interact with the C-terminal domain of $GABA_{B2}$ via a conserved N-terminal tetramerization domain. The KCTD proteins contain a variable C-terminal region comprising homology domains H1 and H2 (H2 present only in KCTD8 and 16). The T1 as well as H1 domains of the KCTDs assemble into pentameric structures (Smaldone et al. 2016; Pinkas et al. 2017; Zheng et al. 2019; Zuo et al. 2019). Apart from $GABA_{B2}$, the KCTDs also bind to Gβγ subunits of the G protein (Turecek et al. 2014). The dual binding of KCTD proteins to the receptor and the G proteins stabilizes the interaction of the receptor with the G protein, which accelerates G protein signaling (Turecek et al. 2014; Fritzius et al. 2024).

KCTD proteins are regarded as accessory proteins conveying important characteristics to the receptor, such as increased affinity for agonists, fast desensitization, and increased cell surface stability (Schwenk et al. 2010; Seddik et al. 2012; Turecek et al. 2014; Zheng et al. 2019; Ivankova et al. 2013; Booker et al. 2017). The differential association of KCTD members with $GABA_B$ receptors is thought to constitute the basis for distinct functional receptor core complexes, which build in combination with various other more stably or transiently associated proteins $GABA_B$ receptor signaling complexes with distinct properties. Currently, such distinct $GABA_B$ receptor signaling complexes remain to be identified. In particular, the

characterization and establishment of $GABA_B$ receptor signaling complexes and their dynamics represent challenging future tasks.

So far, many $GABA_B$ receptor-interacting proteins have been identified, including $GABA_B$ receptor effector proteins. Other interacting proteins have been shown to regulate $GABA_B$ receptor function, trafficking, cell surface stability, and degradation (see Table 1.1 for further information).

1.3 Targeting Disease-Relevant $GABA_B$ Receptor Protein-Protein Interactions: A Novel Approach in $GABA_B$ Receptor Drug Discovery

Proper brain function depends on a precise balance and regulation of excitatory and inhibitory activity. Chronic deviations from the excitatory/inhibitory balance are associated with many neuronal diseases and commonly involve de-regulation of $GABA_B$ receptors (Jacobson et al. 2018; Princivalle 2022; Vlachou 2021; Zhang et al. 2021; Wang et al. 2022). Hence, $GABA_B$ receptors represent a promising drug target for several neurological diseases, ranging from neurodegenerative disorders, anxiety, depression, addiction, and epilepsy to cerebral ischemia. Currently, the $GABA_B$ receptor agonist baclofen is the only clinically approved drug, mainly for the treatment of severe spasticity and its associated pain caused by CNS injury (Slonimski et al. 2004; Bowery 2016; see Chap. 3 of this volume). Although there are a variety of potential indications for the therapeutic use of baclofen, its poor penetration of the blood-brain barrier, the development of tolerance at high concentrations, and numerous reported side effects such as sedation, dizziness, fatigue, insomnia, headache, paresthesia, tinnitus, and restlessness restrict its broad clinical application (Brennan and Whittle 2008; Rolland et al. 2018; see Chap. 7 of this volume). In view of the almost ubiquitous distribution of $GABA_B$ receptors in the CNS (Castelli and Gessa 2016) and their involvement in many important brain functions, it is not surprising that global activation of $GABA_B$ receptors is associated with substantial side effects. Therefore, it is rather unlikely that the development of new orthosteric receptor agonists can solve the shortcomings associated with baclofen. An important step forward to approach this problem was the development of allosteric modulators of $GABA_B$ receptors (Froestl 2010; Urwyler 2011, 2016). Pure allosteric modulators increase (positive modulators) or decrease (negative modulators) the affinity and/or efficacy of the receptor for GABA, resulting in an "use-dependent" modulation of $GABA_B$ receptor activity. This kind of modulating the receptor activity seems to be associated with considerably fewer side effects. However, in disease conditions associated with a severely reduced GABAergic tone, allosteric modulators may display limited efficacy, as shown for *rac*-BHFF in the chronic constriction injury mouse model of neuropathic pain (Zemoura et al. 2016a). In addition, the problem of the widespread targeting of $GABA_B$ receptors not involved in the disease state remains. Therefore, more specific interventions

Table 1.1 Proteins interacting with the minimal functional $GABA_B$ receptor (GBR) core complex consisting of $GABA_{B1}$ (GB1), $GABA_{B2}$ (GB2), and $G_{i/o}$ proteins

	Effect	References
Accessory proteins		
KCTD8	Increase agonist potency and GBR responses	Schwenk et al. (2010)
KCTD12	Increase GBR cell surface stability, required for fast GBR desensitization	Ivankova et al. (2013), Turecek et al. (2014), Schwenk et al. (2010), Seddik et al. (2012), Booker et al. (2017), and Zheng et al. (2019)
KCTD12b	Required for fast GBR desensitization	Schwenk et al. (2010) and Seddik et al. (2012)
KCTD16	Increase agonist potency and GBR responses	Schwenk et al. (2010)
Effector proteins		
N-type voltage-gated Ca^{2+} channels	GBR activation inhibits N- and P/Q-type Ca^{2+} channels via Gβγ in most neurons at presynaptic sites to reduce neurotransmitter release. Postsynaptic GBR activation inhibits dendritic N-P/Q-type channels	Schwenk et al. (2016), Chalifoux and Carter (2011)
P/Q-type voltage-gated Ca^{2+} channels		
L-type voltage-gated Ca^{2+} channels	Co-clustering in dendrites with GBRs. Activation of GBRs inhibits channel activity	Booker et al. (2018), Chalifoux and Carter (2011)
R-type voltage-gated Ca^{2+} channels	Interacts with GBRs via KCTD8 and KCTD12b. KCTD8 increases Ca^{2+} channel activity and basal neurotransmitter release probability; KCTD12b prevents increased basal release	Bhandari et al. (2021), Chalifoux and Carter (2011)
G protein-gated inwardly rectifying K^+ channels (GIRK1–3, $K_{ir}1$–3)	K^+ channels are not co-purified in stringent proteomic screens. However, they are co-immunoprecipitated and co-regulated with GBRs. GBR-activated K^+ channels induce inhibitory postsynaptic currents	David et al. (2006), Fowler et al. (2007), Ciruela et al. (2010), Fernandez-Alacid et al. (2009); Hearing et al. (2013), Padgett et al. (2012), and Luján et al. (2018)
Hyperpolarization-activated cyclic nucleotide-gated channel (HNC1/2)	Associates with GBRs via KCTD16. Activation of HCN currents in VTA dopamine neurons via GBRs shortens inhibitory postsynaptic potentials	Schwenk et al. (2016)
Two-pore domain K^+ channel 2 (TREK1/2) (No direct interaction with GBR is shown)	GBR-mediated reduction of PKA activity activates TREK2 channels via releasing tonic inhibition of the channels by PKA	Deng et al. (2009), Sandoz et al. (2012), and Chang et al. (2021)

(continued)

Table 1.1 (continued)

	Effect	References
Proteins regulating GBR function		
G protein-coupled receptor kinases (GRK4, GRK5)	Involved in GBR desensitization. Caused by interaction but not phosphorylation	Perroy et al. (2003) and Kanaide et al. (2007)
N-ethylmaleimide-sensitive fusion protein (NSF)	Involved in receptor PKC-dependent GBR desensitization	Pontier et al. (2006)
R7-RGS proteins (Regulators of G protein signaling)	Interact with GBRs and GIRK channels to accelerate deactivation kinetics of GIRK channels	Fajardo-Serrano et al. (2013), Fowler et al. (2007), and Xie et al. (2010)
Gα inhibitory interacting protein (GINIP)	Interacts via Gαi proteins with GBRs. Stabilizes GBR-induced G protein signaling	Gaillard et al. (2014)
14-3-3η	Decouples GBRs from $K_{ir}3$ channels after treatment with antidepressants	Workman et al. (2015) and Schwenk et al. (2016)
Syntaxin 1	Interacts with GB1 and N-type Ca^{2+} channels to modulate Gβγ interaction and vesicle release	Vertkin et al. (2015) and Jarvis et al. (2000)
Tenacin-HNK1	Interaction suppresses postsynaptic GBR K^+ currents in CA1 pyramidal neurons	Saghatelyan et al. (2001, 2003)
Proteins regulating GBR trafficking		
Prenylated Rab acceptor 1 domain family—member 2 (PRAF2)	Binds to GB1 for ER retention to regulate forward trafficking of GBRs to the cell surface	Doly et al. (2015)
CCAAT/enhancer-binding protein homologous protein (CHOP)	Binds to GB1 and GB2 in the ER to prevent GBR assembly. Inhibits forward trafficking of the receptors to the cell surface	Sauter et al. (2005) and Maier et al. (2014)
Coat protein I complex (COPI)	Involved in ER retention of GB1. Retrograde transport of unassembled GB1 from cis-Golgi back to the ER	Brock et al. (2005)
Shroom 4 (shrm4)	Involved in intracellular GBR trafficking and control of cell surface receptor expression	Zapata et al. (2017)
Rat homolog of B2-1/cytohesin-1 (msec7-1)	Facilitates cell surface transport	Restituito et al. (2005)
G protein-coupled receptor-interacting scaffolding protein (GISP)	Involved in forward trafficking and stabilization of GBR at the cell surface	Kantamneni et al. (2007, 2008)
Marlin 1	Links GB1 to tubulin cytoskeleton for dendritic transport	Vidal et al. (2007)

(continued)

Table 1.1 (continued)

	Effect	References
β-Amyloid precursor protein (APP)	Binds to N-terminal sushi domain in GB1a. and is involved in axonal trafficking of GBRs. Interaction of GBRs with APP stabilizes APP and inhibits Aβ formation	Schwenk et al. (2016) and Dinamarca et al. (2019)
Protein phosphatase 2A	Dephosphorylation of GB2 Ser783 inhibits constitutive GBR recycling	Terunuma et al. (2010), Hleihil et al. (2022), and Li et al. (2020)
Proteins regulating GBR cell surface residence time		
Ras-associated protein 1 (Rap1)	Agonist-induced stabilization of cell surface GBRs by facilitation of recycling	Zhang et al. (2015)
AMP-activated protein kinase (AMPK)	Interacts with GB1 and phosphorylates GB1 Ser917 and GB2 Ser783. Stabilizes GBR at the cell surface	Kuramoto et al. (2007)
Multi-PDZ domain protein1 (MUPP1)	Interact with the C-terminal PDZ binding motif in GB2. Stabilize cell surface GBRs	Balasubramanian et al. (2007)
14-3-3ζ	Interferes with the GBR heteromer at the cell surface in spinal cord neurons	Laffray et al. (2012) and Couve et al. (2001)
Proteins regulating GBR degradation		
Ubiquitin-specific protease 14 (USP14)	Most likely involved in endocytosis and lysosomal degradation pathways of GBRs	Lahaie et al. (2016)
Ca^{2+} calmodulin-dependent protein kinase IIβ (CaMKIIβ)	Regulates/induces phosphorylation of GB1 by ERK1/2, which is a signal for sorting the receptors to lysosomal degradation	Bhat et al. (2023a), Balakrishnan et al. (2023), Guetg et al. (2010), and Zemoura et al. (2019)
Extracellular signal-regulated protein kinase 1 and 2 (ERK1/2)	Phosphorylates of GB1 Ser867/ Thr872, which sorts the receptors to lysosomal degradation	Bhat et al. (2023a)
Mind bomb 2 (MIP2)	Mediates Lys63-linked ubiquitination of GB1. Signal for lysosomal degradation of GBRs	Zemoura et al. (2016b, 2019)
Hrd1 ubiquitin ligase	Mediates Lys48-linked ubiquitination of GBRs in the ER as signal for proteasomal degradation	Zemoura et al. (2013)
AAA-ATPase p97	Retrotranslocation of GBRs from ER membranes to the cytoplasm for proteasomal degradation	Zemoura et al. (2013)
AAA-ATPase Rpt6	Component of the proteasome. Interaction with the C-terminus of GB2 is required for proteasomal degradation	Zemoura and Benke (2014)

GBR interactions with other receptors are not included. Only proteins with established effects are listed

targeting selectively those receptors involved in the disease state may result in drugs with ideally neglectable unwanted side effects. A promising approach in this direction is the targeting of disease-relevant protein-protein interactions associated with $GABA_B$ receptors.

Most biological processes are primarily regulated via protein-protein interactions. The human interactome has been estimated to cover more than 300,000 protein-protein interactions (Zhang et al. 2012). Therefore, targeting protein-protein interactions is considered a promising opportunity for the development of novel, highly specific therapeutics for even previously "undruggable" classified proteins (Lee et al. 2019; Hashemi et al. 2021; Bruzzoni-Giovanelli et al. 2018; Rosenbaum et al. 2020). There are now the first preclinical endeavors targeting $GABA_B$ receptor protein-protein interactions involved in neurological disease.

1.3.1 Targeting the Interaction of $GABA_B$ Receptors with 14-3-3ζ in Neuropathic Pain

$GABA_B$ receptors play an important role in the modulation of pain signals and chronic pain states (Benke 2022; Enna and McCarson 2016) and are therefore considered promising drug targets for the development of novel non-opioid analgesics. Neuropathic pain is a form of chronic pain originating from nerve injury often associated with diseases that lead to peripheral or central neuronal damage such as diabetes, cancer, multiple sclerosis, and HIV (Schreiber et al. 2015; Davis 2018; Khan and Smith 2014; Aziz-Donnelly and Harrison 2017). The development of neuropathic pain is associated with a multitude of plastic alterations in the CNS, including a prominent reduction of GABAergic inhibitory control (Zeilhofer et al. 2012; Sandkuhler 2009; Gangadharan and Kuner 2013). In various animal models of neuropathic pain administration of baclofen reduced nociceptive responses, documenting the importance of $GABA_B$ receptors in controlling pain signals (Smith et al. 1994; Patel et al. 2001; Magnaghi et al. 2014; Hwang and Yaksh 1997; Malan et al. 2002; Gwak et al. 2006; Bai et al. 2014; Dias and Prado 2016; Zemoura et al. 2016a; Liu et al. 2018; Migita et al. 2018).

A very intriguing mechanism impairing $GABA_B$ receptor activity under conditions of neuropathic pain involves the interaction of $GABA_B$ receptors with 14-3-3ζ (Laffray et al. 2012). 14-3-3 proteins (14-3-3β, γ, ε, ζ, η, σ, and τ) are ubiquitously expressed in eukaryotic cells. They exert regulatory functions by interacting with numerous target proteins such as kinases, phosphatases, or transmembrane receptors and regulate a variety of cellular processes ranging from protein trafficking, apoptosis, cell cycle, signal transduction, and cell adhesion to metabolism. Alterations in the expression levels of 14-3-3 proteins and/or changes in the interaction status with target proteins have been observed in many diseases including neurological disorders (Zhao et al. 2011; Kaplan et al. 2017). In particular, targeting

14-3-3 interactions with G protein-coupled receptors has been proposed as promising drug targets (Kongsamut and Eishingdrelo 2023).

Among 14-3-3 proteins, 14-3-3ζ has been shown to interact with the C-terminal domain of $GABA_{B1}$ and in vitro inhibit the heterodimerization of $GABA_{B1}$ and $GABA_{B2}$ C-terminal domains (Couve et al. 2001). In a rat model of neuropathic pain (spinal nerve ligation), a significant upregulation of 14-3-3ζ and increased interaction with $GABA_B$ receptors in the dorsal horn of the lumbar spinal cord were observed (Laffray et al. 2012). Neurons in the dorsal horn process nociceptive signals in response to the injury. The increased interaction of 14-3-3ζ with $GABA_B$ receptors appeared to disrupt receptor heteromers and consequently diminished $GABA_B$ receptor signaling. Preventing the interaction of $GABA_B$ receptors with 14-3-3ζ using an interfering synthetic peptide restored $GABA_B$ receptor expression and signaling in the plasma membrane. Most importantly, restoration of functional $GABA_B$ receptors enhanced baclofen-mediated analgesia and partially reversed nocifensive behaviors in the absence of baclofen. These findings support a role for diminished $GABA_B$ receptor signaling in neuropathic pain and may be a starting point for the development of a novel generation of analgesics.

1.3.2 Targeting $GABA_B$ Receptor Interactions in Cerebral Ischemia

Although stroke is a major global health problem (Collaborators 2021), an efficient and adequate pharmacological treatment to reduce neuronal death in stroke patients is still lacking. In cerebral ischemia, severely reduced blood supply deprives neurons from oxygen and energy, leading to rapid death of neurons in the ischemic core. The only available pharmacological treatment for acute ischemic stroke is the application of thrombolytic drugs (alteplase, tenecteplase) within 4–5 h after stroke onset to restore blood circulation by resolving the blood clot (Shen et al. 2023; Tsivgoulis et al. 2023). Even after successful restoration of blood flow, neuronal death is spreading to brain tissue surrounding the ischemic core (ischemic penumbra) due to sustained depolarizations and the massive release of glutamate overstimulating glutamate receptors (excitotoxicity) (Neves et al. 2023). It is very unlikely that any pharmacological treatment can significantly reduce neuronal death in the ischemic core, which suffers from the almost immediate death of neurons. Therefore, progressive neuronal death in the ischemic penumbra is the target for the development of neuroprotective approaches.

There is strong preclinical evidence from in vivo models that sustained activation of $GABA_B$ receptors by baclofen provides neuroprotection to some extent (Lal et al. 1995; Jackson-Friedman et al. 1997; Kulinskii and Mikhel'son 2000; Babcock et al. 2002; Dave et al. 2005; Zhang et al. 2007; Xu et al. 2008; Liu et al. 2015; Hleihil et al. 2021). However, the neuroprotective efficiency of baclofen is restricted by the fact that $GABA_B$ receptors are downregulated after an ischemic insult (Kim et al.

2011; Zhu et al. 2015; Huang et al. 2017; Hleihil et al. 2021). One important function of $GABA_B$ receptors is to counteract excessive neuronal activity to prevent neurons from shifting into an excitotoxic condition. Therefore, restoring $GABA_B$ receptor expression after an ischemic insult by interfering with protein interactions that cause the downregulation of the receptors might be a promising neuroprotective approach. A first indication that this indeed might be a valid strategy came from the observation that treatment of mice with baclofen that were subjected to middle cerebral artery occlusion (MCAO) partially restored $GABA_B$ receptor expression in the affected brain areas and reduced progressive neuronal death (Hleihil et al. 2021). Currently, two pathways have been identified causing the downregulation of $GABA_B$ receptors under ischemic conditions (Fig. 1.5).

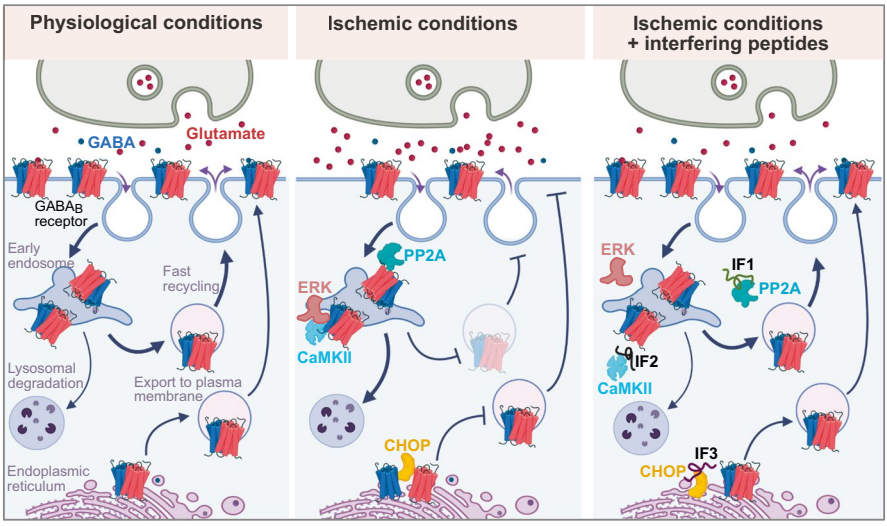

Fig. 1.5 Mechanisms downregulating $GABA_B$ receptors under ischemic conditions and action of interfering peptides to restore receptor expression. $GABA_B$ receptors undergo constitutive internalization under physiological conditions. Most of the receptors are recycled back to the cell surface, and a fraction is sorted into lysosomes for degradation. Degraded receptors are replaced by new receptors exported from the ER. Under ischemic conditions, excitotoxic stress activates PP2A. PP2A dephosphorylates Ser783 in the $GABA_{B2}$ subunit, which inhibits the recycling of the receptors. In addition, raised intracellular Ca^{2+} levels activate CaMKIIβ, which in turn recruits active ERK1/2 to the receptor to phosphorylate Ser867 and Thr872 in the $GABA_{B1}$ subunit of internalized receptors. Phosphorylation of Ser876/Thr872 serves as a signal for sorting the receptors into lysosomes. These events cause rapid downregulation of $GABA_B$ receptors from the plasma membrane. Furthermore, ischemia-induced ER stress upregulates the transcription factor CHOP. Upregulated CHOP interacts with $GABA_B$ receptors in the ER and interferes with receptor heterodimerization and their ER exit. This traps the receptor subunits in the ER and depletes receptors from the plasma membrane because of persisting lysosomal degradation of the receptors. Application of interfering peptides inhibiting the interaction of PP2A, CaMKII, or CHOP with $GABA_B$ receptors normalizes all aberrant trafficking events and restores plasma membrane expression of $GABA_B$ receptors. This inhibits neuronal overexcitation and progressive neuronal death. IF1: interfering peptide 1 targeting PP2A, IF2: interfering peptide 2 targeting CaMKII, IF3: interfering peptide 3 targeting CHOP. (This figure was adapted from Bhat et al. (2023b))

1.3.2.1 Endoplasmic Reticulum Stress-Induced Downregulation of $GABA_B$ Receptors

Cerebral ischemia is associated with severe cellular stress. This impairs ER function, causing the accumulation of proteins in the ER, which in turn activates ER stress response pathways to restore proper function of the ER (Kaneko et al. 2017; Hetz 2012). When the restoration of ER function fails, apoptosis is induced. One major ER stress-induced apoptotic pathway is triggered by the upregulation of the proapoptotic transcription factor C/EBP homologous protein (CHOP, also known as growth arrest and DNA damage-inducible gene 153 (GADD153)) (Hu et al. 2019; Yang et al. 2017; Oyadomari and Mori 2004).

In addition to its function as proapoptotic transcription factor, upregulated CHOP also interacts with $GABA_B$ receptors via the leucine-zipper domain with the intracellularly located C-terminal domain of $GABA_{B2}$ to regulate cell surface expression of the receptors (Sauter et al. 2005). Binding of CHOP to $GABA_B$ receptors prevents dimerization of $GABA_{B1}$ and $GABA_{B2}$ in the ER. This blocks the exit of assembled receptors from the ER and their forward trafficking to the plasma membrane (Maier et al. 2014). Ongoing constitutive internalization and lysosomal degradation of the receptors cause the depletion of $GABA_B$ receptors from the cell surface because the supply of newly synthetized receptors is inhibited. Blocking the interaction of $GABA_B$ receptors with a recently developed interfering peptide restored $GABA_B$ receptor cell surface expression and function in cultured neurons subjected to oxygen and glucose deprivation (OGD) (Bhat et al. 2022). Re-establishing normal $GABA_B$ receptor function was sufficient to reduce enhanced neuronal activity in OGD-stressed neurons almost to normal levels and to inhibit progressive neuronal death (Bhat et al. 2022). Because CHOP is selectively upregulated upon severe ER stress, this is a striking example of a disease-specific receptor-protein interaction. However, the activity of this interfering peptide needs to be verified in an animal model of cerebral ischemia before this protein-protein interaction can be considered a target for the development of a neuroprotective intervention.

1.3.2.2 Overexcitation-Induced Downregulation of $GABA_B$ Receptors

Cell surface expression of $GABA_B$ receptors is determined via trafficking events that are precisely regulated by the activity state of the neuron. Under normal physiological conditions, $GABA_B$ receptors are constitutively internalized, and most of the receptors are recycled back to the plasma membrane while a fraction is sorted to lysosomes for degradation (Fig. 1.5) (Benke 2010). Moderately enhanced neuronal activity that activates synaptic NMDA receptors stabilizes cell surface $GABA_B$ receptors by increasing the rate of receptor recycling (Kantamneni et al. 2014). However, excessive neuronal excitation that activates proapoptotic extrasynaptic NMDA receptors severely downregulates $GABA_B$ receptors by reduced recycling

and increased lysosomal degradation (Guetg et al. 2010; Maier et al. 2010; Terunuma et al. 2010).

So far, several $GABA_B$ receptor protein interactions have been identified as suitable targets for the development of interfering peptides to restore $GABA_B$ receptor expression and receptor-mediated inhibition. In vitro studies on cultured neurons discovered dephosphorylation of serine 783 in $GABA_{B2}$ by protein phosphatase 2A (PP2A) as a critical step involved in the downregulation of the receptors by inhibiting fast recycling of the receptors (Fig. 1.5) (Terunuma et al. 2010; Hleihil et al. 2022).

A second critical step is orchestrated by CaMKIIβ. Elevated intracellular Ca^{2+} levels activate CaMKIIβ, which induces phosphorylation of Ser867 in $GABA_{B1}$ (Guetg et al. 2010; Zemoura et al. 2019). Recent evidence indicates that CaMKIIβ activates ERK1/2, which in turn phosphorylates serine 867 and threonine 872 in $GABA_{B1}$ (Bhat et al. 2023a). Phosphorylation of $GABA_{B1}$ at these sites recruits the E3-ubiquitin ligase mind bomb 2 (MIB2) for K63-linked ubiquitination of the receptors, serving as a signal for their lysosomal degradation (Zemoura et al. 2016b, 2019). In principle, all the identified proteins interacting with $GABA_B$ receptors under ischemic conditions—PP2A, CaMKIIβ, ERK1/2, and MIB2—could serve as targets for the development of interfering peptides to reverse the de-regulated $GABA_B$ receptor trafficking. So far, peptides were developed to inhibit the interaction of $GABA_B$ receptors with PP2A and with CaMKIIβ (Balakrishnan et al. 2023; Hleihil et al. 2022). Treatment of cultured neurons subjected to ischemic conditions or brain slices derived from mice subjected to middle cerebral artery occlusion (MCAO) with either peptide restored cell surface expression of functional $GABA_B$ receptors, normalized aberrant expression of $GABA_B$ receptor effector proteins, reduced neuronal excitability, and inhibited progressive neuronal death (Fig. 1.6) (Hleihil et al. 2022; Balakrishnan et al. 2023). Thus, both peptides can be considered solid candidates for further development.

1.3.3 Targeting $GABA_B$ Receptor Downregulation in Psychostimulant-Induced Addiction

Psychostimulants can mediate addiction by inducing long-lasting plastic neuronal adaptations via enhanced dopamine release in the mesocorticolimbic dopamine pathway (Ostroumov and Dani 2018; Fischer et al. 2021). The mesocorticolimbic system is involved in many cognitive processes, including reward, motivation, learning, and fear (Volman et al. 2013; Baik 2020). Within this system, $GABA_B$ receptors control neuronal excitability and the release of GABA, glutamate and dopamine (Lalive and Lüscher 2016). It is therefore no surprise that baclofen-mediated activation of $GABA_B$ receptors reduced several addictive behaviors in preclinical studies including, motor sensitization, drug seeking, drug self-administration, and drug-induced cognitive impairment (Bartoletti et al. 2004, 2005; Cedillo et al.

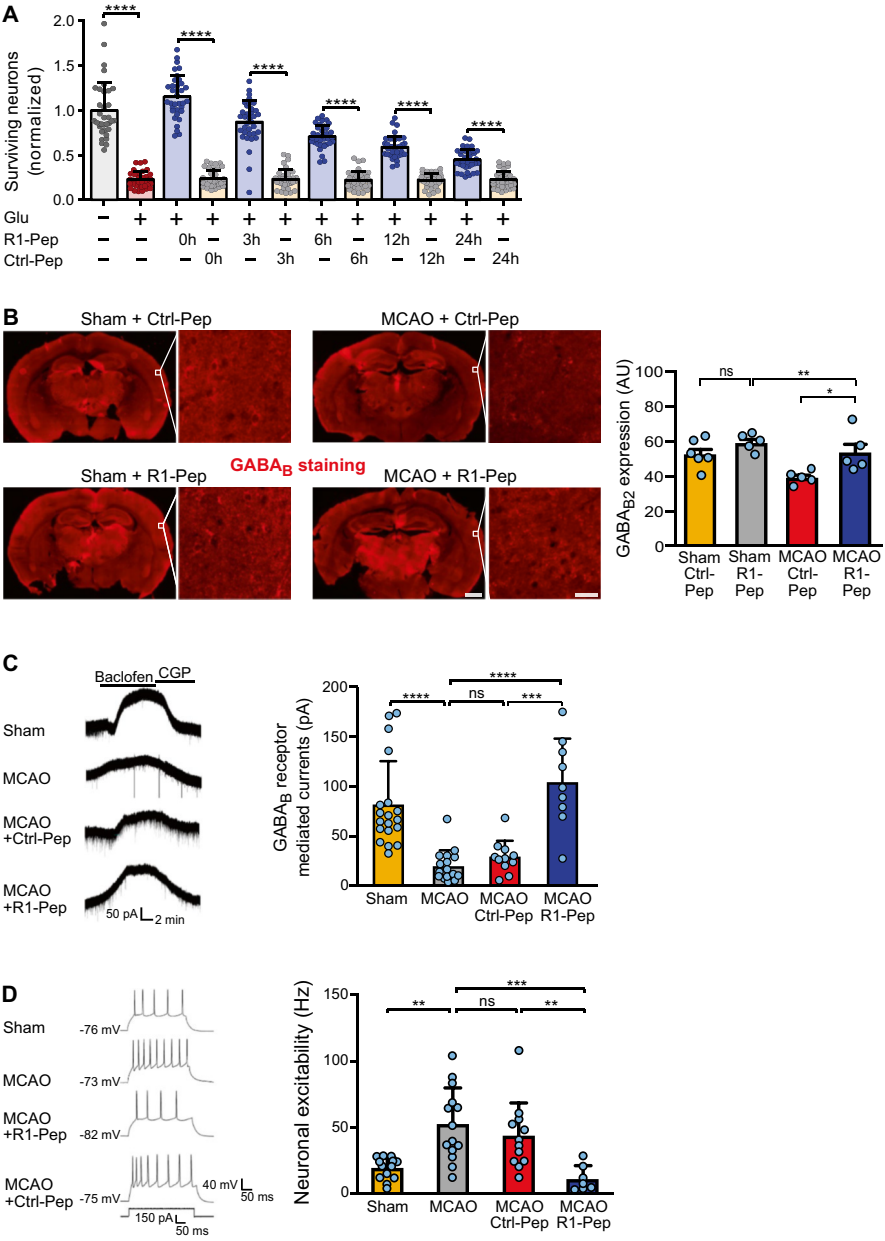

Fig. 1.6 Restoring $GABA_B$ receptor expression by inhibiting the interaction of the receptor with CaMKII after an ischemic insult normalizes neuronal excitability and provides neuroprotection. (**a**) The interfering peptide (R1-Pep), blocking the interaction of $GABA_B$ receptors with CaMKII, inhibits progressive glutamate-induced neuronal death within a wide time frame. Cultured neurons were stressed with glutamate (Glu) and treated with no peptide (Ctrl), R1-Pep, or an inactive control peptide (Ctrl-Pep) 0 h, 3 h, 6 h, 12 h, and 24 h thereafter. After 48 h, the cultures were stained

2019; Li et al. 2001; Roberts and Brebner 2000; Ranaldi and Poeggel 2002; Brebner et al. 2005; Arai et al. 2009; Mizoguchi and Yamada 2011).

Although baclofen can reduce addictive phenotypes, its efficacy might be severely affected by the observation that $GABA_B$ receptor-mediated inhibition is considerably reduced in some key structures of the mesocorticolimbic system after psychostimulant treatment. The observed reduced inhibitory control has been attributed to downregulation of $GABA_B$ receptors and/or GIRK channels (Arora et al. 2011; Padgett et al. 2012; Munoz et al. 2016; Hearing et al. 2013; Sharpe et al. 2015; Lu et al. 2023). In the nucleus accumbens of cocaine-addicted mice, downregulation of $GABA_B$ receptors involved the activation of CaMKII (Lu et al. 2023). In the medial prefrontal cortex (Hearing et al. 2013) and VTA (Padgett et al. 2012), PP2A-mediated downregulation of receptors was detected after cocaine or methamphetamine exposure, respectively.

A recent study tested whether blocking the interaction of $GABA_B$ receptors with PP2A in the VTA, which is a key structure triggering addiction (Nestler 2005), would restore $GABA_B$ receptor expression in methamphetamine-addicted mice and diminish addiction behaviors (Hleihil and Benke 2024). A significantly reduced expression of $GABA_B$ receptors was detected in the VTA of methamphetamine-addicted mice, which was normalized upon infusion of the interfering peptide into the VTA (Fig. 1.7). Restoration of VTA $GABA_B$ receptor expression inhibited methamphetamine-induced locomotor sensitization and reduced drug-seeking behavior (Hleihil and Benke 2024). These observations support the importance of VTA $GABA_B$ receptors in controlling addictive phenotypes and indicate that re-establishing $GABA_B$ receptor expression is sufficient to diminish at least some addictive phenotypes. Therefore, the approach of interfering peptides might be a promising strategy for the development of an anti-addictive treatment.

Fig. 1.6 (continued) with 4′,6-diamidino-2-phenylindole (DAPI) for total cells (glia plus neurons) and with an antibody directed against NeuN for neurons and analyzed for surviving neurons. (**b**) Administration of R1-Pep restored $GABA_B$ receptor expression after MCAO. Brain slices containing the somatosensory cortex prepared from sham and MCAO-treated mice were incubated for 6 h with R1-Pep and then stained for $GABA_B$ receptor expression (scale bar low magnification: 1 mm, higher magnification: 20 μm). (**c**, **d**) R1-Pep restored $GABA_B$ receptor-mediated currents and reduced neuronal excitability. Brain slices prepared from the somatosensory cortex of sham-operated and MCAO mice were analyzed using whole-cell patch-clamp recordings 3–5 h after treatment with peptide. **C** R1-Pep restored $GABA_B$ receptor-mediated currents. Neurons were analyzed for baclofen-induced K^+ currents. Left: representative current traces, right: quantification of data. (**d**) R1-Pep reduced neuronal excitability. Neuronal excitability was determined by injecting increasing current steps. Left: representative traces, right: quantification of data. (This figure was adapted from Balakrishnan et al. (2023))

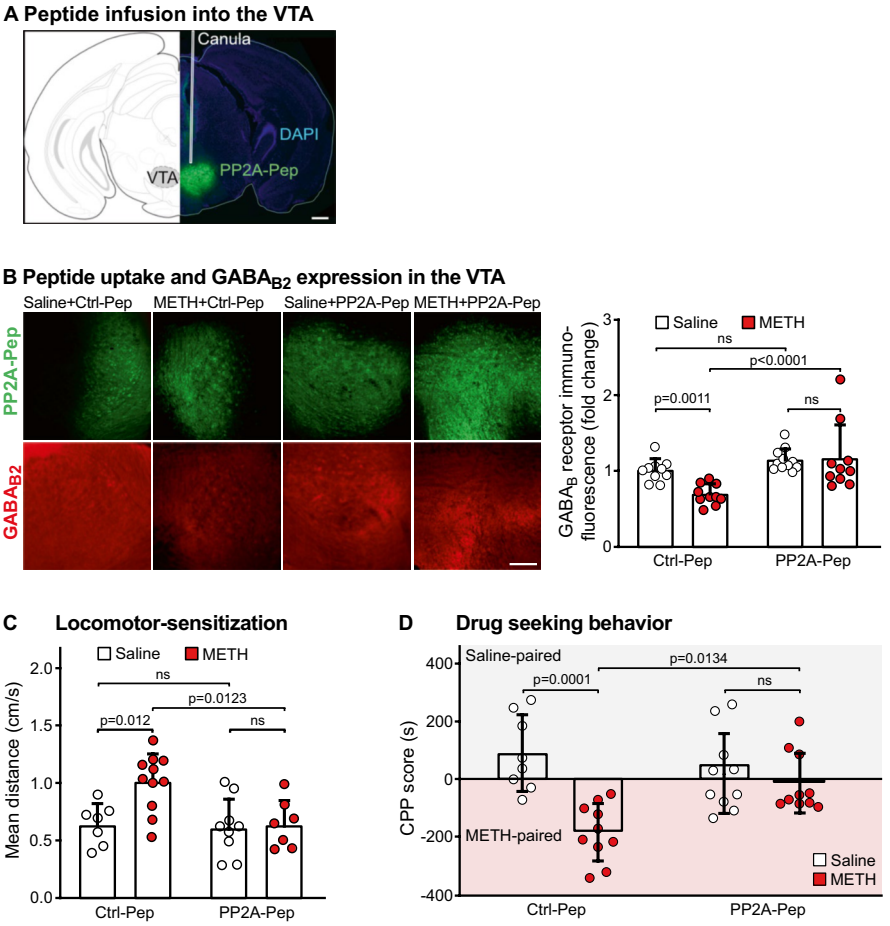

Fig. 1.7 Infusion of an interfering peptide (PP2A-Pep), inhibiting the interaction of PP2A with the $GABA_B$ receptor, into the VTA restored $GABA_B$ receptor expression and reduced methamphetamine-induced locomotor sensitization and drug-seeking behavior. (**a**) Infusion of PP2A-Pep into the VTA. Right: Brain section stained for the infused PP2A-Pep (green) and cell nuclei (DAPI, blue). The placement of the canula is indicated (white lines). Left: Location of section in the mouse brain atlas. Scale bar: 500 µm. (**b**) Infusion of PP2A-Pep into the VTA of METH-addicted mice normalized $GABA_B$ receptor expression. Mice received multiple METH/saline (METH-addicted mice) or saline/saline (control mice) injections and were then either infused with PP2A-Pep or an inactive control peptide (Ctrl-Pep) into the VTA. About 24 h after peptide infusion, the mice were then analyzed for $GABA_B$ receptor expression. Left: representative images display peptide uptake (green) and $GABA_B$ receptor expression (red) in the VTA (scale bar: 100 µm). Right: quantification of fluorescence intensities in the area stained for the peptide. (**c**) METH-addicted mice exhibited increased locomotor activity (Ctrl-Pep condition, red dots) compared to saline injected mice (Ctrl-Pep condition, white dots), which was restored to normal levels after PP2A-Pep infusion into the VTA (PP2A-Pep condition, red dots, assessed by the open field test). (**d**) Infusion of PP2A-Pep into the VTA of METH-addicted mice reduced drug-seeking behavior as tested by the conditioned place preference test (CCP). METH-conditioned mice showed increased preference for the METH-paired chamber (left, Ctrl-Pep condition). Infusion of PP2A-Pep into the VTA reduced the preference for the METH-paired chamber in METH-addicted mice (right, PP2A-Pep condition). (This figure was adapted from Hleihil and Benke (2024))

1.4 Conclusions

The research of the past decade has clearly established that $GABA_B$ receptors are assembled into various modular signaling complexes depending on the cellular context and required functions. A $GABA_B$ receptor "core complex" is built from $GABA_{B1}$ and $GABA_{B2}$ subunits in combination with KCDT proteins and G proteins (Schwenk et al. 2010, 2016). Effector proteins and additional proteins may transiently or weakly interact for a specific function, forming, most likely, temporary $GABA_B$ receptor signalosomes. A largely unresolved and challenging task remains the identification of individual $GABA_B$ receptor signalosomes and dissecting their composition and dynamics under physiological and pathological conditions. This knowledge would vastly widen the prospects for future drug targets for the development of novel therapeutic interventions targeting neurological diseases.

Current advances in dissecting the pathways and protein-protein interactions leading to the aberrant regulation of $GABA_B$ receptor trafficking in cerebral ischemia and addiction have already led to the development of effective interfering peptides. In the first preclinical experiments, application of these peptides restored $GABA_B$ receptor expression and function, resulting in neuroprotection and anti-addictive activity (Balakrishnan et al. 2023; Hleihil et al. 2022; Hleihil and Benke 2024). These are promising results, but there are significant hurdles to overcome associated with peptide drugs, such as lack of cell-type selective uptake, poor proteolytic stability after systemic application, and fast renal excretion of small peptides. Another problem might be the targeting of very strong protein-protein interactions. Attempts to develop an interfering peptide inhibiting the interaction of $GABA_B$ receptors with its accessory protein KCTD12 yielded, after optimization, a peptide with nanomolar affinity for KCTD12 (Sereikaite et al. 2019). Although this peptide exhibited high affinity binding to KCTD, it required high micromolar concentrations to inhibit the interaction of $GABA_B$ receptors/KCTD interaction, limiting its use for further in vivo studies. Weak or transient protein-protein interactions may therefore be more promising candidates for the development of efficient interfering peptides. The current availability of information on protein structures and protein-protein interaction databases will certainly vastly advance the identification, design, and optimization of new peptide drugs (Rosenbaum et al. 2020; Lee et al. 2019; Hashemi et al. 2021).

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Chapter 2
$GABA_B$ Receptor Functioning: Focus on Allosteric Modulation

Philippe Rondard, Julie Kniazeff, and Jean-Philippe Pin

Abstract $GABA_B$ receptors are activated by γ-aminobutyric acid (GABA), an amino acid that serves as the primary inhibitory neurotransmitter in the central nervous system, with links to a range of neurological diseases. This receptor represents a promising drug target in the field of neurological diseases for the treatment of spasticity, alcohol use disorder, anxiety, and insomnia using therapeutic options such as baclofen and phenibut. The $GABA_B$ receptor belongs to class C of the G protein-coupled receptors and acts as an obligate heterodimer made up of two subunits, $GABA_{B1}$ and $GABA_{B2}$. Each subunit is comprised of an extracellular domain in which GABA and the above-mentioned therapeutic drugs bind and a transmembrane domain responsible for G protein activation. $GABA_B$ features a unique allosteric mechanism for signal transduction in which agonist binding in the $GABA_{B1}$ extracellular domain elicits G protein activation via rearrangement of the intracellular face of the $GABA_{B2}$ transmembrane domain. More recently, the $GABA_B$ receptor has been found to display a tendency to form oligomers, thus increasing the complexity of allosteric communications in the receptor. This chapter summarizes the current state-of-the-art relating to the structure, activation mechanism, and allosteric modulation of the $GABA_B$ receptor, which may provide novel opportunities for the development of approaches aimed at regulating receptor activity.

Keywords G protein-coupled receptor · Heterodimer · Oligomer · Allosteric modulator

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2.1 Introduction

Since their discovery in the mid-1980s as the molecular target of the anti-spasticity drug baclofen, γ-aminobutyric acid (GABA) type-B ($GABA_B$) receptors have garnered considerable interest, with studies focused on elucidating their molecular bases (Bowery et al. 2002; Gassmann and Bettler 2012; Pin and Bettler 2016). Subsequently, toward the end of the 1990s, a discovery was made revealing the presence of two genes encoding $GABA_B$ receptors. The first gene encodes two variants, $GABA_{B1a}$ and $GABA_{B1b}$, via an alternative initiation site, adding two sushi domains (SDs) at the N-terminus of the $GABA_{B1a}$ variants (Kaupmann et al. 1997). The second subunit, $GABA_{B2}$, is structurally homologous to $GABA_{B1}$ and is essential in achieving agonist high affinity and effective G-protein coupling of the $GABA_B$ receptor heterodimer (Jones et al. 1998; Kaupmann et al. 1998; White et al. 1998). The $GABA_{B2}$ subunit has likewise proved to be crucial for proper membrane insertion of the $GABA_B$ receptor. Indeed, when expressed alone, $GABA_{B1}$ subunit remains intracellularly retained between the endoplasmic reticulum and Golgi due to an intracellular retention signal in its C-terminal tail (Margeta-Mitrovic et al. 2000; Pagano et al. 2001). However, interaction with the $GABA_{B2}$ subunit masks the retention signal and facilitates transportation of the heterodimer to the cell surface, thus highlighting the unique nature of this receptor in the context of G protein-coupled receptors (GPCRs) known at the time and representing the first obligatory heterodimeric GPCR. This mechanism of retention and trafficking of the $GABA_B$ receptor seems to be highly conserved during evolution, as recently demonstrated with the drosophila $GABA_B$ receptor (Zhang et al. 2020). Moreover, early studies demonstrated the implication of the $GABA_{B1}$ subunit in agonist binding, with $GABA_{B2}$ subunit proving essential in G protein activation (Galvez et al. 2001; Margeta-Mitrovic et al. 2001a, b). These findings represented a major breakthrough not only in the field of $GABA_B$ receptor studies but also in the wider GPCR community, in which the notion of GPCR dimerization was still the subject of intense debate (Sleno and Hébert 2019).

The general organization of the $GABA_B$ receptor is similar to that of other obligate dimeric class C GPCRs (Kniazeff et al. 2011), including receptors activated by glutamate (Koehl et al. 2019; Lin et al. 2021; Seven et al. 2021), calcium (He et al. 2024; Zuo et al. 2024) and sweet, and umami-tasting compounds (Xu et al. 2004) (Fig. 2.1a, b). The extensive $GABA_{B1}$ and $GABA_{B2}$ extracellular domains comprise a Venus flytrap-like (VFT) domain similar to the binding domain of metabotropic glutamate (mGlu) receptors and other class C GPCRs, with the VFT domain linked in both subunits to a heptahelical transmembrane (7TM) domain common to all GPCRs through a 10–15 amino-acid stalk region (Mao et al. 2020; Papasergi-Scott et al. 2020; Park et al. 2020; Shaye et al. 2020; Kim et al. 2020; Shen et al. 2021). The GABA binding site (Galvez et al. 1999) is only present in the VFT domain of the $GABA_{B1}$ subunit, whereas the $GABA_{B2}$ subunit alone is responsible for G protein coupling through rearrangement of the intracellular face of its transmembrane domain. More recent studies reported how the $GABA_B$ receptor displays a tendency

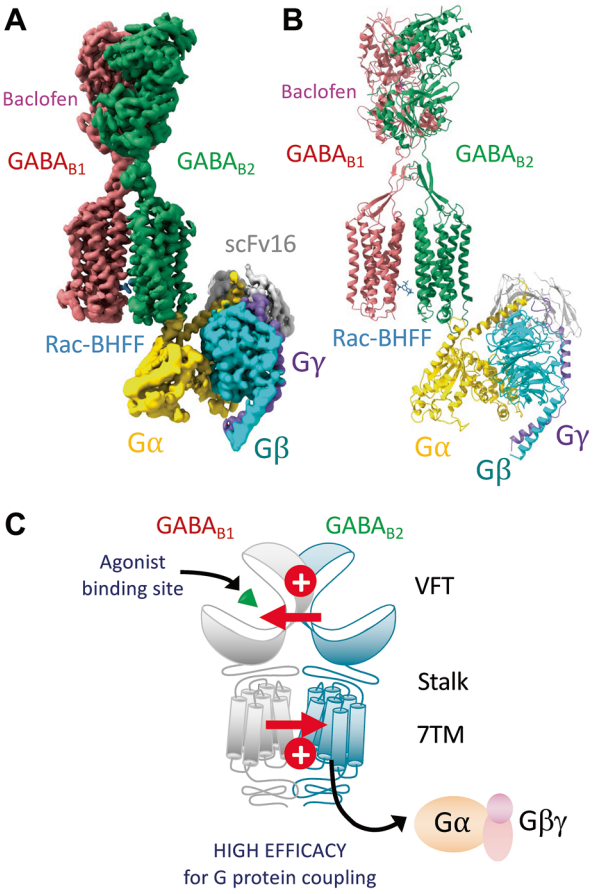

Fig. 2.1 $GABA_B$ receptor structural organization. (**a**, **b**) Cryo-electronic map of the baclofen bound to $GABA_B$ receptor in complex with the positive allosteric modulator, *rac*-BHFF, and the Gi protein (PDB 7EB2) (**a**) and its model ribbon view (**b**), respectively. (**c**) Positive allosteric interactions between $GABA_{B1}$ and $GABA_{B2}$ a Venus flytrap-like (VFT) domains (Liu et al. 2004) and between $GABA_{B1}$ and $GABA_{B2}$ heptahelical transmembrane (7TM) domains (Monnier et al. 2011) are illustrated

to form oligomers, thus increasing the complexity of allosteric communications in the receptor (Maurel et al. 2008; Comps-Agrar et al. 2011; Stewart et al. 2018; Xue et al. 2019); the formation of spontaneous, stable, and well-organized oligomers is a characteristic exclusive to the $GABA_B$ receptor in the GPCR family (Pin et al. 2009).

This unique organization of the $GABA_B$ receptor has prompted the undertaking of extensive studies aimed at elucidating the molecular bases of the activation mechanism and allosteric properties of this receptor. The atomic structures of the full-length $GABA_B$ receptor, including its complex with G protein (Shen et al. 2021), were recently revealed by five independent groups using cryo-electron microscopy (cryo-EM) (Mao et al. 2020; Papasergi-Scott et al. 2020; Park et al. 2020; Shaye et al. 2020, 2021; Kim et al. 2020) (Fig. 2.1a, b). These studies revealed a mode of coupling of the G protein that differed from those of other GPCRs, although it was conserved in other class C GPCRs (Lin et al. 2021; Seven et al. 2021; He et al. 2024; Zuo et al. 2024). These structures also contributed toward

clarifying the mode of action of positive allosteric modulators (PAMs), all of which were found to bind in the transmembrane interface formed by the $GABA_{B1}$ and $GABA_{B2}$ subunits (Mao et al. 2020; Shaye et al. 2020; Kim et al. 2020; Shen et al. 2021), thus at variance with the known binding sites of allosteric modulators of the class C GPCRs (Kumar et al. 2018; Seven et al. 2021; He et al. 2024; Zuo et al. 2024) and most other classes of GPCRs (Thal et al. 2018). These atomic structures will contribute to the development of novel therapeutic applications, including novel allosteric modulators aimed at modulating $GABA_B$ receptor activity.

In this chapter, we discuss the current state of the art relating to the structure and molecular basis of $GABA_B$ receptor activation to enhance our understanding of how this multidomain membrane protein is activated by a small ligand for the control of G protein activity. This chapter will address the issue of how allosteric transitions between the different domains regulate receptor activity and how the latter is controlled by allosteric modulators. A deeper understanding of these issues will undoubtedly provide novel options to be applied in the development of innovative treatments for use in regulating this important brain receptor and may hopefully shed light on possible assembly and allosteric interactions between other GPCRs.

2.2 Structure and Mechanism of Activation of the $GABA_B$ Receptor

Resolution of the crystal structures of the isolated extracellular domains of $GABA_{B1}$ and $GABA_{B2}$ subunits (Geng et al. 2012, 2013) and, more recently, of the full-length receptor (Mao et al. 2020; Papasergi-Scott et al. 2020; Park et al. 2020; Shaye et al. 2020; Kim et al. 2020; Shen et al. 2021) have provided major insight into the molecular mechanism of activation of $GABA_B$ receptors. In the absence of ligand (apo state), $GABA_{B1}$ and $GABA_{B2}$ VFT domains share a good structural homology (Geng et al. 2013). These VFT domains derive from bacterial periplasmic amino acid-binding proteins such as the leucine/isoleucine/valine binding protein (LIVP) (O'Hara et al. 1993). Each VFT domain (approx. 410 residues) (Rondard et al. 2011) is composed of two lobes linked by three short loops, with lobe 1 being the N-terminal lobe and lobe 2 the C-terminal one (Fig. 2.1a, b). The $GABA_{B1}$ VFT domain was observed in two separate conformations, being open in apo and antagonist-bound states and closed in the presence of agonists (Geng et al. 2012, 2013). In comparison, $GABA_{B2}$ VFT domain is invariably observed in an open conformation, with the angle defined by the two lobes remaining virtually constant for $GABA_{B2}$ VFT domain in all structures. The angle defined by the two lobes in $GABA_{B2}$ VFT domain is in line with the large angle observed for the apo state and antagonist-bound conformations of $GABA_{B1}$ VFT domain. The $GABA_{B2}$ VFT domain, however, remains in an open conformation, both alone and when associated with the open or closed $GABA_{B1}$ VFT domain (Geng et al. 2012, 2013). This confirms the observation whereby $GABA_{B1}$ subunit is the sole subunit binding

agonist in the $GABA_B$ receptor, underlying activation of the entire receptor complex (Kniazeff et al. 2002, 2004).

Structural and mutagenesis studies have been performed, describing the GABA binding site in $GABA_{B1}$ VFT domain in detail (Galvez et al. 1999; Galvez et al. 2000; Kniazeff et al. 2002; Geng et al. 2013). The carboxylate moiety of GABA lies 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 $GABA_{B1a}$ subunit 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. Baclofen, a $GABA_B$ receptor-specific agonist, binds in a similar way to GABA but with a conformational flip of Tyr 366 to accommodate the chlorophenyl moiety of the ligand (Galvez et al. 2000; Geng et al. 2013). Orthosteric receptor antagonists are GABA derivatives that also bind to $GABA_{B1}$ VFT domain alone. Co-crystallization of $GABA_B$ VFT domains with antagonists showed how they bind tightly to lobe 1, involving similar residues to GABA binding (Ser 246, Ser 269, His 286, Glu 465, and Trp 181). However, compared to agonist-bound conformation, only sparse interaction with lobe 2 is detected, in line with the greater distance between the two lobes and stabilization of an "open" conformation (Geng et al. 2013). Of note, the residues involved in GABA binding in $GABA_{B1}$ VFT domain are not conserved in $GABA_{B2}$ VFT domain (Kniazeff et al. 2002). In addition, in contrast to the $GABA_{B1}$ VFT domain cleft where agonists bind, the $GABA_{B2}$ cleft displays no specific or high conservation during evolution, strongly suggesting the absence of ligand interaction at this site (Kniazeff et al. 2002).

Upon agonist binding in $GABA_{B1}$ VFT domain, the VFT dimer undergoes relative rearrangement. The lobe 1 interface, situated between the two subunits consisting of a central hydrophobic patch surrounded by hydrogen bonds and a salt bridge, serves as a rotation axis. As a consequence, a higher proximity and interaction between the two lobes 2 is observed in agonist-bound structures, with additional contacts between the lobes 2 involving mainly polar interactions. Of note, this relative reorientation between the two VFT domains in the $GABA_B$ receptor is common to class C GPCRs containing a VFT domain such as that observed in mGlu receptors and calcium sensing (CaS) receptors, although the amplitude of this movement is considerably smaller in $GABA_B$ receptors. Our group has taken advantage of this reorientation of $GABA_B$ to develop fluorescence resonance energy transfer (FRET)-based conformational sensors (Lecat-Guillet et al. 2017) aimed at recording agonist-induced activation of the receptor by means of light measurement in high-throughput assays. However, consistent with the low amplitude reorientation of $GABA_B$ VFT domains, the position of the fluorophores was optimized to observe a change of FRET signal upon agonist activation in these $GABA_B$ receptor conformational sensors (Lecat-Guillet et al. 2017), compared to other sensors developed for mGlu receptors (Doumazane et al. 2013; Scholler et al. 2017; Lecat-Guillet et al. 2023) and CaS receptors (Liu et al. 2020). Indeed, in these latter conformational sensors, the attachment of fluorophores at the N-terminal end of the mGlu and CaS receptors was sufficient to measure a large change in the FRET signal, indicating a major conformational change in the reorientation of the VFT dimer in these receptors.

The cryo-EM structure of the full-length $GABA_B$ receptor also revealed the first atomic structures of $GABA_B$ 7TM domains (Fig. 2.1a, b), homologous to those found in all other GPCRs, including other class C receptors. However, the conformational change observed between the inactive and active states is more subtle, with a smaller movement of the transmembrane helix 6 (TM6) compared to class A and B GPCRs. Indeed, movement of the TM6 linked to receptor activation and G protein coupling represents one of the main characteristics of the activation mechanism of GPCRs (Weis and Kobilka 2018). A similarly low amplitude of this TM6 movement is conserved in other class C GPCRs, including the mGlu and CaS receptors, displaying consistency with a different mode of coupling of the G protein to the $GABA_B$ receptor (Shen et al. 2021). Indeed, surprisingly, the structure of the $GABA_B$ receptor in complex with the G protein has revealed how the latter interacts at a different position to the intracellular face of the class C receptors. In addition, as a consequence of the subtle movement of TM6, the C-terminal end of the Gα subunit does not penetrate deeply into the 7TM core of the $GABA_{B2}$ subunit but engages mainly with intracellular loops i2 and i3, as found in other class C mGlu (Lin et al. 2021; Seven et al. 2021) and CaS (He et al. 2024; Zuo et al. 2024) receptors. It is therefore rather a challenge to grasp how the $GABA_{B2}$ subunit activates the G protein by inducing the release of guanosine 5′-diphosphate (GDP) bound to the Gα subunit.

Another important feature is constituted by the 7TM heterodimer interface mediated by the two transmembrane helices 5 (TM5s) of $GABA_{B1}$ and $GABA_{B2}$ subunits in the inactive state, and TM6s in the active state (Mao et al. 2020; Papasergi-Scott et al. 2020; Park et al. 2020; Shaye et al. 2020; Kim et al. 2020; Shen et al. 2021). This relative orientation of the 7TM domains is in agreement with a recent study conducted in our lab using site-directed mutagenesis and structure-function analysis, in which the interface of dimerization was revealed by means of a cysteine crosslinking approach (Xue et al. 2019). This study showed how it is sufficient to stabilize the interaction between the TM6s of $GABA_{B1}$ and $GABA_{B2}$ subunits to induce constitutive activity of the receptor. Of note, the subtle rearrangement of the 7TM interface in $GABA_B$ receptors between the inactive and active states compared to the mGlu receptors (Xue et al. 2015, 2019; Seven et al. 2021; He et al. 2024; Krishna Kumar et al. 2024; Zuo et al. 2024), is consistent with the subtle rearrangement described above in the VFT heterodimer between the inactive and active states. Finally, an important feature revealed by $GABA_B$ receptor structures is the stalk region that maintains a space between the VFT and 7TM domains in each subunit (Mao et al. 2020; Papasergi-Scott et al. 2020; Park et al. 2020; Shaye et al. 2020; Kim et al. 2020; Shen et al. 2021). The long extracellular loop 2 of each subunit protrudes from the 7TM core and interacts directly with these stalk regions. These interactions are most probably crucial in transmitting activation from VFT to 7TM domains, although the mechanism underlying this coupling remains unclear.

Unfortunately, the C-terminal regions of the $GABA_B$ receptor were not observed, as truncated subunits were used, and also due to the high flexibility of these regions. Indeed, the intracellular C-terminal region of $GABA_{B1}$ and $GABA_{B2}$ subunits is rather long (107 and 200 residues, respectively) and contains a well-structured

coiled-coil domain essential in achieving heterodimerization and ensuring a correct assembly of the heterodimer prior to appropriate targeting of the plasma membrane (Kammerer et al. 1999; Margeta-Mitrovic et al. 2000; Pagano et al. 2001). Heterodimeric interaction of the $GABA_B$ receptor is stabilized by a coiled-coil interaction between the $GABA_{B1}$ and $GABA_{B2}$ C-termini, encompassing approximately 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). The structure of $GABA_B$ receptor coiled-coil domain has been solved by X-ray crystallography, highlighting molecular details of the interaction (Burmakina et al. 2014). The structure of this domain is conserved during evolution, as recently shown by the structure of the coiled-coil domain of the drosophila $GABA_B$ receptor by nuclear magnetic resonance (NMR) (Zhang et al. 2020).

Finally, 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), resulting in the presence of a repeat of two sushi domains (SDs, named SD1 and SD2) at the extracellular N-terminus of $GABA_{B1a}$ subunit alone. SDs, also known as complement control protein (CCP) modules or short consensus repeats (SCR), are approx. 60 residues long and known to be involved in numerous recognition processes, including that of the complement system (Reid and Day 1989). In the case of the $GABA_B$ receptor, SDs control specific targeting of the receptor to excitatory terminals, most probably through interactions with the extracellular matrix (Vigot et al. 2006). SD2 is a typical SD having approximately 60 amino acid residues, including four cysteines forming two disulfide bridges (Blein et al. 2004). NMR analysis revealed the three-dimensional structure of SD2 to be similar to that of previously solved SD structures. 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).

2.3 Allosteric Interaction Within the $GABA_B$ Receptor and with the G Protein

The following section illustrates how allosteric communication between the four main domains in $GABA_B$ receptor concurs toward activation of the receptor (Fig. 2.1c). First, a positive allosteric interaction between $GABA_{B1}$ and $GABA_{B2}$ VFT domains was demonstrated (Liu et al. 2004; Geng et al. 2012), although $GABA_{B2}$ VFT domains also play an important role in facilitating $GABA_{B1}$ VFT closure, thereby resulting in a higher affinity for orthosteric receptor agonists (Liu et al. 2004; Geng et al. 2012). The tighter interaction between $GABA_{B1}$ and $GABA_{B2}$ VFTs in the presence of an agonist stabilizes the closed state of $GABA_{B1}$ VFT domain, increasing agonist potency and revealing a positive allosteric interaction between the two VFT domains (Fig. 2.1c). However, while interactions between the

lobes 2 are strictly required for activation of the wild-type receptor, a $GABA_B$ receptor mutant lacking $GABA_{B2}$ VFT domain is still functional, although displaying low agonist potency and low efficacy (Monnier et al. 2011).

A second major positive allosteric modulation in the $GABA_B$ receptor is represented by functional interaction between the two 7TM domains (Monnier et al. 2011) (Fig. 2.1c). Indeed, agonist-induced activity of the $GABA_B$ mutant lacking the $GABA_{B2}$ VFT domain described above has demonstrated the evidence of a strong allosteric coupling between the two 7TMs, referred to as transactivation. In this mechanism, coupling between the agonist-bound VFT and 7TM domains in $GABA_{B1}$ subunit is sufficient to stabilize the $GABA_{B2}$ 7TM in the active state through the 7TM dimer interface. The latter also highlights the importance of reorientation of the heterodimer interface from TM5-TM5 to TM6-TM6 interface in receptor activation, a conformational change expected to occur prior to activation of $GABA_{B2}$ 7TM core and G protein coupling (Shaye et al. 2020), according to the molecular mechanism of activation proposed for the mGlu receptors (Rondard and Pin 2015). It however remains unclear how this conformational change in the VFT dimer leads to activation of the $GABA_{B2}$ 7TM domain, although most likely implicating stalk domains and an interaction of these with the extracellular loops 2 of the two subunits.

The G protein is known to exert a positive allosteric effect in the context of stabilizing the active conformation of the 7TM domain of GPCRs (DeVree et al. 2016). The latter was well demonstrated using a FRET-based conformation sensor of $GABA_B$ receptor that reports conformational states of the VFT dimer (Lecat-Guillet et al. 2017). G protein mutants that facilitate coupling of the G protein to the $GABA_B$ receptor favor rearrangement of the VFT dimer to the active state upon agonist activation, as previously observed for the $mGlu_2$ receptor (Doumazane et al. 2013). Interestingly, using bioluminescence resonance energy transfer (BRET)-based G protein sensors, we have recently shown that native $GABA_B$ receptors in neurons couple to all Gi/o proteins in the same manner observed in human embryonic kidney 293 (HEK 293) cells, although agonist-induced activation of the G proteins by native receptors in neurons proved to be much more efficient (Xu et al. 2024). These studies also revealed how the native $GABA_B$ receptors in neurons do not seem to possess constitutive activity, in contrast to the recombinant receptors expressed in cells (Xu et al. 2024; Ma et al. 2024). This finding might be explained by the fact that $GABA_B$ receptors expressed in neurons are capable of interacting with both extracellular and intracellular proteins, likely implicated in the control of the conformational state and limitation of activation.

2.4 Allosteric Modulation by Positive Allosteric Modulators

In addition to the orthosteric receptor agonists and antagonists, allosteric modulators were developed to control the activity of the $GABA_B$ receptor by acting on other binding sites in the receptor (Urwyler 2011) (Fig. 2.2a). The $GABA_B$ PAMs are of

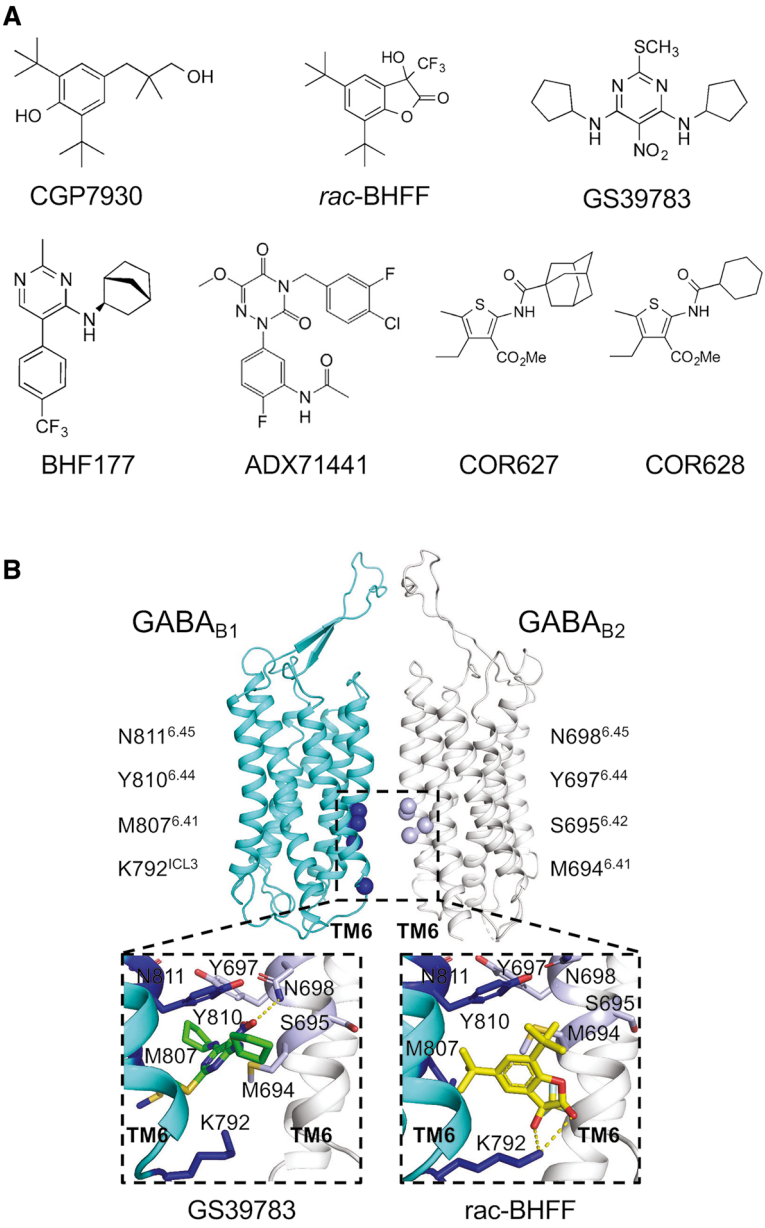

Fig. 2.2 Positive allosteric modulation of the GABA$_B$ receptor. (**a**) Chemical structure of the main positive allosteric modulators (PAMs) of GABA$_B$ receptors is indicated. CGP7930 (Urwyler et al. 2001), *rac*-BHFF (Malherbe et al. 2008), GS39783 (Dupuis et al. 2006), BHF177 (Guery et al. 2007), ADX71441 (Kalinichev et al. 2017), COR627, and COR628 (Castelli et al. 2012). (**b**) Binding site of the indicated GABA$_B$ PAMs at the interface between the heptahelical transmembrane (7TM) domains and, notably, transmembrane helices 6 (TM6s) of GABA$_{B1}$ and GABA$_{B2}$ was validated by site-directed mutagenesis and functional analysis (Liu et al. 2021)

special interest since they enable to favor the active conformation of the receptor. They are also expected to respect the physiological activity of the receptor by potentiating the activation by GABA, without having an agonist activity per se (Pin and Prézeau 2007), so that they may have better therapeutic efficacy, with fewer side effects.

Confirmation of the molecular basis underlying the action of these PAMs has recently been clarified using $GABA_B$ receptor structures (Mao et al. 2020; Shaye et al. 2020; Kim et al. 2020; Shen et al. 2021) together with FRET-based conformational $GABA_B$ sensors (Lecat-Guillet et al. 2017) and BRET-based G protein sensors (Xu et al. 2024). Indeed, while these compounds were expected to bind to the $GABA_{B2}$ 7TM core (Binet et al. 2004; Freyd et al. 2017), surprisingly, the solving of structures revealed how they all bind at the 7TM heterodimer interface, stabilizing the active interface formed by the two TM6 helices (Mao et al. 2020; Shaye et al. 2020; Kim et al. 2020; Shen et al. 2021) (Fig. 2.2b). In addition, the binding site of three of these compounds, CGP7930, GS39783 (Shaye et al. 2020) and *rac*-BHFF (Shen et al. 2021) has been validated by site-directed mutagenesis (Liu et al. 2021). These $GABA_B$ PAMs bind in the same binding pocket located deep inside the transmembrane domain interface, close to the intracellular part of the receptor. This unexpected PAM-binding site might be explained by difficulties encountered in binding to the 7TM core, occupied by phospholipids in the inactive state (Papasergi-Scott et al. 2020; Park et al. 2020; Kim et al. 2020). This is in contrast to PAMs for class C GPCRs, which interact in the 7TM core, with the exception of one PAM that binds at the interface of the heterodimeric $mGlu_{2-4}$ receptor in the vicinity of the extracellular face (Wang et al. 2023).

The intrinsic agonist activity of these compounds has likewise been recently clarified. In transfected cells, CGP7930, and largely *rac*-BHFF, appear to exert intrinsic agonist activity in the absence of GABA (Lecat-Guillet et al. 2017; Liu et al. 2021), while, conversely, GS39783 is a pure $GABA_B$ PAM (Lecat-Guillet et al. 2017). As expected, other compounds such as COR627 and COR628 also behaves as a pure $GABA_B$ PAM (Castelli et al. 2012). Of interest, agonist activity displayed by *rac*-BHFF was not observed for the native $GABA_B$ receptor in neurons, indicating that developed PAMs might act as pure allosteric modulators in the native system (Xu et al. 2024).

In contrast, negative allosteric modulators (NAMs) of $GABA_B$ receptors were poorly developed compared to potentiators, while competitive receptor antagonists were reported to improve cognition in animal models (Froestl et al. 2004; Kleschevnikov et al. 2012; Iqbal and Gillani 2016). So far, only two series of $GABA_B$ NAMs derived from CGP7930 (Sun et al. 2016) and COR compounds (Porcu et al. 2021) have been developed but not tested in vivo.

2.5 Allosteric Modulation Through Oligomerization

The $GABA_B$ receptor has been reported to display a tendency to form organized and stable oligomers at the cell surface (Maurel et al. 2008; Comps-Agrar et al. 2011, 2012; Calebiro et al. 2013), an unexpected property for GPCRs outside rhodopsin receptors in the retina (Jastrzebska 2017). The presence of tetramers and higher-order oligomers has also been proposed based on the findings of FRET (Maurel et al. 2008) and diffusion (Calebiro et al. 2013) studies of the $GABA_B$ receptor performed in transiently transfected mammalian cells expressing even very low expression levels of receptor, compatible with endogenous expression in cortical neurons (Maurel et al. 2008; Comps-Agrar et al. 2011).

Several experiments have demonstrated how the association of $GABA_B$ receptor heterodimers is mediated by $GABA_{B1}$ subunits (Fig. 2.3a). Primarily, in recombinant transfected HEK 293 cells, a high FRET signal is measured between the SNAP-tagged $GABA_{B1}$ subunits labeled with fluorophores compatible with FRET measurement, while this signal is low between the SNAP-tagged $GABA_{B2}$ subunits. Accordingly, a significant FRET signal was measured for the endogenous $GABA_B$ receptor in mouse brain membranes using fluorescent anti-SD antibodies expected to recognize $GABA_{B1a}$ subunits (Tiao et al. 2008; Comps-Agrar et al. 2011).

The proximity between $GABA_{B1}$ VFT domains is also consistent with cysteine crosslinking studies of the $GABA_{B1}$ 7TM domain (Fig. 2.3b). Indeed, specific disulfide crosslinkings were obtained between TM5s in the active state that were further increased following agonist treatment, indicating that $GABA_{B1}$ TM5s may be located in close proximity. Interestingly, in this study (Xue et al. 2019), our group mapped the interface of the interaction between $GABA_B$ heterodimers in higher-order oligomers and revealed the conformational changes of these interfaces upon receptor activation (Fig. 2.3b).

Nevertheless, the functional consequences of $GABA_B$ oligomerization on receptor function should be ascertained. To determine the differential G protein coupling profiles of heterodimers and oligomers, our group has developed a series of strategies aimed at decreasing the level of receptor oligomerization in cells. As a first step, a minimal construct consisting of the 7TM part of $GABA_{B1}$ subunit but lacking the VFT domain and the C-terminal region was used as a competitor of the $GABA_{B1}$/$GABA_{B1}$ interface (Maurel et al. 2008; Comps-Agrar et al. 2011). Subsequently, we engineered mutations in $GABA_{B1}$ VFT domain to produce an impairment at the $GABA_{B1}$/$GABA_{B1}$ VFT interface based on the structure of VFT tetramers in the glutamate receptor, AMPA, involving an interaction with VFT lobe 2 (Sobolevsky et al. 2009; Comps-Agrar et al. 2011; Stewart et al. 2018). Both strategies yielded a $GABA_B$ receptor featuring a higher G protein coupling efficacy without affecting the potency of GABA stimulation. This highlights the critical role played by oligomerization in controlling G protein coupling efficacy, further underlining the superior efficiency of oligomers in limiting G protein coupling compared to heterodimers.

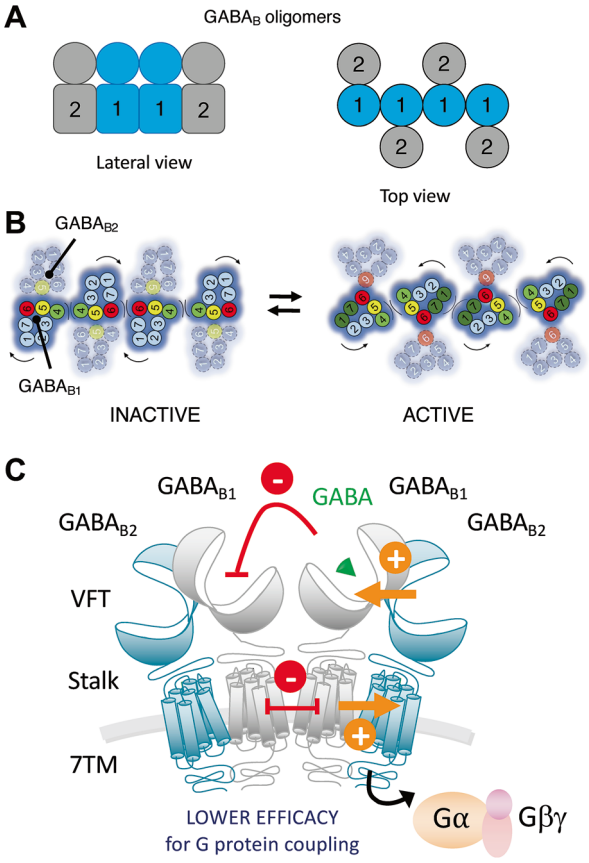

Fig. 2.3 Allosteric modulation in GABA$_B$ receptor oligomers. (**a**) Schemes for the organization of transmembrane domains in the GABA$_B$ oligomer where the GABA$_{B1}$ subunits form the interface between the heterodimers in the inactive and active states, as recently proposed (Maurel et al. 2008; Comps-Agrar et al. 2011; Stewart et al. 2018; Xue et al. 2019). (**b**) Model for the organization of the heptahelical transmembrane (7TM) domains in GABA$_B$ oligomers in the inactive and active states based on a cysteine-crosslinking analysis (Xue et al. 2019). In this study, the close proximity between transmembrane helices at the interface between GABA$_{B1}$ and GABA$_{B2}$ 7TM domains, and between two GABA$_{B1}$ subunits was mapped in absence or presence of GABA. GABA$_{B2}$ subunits are shown in transparency. (**c**), The negative allosteric interactions between GABA$_B$ heterodimers limit the efficacy of G protein activation, as demonstrated (Maurel et al. 2008; Comps-Agrar et al. 2011; Stewart et al. 2018)

Binding experiments performed on GABA$_{B1}$ subunit mutants, in which oligomerization was reduced by the introduction of an N-glycosylation for the purpose of inducing steric hindrance (Stewart et al. 2018), allowed us to propose a model of allosteric interactions with the GABA$_B$ heterodimer in these oligomers (Fig. 2.3c). The GABA$_{B1}$ subunit of one heterodimer was able to interact with a second GABA$_{B1}$ subunit to limit ligand binding. In addition, one of these N-glycosylation mutants

resulted in an increase of G protein efficacy. This further highlights the critical role played by oligomerization in controlling G protein coupling efficacy.

2.6 Conclusion

Recent structural, biophysical, and biochemical studies carried out to study the $GABA_B$ receptor have highlighted the high degree of complexity of this receptor. The receptor indeed appears to be well arranged in organized oligomers aimed at controlling G protein signaling. This organization enables to strictly control allosteric interactions between heterodimers and between domains within heterodimer, which, by all accounts, are implicated in the activation and modulation of the receptor. Finally, the complex structure of the $GABA_B$ receptor is well suited to foster the development of novel allosteric modulators. However, whether binding to the heterodimer interface constitutes the only means of developing these allosteric modulators remains an open question to be discussed in the context of the development of future drugs.

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Part II
Pharmacology of Baclofen

Chapter 3
Historical Overview on Baclofen, the Only $GABA_B$ Receptor Agonist Approved and Available for Clinical Use

Mauro A. M. Carai

Abstract Baclofen was synthesized in 1962 as a component of a group of ß-aryl-halogenated GABA derivatives capable of crossing the blood-brain barrier and, thus, exerting inhibitory effects on the central nervous system. At that time, the role of GABA as a neurotransmitter had not been fully discovered, and nothing was known about its receptors. The clinical development of baclofen was based on its muscle-relaxant effects. Ten years after its synthesis, baclofen was registered in Europe as Lioresal®; registration in the United States occurred in 1977. Over the following decade, baclofen became the drug of choice for the treatment of spasticity and muscle spasms. Anecdotal reports from patients taking baclofen for its approved indications led to the identification of additional, possible uses (often supported by accumulating findings on the central and peripheral functions of GABA neurotransmission). Among these new indications, it is worth mentioning the treatment of substance use disorder, which actually represents the sole, recent advancement in terms of authorizations. Since the early 1980s, baclofen actions at specific GABA receptors (named $GABA_B$) were discovered. It is now hoped that future research will focus on (i) possible new clinical applications of baclofen and (ii) new $GABA_B$ receptor agonists, including those naturally occurring, that would hopefully come alongside baclofen and possibly extend its therapeutic prospects.

Keywords GABA derivatives · Baclofen · Lioresal® · Therapeutic use · Off-label use · Clinical development

3.1 From GABA to Baclofen

Since the eighteenth century, at a time when methodological and scientific findings were starting to lay the groundwork for the future development of pharmacology, plant-derived alkaloids have repeatedly been associated with novel discoveries of

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major importance (see Holmstedt and Liljestrand 1981). Indeed, relevant insight into the mechanism of action of baclofen was unequivocally linked to one such alkaloid. In 1932, Richard H.F. Manske, an outstanding Canadian chemist who provided a fundamental contribution to the isolation and characterization of numerous alkaloids, published the findings of his studies on a series of alkaloids extracted from *Dicentra cucullaria* (Manske 1932). At the time, γ-aminobutyric acid (GABA) was already well known, with the synthesis of the compound being described for the first time in 1883 (Schotten 1883), followed by the demonstration in 1910 of the presence of GABA in biological tissues (see Roberts and Frankel 1950). However, the presence of GABA in the central nervous system (CNS) was only identified in the 1950s (Roberts and Frankel 1950), and it was not until 1959 that a study was published affirming that "*These observations on the effects of GABA on the guinea-pig ileum have been confirmed in the present series of experiments; however, no inhibitory effect of GABA on induced contractions of rabbit ileum has been detected. Factor I has been found to have effects qualitatively similar to GABA*" (Florey and McLennan 1959). Factor I was indeed an inhibitory factor extracted from the brain, and this important finding led scientists to hypothesize the likely role of GABA as a neurotransmitter (Florey and McLennan 1959). However, despite these observations, a widespread debate focused on whether or not GABA acted as a neurotransmitter was ongoing until 1967. In the same year, a study entitled, "The action of gamma-aminobutyric acid on cortical neurons" was published, demonstrating in a series of convincing electrophysiology experiments that GABA was indeed an inhibitory neurotransmitter, with the authors stating, "*When γ-aminobutyric acid is applied to single cortical neurons, it causes changes in membrane potential and conductance that are similar to the effects of synaptic inhibition. It is therefore concluded that this normal constituent of the brain could be the physiological transmitter at inhibitory synapses in the cerebral cortex*" (Krnjević and Schwartz 1967). It was at this point that bicuculline, one of the alkaloids isolated and characterized by Manske, first appeared on the scene; no therapeutic indications had been proposed for this alkaloid, and indeed, this remains the case today, although the role of bicuculline as a GABA antagonist gained rapid consensus. Four detailed reports were published in 1970 demonstrating the antagonistic activity of bicuculline (Curtis et al. 1970a, b, c; Godfraind et al. 1970) and indicating this organic compound as a powerful tool for use in studying GABA-mediated inhibitory transmission. One of these studies successfully determined that bicuculline antagonism of GABA effects was not, in turn, inhibited by strychnine (Eccles 1969; Curtis et al. 1970c), thus helping to discern between the effects of GABA and those produced by glycine when the latter acted as a neurotransmitter. This paved the way for a period of rapid advancement in the knowledge of GABA-mediated transmission and GABA receptors and led to GABA being deemed responsible for 40% of inhibitory synaptic transmission in the CNS. Moreover, further studies clarified how the action of GABA increased neuronal membrane permeability to chloride. Benzodiazepines, an emerging class of novel hypno-sedative drugs, were found to act by potentiating the effects of GABA-mediated transmission (Haefely et al. 1975).

During the 1970s, in the midst of this period of increased awareness, several researchers observed how neurons of the sympathetic or dorsal root ganglia were implicated in producing GABA effects. Indeed, although no significant differences were present between the CNS and the peripheral nervous system (PNS), with the effects of GABA invariably mediated by an increased influx of Cl^- ions into intracellular compartments, the potential remained markedly lower in sympathetic neurons compared to the CNS, with values of −42 mV and −75 mV, respectively (see Bowery 2010). Additional studies aimed at clarifying this discrepancy led to the differentiation of GABA receptors. In a series of key experiments performed in rat heart atria, GABA effects were found to underlie a reduction in evoked release of tritiated noradrenaline; surprisingly, although not antagonized by bicuculline, this effect was substantially, and in a stereospecific manner, reproduced by β-chlorophenyl-GABA or baclofen (Fig. 3.1), thus persuading Norman G. Bowery and colleagues that they had identified a new GABA receptor (Bowery et al. 1979, 1980; see also Chap. 15 of this volume). These findings were subsequently followed by a series of accomplished studies resulting in the definition of GABA type-A ($GABA_A$) and type-B ($GABA_B$) receptors, which, in turn, led to the discovery and fundamental understanding of their mechanisms of action and composition (see Bowery 2010, 2016).

At this point, however, we should take a step back in an attempt to grasp how Bowery and his collaborators were able to identify an agonist for a receptor that had yet to be discovered. Based on the history of pharmacology, this question may appear rather banal, as it is indeed an acknowledged fact that man has invariably succeeded in identifying naturally-occurring substances prior to the identification, at a later date, of synthetic compounds, with mechanisms of action only being revealed in more recent decades, following thousands of years of therapeutic or recreational use (see Holmstedt and Liljestrand 1981; Efron et al. 1967). The history of baclofen dates back to the early 1960s, when, in anticipation of clarification of the neurotransmitter role played by GABA, the race had begun to obtain derivatives capable of penetrating the CNS, i.e., molecules capable of crossing the blood-brain barrier (BBB) and thus exerting a therapeutic action on the CNS. One means of augmenting the lipophilicity of GABA lay in the potential insertion of lipophilic substituents into the GABA molecule, thus giving rise to a dedicated research program led by a group of researchers at Ciba in Basel,[1] and baclofen (initially known as Ciba 34647-BA) was subsequently synthesized in September 1962. The research group involved in these discoveries was led by Heinrich Keberle, an investigator who had worked for more than 30 years for the company that would become Ciba Geigy in 1970; Johann Werner Faigle and Max Wilhelm were also members of

[1] Ciba AG (Chemische Industrie Basel) was a Swiss chemical and pharmaceutical company established in 1859. Following the merger of Ciba AG and J.R. Geigy SA in 1970, Ciba Geigy was formed.

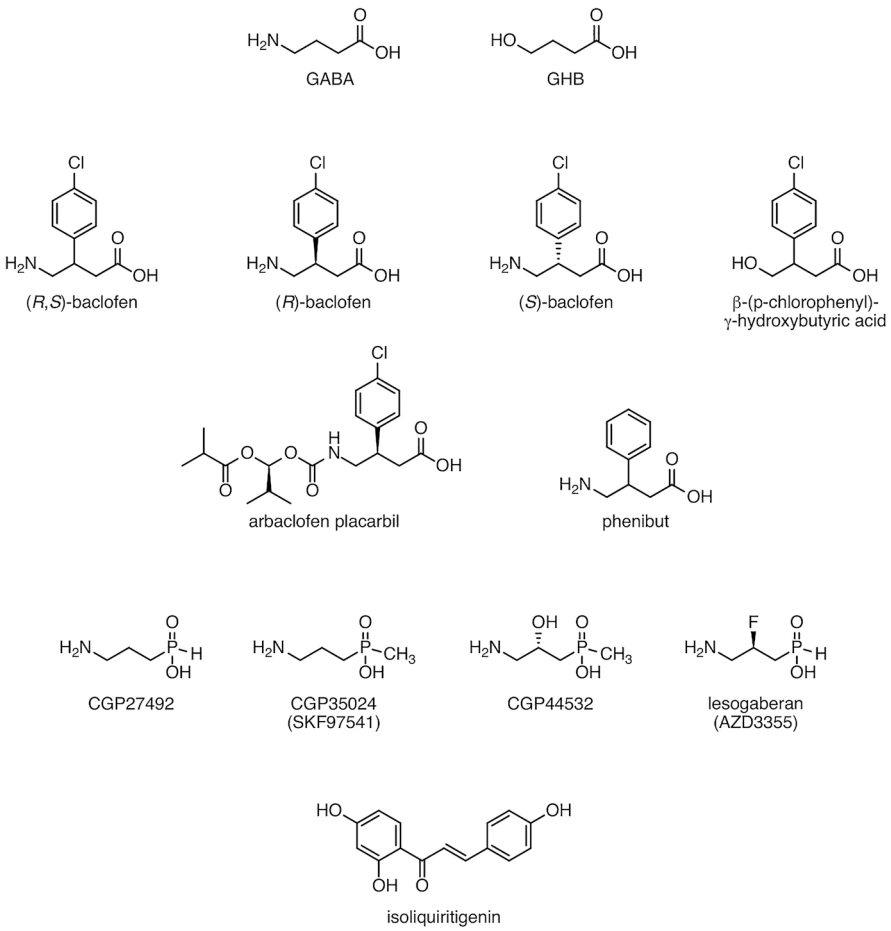
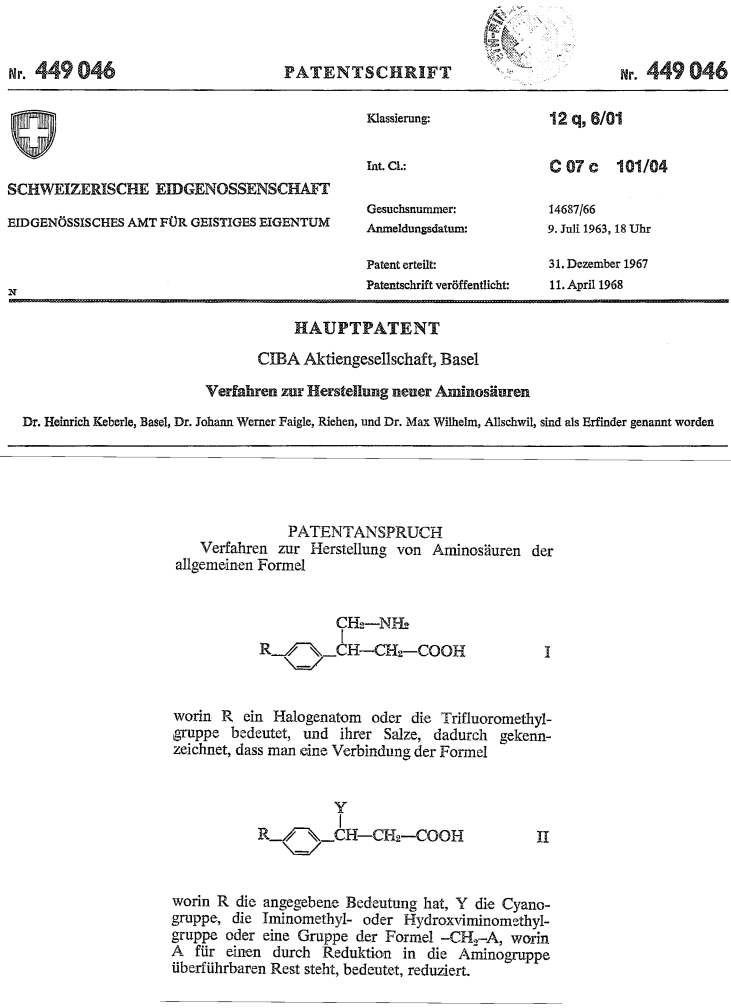


Fig. 3.1 Chemical structure of GABA, baclofen (including enantiomers, pro-drugs, metabolites, and analogs), γ-hydroxybutyric acid (GHB), and $GABA_B$ receptor agonists

Keberle's lab and were included on the list of authors for a patent deposited by Ciba on July 9, 1963, entitled *Verfahren zur Herstellung neuer Aminosäuren* "Process for producing new amino acids"[2] (Fig. 3.2) (Espacenet 2024).

[2] Heinrich Keberle had come up with the idea of synthesizing a GABA analog featuring the characteristics cited following his participation in a conference organized by Wilhelm Feldberg at the Royal Society in London (see Bowery 2016). As the history of the twentieth century raged, the fractures started to appear: Feldberg was a German pharmacologist of Jewish origin who was banished from the Physiological Institute in Berlin in 1933 and subsequently admitted, in 1934, to the National Institute for Medical Research, situated on the outskirts of London, where he embarked on a close collaboration with Henry Hallett Dale, a distinguished twentieth-century British pharmacologist who, in 1936, shared the Nobel prize with Otto Loewi for their research on the role of acetylcholine in neurotransmission (Feldberg 1969).



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HAUPTPATENT

CIBA Aktiengesellschaft, Basel

Verfahren zur Herstellung neuer Aminosäuren

Dr. Heinrich Keberle, Basel, Dr. Johann Werner Faigle, Riehen, und Dr. Max Wilhelm, Allschwil, sind als Erfinder genannt worden

PATENTANSPRUCH

Verfahren zur Herstellung von Aminosäuren der allgemeinen Formel

I

worin R ein Halogenatom oder die Trifluoromethylgruppe bedeutet, und ihrer Salze, dadurch gekennzeichnet, dass man eine Verbindung der Formel

II

worin R die angegebene Bedeutung hat, Y die Cyanogruppe, die Iminomethyl- oder Hydroxviminomethylgruppe oder eine Gruppe der Formel $-CH_2-A$, worin A für einen durch Reduktion in die Aminogruppe überführbaren Rest steht, bedeutet, reduziert.

Fig. 3.2 Title page and claims of Swiss patent no. 449046. Date of application filling: July 9, 1963, 18:00; date of patent grant: December 31, 1967; date of patent publication: April 11, 1968. Claims provide as follows: "*Process for producing amino acids of the general formula I, where R means a halogen atom or the trifluoromethyl group, and their salts, characterized in that one is a compound of the formula II, where R has the meaning given, Y is the cyano group, the iminomethyl or hydroxyiminomethyl group or a group of the formula -CH_2-A, where A stands for a residue that can be converted into the amino group by reduction*"

The Basel chemists had successfully met their objective of improving, at least in part, GABA lipophilicity; GABA has an estimated value of log of partition coefficient (logP) of −3.17, while baclofen has a logP of 1.3 (PubChem 2024a, b). Consequently, the lipophilicity of baclofen was not sufficient to cross the BBB by means of passive diffusion, and it was subsequently reported that this occurred by means of a carrier-mediated transport system (van Bree et al. 1988). In 1972, Keberle and Faigle published partial data obtained from their baclofen studies

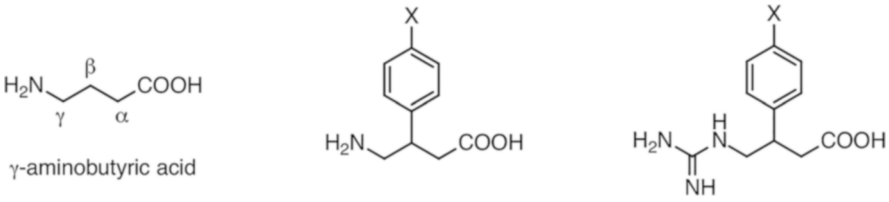

Fig. 3.3 Chemical structure of some of the GABA-related compounds, including baclofen, tested by Faigle and Keberle (1972) for biological activity. X stands for a generic substituent

(Faigle and Keberle 1972), highlighting how only GABA derivatives featuring an aromatic ring in the β position of the amino acid displayed "central inhibitory effects," with the γ-guanidine GABA analog representing the only other active compound (Fig. 3.3). Moreover, Keberle and Faigle were in charge of setting up pioneering pharmacokinetic studies in animals and humans in which, further to establishing the ready absorption of baclofen in the gastrointestinal tract, they demonstrated how the compound was excreted largely unchanged, identifying β-(4-chlorophenyl)-γ-hydroxybutyric acid (Fig. 3.1) as one of the main metabolites (Faigle and Keberle 1972).

However, the limited ability of baclofen to cross the BBB was not the only problem; indeed, although baclofen had been developed as an anti-convulsant drug, the studies performed failed to yield promising results. Unfortunately, the findings of experimental in vivo studies carried out by Ciba were never published, although the highly complex role of the $GABA_B$ receptor in epilepsy is now widely acknowledged. Put simplistically, as a $GABA_B$ receptor agonist, baclofen elicits the onset of typical and atypical absence seizures, which, on the contrary, are prevented by $GABA_B$ receptor antagonists. However, to cite a mere example, in animal models of focal seizures, low doses of baclofen, i.e., 1 mg/kg, lower the seizure threshold, while doses exceeding 10 mg/kg raise the threshold (see Joshi et al. 2016). Accordingly, efforts to introduce baclofen as a therapeutic option in this field were largely thwarted. However, while investigating potential therapeutic applications for baclofen, scientists became aware of its muscle-relaxant properties, placing increased focus on this aspect during the drug development process.

3.2 Clinical Use of Baclofen

Long before Bowery and co-workers identified the $GABA_B$ receptors and the specific actions of baclofen on these receptors, baclofen was marketed as a drug by Ciba Geigy under the brand name Lioresal® (Fig. 3.4). Wolfgang Froestl refers to the marketing of Lioresal® occurring in 1972 (see Froestl 2010), while Hudgson and Weightman (1971) refer to Lioresal® being introduced in 1966.

The first clinical studies published on the use of baclofen in the treatment of spasticity were performed by Bergamini and co-workers in 1966 (Bergamini et al.

Fig. 3.4 Lioresal® on sale in Spain in the 1970s. (From http://www.ub.edu/pharmakoteka/node/27349)

1966) and Birkmayer and coworkers in 1967 (Birkmayer et al. 1967); these preliminary studies provided proof of the efficacy of baclofen in regulating spasticity, particularly in the context of spinal cord lesions. In 1971, in the wake of a pilot study conducted in six patients affected by severe spasticity of the lower limbs induced by multiple sclerosis or as *sequelae* of previous surgery, Hudgson and Weightman (1971) published the findings of a double-blind, placebo-controlled, cross-over trial conducted on 23 patients affected by lower limb spasticity as a result of spinal cord disease, although caused by a wider range of neurological disorders compared to previous studies. At the time, the sole option available for the treatment of spasticity resulting from spinal cord disorders or other neurological lesions was neurolysis, achieved using intrathecal injections of phenol or oral administration of the benzodiazepines, chlordiazepoxide, and diazepam, at the time considered the most effective drugs in the treatment of spasticity. However, the excess sedation caused by these drugs when administered at the doses required to treat spasticity was well-known. Hudgson and Weightman (1971) treated patients with 10 mg of baclofen t.i.d. or placebo for a period of 10 days, followed by a 7-day washout period, subsequently moving each patient to the opposite arm of the trial. The results obtained demonstrated the significantly higher efficacy of baclofen over placebo in a comparison of mean changes in spasticity (Table 3.1). No major side effects were observed, including drowsiness (Table 3.1). Over the next 2 years (1972–1973), baclofen was granted authorization in Europe under the brand name Lioresal® for use in the treatment of spasticity and muscle spasms caused by a range of neurological disorders, followed in 1977 by US authorization (Agenzia Italiana del Farmaco 2024; Food and Drug Administration 2024).

In a similar time frame, the structure of baclofen was solved as comprising two enantiomers, R(−)baclofen and S(+)baclofen (Fig. 3.1) by a researcher at Ciba Geigy, William Bencze. Isomer studies revealed the stereospecificity of baclofen, with its major effects being attributed to R(−)baclofen (see Froestl 2010). No evidence had however been provided to confirm that baclofen was a GABA-mimetic

Table 3.1 Beneficial effect of treatment with baclofen on spasticity and episodes of side effects

Improvement on baclofen					
N. of patients	Mean spasticity score		Mean improvement	SEM improvement (within patients)	*P* value
	Before baclofen	After baclofen			
23	3.74	2.30	1.44	0.230	<0.001
The mean improvement of 1.44 in patients while taking the drug is highly significant					

Improvement on placebo					
N. of patients	Mean spasticity score		Mean improvement	SEM improvement (within patients)	*P* value
	Before placebo	After placebo			
23	3.65	3.11	0.54	0.231	<0.05
The mean improvement of 0.54 in patients while taking the placebo is statistically significant at the 5% level					

	Side effects experienced by patients during trials	
	N. of patients on	
	Baclofen	Placebo
Nausea	3	1
Transient vertigo	1	–
Blurring of vision	–	1
Upper respiratory tract infection	–	1
Supraorbital pain	1	–
Sleepiness	1	–
	6	**3**

Adapted from Hudgson and Weightman (1971) with permission from The British Medical Journal
Spasticity was arbitrarily graded from 0 (normal) to 4 according to clinical evaluation. Patients were randomly allocated to placebo or baclofen (10 mg thrice daily for 10 days) under a crossover design with a 7-day washout period

drug, despite marketing authorization being granted for the compound in the 1970s as a "GABA-related drug" (see Bowery and Smart 2006).

As the use of baclofen became more widespread, limitations of the drug became evident, highlighting how, despite its rapid absorption in the upper small intestine, with a bioavailability of 75–85% and a half-life of 2–6 h (see Kent et al. 2020; see also Chap. 7 of this volume), the relatively poor ability of baclofen to cross the BBB implied a need for repeated doses and, particularly, the use of higher doses to achieve a therapeutic effect in specific patient populations, resulting in the onset of side effects including drowsiness, lethargy, convulsions, nausea, vomiting, hypotension, and respiratory depression, thus affecting baclofen manageability in these patients. These problems were, however, overcome following the introduction of the intrathecal (IT) baclofen administration. The first studies in this regard were performed by Penn and Kroin (1984), who highlighted how this new route of administration facilitated the delivery of baclofen to its sites of action within the CNS, thus yielding concentrations of the drug in cerebrospinal fluid (CSF) that would have been unachievable via an oral route of baclofen administration (see Chaps. 4

and 7 of this volume). IT baclofen at doses of 12–400 μg per day was more effective than oral treatment in restoring normal muscle tone and eliminating spontaneous spasms (Penn and Kroin 1985). Froestl (2010) reported how intrathecal doses of 600 μg baclofen produced higher CSF concentrations compared to those obtained following a 100-mg oral dose. Moreover, in addition to increased efficacy, this route of administration also proved beneficial in markedly reducing side effects typically associated with high doses of oral baclofen (see Bowery 2010; Froestl 2010). However, the use of IT baclofen unraveled a series of issues, present to a lower degree also with oral treatment, including the risk of potentially fatal overdose, generally linked to pump malfunction, and the onset of withdrawal syndrome on abrupt discontinuation of intrathecal treatment linked to pump malfunction or recharging errors (see Romito et al. 2021; see also Chap. 7 of this volume).

Almost 10 years after the introduction of baclofen in clinical practice, Bowery and co-workers made their crucial discoveries relating to the $GABA_B$ receptor, which revealed the mechanism of action of baclofen. From that time onward, the availability of radioactives proved to be a formidable tool, facilitating in-depth studies of the $GABA_B$ receptor and its distribution. In this regard, Bowery mentioned how he had approached Helmut Bittiger at Ciba Geigy to enquire into the possibility of receiving tritiated baclofen for use in receptor binding studies. One year later, Bittiger supplied the ^{3}H-baclofen, alerting Bowery to the fact that his group had failed to elucidate the binding site and attaching a detailed description of the experiments undertaken. Bowery, together with his close collaborator, David R. Hill, focused intensely on the buffer solution used by Bittiger and co-workers: a TRIS-containing solution that was widely used at the time in radio-receptor binding assays. Bowery and Hill's instincts led them to replace the latter with saline in a solution using Kreb's solution instead of TRIS as a buffer, resulting in the observation of saturable binding characterizing bicuculline-insensitive $GABA_B$ sites, in line with the hypothesis acknowledging the involvement of alkaloids in all pharmacological discoveries (Hill and Bowery 1981; see Bowery 2016).

Baclofen today plays a major role in the treatment of conditions relating to traditional indications for the drug, including spasticity resulting from multiple sclerosis, particularly for the relief of flexor spasms and concomitant pain, clonus, and muscular rigidity; baclofen may also be of some value in patients with spinal cord injuries and other spinal cord diseases. More widely, the drug may be indicated in the treatment of spastic hypertonia of the striated muscles in multiple sclerosis and spastic muscular hypertonia in spinal cord disorders of infectious, degenerative, traumatic, neoplastic, or unknown origin, i.e., spastic spinal paralysis, amyotrophic lateral sclerosis, syringomyelia, transverse myelitis, traumatic paraplegia or paraparesis, spinal cord compression, and spastic hypertonia of cerebral origin, particularly in cases of infantile encephalopathy, following cerebral vasculopathy, or associated with neoplastic or degenerative cerebellar disorders. In children, baclofen is indicated in the symptomatic treatment of cerebral spasticity in patients between the ages of 0 and <18 years, particularly when caused by infantile cerebral palsy, subsequent to cerebrovascular injury, or in the presence of neoplastic or degenerative cerebellar disorders (see Chap. 4 of this volume). Baclofen is also indicated in

the symptomatic treatment of muscle spasms manifested in the course of spinal cord disorders of infectious, degenerative, traumatic, neoplastic, or unknown origin, such as multiple sclerosis, spastic spinal paralysis, amyotrophic lateral sclerosis, syringomyelia, transverse myelitis, traumatic paraplegia or paraparesis, and spinal cord compression (Agenzia Italiana del Farmaco 2024; Food and Drug Administration 2024).

3.3 Baclofen Between Off-Label Use and New Indications

A short deliberation into a series of clinical off-label or experimental uses put forward for baclofen over the 40-year period since its first introduction as a therapeutic option, although failing to obtain marketing authorization, may be of interest.

3.3.1 Bronchospasm and Cough

Several studies have suggested that the presence of $GABA_B$ receptors in the CNS and PNS, as well as in the lungs, may lay the foundation for the use of $GABA_B$ receptor agonists in the regulation of cough and bronchospasm. Recent studies conducted with baclofen and lesogaberan (Fig. 3.1) have yielded positive findings in support of these hypotheses (see Chapman et al. 1993; Prakash 2009; Badri et al. 2021; see also Chap. 5 of this volume).

3.3.2 Stiff-man Syndrome

Since 1980, the ability of oral or intrathecal administration of baclofen to produce a marked therapeutic effect in patients affected by this rare neurological syndrome, the main characteristic of which is progressive muscle rigidity, has been well known (Whelan 1980; Geffen and Chiang 2019).

3.3.3 Chronic Hiccup

A rare condition resulting in potentially severe consequences, including inevitable weight loss and lack of sleep, the etiology of the disorder remains unknown, although it is frequently associated with other severe pathological conditions. Routine treatments include metoclopramide and chlorpromazine, although these are not always effective, with the use of baclofen over the last 30 years yielding satisfactory results (see Grant et al. 1991; Launois et al. 1993; Polito and Fellows 2017).

3.3.4 Tetanus

Nowadays, a rare condition is characterized, in addition to other symptoms, by muscle rigidity and reflex spasms. Initial observations relating to the use of intrathecal baclofen date back 40 years, with baclofen remaining one of the main therapeutic options in the treatment of tetanus (Müller et al. 1986; see Rodrigo et al. 2014).

3.3.5 Charcot-Marie-Tooth Disease, Type 1A

A rare inherited neuropathy is characterized by abnormal Schwann cell differentiation, which produces a cascade of events resulting in neuronal and, subsequently, muscle lesions. Several clinical studies have demonstrated the efficacy of baclofen, in combination with naltrexone hydrochloride and D-sorbitol, in reducing myelin defects typically manifested in this disease (Attarian et al. 2014). Since 2014, the above combination of drugs has been given orphan designation by the European Medicines Agency (EMA) (European Medicines Agency 2024). However, no official marketing authorization has yet been granted.

3.3.6 Pain

Numerous bodies of evidence confirm the analgesic effect of baclofen, supported by the discovery of the siting of $GABA_B$ receptors along pain pathways. The analgesic action of baclofen is generally thought to be elicited by an inhibitory action, largely at the spinal level, on synaptic release of L-glutamate, tachykinin, substance P, and calcitonin gene-related peptide (CGRP) (see Enna and McCarson 2016).

3.3.7 Trigeminal Neuralgia

To delve further into the analgesic effect of baclofen, in 1980, Fromm and coworkers published the findings of a study conducted to evaluate the effect of baclofen on trigeminal neuralgia. This pilot study included 14 patients who received baclofen at doses of 60–80 mg/day, reporting satisfactory results and relief from paroxysmal episodes of *tic douloureux* in two-thirds of patients (Fromm et al. 1980). Subsequently, the same group of clinical researchers demonstrated a more than five-fold higher potency of the baclofen (R)-isomer compared to racemic baclofen in 9 out of 15 patients affected by trigeminal neuralgia (Fromm and Terrence 1987). Baclofen still today represents a crucial second-line drug for use in the treatment of trigeminal neuralgia in patients who fail to respond to carbamazepine and oxcarbazepine; in these cases, baclofen may be administered alone or in combination with carbamazepine, lamotrigine, or gabapentin (see Rana et al. 2023).

3.3.8 Migraine

The use of baclofen in the treatment of migraine is also worthy of mention. The first clinical study, conducted on 44 patients affected by migraine with and without *aura*, was performed in 1999 with administration of baclofen at doses ranging from 15 to 40 mg t.i.d. for a period of 12 weeks (Hering-Hanit 1999). Treatment efficacy was assessed based on headache frequency and severity, with results confirming the effectiveness of baclofen in more than 80% of patients enrolled in the study.

3.3.9 Cluster Headache

A few years later, in the year 2000, baclofen yielded interesting results in a study performed to investigate a severe, and relatively rare, condition known as cluster headache; indeed, when administered at fairly low doses (5–10 mg t.i.d.), baclofen was able to successfully prevent attacks in two-thirds of patients after 1 week of treatment (Hering-Hanit and Gadoth 2000). Baclofen is currently used as a second-line option when treatment with sumatriptan injection or oxygen inhalation, or preventative treatment with verapamil is not effective (Ashkenazi and Schwedt 2011).

3.3.10 Unstable Bladder Syndrome and Overactive Bladder

Baclofen was suggested as a therapeutic option for use in the treatment of unstable bladder syndrome and overactive bladder. The first clinical study was carried out by Taylor and Bates prior to the discovery of the $GABA_B$ receptor in 40 patients treated with a 5-mg dose of the drug. The authors reported how baclofen produced a significant improvement in all parameters assessed: degree of incontinence and frequency of nocturnal and daytime urination (Taylor and Bates 1979). Other studies were performed on patients affected by hyperactive bladder, with numerous observations reporting an improvement in bladder function in patients treated with IT baclofen (Chin et al. 2012; Abboud et al. 2017; Konstantinidis et al. 2022).

3.3.11 Gastroesophageal Reflux Disease

The premise for these studies was based on observations relating to the effect of baclofen on cough, together with those produced by transient lower esophageal sphincter relaxation (TLOSR). The pioneer of these studies was Anders Lehmann, a group leader at AstraZeneca and author of a detailed description of the use of $GABA_B$ receptor agonists in gastroesophageal reflux disease (GERD) (see Lehmann

et al. 2016; see also Chap. 5 of this volume). Preliminary findings were published in two separate studies in 1999, demonstrating the ability of baclofen and the other $GABA_B$ receptor agonists, CGP44532 and SKF97541, to reduce TLOSR in different animal models of GERD (Blackshaw et al. 1999; Lehmann et al. 1999). The following year, the first human study was conducted on healthy volunteers, indicating how single administration of a 40-mg dose of baclofen represented an alternative therapeutic option in the treatment of GERD (Lidums et al. 2000). A relatively ample body of literature supports the use of baclofen in patients affected by GERD, particularly in the presence of concomitant conditions such as alcohol use disorder (AUD), non-acid reflux, and obesity (see Arabpour et al. 2023). Other $GABA_B$ receptor agonists tested in the treatment of GERD include the prodrug of R(−) baclofen, arbaclofen placarbil (Fig. 3.1) (Gerson et al. 2010), and lesogaberan, the latter acting mainly at a peripheral level (Lehmann et al. 2009; Boeckxstaens et al. 2010; Miner Jr et al. 2014; see also Chap. 4 of this volume).

3.3.12 Fragile X Syndrome and Autism Spectrum Disorder

These two conditions are more closely linked to CNS disorders, for which baclofen has been put forward as a therapeutic option (see Haile 2016). Fragile X syndrome (FXS) constitutes the major genetic cause of autism, with the majority of individuals affected by FXS meeting criteria established for a diagnosis of autism or autism spectrum disorder (ASD). Based on anecdotal observations reporting an improvement in these conditions following the administration of baclofen to treat spasticity and the consideration that $GABA_B$ receptor agonists possess the ability to control overactivation of brain regions involved in regulating behavior, a series of clinical trials using arbaclofen was set up from 2012 onward. These clinical trials, which saw the recruitment of hundreds of patients in double-blind, randomized, and placebo-controlled studies, many of which were sponsored by the company Seaside Therapeutics, seemingly failed to show any effect of arbaclofen on main endpoints but yielded interesting cues as to its efficacy on secondary endpoints (Erickson et al. 2014; Berry-Kravis et al. 2012, 2017). The findings of another clinical trial set up in 2021, as a multi-site, double-blind, parallel group, Phase II randomized clinical trial on 130 children and adolescents are expected forthwith (Parellada et al. 2021).

3.3.13 Panic Attacks

The findings of a double-blind, placebo-controlled, crossover trial were published in 1989, reporting the effects obtained by a 30-mg dose of baclofen once daily for 4 weeks; baclofen was found to be more effective than placebo in preventing panic attacks and improving scores obtained at a series of rating scales used to measure anxiety (Breslow et al. 1989).

3.3.14 Post-Traumatic Stress Disorder

In a small pilot study published in 2003, 14 patients affected by chronic, combat-related post-traumatic stress disorder (PTSD) were treated with doses of baclofen up to 80 mg daily. The authors reported how, compared to baseline values, following 8-week treatment with baclofen, lower scores were obtained at the Clinician-Administered PTSD Scale (CAPS) (Drake et al. 2003). In another double-blind clinical trial, a daily dose of 40 mg of baclofen was combined with either citalopram or placebo over an 8-week treatment period (Manteghi et al. 2014). This study likewise observed a significant improvement in the CAPS score and score obtained at the Global Assessment of Functioning with regard to depression and anxiety compared to placebo.

3.3.15 Anxiety

Preclinical and clinical studies have implicated $GABA_B$ receptors in anxiety disorders (Felice et al. 2016). Nevertheless, to date, no indications or specific drugs based on manipulation of the $GABA_B$ receptor have been approved for use in the treatment of anxiety. Historically, it is noteworthy how the authors of a pilot study conducted in 1976, in the wake of the recent approval of baclofen in Europe and prior to the discovery of the $GABA_B$ receptor, attributed the interest in baclofen and benzodiazepines to the potential presence of common mechanisms of action. This observational double-blind, between-patient, placebo-controlled study conducted on 20 patients with schizophrenia reported an absence of differences between the two groups with regard to psychiatric conditions, observing the superiority of baclofen over placebo in the treatment of anxiety (Gulmann et al. 1976). The effects of baclofen on anxiety have recently been observed in the context of treatment for AUD (Agabio et al. 2021).

3.3.16 Substance Use Disorder

Interest in the clinical use of baclofen in substance use disorder (SUD) was fostered by a detailed body of preclinical literature and knowledge relating to activation of the $GABA_B$ receptor and inhibition of the dopaminergic brain "reward" system (see Frankowska et al. 2016). This interest, stemming from studies involving orthosteric ligands of the $GABA_B$ receptor, was further boosted by the discovery of $GABA_B$ receptor positive allosteric modulators (PAMs; see Chap. 11 of this volume). Despite the abundance of experimental literature, to date, clinical trials have only yielded partial and preliminary findings. To briefly summarize, the clinical history of the evaluation of baclofen efficacy in the treatment of SUD commenced in 1998 with a

small and open-label trial in which 10 patients with cocaine use disorder (CUD) received baclofen (20 mg, t.i.d.) in conjunction with counseling sessions for 16 weeks (Ling et al. 1998). The results of this study suggested that baclofen could be useful in reducing both cocaine craving and use (Ling et al. 1998). However, subsequent RCTs did not confirm these preliminary results; a systematic review reported that baclofen did not differ from placebo on CUD, opioid, and methamphetamine use disorders (see Agabio et al. 2013). Conversely, baclofen resulted to be effective in the treatment of nicotine use disorder and heroin withdrawal syndrome (see Agabio et al. 2013). However, since evidence on each SUD comes from a single RCT, additional surveys are needed to draw definitive conclusions.

A robust reduction in craving score was obtained in patients affected by polysubstance use disorder (PsUD) who were treated with a combination of baclofen and opipramol (Bareli et al. 2021). Lastly, several smaller clinical trials were conducted in patients with binge eating disorder and obesity. In a small open-label trial, baclofen was found to reduce the frequency of binge eating (see Agabio et al. 2013). In agreement with these findings, the findings of a study performed on obese patients revealed a significant reduction in body weight, waist circumference, appetite, and sugar cravings during treatment with baclofen (Arima and Oiso 2010).

3.3.17 Alcohol Use Disorder

A separate paragraph should be devoted to the clinical use of baclofen in AUD, in which the first studies were set up in the same time frame as clinical trials relating to the use of baclofen in SUD. Chapter 6 of this volume provides a detailed description of the therapeutic aspects and efficacy of baclofen in the treatment of AUD (see also Agabio et al. 2018, 2023).

Herein, we describe a series of aspects relating to the development of a specific baclofen-based drug, Baclocur®, currently available in France for the treatment of AUD. Baclocur®, available in 10, 20, 30, and 40 mg tablets, is a drug approved for use in France by the regulatory agency ANSM (*Agence Nationale de Sécurité du Médicament et des produits de santé*, National Agency for the Safety of Medicines and Health Products). Events leading up to the granting of marketing authorization are of particular interest in view of the singular means of dissemination of this drug. The marketing authorization process routinely adheres to a top-down approach in which an entity, usually a pharmaceutical industry, develops a drug for which marketing authorization is granted and then disseminates the product to healthcare operators, who, in turn, pass it on to their patients. However, in France, the dissemination of baclofen for use in the treatment of AUD followed a bottom-up trend and was subjected to strong patient pressure from the early stages. Starting in 2001, ANSM detected an increased off-label use of baclofen in the treatment of AUD, with this increase gradually escalating from 2008 onward. In 2011, ANSM estimated that AUD treatment accounted for 96% of off-label uses of baclofen (Villier et al. 2012; Agence Nationale de Sécurité du Médicament et des produits de santé

2017a, b). Indeed, in a retrospective analysis dated 2017, ANSM associated the increase in use of baclofen with the publication of the book entitled *Le dernier verre* (The End of My Addiction in English) by Olivier Ameisen (1953–2013), a French-American cardiologist affected by AUD.[3] In his widely circulated book, as well as in a series of articles published in scientific journals, Ameisen described how he took high doses of baclofen up to a maximum of 270 mg daily, which he subsequently reduced to 120 mg daily, ultimately resulting in him being "free of alcohol dependence symptoms effortlessly for the ninth consecutive month" (Ameisen 2005, 2008). Indeed, following the publication of Ameisen's book, data provided by both ANSM and health insurance companies responsible for reimbursing patients for medical expenses highlighted how the overall number of patients using baclofen in France had risen from approx. 45,000 in 2007 to approx. 120,000 in 2014. This increase was attributed to use of the drug in AUD, as confirmed by additional statistical analyses performed by ANSM and included in the same report (Villier et al. 2012; Agence Nationale de Sécurité du Médicament et des produits de santé 2017a). The health authorities endeavored to regulate this spontaneous phenomenon, in particular by placing emphasis on the fact that the recommended dose of 75 mg of baclofen per day should only be increased to 120 mg per day if deemed necessary in hospitalized patients (Agence Française de Sécurité Sanitaire des produits Santé 2021).

Following the authorization by ANSM of two new clinical trials, in April 2012, a multicenter, pragmatic, double-blind, randomized placebo-controlled trial (RCT) known as Bacloville was set up, providing for the recruitment of 320 outpatients from 62 French primary care centers. Patients were treated for a period of 52 weeks with doses of up to 300 mg of baclofen per day or placebo. The trial was sponsored by Assistance Publique—Hôpitaux de Paris, thus underlining the strong interest displayed by the French health authorities in regulating the use of baclofen in AUD (ClinicalTrial.gov 2014, 2017). In October 2012, ANSM authorized the setup of a second clinical trial, ALPADIR, across 32 French hospital centers. The study was designed as a multicentric RCT aimed at establishing, versus placebo, the efficacy of baclofen treatment at doses of 180 mg daily over a 20-week treatment period. A total of 316 patients were recruited to this second trial, which was sponsored by a French pharmaceutical company, Ethypharm (Reynaud et al. 2017; Rigal et al. 2020). In March 2013, ANSM announced they were preparing to grant an application for what, within the French legal framework, is known as a "*recommandations temporaires d'utilisation*" (RTU), i.e., temporary recommendations for use. This is a regulatory framework, which also exists in other countries, allowing authorities in

[3] Ameisen first heard about the use of baclofen in SUD in 2001, when a friend gave him an article published in the year 2000 in the New York Times to read. The article described baclofen as a drug candidate suitable for testing in CUD and a detail that left an impression on Ameisen, in the closing paragraph, the article cited the testimony of Mr. Coleman, a patient who used baclofen to treat spasms in his legs. Mr. Coleman had discovered that when taken a short time after cocaine, baclofen blocked the effects of and reduced craving not only for cocaine but also for for alcohol and cigarettes (Ameisen 2008).

the pharmaceutical field to authorize the prescription of a given drug to treat a prespecified indication, even in the absence of marketing authorization for the relevant drug or indication. This tool consists of an interim ruling made in the presence of strong public interest relating to the use of a given drug for which the pharmaceutical company is expected to subsequently request marketing authorization. On March 14, 2014, ANSM authorized an RTU for the use of baclofen in AUD following the publication, on March 1, 2014, of a detailed 64-page protocol establishing how patients covered by the RTU should be cared for, with the aim of providing a series of guidelines for prescribing physicians and a safety measure for patients. Ultimately, on June 13, 2014, Marisol Touraine, minister of *Affaires Sociales et de la Santé*, authorized the reimbursement of baclofen for patients who were prescribed the drug for treatment of their AUD, stating that "*se réjouit de cette grande avancée pour les patients, qui permet de répondre à une préoccupation majeure de santé publique. La France est ainsi le premier pays à reconnaître la réalité de l'utilisation de ce médicament, et son efficacité dans la lutte contre l'alcoolisme*" ("welcomes this major step forward for patients, which addresses a concern major in public health. France therefore became the first country to recognize the reality of use of this medication, and its effectiveness in the fight against alcoholism") (Agence Nationale de Sécurité du Médicament et des produits de santé 2014a, b; Journal officiel de la République française 2014; Ministère des Affaires sociales et de la Santé 2014). Later that year, once the RTU had come into force, the number of patients using baclofen to treat AUD in France rose even further. Indeed, the number of patients taking baclofen for all types of indications had risen from approx. 45,000 in 2008 to 90,000 in 2013, exceeding 120,000 by the end of 2014. These figures refer to patients to whom the drug was prescribed in line with some form of reimbursement, either by the national health service or health insurance funds, although they are likely somewhat underestimated, as some people undoubtedly paid for the drug upfront despite not being eligible for reimbursement. A recent publication examined data from the "Temporary Recommendation for Use register," indicating a discrepancy between the number of patients present on the register and the number of estimated patients mentioned above, and acknowledged by ANSM on renewal of the RTU on March 16, 2017 (Agence Nationale de Sécurité du Médicament et des produits de santé 2016, 2017b; de Beaurepaire and Jaury 2024). In October 2018, 4 years after the RTU had come into force, ANSM granted marketing authorization for Baclocur® in the wake of discussions held between ANSM, scientific organizations devoted to the study and care of AUD and patient associations; it is of interest to note how only a few months previously, the Cagliari Statement (Agabio et al. 2018) had contributed toward defining the conditions of use of baclofen in AUD. The authorization granted for Baclocur® provided for the administration of baclofen at doses not exceeding 80 mg per day. However, in June 2020, following the marketing of Baclocur®, a problem arose due to an upward tick in the bottom-up thrust, with several patient associations lodging an appeal with the *tribunal administratif de* Cergy Pontoise, demanding the withdrawal of marketing authorization due to the maximum prescribable dose of 80 mg per day representing a notable constraint for prescribing physicians. The appeal was based on the

premise that within the framework of the French experience with baclofen in AUD, the drug had habitually been administered at much higher doses, ultimately therefore placing patients at risk if physicians were bound to comply with an 80 mg per day limit. Indeed, as the series of events evolved into a legally binding process, the spontaneous patient movement that had accompanied the use of baclofen in AUD throughout France, once again came to the helm to influence proceedings. Indeed, the judges in Cergy Pontoise granted their appeal and marketing authorization for Baclocur® was withdrawn. Subsequently, the RTU framework was once again set up, with the ANSM providing recommendations to not exceed the maximum dose of 300 mg daily. This first sentence was followed by further undertakings in the administrative courts, by now the designated location in which to discuss the maximum dosage to be adopted for baclofen in the treatment of AUD. The High Court, le *Conseil d'état* subsequently passed sentence, followed by a further sentence from the *tribunal administratif de* Cergy Pontoise, finally putting an end to this process on March 4 2021, establishing that Baclocur® could be prescribed and distributed throughout all French pharmacies. A few days earlier, on February 8, 2021, the RTU had been revoked, and, lastly, on November 18, 2021, ANSM published a new set of recommendations, listed on the summary of product characteristics for Baclocur®, specifying that a daily dose of 80 mg should not be routinely exceeded, although should this not be sufficient, the patient should be referred to a multi-disciplinary team specialized in the treatment of AUD, who would be permitted to increase the dose up to a maximum of 300 mg per day (Agence Nationale de Sécurité du Médicament et des produits de santé 2021; Ministère et des Solidarités et de la Santé 2023).

3.4 The Solitude of Baclofen

Throughout the 50-year period leading up to the marketing of baclofen, no other comparable drugs have emerged for use in the treatment of consolidated or novel indications. A comparison of the pharmacology of $GABA_B$ versus $GABA_A$ receptors highlights the huge difference between the number of drugs that have been developed for the two receptors and used in the clinic, even assuming that a similar comparison may hold some significance. The pharmacology of the $GABA_A$ receptor substantially predates that of the $GABA_B$ receptor; barbital treatment was first introduced in 1902, phenobarbital, still in use today, in 1912, and chlordiazepoxide, the first benzodiazepine, was identified in the mid-1950s and introduced into clinical use in 1961, with a current estimation pointing to the synthesis of approximately 3000 benzodiazepines, 35 of which currently registered for clinical use both in Europe and the USA (Randall et al. 1960; Food and Drug Administration 2020; European Monitoring Centre for Drugs and Drug Addiction 2024). Baclofen was indeed introduced into clinical practice only a few years after the advent of benzodiazepines. A direct comparison of the therapeutic applications relating to $GABA_A$ versus $GABA_B$ receptor pharmacology is of course impossible, but it is likewise equally hard to comprehend how, over a period of 60 years, no other $GABA_B$

receptor agonists have been identified and approved for clinical use, how no therapeutic advances linked to new chemical compounds have been made. As reported by Wolfgang Froestl, "a founding father of the $GABA_B$ receptor research," from 1980 and spanning a period of approximately 20 years, researchers at Ciba Geigy/Novartis in Basel and Manchester developed a remarkable program for the synthesis and characterization of a library of $GABA_B$ receptor ligands, mainly phosphinic acid derivatives, which Froestl classified as: $GABA_B$ receptor agonists, $GABA_B$ receptor partial agonists, first generation $GABA_B$ receptor antagonists, second generation $GABA_B$ receptor antagonists, third generation $GABA_B$ receptor antagonists, as well as the first $GABA_B$ PAMs (Froestl 2010). Finally, in 1997, the Novartis scientists succeeded in cloning the $GABA_B$ receptor for the first time (Kaupmann et al. 1997). Following this extraordinary feat, baclofen has continued to stand as the sole $GABA_B$ receptor agonist approved for clinical use. Undoubtedly, significant progress has been made on a cultural and scientific level, although, to date, no further advances linked to the therapeutic use of $GABA_B$ receptor agonists have been made.

Compounds including CGP27492, CGP44532, and CGP35024 (also known as SKF97541) (Fig. 3.1) have been widely studied and employed to better characterize the action of $GABA_B$ receptor agonists both in vitro and in animal models. It might be helpful if Novartis and other pharmaceutical companies involved in the undertaking of research and development programs to investigate $GABA_B$ receptor pharmacology, were to disclose the motives that have prevented novel $GABA_B$ receptor agonists from undergoing or completing clinical development. We are currently therefore only in a position to confirm that no $GABA_B$ receptor antagonists have yet been developed for the purpose of clinical use, despite the interesting findings yielded in preliminary clinical trials (Froestl et al. 2004; see Iqbal and Gillani 2016). At the present time, therefore, a major focus is represented by the potential therapeutic application of $GABA_B$ PAMs (see Chaps. 8, 9, 10, 11, and 12 of this volume).

3.5 Prodrug and Analogs of Baclofen

It is noteworthy to underline, as mentioned previously, how both baclofen prodrugs and other drugs are assumed to act, at least partially, by interacting with the $GABA_B$ receptor.

3.5.1 Baclofen Methyl Ester

The first baclofen prodrug to be discovered was baclofen methyl ester. Studies conducted following intraperitoneal administration of this drug in the rat brain to measure brain concentrations of baclofen and its methyl ester demonstrated higher levels of methyl ester in the brain, although higher baclofen concentrations were detected in the cerebellum, thus indicating partial methyl ester hydrolysis (Leisen et al. 2003).

3.5.2 Arbaclofen Placarbil

More extensive studies have been conducted using the other baclofen prodrug, arbaclofen placarbil, a carbamate derivative of R(−)baclofen linking two specific traits to foster a more effective intestinal absorption of this active isomer of baclofen (see Corelli and Mugnaini 2016), as cited previously, a technique initially developed by Xenoport in FXS and ASD. However, although promising results were obtained in preliminary clinical trials, the development of the drug seems to have come to a halt. As of April 2024, a total of 11 clinical trials were registered both in Europe and the United States between the years 2016 and 2021, all of which aimed at treating conditions for which baclofen approval has been granted (ClinicalTrials.gov 2021; EU Clinical Trials Register 2024).

3.5.3 Lesogaberan

Another $GABA_B$ receptor agonist subjected to extensive investigation in clinical trials is lesogaberan (AZD3355), a fluorinated derivative of CGP27492 (Fig. 3.1). As mentioned above (Sect. 3.3.11), lesogaberan was first characterized in 2008 by scientists working for AstraZeneca, a company that had previously undertaken extensive research on the use of baclofen in GERD. It is noteworthy that lesogaberan was defined as a "predominately peripherally restricted $GABA_B$ receptor agonist," and consequently did not elicit the typical CNS side effects of baclofen, thus indicating lesogaberan as a potential candidate for use in the treatment of GERD. However, the effect produced by the drug was deemed insufficient, and clinical development was suspended in 2013 (see Corelli and Mugnaini 2016; Lehmann et al. 2016). More recently, the drug has been proposed for use in the treatment of non-alcoholic steatohepatitis and refractory chronic cough (Badri et al. 2021, 2022; Bhattacharya et al. 2021; see also Chap. 5 of this volume). Indeed, studies conducted with levogaberan underline an urgent need to gain further insights into the distribution and function of peripheral $GABA_B$ receptors and to investigate the therapeutic potential of pharmacological manipulation.

3.5.4 GHB

γ-Hydroxybutyric acid (GHB) is a structurally related, secondary metabolite of GABA (Fig. 3.1) known to exert effects, as shown over the last 20 years, mediated in part by $GABA_B$ receptors, although specific receptors acting as high affinity GHB binding sites have been identified in the mammalian brain (see Chap. 13 of this volume). The history of GHB marginally predates that of baclofen, being first introduced for clinical use as an anesthetic in 1964, although this use was subsequently

rejected. The drug is currently registered under the brand name Xyrem® in the EU and United States for the treatment of narcolepsy, while also being registered for use in the treatment of AUD in several countries. GHB is subject to widespread recreational use and is a potential drug of abuse. Despite the complex relationship between the effects produced by GHB and $GABA_B$ receptors, GHB does not represent an alternative $GABA_B$ receptor agonist to baclofen for use in a therapeutic setting (see Agabio et al. 2010; Baladi and Carter 2016; see also Chap. 13 of this volume).

3.5.5 Phenibut

The des-chloro analog of baclofen known as phenibut (Fig. 3.1) (see Corelli and Mugnaini 2016), a close analog of baclofen, was first synthesized as a GABA analog in Russia by Perekalin and co-workers (see Lapin 2001), although featuring a superior ability to cross the BBB than the latter. The drug was introduced into clinical use in Russia during the 1960s, where it was administered at doses ranging from 0.25 to 2 g per day as a nootropic tranquilizer-anxiolytic based on its claimed cognition-enhancing effects; it has also been used in the treatment of PTSD, depressive syndromes, and, in children, to treat hyperactivity disorders (see Lapin 2001; Kupats et al. 2020). Scarce experimental data have been made available to date, and it remains unclear whether phenibut has any other CNS binding sites in addition to $GABA_B$ receptors, with which it is known to interact. In one of the very few binding studies performed using ^{3}[H]-CGP54626 on membranes obtained from rat neurons, the K_i of racemic baclofen and racemic phenibut corresponded to 6 μM and 177 μM, respectively (Dambrova et al. 2008). More recently, phenibut has started to emerge as a potential substance of abuse capable of producing dependence and rapid withdrawal on discontinuation of the drug (see Feldman et al. 2023). Baclofen combined with a benzodiazepine is frequently administered to treat phenibut withdrawal (Kupats et al. 2020; Feldman et al. 2023). At the current state of the art, it appears unlikely that phenibut will be adopted in clinical practice as a $GABA_B$ receptor agonist alongside baclofen.

Lastly, it should be highlighted how, since their emergence in 2001, no appropriate therapeutic applications capable of paving the way to full clinical development have yet been identified for the most recent major discovery—$GABA_B$ receptor PAMs (see Chaps. 10, 11, and 12 of this volume).

3.6 Conclusions

Almost 60 years after its first synthesis, baclofen continues to represent the only compound currently in use in clinical practice in the pharmacology of the $GABA_B$ receptor. This persistent position of superiority may well be evidence of the

intuition of Heinrich Keberle and, therefore, of the fact that baclofen possesses highly singular characteristics with which no other molecule has proven capable of competing. Or indeed, might the uniqueness of baclofen be the consequence of a scarcity of extensive research programs in the field? Indeed, the most recent program was set up by Ciba Geigy in the 1980s and, while failing to identify new drugs to be used in clinical practice, led, however, to the major discovery of $GABA_B$ PAMs. Indeed, our current knowledge of the physiological functions of $GABA_B$ receptors should be closely scrutinized to pinpoint the requisites needed to attract public and private investments in research programs, as well as to further elucidate our basic knowledge of the role of central and peripheral $GABA_B$ receptors.

To reiterate a notion expressed at the beginning of this chapter, we need to verify whether the natural alkaloid for the $GABA_B$ receptor, a molecule existing in nature capable of disclosing its full potential, indeed exists and remains to be found. As an example, isoliquiritigenin (Fig. 3.1), one of a range of molecules isolated from a number of plant species present in the traditional Eastern and Western pharmacopoeia and traditionally used in Africa and Oceania, might well fit the bill, particularly as some form of activity on $GABA_B$ receptors might be justified based on the active ingredients of the compound and their traditional therapeutic use, as well as the available data indicating the apparent presence of active ingredients displaying high affinity for $GABA_B$ receptors (see Chap. 14 of this volume). It is, however, undeniable that major breakthroughs will need to be witnessed before we find ourselves once again in the position of further developing the history and pharmacology of $GABA_B$ receptors.

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Chapter 4
Baclofen for the Treatment of Spasticity and Dystonia in Cerebral Palsy

Laura A. Bonouvrié

Abstract Movement disorders in childhood, notably cerebral palsy (CP), stem largely from brain dysfunction during development, manifesting as non-progressive deficits. CP encompasses various movement- and posture-related disorders, often accompanied by cognitive, behavioral, and sensory disruptions, as well as epilepsy and musculoskeletal issues. The CP classification is gauged by the severity of motor function impairment and the dominant movement disorder type.

Spasticity, the most common CP type, involves increased muscle resistance caused by central nervous system lesions. Dyskinetic CP, the second most prevalent form, features involuntary movements and fluctuating muscle tone.

Treatment of disabling movement disorders such as spasticity or dyskinesia in children is challenging. Oral pharmacological treatment options, including oral baclofen, are often insufficient and/or cause side effects.

Intrathecal (IT) baclofen treatment represents a therapeutic option for some of these patients. There is some evidence, mostly provided by single-bolus randomized trials, of the effectiveness of IT baclofen in the treatment of spasticity in children with spastic CP at the level of body functions and structures (spasticity). However, a low level of evidence of the effectiveness of continuous IT baclofen, particularly with regard to activities/participation, has been reported.

One randomized controlled trial (RCT) and related follow-up study reported evidence of the effect of IT baclofen treatment on dystonia in children with dyskinetic CP, including attainment of individual goals. However, other studies yielding similar results provide lower levels of evidence.

Despite the reported levels of evidence, there are frequently no alternative treatment options for these children; therefore, when all other options fail, IT baclofen should be considered. Future studies should be conducted to specifically investigate outcome measures at the level of activities and participation.

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4.1 Introduction

Movement disorders in childhood are caused largely by dysfunction of the developing brain due to brain lesions or brain abnormalities. The most common cause of cerebral movement disorders and physical disability in childhood is cerebral palsy (CP) (Monbaliu et al. 2017). The prevalence of CP in Western countries has remained fairly stable for many years, with a prevalence fluctuating around 2 per 1000 live births (Himmelmann and Uvebrant 2018; Oskoui et al. 2013). CP is not a diagnosis per se but comprises a group of movement and posture-related developmental disorders that may be attributed to the manifestation of non-progressive deficits in the developing fetal or young infant brain (<1 year old) (Himmelmann et al. 2006). Motor impairments are frequently not the only symptoms observed in CP, with cognitive and behavioral disorders and disruptions to sensation, communication, and perception also manifested. Furthermore, epilepsy may also be present, with secondary musculoskeletal problems arising during growth (Himmelmann et al. 2006; Odding et al. 2006; Rosenbaum et al. 2007).

CP is categorized by severity according to gross motor function, manual ability, and communication function and based on the type of dominant movement disorder involved (spasticity, dyskinesia, or ataxia) (Rosenbaum et al. 2007).

4.1.1 Severity of Cerebral Palsy

The Gross Motor Function Classification System (GMFCS) is used to assess severity based on impairment of functional mobility in CP (Palisano et al. 1997). Five levels of the GMFCS are applied across four age bands on the basis of abilities and limitations in self-initiated movement. Levels are distinguished by differences in functional ability and the need for assistive devices such as a walker or wheelchair. The higher the GMFCS level, the more severely mobility is impaired (Palisano et al. 1997, 2008).

Similar 5-point scales are used to describe the level of manual function (Manual Ability Classification System, MACS) and the level of communication function (Communication Function Classification System, CFCS) (Cooley Hidecker et al. 2011; Elisasson et al. 2006).

4.1.2 Types of Cerebral Palsy

CP may present in three different forms: spastic (80%), dyskinetic (15%), or ataxic (5%) (Rosenbaum et al. 2007), with classification of the type of CP based on the dominant movement disorder.

Of these three movement disorders, spasticity is the most frequently observed in individuals with CP (72–91%) (Himmelmann et al. 2005; Odding et al. 2006). Spasticity is defined as a velocity-dependent, increased resistance to externally applied passive muscle stretch detected on physical examination (Delgado and Albright 2003; Sanger et al. 2003). Spasticity results from an imbalance in excitatory and inhibitory responses to a sensory input signal. In a healthy person, inhibitory commands from the brain ensure that the response to ongoing sensory input signals via the stretch reflex arch is well balanced. However, when inhibitory commands are omitted due to lesions in the central nervous system, manifestation of abnormal response via the stretch reflex pathway may occur, leading to overactive and spastic muscles (Ivanhoe and Reistetter 2004). The severity of spasticity may vary depending on the localization and extent of the brain lesions and may also vary within the same person depending on posture, activity, emotional state, pain, or other triggers (Sanger et al. 2003).

Dyskinetic CP is the second most common type, as well as the most disabling form, of CP, with 59–80% of patients being scored as GMFCS level IV or V (Himmelmann et al. 2007, 2009). This is substantially higher compared to children affected by bilateral spastic CP, in whom GMFCS IV and V account for less than 20% of cases (Himmelmann et al. 2007). Dyskinesia is defined as involuntary, uncontrolled, recurring, and occasionally stereotyped movements with fluctuating muscle tone (Krägeloh-Mann et al. 2005). Dyskinesia may be further divided into dystonia and choreoathetosis (Krägeloh-Mann et al. 2005; Sanger et al. 2010). Dystonia is defined as involuntary movements, distorted voluntary movements, and abnormal postures due to sustained or intermittent muscle contractions (Krägeloh-Mann et al. 2005; Sanger et al. 2010). Tone fluctuates but is easily increased (hypertonia) (Krägeloh-Mann et al. 2005). Choreoathetosis is characterized by (faster) hyperkinetic movements and tone fluctuation (mainly hypotonia) (Krägeloh-Mann et al. 2005). Dystonia and choreoathetosis may be simultaneously present, often with dystonia as the more dominant feature. Manifestation of dystonia and choreoathetosis is seen at rest and increases with activity (Monbaliu et al. 2016, 2017).

4.2 Baclofen in Cerebral Palsy

4.2.1 Mechanism of Action

Baclofen is a γ-aminobutyric acid (GABA) type-B ($GABA_B$) receptor agonist that binds to pre- and postsynaptic neurons (Albright 1996b; Bowery 2016; Brennan and Whittle 2008). Baclofen inhibits the release of the excitatory neurotransmitter

glutamate by binding to the presynaptic receptors. Calcium channel downregulation occurs, inhibiting the release of the excitatory neurotransmitters and thus preventing neuronal signal transmission onto spinal motor neurons (Albright 1996b; Bowery 2016; Brennan and Whittle 2008).

The site of action of baclofen in the treatment of spasticity is thought to lie in the superficial layers of the dorsal spinal cord (Albright 1996a; Bowery 2016; Enna and McCarson 2016). This hypothesis is supported by clinical findings: a single intrathecal bolus injection of baclofen (dosage range 12.5–50 mg) at the lumbar region is usually successful in achieving a temporary decrease in spasticity (Buizer et al. 2019; Hoving et al. 2007).

In people with dystonic CP, a single intrathecal bolus of baclofen is not sufficient however to decrease dystonia. A prolonged period (several days) of continuous intrathecal baclofen infusion is required to achieve improvement in dystonia. This length of time is presumably needed for baclofen to spread to the intracranial level (Albright et al. 2001). In subjects affected by dystonic CP, the catheter is routinely placed at cervical level, often requiring administration of higher daily dosages using a flexed dosing method (Gober et al. 2022). The site of action for baclofen in the treatment of dystonia is hypothesized as being on an intracranial level (Albright 1996b; Albright et al. 2001).

Intrathecal (IT) baclofen is hypothetically capable of acting at different intracranial levels, with $GABA_B$ receptors being present at varying densities throughout the mammalian brain (Bowery 2016; Enna and McCarson 2016). In dyskinetic CP, lesions of the putamen and globus pallidus induce decreased inhibition of the thalamus, subsequently resulting in the thalamus stimulating, instead of inhibiting, the supplementary motor and premotor cortex, thus producing excessive movements (e.g., dystonia). When investigating the mechanism of action of baclofen in dystonia, the first option points to baclofen exerting an inhibitory effect on the external globus pallidus. This would result in normalization of the indirect basal ganglia pathway, thus eliciting an inhibitory effect of the external globus pallidus on the thalamus and a subsequent inhibitory effect on the supplementary motor and premotor cortex, ultimately resulting in a reduction of dystonia (Albright 1996b). A potential second mechanism of action would appear to imply GABA acting as an inhibitory neurotransmitter in the human cerebral cortex, with baclofen directly inhibiting postsynaptic signal transmission from the cortex (Albright et al. 2001; McCormick 1989). A third option may involve the exertion of an inhibitory action by baclofen at the level of the thalamus. There is a high $GABA_B$ receptor density in different regions of the central nervous system, including the dorsal horn of the spinal cord, the thalamic nuclei, and the cerebral cortex (Bowery 2016), thus lending support to the described theory of the mechanism of action.

An overview of different mechanisms of action is presented in Fig. 4.1.

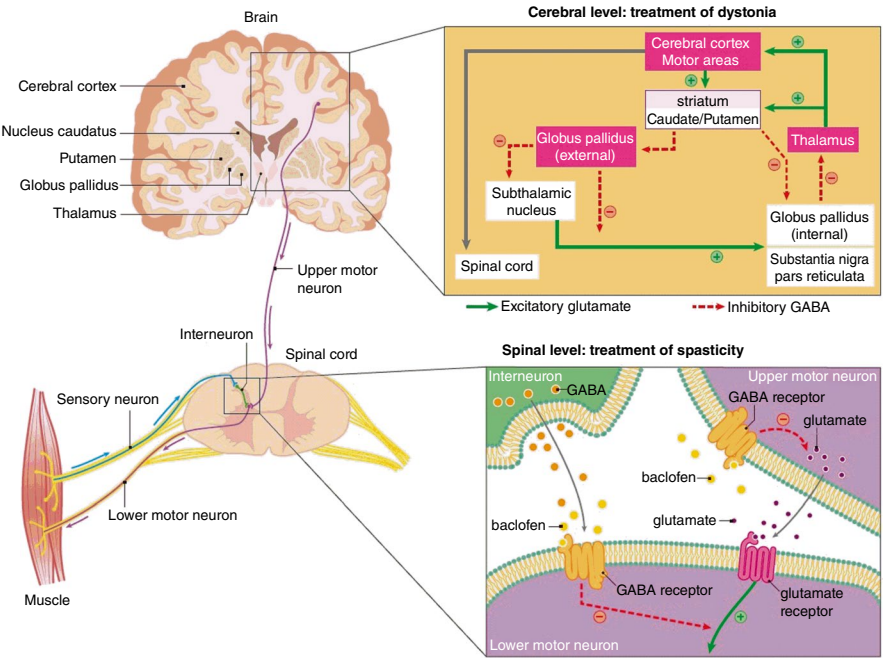

Fig. 4.1 Baclofen mechanism of actionAt the spinal cord level, baclofen binds to the presynaptic $GABA_B$ receptors, thereby inhibiting the release of the neurotransmitter glutamate. As a consequence, neuronal transmission onto the lower motor neurons is inhibited. It also binds to postsynaptic $GABA_B$ receptors, inhibiting signal transmission in the lower motor neuron. This is the mechanism of action in the treatment of spasticity (lower right image). On a cerebral level, baclofen inhibits excessive stimulation of the cortex by inhibiting the globus pallidus, thalamus, or supplementary motor and premotor cortex (upper right image). This is the hypothesized mechanism of action of intrathecal baclofen involved in the treatment of dystonia. (Figure by courtesy of Bonouvrie (2019))

4.2.2 Oral Baclofen Treatment

Despite oral baclofen being the most commonly prescribed agent in clinical practice for the treatment of spasticity in CP, the described effectiveness varies and level of evidence for the effect is very low (Navarerete-Opazo et al. 2016). In severe cases, however, the therapeutic effect achieved is insufficient and accompanied by additional adverse effects such as sedation (Albright 1996a; Knutsson et al. 1974).

Due to its low lipid solubility, orally administered baclofen does not effectively pass the blood-brain barrier (Albright 1996a) (see Chap. 7 of this volume). Plasma concentration levels ranging from 50 to 445 ng/ml yield cerebrospinal fluid (CSF) concentration levels of between <12 and 64 ng/ml. A higher plasma concentration does not correspond to a higher CSF concentration (Knutsson et al. 1974), thus implying that in many cases, high oral dosages are needed to acquire sufficient CSF

concentrations, with a consequently high risk of adverse effects (Albright 1996a; Knutsson et al. 1974).

4.2.3 Intrathecal Baclofen Treatment

Patients with generalized disabling spasticity who are refractory to oral medication are eligible for IT baclofen treatment (Biering-Sorensen et al. 2022; Dan et al. 2010). Furthermore, treatment goals should be clear and realistic, candidates should be able and motivated to attend follow-up and monitoring, and they should be of sufficient body size to allow pump implantation (Dan et al. 2010). No specific selection criteria have been formulated to date for use in the treatment of dystonia; therefore, in clinical practice, criteria similar to those described below are used.

To obtain higher levels of CSF baclofen concentration and low plasma concentration, the blood-brain barrier can be bypassed by delivering baclofen intrathecally via an implanted micro-infusion pump. This increases the effect and limits adverse effects produced by oral administration (Albright 1996a; Albright and Shultz 1999) (see Chap. 7 of this volume).

When providing IT baclofen, a significant reduction in drug concentration in the CSF is observed cranially along the spinal canal from the place of insertion (Kroin et al. 1993), thus indicating that the level at which the tip of the catheter is placed corresponds with the desired effect. In the treatment of spasticity, a low thoracic level is chosen to produce an effect on the legs and a mid-thoracic level for an effect on both arms and legs. For the treatment of dyskinesia, the tip is placed at cervical level (Albright 1996b; Albright et al. 2006).

The pump is usually implanted subcutaneously in the left lower abdomen (Albright et al. 2006), and, once implanted, an external pump programmer is used to adjust dosage as needed for the individual patient. Effective therapeutic dosage varies enormously between patients (range 30–1740 μg/day) (Lodh et al. 2021) and is not predictable: a low dosage for one patient may cause symptoms of overdose in another (see Chap. 7 of this volume).

4.2.3.1 Effect of Intrathecal Baclofen in Spastic Cerebral Palsy

A systematic review revealed how the level of evidence obtained in the 33 included studies investigating the effect of continuous IT baclofen in children with CP is generally low (Buizer et al. 2019). A higher level of evidence is provided by studies investigating the effect of intrathecal bolus injections compared to placebo (Albright et al. 1991; Hoving et al. 2007; van Schaeybroeck et al. 2000).

Outcome measures in the different studies vary, with the majority focused on the level of body function (Buizer et al. 2019). A commonality of all studies is their reporting of a decrease in spasticity with IT baclofen (Albright et al. 1991; Buizer et al. 2019; Hoving et al. 2007; van Schaeybroeck et al. 2000). Furthermore, pain,

discomfort, and a decreased quality of life, often reported in children with severe spastic CP, improve with IT baclofen (Buizer et al. 2019; Campbell et al. 2002; Hoving et al. 2009; Kraus et al. 2017; Vles et al. 2013).

Outcomes relating to level of activities and participation are heterogeneous, although frequently not systematically measured (Buizer et al. 2019). Children with IT baclofen are often severely affected and classified at higher GMFCS levels (needing a wheelchair for mobilization). Some studies, with low levels of evidence, reported an improvement in gross motor function; however, effect sizes were small—an expected outcome due to the majority of patients being severely affected, with improvement of motor function unlikely (Buizer et al. 2019).

Individually formulated problems in daily life measured on a visual analogue scale (VAS) are described as having improved significantly following IT baclofen bolus test treatment compared to placebo, in the same way as VAS scores obtained for ease of care (Hoving et al. 2007). Prospective cohort studies reported how improvements were maintained following long-term, continuous IT baclofen treatment. The majority of these issues were related to the severity of CP, and treatment was aimed at enhancing ease of care by caregivers (Hoving et al. 2009; Vles et al. 2013). Taking care of a child with a severe disability places a remarkable burden on caregivers, and a prospective cohort study showed how, following administration of long-term IT baclofen treatment, caregivers reported fewer emotional concerns and fewer limitations in time (Vles et al. 2013). Caregiver satisfaction was high, with the majority prepared to opt for IT baclofen treatment again in the future (Campbell et al. 2002; Kraus et al. 2017; Vles et al. 2013).

4.2.3.2 Effect of Intrathecal Baclofen in Dyskinetic Cerebral Palsy

Few studies have been conducted to investigate the effect of IT baclofen in children with dyskinetic CP, the majority of which are case series: one multicenter double-blind placebo-controlled randomized clinical trial (RCT), the IDYS trial, the follow-up study of this trial, and a prospective cohort study (Albright et al. 2001; Bonouvrie et al. 2019, 2023; Eek et al. 2018; Motta et al. 2008).

To evaluate the effect on the level of body functions and structures, the Barry Albright Dystonia Scale (BADS) is the most common outcome measure used (Barry et al. 1999). BADS scores were reported to decrease in most studies, of which the majority are case series (Albright et al. 2001; Eek et al. 2018; Motta et al. 2008). In contrast, the RCT comparing IT baclofen to placebo after 3 months of blinded treatment detected no changes in the BADS (Bonouvrie et al. 2019). At study follow-up, however, within 9–12 months of using IT baclofen, a significant change was observed at the BADS (Bonouvrie et al. 2023). The RCT and follow-up also both adopted the Dyskinesia Impairment Scale as a measure of the severity of dyskinesia (Monbaliu et al. 2012). In both studies, a significant difference was found on the dystonia subscale compared to placebo and to baseline, respectively (Bonouvrie et al. 2019, 2023). One study showed the effect of catheter tip placement, in which a significantly higher decrease in BADS scores was observed with a higher placed

catheter tip (Thoracic 4 and higher) compared to a lower placed tip (Thoracic 6 and lower) (Albright et al. 2001).

With regard to pain, comfort, and quality of life, three studies reported a potential improvement in these domains (Albright et al. 2001; Eek et al. 2018; Motta et al. 2008). The previously described RCT found no difference in pain and comfort compared to placebo after 3 months of blinded treatment (Bonouvrie et al. 2019). However, in the follow-up study, after 9–12 months of IT baclofen, pain decreased in patients who had pain reduction as a treatment goal (Bonouvrie et al. 2023).

Based on the findings of structured interviews and questionnaires, the case series reported improvements in several areas of activities and participation. An improvement was reported for feeding and swallowing, sitting and posture control, upper limb use, and communication/speech (Albright et al. 2001; Eek et al. 2018; Motta et al. 2008). However, no change in autonomy in carrying out daily activities was observed in the majority of patients (Motta et al. 2008), similar to the findings obtained in patients with spasticity, as expected in view of the severity of CP in the majority of patients with IT baclofen.

Ease of care represented the main treatment goal for the majority of patients with dyskinetic CP and IT baclofen, with an improvement in caregiving reported in most patients (Albright et al. 2001; Eek et al. 2018; Motta et al. 2008). The IDYS trial used the Goals Attainment Scale (GAS) to assess the fulfillment of individual treatment goals (Steenbeek et al. 2007). After 3 months of blinded treatment, a significant difference was detected between the placebo and IT baclofen groups, in favor of the IT baclofen group (Bonouvrie et al. 2019). This effect remained consistent even after 9–12 months of treatment, with 71% of patients with dyskinetic CP fully achieving one or more treatment goals, and partial achievement of one of more treatment goals reported in 97% (Bonouvrie et al. 2023).

Caregiver satisfaction was high (80%), and 74% of patients reported they would choose IT baclofen treatment again (Motta et al. 2008).

4.3 Conclusions

Treatment of disabling movement disorders such as spasticity or dyskinesia in children is challenging. Oral pharmacological treatment options, including oral baclofen, are often insufficient and/or cause side effects.

Intrathecal baclofen treatment represents a therapeutic option for some of these patients. There is some evidence, mostly provided by single-bolus randomized trials, of the effectiveness of IT baclofen in the treatment of spasticity in children with spastic CP at the level of body functions and structures (spasticity). However, a low level of evidence of the effectiveness of continuous IT baclofen, particularly at the level of activities/participation, has been reported.

One RCT and related follow-up study reported evidence of the effect of IT baclofen treatment on dystonia in children with dyskinetic CP, including attainment

of individual goals. However, other studies yielding similar results provide lower levels of evidence.

Despite the reported levels of evidence, there are frequently no alternative treatment options for these children; therefore, when all other options fail, IT baclofen should be considered. Future studies should be conducted to specifically investigate outcome measures at the level of activities and participation.

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Chapter 5
Baclofen for the Treatment of Cough

Huda Badri and Jacky A. Smith

Abstract $GABA_B$ receptor agonists have long been implicated in the control of the cough reflex. Animal studies have demonstrated the presence of $GABA_B$ receptors in the upper airways, the trachea, and the dorsal root ganglia. Several preclinical and clinical studies have demonstrated the antitussive effect of the $GABA_B$ agonist baclofen. However, its use in clinical practice has been limited due to significant central nervous system side effects. In this chapter, we review the role of GABA and $GABA_B$ receptor agonists in cough.

Keywords Baclofen · GABAB receptor · Cough · Antitussive effects

5.1 Introduction

Cough is a complex sensorimotor phenomenon critical to the protection of the lungs and airways from aspirate, inhaled particulate matter, accumulated secretions and irritants (Chung et al. 2022). Impairment of the cough reflex has severe consequences, such as the inability to clear the airways, leading to aspiration and recurrent infections, causing significant co-morbidity and even death (Won et al. 2018). Cough can also be indicative of an underlying condition. For example, acute cough, defined as lasting 3 weeks or less, is often due to upper or lower respiratory tract infections, and although it may persist for several weeks after the resolution of the infection, it is usually self-limiting (Morice et al. 2020). Chronic cough, on the other hand, is present for longer than 8 weeks and can be due to an underlying chronic respiratory illness such as asthma (Morice et al. 2020).

Approximately 5% of adult patients with cough have refractory chronic cough (RCC), meaning that their symptoms are unexplained or do not respond to treatment

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for underlying conditions (Smith 2024). In these patients, the cough becomes intractable, with patients coughing hundreds and even thousands of times per day. These are similar rates of cough to those during an acute viral infection, except persististing for years (Kelsall et al. 2009; Sunger et al. 2013) and causing significant morbidity. Patients in chronic cough clinics often report complications such as inguinalor incisional hernias, urinary and fecal incontinence, rib fractures, and even cough syncope in extreme cases (French et al. 1998; Hrisanfow and Hagglund 2013). Adams and colleagues found that 34% of chronic cough patients suffered from depression, which is comparable to the rates of depression in serious medical conditions (Adams et al. 2009). In addition to health and psychological impairment, there are significant socio-economic implications related to chronic cough, accounting for significant absenteeism from work (Song et al. 2015; Colak et al. 2017; Jenson et al. 2001).

Gefapixant, a P2X3 purinergic receptor antagonist, the first developed and licensed for the treatment of RCC, has recently been licensed in the European Union, Switzerland, and Japan (Smith 2024; McGarvey et al. 2022). Although the US Food and Drug Administration (FDA) has declined to approve it for a license at present, gefapixant is still not widely available. Current pharmacological management options for RCC are based on cough suppression therapy via off-license use of centrally active drugs such as opioids and gabapentin (Morice et al. 2020). γ-Aminobutyric (GABA) type-B ($GABA_B$) receptor agonists have long been thought to possess antitussive properties (Bolser et al. 1993). In this chapter, we review the evidence for the mechanisms of $GABA_B$ receptor agonism in cough neurophysiology, as baclofen remains the only clinically available $GABA_B$ receptor agonist.

5.2 Neuronal Mechanisms Underlying Cough

Detailed descriptions of the anatomy and neurophysiology of cough are well described elsewhere (Canning et al. 2014). In summary, the neurophysiological processes underlying cough initiation and control are complex and involve a delicate interplay between the peripheral nervous system, the brain stem, and higher cortical control. Involuntary cough is typically initiated by irritant stimuli that activate sensory vagal afferent fibers in the airways (larynx, trachea, and large bronchi) (Canning and Chou 2009). Once activated, the action potential travels to either the nodose or jugular ganglion. The first-order neurons then synapse in the nucleus tractus solitarius (NTS), or paratrigeminal nucleus of the brainstem (Chung and Pavord 2008), depending on the type of fiber activated by the stimuli. Neurotransmitters are then subsequently released, leading to the activation of vagal efferent nerves supplying the muscles of the diaphragm, larynx, and chest wall, resulting in the alteration of a normal breathing pattern to a cough motor pattern (Fig. 5.1).

The sensory vagal fibers involved in initiating cough express a wide range of G protein-coupled cell surface receptors and ion channels and are mainly divided into chemically sensitive unmyelinated C-fibers or mechanically sensitive, thinly

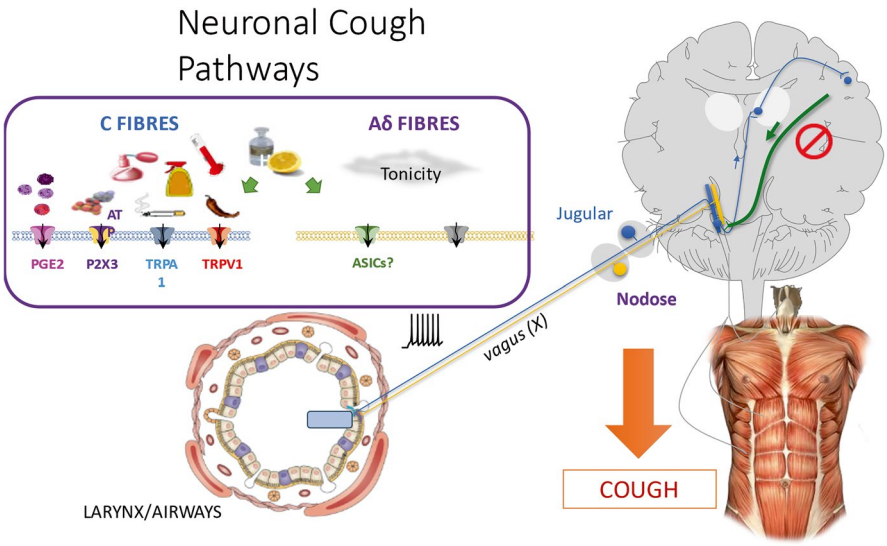

Fig. 5.1 Neuronal cough pathways. The mechanisms underlying cough are complex, but we now recognize that chronic cough is due to neuronal hypersensitivity, involving peripheral sensory (via C-fibers and Aδ fires) afferent vagal nerve fibers, the midbrain, and the CNS. With various immune mediators, particulate matter, noxious chemicals, and mucus playing a role in activating these cell surface receptors. Inhibitory mechanisms are depicted in green (PGE2: Prostaglandin E2; P2X3: Purinoceptor 3; TRPA1: transient receptor potential ankyrin 1; TRPV1: transient receptor potential vanilloid 1; ASICs: acid sensing ion channels)

myelinated Aδ fibers (Mazzone and Undem 2016). C-fibers are also activated by the chili pepper extract, capsaicin, via the transient receptor potential vanilloid 1 (TRPV1), and this mechanism is used in preclinical models and human studies of evoked cough (Mazzone et al. 2005). Conversely, Aδ fibers are activated by punctuate mechanical stimuli, aspirated particles, accumulated secretions, and mucosal acidification (Canning et al. 2004). Aδ fibers are sensitive to changes in osmolarity and low pH.

Cough can be voluntarily induced, enhanced, or suppressed via higher brain motor control pathways (Hutchings et al. 1993; Young et al. 2009; Mazzone et al. 2011). Anatomical and functional studies suggest that vagal afferents converge and interact in the central nervous system (CNS) to regulate cough. In anesthetized guinea pigs, C-fiber activation does not evoke cough but substantially reduces the threshold for initiating the cough reflex (Mazzone et al. 2005). This central sensitization process is well described in pain studies (Curatolo 2024), with the findings being mirrored in chronic cough (Ando et al. 2016; Canning 2009). There is also evidence that suggests that impairment in inhibitory control mechanisms is implicated in cough suppression (Hilton et al. 2020). Patients with refractory chronic cough have attenuated volitional cough suppression and impaired inhibitory cough networks (Hilton et al. 2020). Components of these inhibitory systems that regulate

cough involve the neurotransmitter GABA, and $GABA_B$ receptor agonists modify evoked cough in animals and humans (Canning 2009).

5.3 $GABA_B$ Receptor Agonism in Cough

$GABA_B$ receptor agonists are thought to possess antitussive properties via two main mechanisms:

1. Direct action through the inhibitory neuronal pathways mediating cough
2. Action on transient lower esophageal sphincter relaxations resulting in reduced gastroesophageal reflux disease (GERD) events

Baclofen and arbaclofen are predominantly centrally acting $GABA_B$ receptor agonists. Lesogaberan and 3-aminopropylphosphinic acid are predominantly peripherally acting $GABA_B$ receptor agonists (Corelli and Mugnaini 2016). However, only baclofen is clinically available (see Chap. 3 of this volume).

5.3.1 Evidence of $GABA_B$ Receptors in the Airways

The presence of both GABA type-A ($GABA_A$) and $GABA_B$ receptors in the upper airways, trachea, dorsal root ganglia (DRG), and lower esophageal sphincter is well described in immunohistochemistry studies of small rodents (Yabumoto et al. 2008; Gentilini et al. 1995). These findings are supported by pharmacological studies of GABA-induced modulation of acetylcholine release in the lung (Shirakawa et al. 1987). In particular, guinea pigs and human studies have demonstrated that $GABA_B$ receptors are expressed on airway epithelial cells (Mizuta et al. 2008).

Several functions have been attributed to $GABA_B$ receptors in the airways, including inhibition of smooth muscle contraction, mucus hypersecretion in asthma, and regulation of parasympathetic ganglia neurons (Chapman et al. 1993). Tamaoki et al. (1987) found that baclofen decreased the contractile response of airway smooth muscle by inhibiting the release of acetylcholine (Tamaoki et al. 1987). These findings were later supported by Chapman et al. (1991). Studies with selective $GABA_B$ receptor agonists have demonstrated attenuated airway hyper-reactivity and reversal of bronchospasm both in animals and humans (Grimm et al. 1997; Gleason et al. 2009; Gentilini et al. 1995; Dicpinigaitis et al. 1994; Tohda et al. 1998). Further, smaller studies have also suggested that baclofen can reduce airway reactivity and obstruction, likely via inhibition of cholinergic tone (Grimm et al. 1997; Dicpinigaitis et al. 1994).

Belvisi et al. (1989) and Fawley et al. (2011) investigated whether $GABA_B$ receptor agonists inhibit both evoked and TRPV1-mediated release mechanisms on second-order NTS neurons in the midbrain of rats. They found that baclofen inhibited action potential-triggered and TRPV1-coupled glutamate pathways.

5.4 Antitussive Properties of Baclofen

The work of Bolser et al. in the 1990s demonstrated the antitussive effects of both central and peripheral $GABA_B$ receptor agonists in cats and guinea pigs. They induced cough in cats via mechanical stimulation of the intrathoracic trachea and in guinea pigs via aerosolized capsaicin. In both animal groups, they found that baclofen inhibited cough via a central site of action (Bolser et al. 1993). Indeed, baclofen was comparable to codeine and dextromethorphan in its ability to suppress capsaicin-induced cough. Similar findings in guinea pigs, cats, and rabbits were reported by other groups (Mutolo et al. 2010; Canning et al. 2012).

There are a small number of clinical studies of the effect of baclofen on evoked cough responses. Dicpinigaitis et al. studied the effects of baclofen on evoked cough in healthy volunteers, spinal cord injury patients, and chronic cough patients (Dicpinigaitis 1996; Dicpinigaitis and Dobkin 1997; Dicpinigaitis et al. 1998, 2000). In the healthy volunteer studies, the effect of treatment with baclofen (10–20 mg) versus placebo for a duration of 14–28 days on capsaicin-evoked cough was assessed. In all three studies, treatment with baclofen significantly increased the cough threshold, i.e., the concentration of capsaicin eliciting 5 or more coughs (C5), compared with placebo (Dicpinigaitis and Dobkin 1997, Dicpinigaitis et al. 1998, 2000).

Dicpinigaitis and Dobkin (1997) carried out a double-blind, randomized controlled trial (RCT) in 20 healthy, non-smoking volunteers. The subjects were randomly assigned to take oral baclofen (10 mg) or placebo three times a day for 14 days. Baseline and day 14 capsaicin cough challenges were performed. They found that the baclofen group had a much higher capsaicin cough threshold (pre-therapy and post-therapy log C5, 0.93 ± 0.27 and 1.41 ± 0.36, respectively). In the placebo group, there was no change in the cough sensitivity (pre-study and post-study log C5, 1.44 ± 0.24 and 1.38 ± 0.26, respectively). When the two groups were compared, the change in C5 after treatment was significantly higher in the baclofen group. Four out of the 10 subjects in the baclofen group complained of mild fatigue and drowsiness, interestingly, as did two of the subjects in the placebo arm.

Dicpinigaitis et al. (1998) then went on to look at the effect of a longer period of treatment with baclofen. Forty-one healthy volunteers were recruited, who were randomly assigned to receive either baclofen 10 mg once daily, baclofen 20 mg once daily, or placebo. The subjects then underwent baseline, day 14, and day 28 cough reflex sensitivity testing. The subjects receiving baclofen 20 mg daily demonstrated significant inhibition of their cough reflex sensitivity at days 14 and 28 compared with baseline. In addition, after 28 days of baclofen (20 mg), C5 was significantly greater than after 14 days of therapy. Interestingly, neither the subjects receiving baclofen 10 mg daily nor those receiving placebo had a significant change in their cough sensitivity.

Dicipinigatis et al. also studied patients who were chronically maintained on baclofen for painful muscle spasms secondary to spinal cord injury (Dicpinigaitis et al. 2000). The same study protocol was used as in the previous studies by Dicipinigaitis (Dicpinigaitis 1996; Dicpinigaitis and Dobkin 1997; Dicpinigaitis et al. 1998). Again, they found that patients chronically maintained on baclofen had significantly higher C5. Of note, though this study was very small (n = 12), and a significant number of patients were smokers.

More recently, our group investigated the effect of baclofen 40 mg versus lesogaberan 120 mg (as mentioned above, a peripherally acting $GABA_B$ receptor agonist) versus placebo on capsaicin-evoked cough in healthy volunteers. Baclofen did not reduce cough frequency but did improve cough responses. Interestingly, lesogaberan produced a small (non-statistically significant) effect on cough frequency but had no effect on evoked cough responses (Badri et al. 2021).

There are no clinical trials of baclofen in RCC; however, there are a few case studies. For example, Dicpinigaitis and Rauf (1998) reported two cases of chronic cough patients being maintained on a 14-day course of low-dose baclofen. They found that cough reflex sensitivity threshold in these two patients was significantly increased after treatment with baclofen, and the patients also reported symptomatic improvement. A more recent report in the literature by Xu et al. (2012) describes three cases of chronic cough patients being treated with baclofen. All were treated with baclofen, and the cough improved within 2–4 weeks of treatment. Anecdotal use of baclofen in RCC does occur in some chronic cough clinics, but this is in highly selected patients with close monitoring.

5.5 Gastroesophageal Reflux Disease-Related Cough

Gastroesophageal reflux has been suggested to cause cough either via direct stimulation of the airway nerve terminals or indirect stimulation by activation of the neural pathways linking the esophagus to the airway (Smith and Houghton 2013) (Fig. 5.2). Impairment in the tone or function of the lower esophageal sphincter (LOS) is an important mechanism leading to gastroesophageal reflux. $GABA_B$ receptor agonists such as baclofen have been shown to act on LOS and thus reduce reflux episodes.

The direct mechanisms are based on the theory that, due to the anatomical proximity of the esophagus to the trachea, microscopic acid refluxate can overspill into the pharynx and upper airways, activating the terminal sensory nerve fibers and causing cough (Houghton et al. 2016). Another hypothesis is that neuronal stimulation of the proximal esophagus by refluxate can lead to an increase in cough events via "neuronal cross talk" (Houghton et al. 2016). However, there is no conclusive data to support these mechanisms, and many are difficult to study.

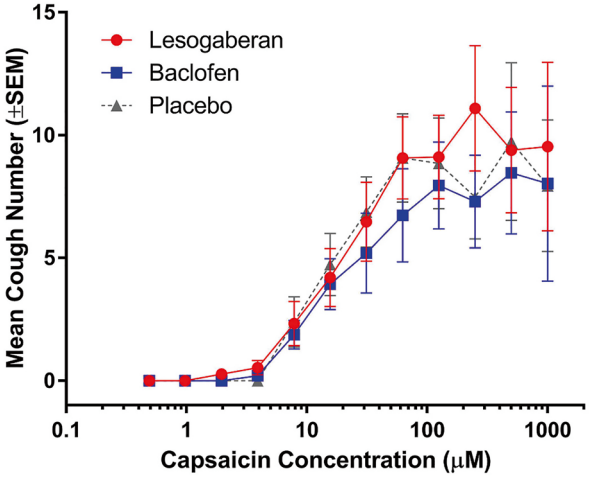

Fig. 5.2 Cough responses to capsaicin challenge showing dose response curves when subjects were treated with lesogaberan, baclofen, and placebo. (Reproduced from Badri et al. (2021) with permission from Elsevier)

5.5.1 Esophageal-Bronchial Reflex

Studies using immunohistochemical techniques (Altschuler et al. 1991) and esophageal infusion of acid/saline infusions (Mansfield et al. 1981) have supported the concept of a neuronal link between esophageal stimulation and cough. These studies included patients with: (i) GERD and chronic cough; (ii) GERD and no cough; (iii) chronic cough but no GERD; and (iv) healthy volunteers. They found that acid/saline infusions in all these groups (apart from the patients with GERD and chronic cough) did not affect cough reflex sensitivity. In contrast, patients with GERD and chronic cough had significantly lower cough reflex sensitivity. This is an interesting finding, as one would have assumed that proton pump inhibitors (PPIs) would be more successful at suppressing cough in GERD patients, yet this is not always the case (Shaheen et al. 2011). This would support the idea of sensitization of the esophageal-bronchial reflex in patients with GERD and chronic cough. Fouad et al. found that the most common abnormality in their patient with GERD associated cough was ineffective esophageal motility (48% of their cough patients) (Fouad et al. 1999). This can result in prolonged exposure of the esophagus to reflux which may provide a larger esophageal stimulus and might be more likely to provoke coughing.

5.5.2 Temporal Associations Between Reflux and Cough

Studies measuring pH impedance have shown that there is a relationship between cough episodes and distal reflux events (Decalmer et al. 2012). Smith et al. looked at 71 unselected chronic cough patients aged 51–64 years old. Cough reflex sensitivity was measured using citric acid inhalational cough challenge. They found that

70% of patients demonstrated a temporal association, with 48% having a positive symptom-associated probability (SAP) for cough preceded by reflux and 56% having a positive SAP for reflux preceded by cough.

Vagal sensory afferents can also be found in other organs, such as the esophagus/stomach. Stimuli within these organs activate these fibers, and impulses are then carried to the NTS, where all the peripheral sensory afferents converge. It is thought that the brain can misinterpret or confuse these signals, and thus the efferent response can occur in a different organ from where the stimulus arose. Evidence for this comes from studies where infusing acid into the esophagus resulted in increased cough frequency and cough reflex sensitivity (Javorkova et al. 2008; Ing et al. 1994).

Persistent cough has long been associated with gastroesophageal reflux, as reported in numerous studies. However, the overall findings from RCTs investigating antacid therapy in patients with chronic cough have been largely negative. Consequently, guidelines for chronic cough management now discourage the routine prescription of acid suppression therapy in the absence of reflux symptoms (Morice et al. 2020).

5.5.3 Inhibition of Transient Lower Esophageal Sphincter Relaxations by Baclofen

Several studies have shown that baclofen is effective at increasing LOS pressure and reducing the number of transient LOS relaxations (TLOSRs). Lidums et al. (2000) and Lee et al. (2003) showed a reduction of the incidence of TLOSRs of 60% by baclofen. Multiple other studies have also demonstrated the effectiveness of baclofen as an anti-reflux agent (Zhang et al. 2002; Omari et al. 2006; Grossi et al. 2008).

There are two studies in the GERD literature assessing the effect of baclofen on TLOSRs. The first study (Zhang et al. 2002) was a placebo-controlled crossover RCT investigating the control of TLOSRs by baclofen. Twenty patients with known GERD were given baclofen 40 mg once-only dose or placebo with a 1-week washout. Baseline esophageal studies were carried out prior to drug administration and then 60 min later. The patients then ate a 750-kcal meal and underwent 3-h pH recordings. Baclofen administration reduced the rate of TLOSRs from a median of 15 (13.8–18.3) per 3 h to 9 (5.8–13.3) per 3 h. The basal LOS pressure was also significantly higher during the baclofen administration phase. There was no significant difference in adverse events between the placebo and baclofen phase, and all were minor.

The second study by Grossi et al. (2008) recruited 21 patients into two groups, and esophageal studies were carried out over a 48-h period. Baclofen 10 mg or placebo was administered four times in the second 24-h period. They also found that basal LOS tone was significantly increased in the baclofen group versus placebo.

The baclofen group also had significantly fewer swallows compared to the placebo group.

Unfortunately, due to the significant side effect profile of baclofen, the use of baclofen in GERD has been limited to add-on therapy to standard treatment with PPIs in limited cases of refractory GERD under specialist review (Katz et al. 2022).

5.5.3.1 Lesogaberan

Lesogaberan has a high affinity for the GABA carrier, which results in reduced extracellular levels of lesogaberan in the CNS (Boeckxstaens et al. 2011a) and is rapidly absorbed, with peak plasma concentrations occurring around 1–2 h after oral dosing (Miner Jr. et al. 2014). Most of the evidence on lesogaberan in the literature comes from GERD studies (Boeckxstaens et al. 2010a; Shaheen et al. 2013; Miner Jr. et al. 2014). Lesogaberan was initially developed as an add-on to PPIs for the treatment of persistent GERD symptoms despite PPIs (Shaheen et al. 2013; Boeckxstaens et al. 2011a). It is thought that lesogaberan reduces the frequency of TLOSRs, which is thought to be an important mechanism in GERD (Boeckxstaens et al. 2011b).

Studies in dogs provided evidence of lesogaberan inhibiting the rate of TLOSRs on average by 39% and reducing esophageal acid exposure (Beaumont et al. 2008; Lehmann et al. 2009; Branden et al. 2010). Lehmann et al. and Brändén et al. demonstrated that lesogaberan reduced the number of reflux episodes and esophageal acid exposures in dogs (Lehmann 2009; Beaumont et al. 2008). Since then, 17 healthy volunteer studies of 489 participants have been carried out (AZ Lesogaberan information brochure). These studies suggested that lesogaberan reduces the frequency of TLOSRs on average by 36%, in keeping with the animal models (Boeckxstaens et al. 2010b). In another human study by Boeckxstaens et al. (2010b) they found that lesogaberan increased transient LOS pressure by 39% compared to placebo (Boeckxstaens et al. 2011a). Although this was lower than the 47% increase in transient LOS pressure by baclofen, the lesogaberan group had less than half the side effect profile of baclofen.

In the two main Phase IIb, double-blind, placebo-controlled, RCTs of lesogaberan in patients with GERD partially responsive to PPI therapy, a positive response was based in reduction of heartburn and regurgitation symptoms (Shaheen et al. 2013, Miner Jr. et al. 2014). In the first study, lesogaberan had a clinically significant positive response over placebo (16% vs. 8%). However, in the second, much larger study, the difference in response between and placebo was not clinically significant. Thus, on the basis of these two studies, the development of lesogaberan for the treatment of GERD was ceased. These studies have shown that there is a significantly reduced CNS side effect profile for lesogaberan compared to baclofen. Boeckxstaens (2009) showed that lesogaberan had half as many CNS side effects compared to baclofen. This was reproduced in their later work (Boeckxstaens et al. 2011a).

Canning et al. found that lesogaberan was as effective as baclofen in reducing the cough reflex in conscious guinea pigs (Canning et al. 2012). They evoked coughing in conscious guinea pigs after pre-treating them with lesogaberan. They found that lesogaberan reduced the cough response, in a dose-dependent manner, with fewer side effects compared to baclofen. Interestingly, in a healthy human volunteer RCT of baclofen versus lesogaberan. Lesogaberan did not reduce capsaicin-induced cough responses, although baclofen improved cough reflex sensitivity (Badri et al. 2021) (Fig. 5.2).

However, in the only clinical trial of lesogaberan chronic cough patients, after 2 weeks of treatment with lesogaberan 120 mg versus placebo, there was a reduction in 24-h cough frequency by 26% over placebo, although this did not reach statistical significance (Badri et al. 2022). However, capsaicin-evoked cough responses did significantly improve (Fig. 5.3). Although this study did assess objective gastro-esophageal reflux episodes, only a small number of participants had reflux-associated cough events, and thus it was difficult to draw any conclusions regarding whether the reduction of evoked coughs was related to a reduction in TLOSRs.

There are several possible explanations for this difference in cough responses between healthy participants and RCC patients; it is possible that in the healthy human cough reflex, $GABA_B$ receptor agonists inhibitory action is centrally mediated whereas in the hyper sensitized state it is peripherally mediated.

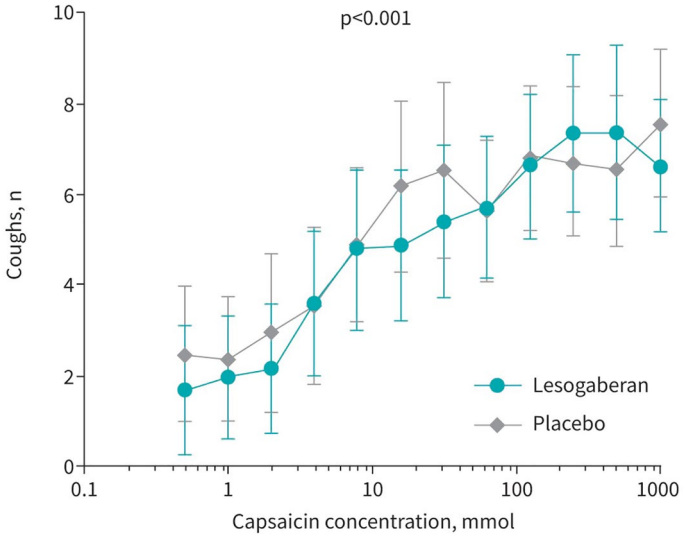

Fig. 5.3 Capsaicin-evoked coughs in chronic cough patients after 2-week treatment with placebo versus lesogaberan. (Reproduced from Badri et al. (2022) with permission from European Respiratory Society Publications)

5.6 Conclusions

The presence of the $GABA_B$ receptors is well documented throughout the lungs and airways. The baclofen studies described above support a role for $GABA_B$ receptor agonists in the suppression of the cough reflex. The antitussive activity of $GABA_B$ receptor agonists is more likely to be due to a direct effect on the neuronal pathways controlling cough than via an indirect effect on gastroesophageal reflux. However, the main limitation for the use of baclofen is that higher doses are needed to suppress chronic cough, and this results in significant CNS side effects (drowsiness, fatigue, dizziness, risk of seizures on withdrawal), which makes this drug unsuitable for most patients (see Chap. 7 of this volume). The use of baclofen in chronic cough is currently off-license and should be limited to expert clinic use in a carefully selected group of patients who are fully informed regarding the side effect profile. The effect of peripheral $GABA_B$ receptor agonists on cough reflex sensitivity, although it did not reach the primary endpoint, suggests a potential target for therapeutic development and requires further work.

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Chapter 6
Baclofen for the Treatment of Alcohol Use Disorder

Roberta Agabio, Benjamin Rolland, and Lorenzo Leggio

Abstract Baclofen is the only GABA type-B ($GABA_B$) receptor agonist available for use in clinical practice; it has been approved worldwide for the treatment of spasticity for more than 50 years. After the promising results of preclinical studies suggesting a potential role of baclofen in the treatment of alcohol use disorder (AUD), several randomized controlled trials (RCTs) have been conducted to evaluate its safety, tolerability, and efficacy in people with AUD. While the results of these RCTs are contrasting, the off-label use of baclofen in the treatment of AUD has spread, especially in some European countries and in specific settings (e.g., in liver settings). Globally, these efforts have led to the approval of baclofen for AUD in France. Systematic reviews and meta-analyses confirm its efficacy in helping people with AUD to abstain from alcohol, especially those with liver disease or high levels of anxiety. However, the lack of large RCTs limits the conclusions that may be drawn on the potential effectiveness of baclofen in AUD.

Keywords Alcohol use disorder · Baclofen · Liver cirrhosis · Efficacy · Safety

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6.1 Introduction

Baclofen is a γ-aminobutyric acid (GABA) type-B ($GABA_B$) receptor agonist, approved by the US Food and Drug Administration (FDA) in 1977 for the treatment of spasticity (Bowery 2016; Kent et al. 2020; Nieto et al. 2022). It was synthesized in 1962 in an attempt to develop a more lipophilic analog of GABA, and, at present, it is still the only $GABA_B$ receptor agonist approved in clinical practice (Bowery 2016; see Chap. 3 of this volume). Besides its formally approved indication for spasticity, baclofen is occasionally prescribed off-label for a few other medical conditions like depression and anxiety (Felice et al. 2016), pain (Enna and McCarson 2016), gastroesophageal reflux, and alcohol withdrawal syndrome (AWS) and alcohol use disorder (AUD) (Bowery 2016; Kent et al. 2020; Nieto et al. 2022). In detail, the use of baclofen in the treatment of AUD and AWS was initially suggested by the promising results of preclinical studies (Colombo and Gessa 2018) that led to a series of clinical trials to investigate its potential efficacy and safety in humans (Agabio et al. 2016a). Interestingly, the first randomized placebo-controlled trial (RCT) was published in 2002 (Addolorato et al. 2002). This study was followed by other RCTs that have been recently investigated by a Cochrane systematic review (Agabio et al. 2023). Twelve years after the first RCT (Addolorato et al. 2002), and even if the RCTs did not lead to consistent results in terms of efficacy (Agabio et al. 2023), still in 2014, the French Medicines Agency (Agence Nationale de Sécurité du Médicament et des Produits de Santé, ANSM) assigned to baclofen a special temporary recommendation for AUD treatment (De Beaurepaire and Jaury 2024); after 4 years, in 2018, the same French agency provided definitive approval for the use of baclofen as a treatment for AUD (De Beaurepaire and Jaury 2024). In 2023, baclofen was included among the medications recommended for AUD treatment by the guidelines of the Mental Health Gap Action Programme for mental, neurological, and substance use disorders of the World Health Organization (2023). Nevertheless, at present, baclofen is approved for AUD treatment only in France and sometimes used as an off-label medication in other countries (Agabio et al. 2018; De Beaurepaire and Jaury 2024). This chapter is aimed at updating our previous chapter on the same topic (Agabio et al. 2016a).

6.2 Definitions of Alcohol Use Disorder and Alcohol Withdrawal Syndrome

Alcohol is one of the most widely consumed psychoactive substances globally, with over 80% of adults using alcohol at some point in their lives (World Health Organization 2018). In Western countries, it has been estimated that more than half of the general population consumes alcohol annually (World Health Organization

2018). Despite its wide use globally, including its role in facilitating social interactions, alcohol represents a major risk factor for injuries, mortality, and the global burden of disease (GBD) (GBD 2016 Alcohol Collaborators 2018). When consumed acutely, it increases the risk of injuries, accidents, aggression, violence, and even death; when consumed chronically, it increases the risk of developing several medical conditions, including cancers, liver diseases, and other physical and mental conditions (GBD 2016 Alcohol Collaborators 2018). When consumed during pregnancy, it can cause negative consequences for the embryo and fetus, such as the fetal alcohol spectrum disorders, characterized by mental impairment, craniofacial anomalies, and other physical defects like cardiac defects (Popova et al. 2021). The risk of developing negative consequences is directly related to the amounts of alcohol consumed, and the consumption with the lowest risk for health loss is zero (GBD 2016 Alcohol Collaborators 2018). These harms represent a significant public health challenge (GBD 2016 Alcohol Collaborators 2018).

AUD is a severe and chronic brain disorder characterized by the inability to control alcohol consumption with subsequent and devastating consequences due to the elevated amounts of alcohol consumed (American Psychiatric Association 2013, 2022). In the last editions of the Diagnostic and Statistical Manual of Mental Disorders (DSM), DSM-5, and DSM-5-TR, AUD diagnosis has replaced the two previous diagnoses of "alcohol abuse" and "alcohol dependence" (e.g., DSM-IV, American Psychiatric Association 1994). AUD diagnosis requires meeting at least two out of a list of 11 criteria, and the number of criteria met is used to classify severity into mild (2–3), moderate (4–5), and severe (6 or more; see Box 6.1; American Psychiatric Association 2013, 2022).

The AUD equivalent diagnosis of the International Classification of Diseases (ICD-11) is alcohol dependence (World Health Organization 2019), even if the AUD diagnosis by DSM-5 and the alcohol dependence diagnosis by ICD-11 do not overlap completely (Saunders et al. 2019). The etiology of AUD is multifactorial, including both genetic and psychological and social environmental influences, with the heritability of developing an AUD being estimated in the range of 40–60% (MacKillop et al. 2022).

Worldwide, the lifetime prevalence of AUD achieves almost 10% of the general population, with large variability between countries related to drinking cultures, social norms, and income levels (Glantz et al. 2020; Rehm et al. 2015; Kranzler 2023). As an example, it ranges from approximately 6% in low-to-middle-income countries to more than 10% in high-income countries (Glantz et al. 2020). AUD prevalence is also higher in men than in women (Glantz et al. 2020). In the United States, it has been estimated to be equal to 36% and almost 23% of male and female general populations, respectively (Grant et al. 2015). However, the gender gap in AUD is narrowing, particularly in the younger population (Slade et al. 2016; Agabio et al. 2021a).

Box 6.1 Diagnostic Criteria for Alcohol Use Disorder

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5; American Psychiatric Association 2013), the diagnosis of alcohol use disorder (AUD) requires that, within a 12-month period, an individual meets at least 2 criteria from a list of 11, which can be grouped into the following 4 categories:

Category 1: Impaired control (Criteria 1–4)

1. Alcohol is consumed in larger amounts or over a longer period than intended.
2. Persistent and unsuccessful desire to cut down on or control alcohol use.
3. Significant time spent obtaining, using, or recovering from the alcohol effects.
4. Craving, or a strong desire or urge to use alcohol.

Category 2: Social Impairment (Criteria 5–7)

5. Failure to fulfill major roles at work, school, or home due to recurrent alcohol use.
6. Continued alcohol use despite persistent social or interpersonal problems.
7. Important social, occupational, or recreational activities are given up or reduced due to alcohol use.

Category 3: Risky Use (Criteria 8–9)

8. Recurrent alcohol use occurs in situations where it is physically hazardous.
9. Continued alcohol use despite knowledge of having a persistent physical or psychological problem.

Category 4: Pharmacological Criteria (Criteria 10–11)

10. Tolerance, as defined by either:
    - Need for increased amounts of alcohol to achieve the desired effect.
    - Markedly diminished effect with continued use of the same amount of alcohol.
11. Withdrawal, as manifested by either:
    - The characteristic alcohol withdrawal syndrome.
    - Alcohol is taken to relieve or avoid withdrawal symptoms.

AUD is a chronic relapsing disorder with alternating periods of abstinence and relapse; when people with AUD, after heavy and prolonged periods of alcohol use, strongly reduce or cease alcohol use, they can develop AWS (American Psychiatric Association 2013, 2022; see Box 6.2).

Box 6.2 Diagnostic Criteria for Alcohol Withdrawal Syndrome
According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5, American Psychiatric Association 2013; DSM-5-TR, American Psychiatric Association 2022), the diagnosis of alcohol withdrawal syndrome (AWS):

1. After heavy and prolonged alcohol use, cessation or strong reduction.
2. Within a few days after cessation (or reduction), developing of two or more of:
   (a) Autonomic hyperactivity (e.g., sweating or pulse rate > 100 bpm)
   (b) Hand tremor
   (c) Insomnia
   (d) Nausea or vomiting
   (e) Visual, tactile, or auditory hallucinations or illusions
   (f) Psychomotor agitation
   (g) Anxiety
   (h) Generalized tonic-clonic seizures
3. These signs and/or symptoms cause significant distress or impairment.
4. The signs and/or symptoms are not attributable to another medical condition.

AWS is characterized by opposite symptoms to those induced by acute alcohol use, like hyperactivity of the autonomic nervous system, irritability, anxiety, tremors, and insomnia (American Psychiatric Association 2013, 2022). It has been estimated that approximately 50% of people with AUD experience a clinically significant AWS (American Psychiatric Association 2013, 2022). Less than 3% and 10% of people who develop AWS may also develop complications like seizures and delirium tremens, respectively (American Psychiatric Association 2013, 2022).

6.3 Approved Pharmacotherapies for the Treatment of Alcohol Withdrawal Syndrome and Alcohol Use Disorder

Medical treatment of AWS is aimed at reducing the severity of signs and symptoms and protecting against the development of life-threatening complications like delirium and seizures (MacKillop et al. 2022). The gold-standard and first-line medications for the treatment of AWS are benzodiazepines, effective in both reducing the severity of AWS and preventing the risk of developing delirium and/or seizures (Amato et al. 2011). Benzodiazepines have dependence liability and increase the

risk of sedation, memory deficits, and respiratory depression; hence, their use should be limited to the treatment of acute AWS in people with AUD (Leggio et al. 2008; Mirijello et al. 2015).

Medical treatment of AUD is aimed at helping people with this disorder to reduce alcohol consumption, achieve and maintain abstinence (Reus et al. 2018). Current guidelines for the treatment of psychiatric disorders recommend a comprehensive and person-centered treatment including both psychosocial treatments (e.g., behavioral therapies, mutual help groups, 12-step facilitation) and pharmacological interventions (Brohan et al. 2023; Haber et al. 2021; Reus et al. 2018; World Health Organization 2023). Medications approved for AUD treatment by the main regulatory agencies are naltrexone, acamprosate, disulfiram, and nalmefene; the latter is approved in Europe but not in the United States (MacKillop et al. 2022; Kranzler 2023).

Briefly, naltrexone, a nonselective antagonist of μ-, κ-, and δ-opioid receptors, acts by blocking the interaction between brain receptors and endogenous opioid peptides involved in the rewarding effects of alcohol (MacKillop et al. 2022). Less intensely rewarding effects progressively reduce alcohol consumption through an extinction mechanism of action (Sinclair 2001). Naltrexone may be used both to maintain abstinence and to reduce alcohol consumption (MacKillop et al. 2022), with modest side effects (Sinclair et al. 2016). Nevertheless, naltrexone is contraindicated in patients requiring opioids for analgesia and those with opioid use disorder who actively consume opioids, as it may reduce the effectiveness of opioids or induce opioid withdrawal (Reus et al. 2018). Furthermore, it needs to be used with caution in patients with severe liver disease due to the potential for accumulation because of reduced metabolism (Reus et al. 2018). Clinical use is also hampered by low adherence, and a long-acting, injectable formulation has been developed to help overcome this problem (MacKillop et al. 2022). Unfortunately, this formulation is not available worldwide (MacKillop et al. 2022).

Acamprosate, derived from the amino acid taurine, is recommended in patients with AUD who are abstinent and aimed at maintaining abstinence from alcohol; it can also be used to reduce alcohol consumption (MacKillop et al. 2022). Acamprosate seems to act by modulating the altered glutamatergic neurotransmission state in patients with AUD, but the exact mechanism of action is still unclear (Reus et al. 2018). Its most common side effect is diarrhea (Sinclair et al. 2016); the dose may need to be adjusted in patients with reduced kidney function, and acamprosate should be avoided in patients with severe renal impairment (Reus et al. 2018). As naltrexone has better evidence in controlling heavy drinking and acamprosate in maintaining abstinence, the choice between these two medications should be based on the intended goals of treatment and/or the presence of contraindications (MacKillop et al. 2022).

Disulfiram inhibits the enzyme aldehyde dehydrogenase, which metabolizes acetaldehyde, a toxic metabolite of alcohol (Kranzler 2023). When people with AUD taking disulfiram consume alcohol, the accumulation of acetaldehyde induces distressing signs and symptoms ranging from facial flush, nausea, and vomiting to severe and rare bradypnea, shock, and death (Reus et al. 2018). Fear of this reaction

acts as a deterrent to alcohol use (Reus et al. 2018). However, its efficacy depends on awareness of the aversive effects and supervision of administration (Saitz 2018); disulfiram should not be administered without supervision (Skinner et al. 2014). Disulfiram is prescribed particularly to maintain abstinence in people with AUD who are already abstinent and are seeking protection against relapses (Reus et al. 2018). People should be abstinent for at least 12 h before starting treatment with disulfiram, and detoxification is required for those unable to achieve abstinence (Reus et al. 2018). Clinical use of acamprosate and disulfiram is also hampered by low adherence, and unlike naltrexone, no long-acting formulations are available for either medication (MacKillop et al. 2022). Nalmefene, a μ- and δ-opioid receptor antagonist and a κ-opioid receptor partial agonist, shares with naltrexone the mechanism of action and contraindications (Mann et al. 2016). Unlike naltrexone, nalmefene has a longer duration of action and is approved in Europe as needed by people aiming to reduce alcohol consumption (Mann et al. 2016).

Not all individuals with AUD respond to these medications, and others present contraindications to their use (MacKillop et al. 2022); furthermore, knowledge of their efficacy and safety for women with AUD is even more limited (Agabio et al. 2016b). Another challenge is the underutilization of AUD medications. For example, a national survey conducted in the United States found that less than 2% of people with AUD receive an FDA-approved medication for AUD (Han et al. 2021). Other countries have estimated similar rates, like Canada (less than 0.4%; Spithoff et al. 2017) and Australia (less than 3%; Morley et al. 2016). Therefore, efforts are needed to develop new, effective medications and more personalized treatment approaches for AUD.

6.4 Baclofen for the Treatment of Alcohol Withdrawal Syndrome

6.4.1 Previous Chapter

Our previous chapter reviewed the results of the case reports, comparative studies, and RCTs that investigated the potential efficacy and safety of baclofen in AWS treatment, concluding that there was insufficient evidence to recommend baclofen for AWS treatment (Agabio et al. 2016a). The same conclusions were drawn by a Cochrane systematic review in 2019 (Liu and Wang 2019), including three of the studies reviewed by our previous chapter (Addolorato et al. 2006; Girish et al. 2016 resulted in a replication of Reddy et al. 2014; Lyon et al. 2011; see Table 6.1) and another study (Jhanwar et al. 2014; see Table 6.1).

Briefly, three trials compared baclofen to benzodiazepines (Addolorato et al. 2006; Girish et al. 2016; Reddy et al. 2014; Jhanwar et al. 2014) and one study compared baclofen to placebo using benzodiazepines as a rescue protocol (Lyon et al. 2011). Among the three studies that compared baclofen to benzodiazepines,

Table 6.1 Baclofen in the treatment of alcohol withdrawal syndrome: comparative studies

#	Study	Baclofen group		Control group		Duration	Outcome	Results (favor of)
		Dose	*n*	Medication	*n*	Days		
1	Addolorato et al. (2006)	30 mg/day	18	Diazepam	19	10	Total CIWA-Ar	nd
2	Girish et al. (2016) Reddy et al. (2014)	30 mg/day	30	Chlordiazepoxide	30	9	Total CIWA-Ar	C
3	Jhanwar et al. (2014)	20 mg/day	24	Diazepam	24	15	Total CIWA-Ar	nd
4	Lyon et al. (2011)	30 mg/day	18	Placebo	13	3	Use of BDZ	B
5	Gulati et al. (2019)	30 mg/day	34	Lorazepam	32	8	Total CIWA-Ar	nd
6	Vourc'h et al. (2021)	50–150 mg/day	159	Placebo	155	15	Agitation-related events	B
7	Crunelle et al. (2023)	30 mg/day	20	Placebo	20	7	Use of BDZs	B
		60 mg/day	25					

B baclofen, *BDZ* benzodiazepines, *CIWA-Ar* Clinical Institute Withdrawal Assessment for Alcohol-revised scale (CIWA-Ar), *C* chlordiazepoxide, *n* number, *nd* no difference

two trials compared baclofen to diazepam (Addolorato et al. 2006; Jhanwar et al. 2014), finding that both medications produced a significant and similar decrease in AWS severity, measured using the Clinical Institute Withdrawal Assessment for Alcohol-revised scale (CIWA-Ar). The third study (Girish et al. 2016; Reddy et al. 2014) compared baclofen to chlordiazepoxide, finding similar results except that baclofen was resulting relatively less effective than chlordiazepoxide. The only trial that compared baclofen to placebo used benzodiazepines as rescue medication and found that participants treated with baclofen required lower amounts of benzodiazepines than participants treated with placebo over the 72-h observation period (Lyon et al. 2011).

6.4.2 Recent RCTs

More recently, other three studies (Gulati et al. 2019; Vourc'h et al. 2021; Crunelle et al. 2023) evaluated the potential efficacy of baclofen in the treatment of AWS (see Table 6.1). The first one compared baclofen (34 participants; 30 mg/day) to a benzodiazepine (lorazepam; 32 participants; 8–12 mg/day), confirming that both medications decreased AWS severity, measured using the CIWA-Ar (Gulati et al. 2019). Another study compared two doses of baclofen (20 participants: 30 mg/day; 25 participants: 60 mg/day) to placebo, indicating that less participants treated with

baclofen (35% and 32% of participants treated with 30 and 60 mg/day, respectively) required rescue treatment of benzodiazepines than participants treated with placebo (72%; Crunelle et al. 2023). The last study evaluated the potential efficacy of relatively high doses of baclofen in reducing agitation-related events among patients with "unhealthy" alcohol use admitted in the intensive care unit (Vourc'h et al. 2021). In that study, "unhealthy" use was defined by a consumption higher than 7 and 14 units per week for female and male patients, respectively. Patients admitted into the intensive care unit with "unhealthy" alcohol use were recruited to receive baclofen (159 participants: 50–150 mg/day) or placebo (155 participants) during mechanical ventilation for a maximum of 15 days (before gradual dose reduction). The results of this study showed that less participants who received baclofen developed one or more agitation-related events (19.7%) compared to participants who received placebo (29.7%; Vourc'h et al. 2021). Overall, these results indicate a potential role of baclofen in the treatment of AWS and suggest the need to conduct a new systematic review to include the more recent studies and evaluate the results using a meta-analytic approach.

6.5 Baclofen for Helping People with Alcohol Use Disorder to Achieve and Maintain Abstinence or Reducing Alcohol Use

6.5.1 Previous Chapter

In our previous chapter, we reviewed the results of case reports, retrospective studies, open studies, human laboratories studies, and RCTs that investigated the potential efficacy and safety of baclofen in helping people with AUD to reduce alcohol consumption, and/or to achieve and maintain abstinence (Agabio et al. 2016a). In this updated chapter, we focused on RCTs (see Table 6.2) and recent systematic reviews that analyzed the results of these RCTs.

6.5.2 Fixed and Low-to-Moderate Daily Doses of Baclofen

The first RCTs, published between 2002 and 2015, recruited AUD patients who received fixed low-to-moderate daily doses of baclofen (Addolorato et al. 2002; Garbutt et al. 2010a, b; Addolorato et al. 2011; Morley et al. 2014; Krupitsky et al. 2015; Ponizovsky et al. 2015; see Table 6.2). These doses were selected according to both baclofen pharmacokinetic characteristics and formulations available for spasticity treatment (de Beaurepaire et al. 2019; Kent et al. 2020). Briefly, after oral administration, baclofen is rapidly absorbed from the gastrointestinal tract with a bioavailability (the rate of an administered dose that reaches the systemic

Table 6.2 Baclofen in the treatment of alcohol use disorder: RCTs

	Baseline characteristics		Duration (weeks)	Baclofen daily dose (mg)	Results		
	Patients (*n*)	Drinks per day			Baclofen	Placebo	Favor of
Low-to-moderate and fixed doses of baclofen (30–60 mg/day)							
Addolorato et al. (2002)	39	18[a]	4	30	Ab pt = 70%	Ab pt = 21%	Bac
Garbutt et al. (2010a)	80	7[b]	12	30	Ab days = 52%	Ab days = 52%	nd
Garbutt et al. (2010b)	20	N.A.	12	30	Ab days = 55%	Ab days = 59%	nd
Krupitsky et al. (2015)	32	0	12	50	Ab days = 100%	Ab days = 100%	nd
Ponizovsky et al. (2015)	64	7[a]	12	50	Ab days = 46%	Ab days = 47%	nd
Addolorato et al. (2011)	42	14[a]	12	30 & 60	Ab pt = 57%	Ab pt = 36%	Bac
Morley et al. (2014)	42	15[c]	12	30 & 60	DD = 6% & 6%	DD = 3%	nd (Bac in anxious pt)
Higher fixed doses of baclofen (75–90 mg/day)							
Leggio et al. (2015)	30	N.A.	12	80	Ab days alc & tob = 12%	Ab days alc & tob = 3.5%	Bac
Morley et al. (2018)[d]	104	17[c]	12	30 & 75	Ab days = 68 & 65%	Ab days = 43%	Bac
Garbutt et al. (2021)	120	10[b]	16	30 & 90	Ab days = 48 & 59%	Ab days = 47%	Bac
Flexible doses of baclofen (162–270 mg/day)							
Müller et al. (2015)	56	17[a]	12	Up to 270	Ab pt = 68%	Ab pt = 24%	Bac
Beraha et al. (2016)	151	11[a]	16	30 & up to 150	Ab days = 42 & 43%	Ab days = 47%	nd
Reynaud et al. (2017)	310	8[a]	26	Up to 180	Ab pt = 12%	Ab pt = 10%	nd
Rigal et al. (2020)	158	11[a]	48	Up to 300	Low risk =57%	Low risk = 34%	Bac
Participants with liver disease							
Addolorato et al. (2007)	84	N.A.	12	30	Ab pt = 71%	Ab pt = 29%	Bac
Hauser et al. (2017)	180	7[b]	12	30	Ab days = 32%	Ab days = 31%	nd
Morley et al. (2018)[d]	104	17[c]	12	30 & 75	Ab days = 68 & 65%	Ab days = 43%	Bac

(continued)

Table 6.2 (continued)

Ab days abstinent days, *Ab pt* abstinent patients, *alc & tob* alcohol and tobacco, *Bac* baclofen, *Low risk* participants with alcohol consumption at low risk, *nd* no difference, *nr* not reported, *RCTs* randomized, controlled trials

[a]Standard drink = 12 g of alcohol

[b]Standard drink = 14 g of alcohol

[c]Standard drink = 10 g of alcohol

[d]Included participants with and without liver disease

circulation) equal to 70–85% and a half-life (the time required for the blood concentration to reduce to half) of 2–6 h; accordingly, baclofen needs to be administered three to four times a day in order to maintain clinically relevant concentrations (Kent et al. 2020; see Chap. 7 of this volume). Nevertheless, only a small portion of the oral doses crosses the blood-brain barrier and enters the central nervous system (CNS). In 1992, an intrathecal (IT) baclofen formulation was developed to infuse baclofen directly into the cerebral spinal fluid, gain rapid access to the CNS, and overcome the pharmacokinetic limitations mentioned above (see Chap. 7 of this volume). The IT formulation was approved by the FDA for spasticity, but only for those cases unresponsive to maximum oral doses of baclofen. The therapeutic daily doses of baclofen for spasticity treatment are comprised between 20 and 80 mg (Kent et al. 2020).

The first RCTs aimed at investigating the potential efficacy and safety of baclofen in AUD treatment used fixed doses of baclofen ranging from 30 mg/day (10 mg three times a day) to 60 mg/day (10 mg three times a day). Some RCTs divided participants into three groups to receive two different doses of baclofen and placebo. In this chapter, they are classified according to the higher doses of baclofen administered. As outlined in Table 6.2, the results on the alcohol outcomes of the RCTs that administered fixed low-to-moderate doses of baclofen were contrasting, with four studies finding no differences between baclofen and placebo (Garbutt et al. 2010a, b; Krupitsky et al. 2015; Ponizovsky et al. 2015) and three studies finding that participants who received baclofen significantly reduced their alcohol consumption (Addolorato et al. 2002, 2011; Morley et al. 2014, only among anxious participants). As these studies used similar doses of baclofen, differences in their results have been attributed to potential differences in the recruited participants. As an example, alcohol consumption at baseline largely varied among participants of these RCTs, ranging from complete abstinence (Krupitsky et al. 2015) and seven drinks per drinking day in the RCTs that did not observe differences between baclofen and placebo (Garbutt et al. 2010a; Ponizovsky et al. 2015) to 14 to 18 drinks per drinking day in the RCTs that found that baclofen reduced alcohol consumption (Addolorato et al. 2002, 2011; Morley et al. 2014) (see Table 6.2). Based on these findings, it has been hypothesized that baclofen may be more effective in people with more severe AUD with high alcohol consumption at baseline than in people with less severe AUD with low alcohol consumption at baseline (Leggio et al. 2010; de Beaurepaire et al. 2019). The anxiety levels of participants at baseline were also hypothesized to influence baclofen efficacy (Addolorato et al. 2002; Morley et al. 2014). A recent systematic review supported this hypothesis, finding

higher rates of abstinent days among participants who received baclofen with higher anxiety levels at baseline compared to those with lower anxiety levels at baseline (Agabio et al. 2021b). Many other potential influencing factors have been investigated, like potential gender differences in the response to baclofen and the presence of other medical and physical comorbidities (de Beaurepaire et al. 2019). However, after the publication of the clinical case of Dr. Olivier Ameisen (2005), who described how he successfully treated his own AUD using high daily doses of baclofen (up to 270 mg; see Sect. 6.7), several RCTs started to investigate the efficacy of higher daily doses of baclofen.

6.5.3 Fixed and Higher Doses of Baclofen

Three different RCTs investigated the efficacy of fixed daily doses of baclofen comprised from 75 to 90 mg/day (Leggio et al. 2015; Morley et al. 2018; Garbutt et al. 2021; see Table 6.2). One RCT (Morley et al. 2018) recruited participants with and without alcohol-associated liver disease (ALD). Accordingly, it is described both in this paragraph and in Sect. 6.5.5. Briefly, one RCT found that participants who received 80 mg/day (20 mg, four times a day) abstained from alcohol and tobacco co-use for a higher number of days compared to participants who received placebo (Leggio et al. 2015). Other two RCTs confirmed that similar doses of baclofen (75 mg/day: Morley et al. 2018; 90 mg/day: Garbutt et al. 2021) resulted to be effective in helping people with AUD to abstain from alcohol use (see Table 6.2).

6.5.4 Flexible Doses of Baclofen

Four RCTs adopted a different approach to investigate and determine the optimal baclofen daily dose (de Beaurepaire et al. 2019). These RCTs used flexible doses and progressively increased the daily doses pending an evaluation of the balance between beneficial and side effects for each participant (de Beaurepaire et al. 2019). Unfortunately, as shown in Table 6.2, these RCTs found contrasting results. In detail, two RCTs found that participants who received flexible doses of baclofen up to 270 mg/day (Müller et al. 2015) and up to 300 mg/day (Rigal et al. 2020, named BACLOVILLE, see Sect. 6.7) reduced their alcohol consumption compared to those who received placebo; on the other hand, two other RCTs (up to 150 mg/day: Beraha et al. 2016; up to 180 mg/day: Reynaud et al. 2017, named ALPADIR, see Sect. 6.7) did not observe differences in alcohol consumption of participants who received baclofen and placebo.

6.5.5 *People with Alcohol-Associated Liver Disease*

Three RCTs investigated the efficacy and safety of baclofen in the treatment of AUD patients with ALD (Addolorato et al. 2007; Hauser et al. 2017; Morley et al. 2018; see Table 6.2). Alcohol is the most frequent cause of liver cirrhosis in the Western world (Leggio and Lee 2017). Persistent alcohol consumption increases mortality in patients with liver cirrhosis; conversely, abstinence improves survival in patients with any stage of ALD (Leggio and Lee 2017; Leggio and Mellinger 2023). In these patients, medical and surgical treatments for ALD have limited success when alcohol use continues; the most effective management strategy consists in achieving complete alcohol abstinence. However, similar to the general population with AUD, those with AUD and ALD rarely receive a standard treatment for their AUD; indeed, the lack of integration between the addiction and the hepatology fields leads to the lack of multidisciplinary approaches to provide a comprehensive treatment to patients with AUD and ALD; as a consequence, those patients quite often do not receive a treatment for AUD, despite this being the underlying reason for their ALD (Leggio and Mellinger 2023; DiMartini et al. 2022).

There is a lack of formal RCTs testing medications for AUD in people with AUD and ALD; indeed, baclofen is the only exception to this. As baclofen biotransformation is low, with up to 85% of oral doses being eliminated through the kidneys (Kent et al. 2020; see Chap. 7 of this volume), it has been hypothesized that it is safe in people with AUD and severe liver disease (Addolorato et al. 2007; see Table 6.2). The first RCT found that participants with alcohol dependence and liver cirrhosis who received baclofen were more likely to be abstinent and significantly reduced their alcohol consumption compared to those who received placebo (Addolorato et al. 2007). These results were largely replicated by Morley et al. (2018), who found an effect of baclofen in AUD people with ALD but not in those without ALD (see also Sect. 6.5.3). On the other hand, Hauser et al. (2017) did not find differences between participants who received baclofen and those who received placebo in their RCT (Hauser et al. 2017). Of note, Hauser et al. (2017) enrolled people with low baseline drinking and with liver diseases related to alcohol use and hepatitis C virus (HCV) infection; by contrast, Morley et al. (2018) enrolled people with more severe ALD, and Addolorato et al. (2007) enrolled people with liver cirrhosis and alcohol dependence. As such, it has been hypothesized that, similar to what was mentioned above (see Sect. 6.5.2), the differences among these studies may be related to differences in the severity with AUD, with the degree of liver disease also reflecting, albeit indirectly, the severity of AUD. This hypothesis would explain why baclofen did not show a significant effect, as compared to placebo, in Hauser et al. (2017), while it did show an effect in the other two RCTs (Addolorato et al. 2007; Morley et al. 2018). Of note, despite the number of RCTs testing baclofen in people with AUD and ALD is limited, this is an area where baclofen may indeed be the most helpful. Indeed, the potential for baclofen to be used in liver settings has been

highlighted in a recent International Consensus (see Sect. 6.8; Agabio et al. 2018). Furthermore, baclofen has been indicated, together with acamprosate, as a medication that should be considered, despite low evidence, in treating AUD in people with ALD, as indicated by both the American College of Gastroenterology (Jophlin et al. 2024) and the American Association for the Study of Liver Diseases (Crabb et al. 2020).

6.5.6 Systematic Reviews and Meta-Analyses

The contrasting results of these RCTs make it difficult to draw clear conclusions on the efficacy and safety of baclofen in the treatment of AUD. Systematic reviews, using a meta-analytic approach, allow to gather the results of different studies and address specific questions, like, in our case, whether baclofen is effective and safe in AUD treatment (Uman 2011). To date, a series of systematic reviews have addressed this question, including the RCTs available at the time of their publication (de Beaurepaire et al. 2019). The two most recent systematic reviews have been published in 2023. The first was aimed at investigating baclofen efficacy and safety among AUD people with severe liver disease (Duan et al. 2023). This review included only two RCTs with 264 participants, concluding that this limited number of RCTs and participants did not allow to draw clear conclusion regarding baclofen efficacy in AUD patients with ALD (Duan et al. 2023). Nevertheless, this review also included open and retrospective trials and did not observe any liver-related side effects or worsening of the liver function (see Chap. 7 of this volume). These findings confirm baclofen safety among patients with ALD (Duan et al. 2023).

The second systematic review investigated the efficacy and safety of baclofen in AUD treatment without limits related to the characteristics of participants (Agabio et al. 2023). This systematic review included 17 RCTs (1,818 participants) and investigated also the role of potential influencing factors like the alcohol consumption of participants at baseline (i.e., if participants were detoxified or whether they were still drinking before the beginning of baclofen treatment), the different daily doses of baclofen administered to participants divided into low ($\leq$30 mg/day), medium (30–100 mg/day), and high (above 100 mg/day), and the duration of treatment (shorter or equal to 12 weeks or longer than 12 weeks). It is worth noting that Cochrane systematic reviews also use a system for grading the certainty of evidence, the Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) (Atkins et al. 2004). The GRADE system uses the following criteria to assign a grade of evidence certainty: high (when the authors are very confident that the true effect lies close to that of the estimated effect), moderate (when the authors are moderately confident in the estimated effect), low (when the authors are limitedly confident in the estimated effect), and very low (when

the authors are very little confident in the estimated effect) (Atkins et al. 2004). This systematic review found evidence that, compared to placebo, baclofen significantly reduced the risk of relapse (meta-analysis conducted in a sample of 1,057 participants) and increased the rate of abstinent days (1,253 participants), with moderate and high certainty of evidence, respectively (Agabio et al. 2023). These findings were not modified by dividing the RCTs according to the daily doses of baclofen (i.e., any dose of baclofen resulted to be effective) or according to the duration of baclofen treatment (baclofen resulted to be effective either in RCTs equal to 12 weeks or longer than 12 weeks). On the other hand, dividing RCTs according to the alcohol use of participants at baseline or not, baclofen resulted to be effective among participants who were not drinking for at least 3 days before the beginning of treatment. Overall, these findings suggest a potential role of baclofen in AUD pharmacotherapy, especially for people who do not respond to approved AUD medications or for whom approved medications are contraindicated (e.g., for the presence of ALD), or with high anxiety levels at baseline (Agabio et al. 2021b).

6.6 Safety Profile

Baclofen's safety profile is similar to that of other GABAergic drugs. Primarily, this includes the possible occurrence of dose-related sedative symptoms, i.e., drowsiness, dizziness, and confusion (Agabio et al. 2023; see Chap. 7 of this volume). 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; Rolland et al. 2015). A randomized, placebo-controlled human laboratory study demonstrated the relatively safe profile of intermediate doses of baclofen, even when people continued to drink (Evans and Bisaga 2009). In this study, 18 heavy drinkers were given doses of baclofen ranging from 40 to 80 mg, 2.5 h before the consumption of approximately 4 alcohol-containing 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 combination of baclofen and alcohol did not result in further performance impairment. Other human laboratory studies conducted under well-controlled experimental conditions have further highlighted the overall safety of the co-administration of baclofen and alcohol in people with AUD (Leggio et al. 2013; Farokhnia et al. 2017, 2018).

However, a prospective cohort study found that the risk of episodes of major sedation was directly related to both the dose of baclofen and the amount of alcohol consumed (Rolland et al. 2015). Baclofen-related sedation can lead to coma in some cases, especially when baclofen intoxication is associated with other sedative drugs, such as alcohol, benzodiazepines, or opioids (Franchitto et al. 2018).

Baclofen can induce other types of adverse drug reactions (ADRs) in AUD patients, especially at high doses (i.e., 100–240 mg/day). Such ADRs consist of insomnia (Rolland et al. 2018), tinnitus (Auffret et al. 2014), increased triglyceride levels (Clarisse et al. 2013), and, more rarely, seizures (Rolland et al. 2012a), manic symptoms (Geoffroy et al. 2014), and central sleep apnea (Perogamvros et al. 2015). Furthermore, when used at high doses, baclofen can induce a specific withdrawal syndrome that somewhat resembles alcohol or benzodiazepine withdrawal syndrome, that is, by the occurrence of agitation, confusion, delirium, and, in some cases, seizures (Akosile and Klan 2016; Nasti and Brakoulias 2011; Rolland et al. 2014; see Chap. 7 of this volume). However, baclofen withdrawal syndrome may last for several days or even more than 1 week and may not be responsive to $GABA_A$ receptor modulators, such as benzodiazepines (Rolland et al. 2014). The best treatment of baclofen withdrawal syndrome consists of reintroducing baclofen (see Chap. 7 of this volume). Other adjunctive medications (i.e., benzodiazepines, cyproheptadine, propofol, dantrolene, dexmedetomidine, and tizanidine) may also be needed for the management of severe withdrawal symptoms (see Chap. 7 of this volume). For all these reasons, it is recommended to handle baclofen dosing smoothly, that is, using a slow titration procedure and small levels of dose increase or decrease (see Sect. 6.8; Agabio et al. 2018). Baclofen treatment should be conducted under medical supervision.

As baclofen is essentially eliminated by the kidneys, it is contraindicated in patients with severely impaired renal function (Reichmuth et al. 2015; see Chap. 7 of this volume). Though not formally contraindicated, baclofen should be used with caution and strict monitoring in clinical scenarios like urinary retention, respiratory disease (because of the risk of sedation), previous history of seizure (because of the risk of baclofen-induced seizures), autonomic dysreflexia, or myopathy (because of the myorelaxant action of baclofen). In view of the risk of sedation, patients taking baclofen should refrain from operating automobiles or other dangerous machinery (Ghanavatian and Derian 2022). Furthermore, baclofen is not recommended in pregnancy, as the impact of baclofen on the fetus is still unclear (see Chap. 7 of this volume), and oral baclofen could even be teratogenic (Prescrire Int 2015). However, alcohol is very harmful for the fetus (see, e.g., Broccia et al. 2023). Thus, maintaining or stopping baclofen in a woman successively treated for AUD and who becomes pregnant should be a case-by-case multidisciplinary decision based on the individual risk-benefit ratio of the dyad.

6.7 The Unique Experience in France

While the first human studies on baclofen for AUD were conducted in the early 2000s, its use for AUD (off-label) in France was not documented until 2008. That year, Dr. Olivier Ameisen, a French cardiologist, published a lay public book, “Le Dernier Verre” (Ameisen 2008), i.e., “The Last Drink,” or in the English version,

"The End of My Addiction" (Ameisen 2010), in which he described how he used progressively higher doses of baclofen for suppressing his craving for alcohol to treat his severe, long-lasting, and treatment-resistant AUD. A few years before the publication of his book, Ameisen published a self-case report in an international scientific journal (Ameisen 2005), but his case went largely unnoticed until the publication of the book.

Compared to international studies, Ameisen's way of handling baclofen was very different and original, as he did not use baclofen at a stable dose and after alcohol detoxification. Rather, he used baclofen as a controlled drinking treatment, a therapeutic strategy which had never been investigated at that time. Ameisen reached the dose of baclofen of 270 mg per day, which was more than three-fold higher than the maximum approved dose of oral baclofen for neurological indications (Rolland et al. 2012b; see also Sect. 6.5.2). At that dose, he reported that baclofen could suppress his craving for alcohol and that he was able to take back control of alcohol consumption. Following the publication of his book, Ameisen quickly attracted wide media attention, and his story was very popular in France at the end of the 2000s and beginning of the 2010s. Many patients with AUD asked for the same therapy, and some physicians dared to prescribe them off-label doses of baclofen. Several patient-physician associations were created at that time and strongly lobbied for the use of high-dose baclofen in the media (Rolland et al. 2012b).

As early safety concerns were reported by the French pharmacovigilance system regarding this prescribing practice, the French Medicines Agency initially advised against the off-label use of baclofen for AUD (ANSM 2011), but, between 2008 and 2013, it was estimated that 200,000 people received baclofen prescriptions for AUD (Chaignot et al. 2015). In this context, the French authorities could not let such overwhelming practices go unsupervised, and they decided in March 2014 to issue a specific regulatory measure, named "temporary recommendation for use" (TRU; Emmerich et al. 2012), which aimed to frame and monitor baclofen off-label prescriptions (ANSM 2014). Based on the TRU, physicians could prescribe up to 300 mg per day of baclofen for AUD, but they were asked to follow strict rules related to patient supervision and collect longitudinal clinical data into a national online register. As its name indicates, the TRU was meant to be a temporary measure, as two clinical trials started in France in 2014 to assess the efficacy and safety of high-dose baclofen in AUD. Based on the results of these trials, the French health authorities planned to accept the approval of baclofen for AUD but not to let the TRU measure last in time.

These two clinical trials, named ALPADIR and BACLOVILLE, had two different developments and designs (see also Sect. 6.5.4 and Table 6.2). ALPADIR was a partially industry-sponsored 20-week clinical trial assessing the efficacy of baclofen up to the daily dose of 180 mg for maintaining abstinence following an inpatient alcohol detoxification among 320 participants with AUD. By contrast, BACLOVILLE was a clinical trial funded by the French government to assess the efficacy of baclofen up to the daily dose of 300 mg in 320 participants with AUD or hazardous drinking. The goal of BACLOVILLE was to test the efficacy of baclofen in reaching

controlled drinking levels after 1 year of follow-up in primary care settings. BACLOVILLE was supported by the patient-physician associations that lobbied for the use of high-dose baclofen for AUD in France. Baclofen did not show efficacy, compared to placebo, in maintaining abstinence or reducing drinking or cravings, in the ALPADIR study (Reynaud et al. 2017). Positive results, with 57% vs. 36% of participants reaching controlled drinking in the baclofen group vs. placebo, respectively, at 1 year, were claimed in the BACLOVILLE trial (Rigal et al. 2020). However, this study was severely criticized for methodological concerns that were mainly related to the blinding procedure (Naudet et al. 2020).

Many concerns were also raised regarding the potential harms of high-dose baclofen, as a nation-wide pharmacoepidemiologic study found a dose-related risk of hospitalization and mortality in patients treated with baclofen (Chaignot et al. 2018). Once again, this study was criticized, as it was impossible to take into account the actual amount of alcohol consumption in the analyses (de Beaurepaire and Rolland 2022).

Despite the many remaining scientific uncertainties, the French Medicines Agency issued an official approval for baclofen in 2017 but restricted the maximum dosage to 80 mg/day. This decision was sued by the pro-baclofen associations, who were successful in the end, as the court forced the French authorities to remove the maximum dosage cutoff, as they deemed that this decision was not supported by sufficient scientific evidence. Consequently, baclofen is now approved with no dose restriction for AUD in France. Recent data did not confirm that higher doses, as compared to lower doses, were more responsible for serious safety concerns (de Beaurepaire and Rolland 2022).

6.8 Consensus Statement on the Use of Baclofen for Alcohol Use Disorder

In 2018, a $GABA_B$ Receptor Conference was held in Cagliari, Italy. In a post-conference closed session, a group of international clinical experts had a meeting to develop a Consensus on the use of baclofen in AUD treatment (Agabio et al. 2018). Most of the experts were investigators who contributed to both positive and negative RCTs on baclofen for AUD treatment. This group discussed the very initial draft of the Consensus in Cagliari, then a working group coordinated and submitted the following drafts to the other members by emails. Statements of the following drafts of the Consensus were voted by each member who could assign a vote ranging from 1 (I strongly disagree) to 5 (I strongly agree); statements rated lower than 3 were discussed, revised, and resubmitted until approval by the entire group. The final draft of the Consensus contains 18 statements approved by all the members (see Box 6.3), divided into general statements (1–3), effectiveness statements (4–12), and safety statements (13–18). This Consensus is a useful tool for physicians involved in the decision-making process of the pharmacological treatment for people with AUD.

Box 6.3 Consensus Statement of the Cagliari Expert Consensus Group on the Use of Baclofen to Treat Patients with Moderate-to-Severe Alcohol Use Disorder

I. General statements on the treatment of patients with AUD	
1	Each country differs regarding medication regulations, laws, models of care, and reimbursement systems that need to be considered in the prescribing of medications and the provision of treatment.
2	Pharmacotherapy is only one component of the treatment of moderate-to-severe AUD. Patient-centered, individualized treatment plans should be used. These plans should also include psychotherapy, in-person and/or web-based treatments, and/or community and peer support groups.
3	The goal of a pharmacological treatment for patients with AUD can be both abstinence and reducing alcohol consumption, ideally below harmful levels. However, in certain subgroups of patients, the goal should be complete abstinence.
II. Effectiveness of baclofen in the treatment of patients with AUD	
4	Baclofen is not licensed as an approved treatment of AUD, and its use is therefore "off-label," except in France*.
5	Clinical research evidence is not clear about the most effective treatment setting for baclofen treatment, but AUD patients may be treated in a range of treatment settings by clinicians with appropriate experience and training.
6	The majority of clinical trials started with baclofen after detoxification and obtaining abstinence. In clinical practice, some physicians prescribe off-label baclofen while the patient is still drinking. These patients should be warned of the risks of side effects (e.g., excessive sedation) due to the pharmacological interaction of baclofen and alcohol.
7	Baclofen should be considered a second-line pharmacotherapy in patients who have not responded to approved pharmacological treatments for AUD. However, the off-label use of baclofen may be considered among the first-line pharmacological treatments in patients with contraindication to approved medications (e.g., patients with advanced liver disease for whom the use of disulfiram or naltrexone may be contraindicated).
8	The daily baclofen dose should be based on safety, tolerability, and the patient's response.
9	The daily dose of baclofen required to achieve abstinence, a significant reduction in alcohol consumption, or a substantial decrease in craving for alcohol can vary widely between patients, over a ten-fold range.
10	Baclofen should be started at a low dose (5 mg three times per day) and slowly titrated upwards (e.g., 5–10 mg/day, every 3 days) to minimize possible side effects, including sedation and overdose.
11	There is no evidence on the use of baclofen in combination with other medications for AUD (e.g., disulfiram, naltrexone, acamprosate, or nalmefene).
12	Baclofen should not be used instead of benzodiazepines in the treatment of alcohol withdrawal syndrome (AWS), as there is no evidence of its efficacy in preventing the development of potentially life-threatening complications of AWS like seizures and delirium tremens.

(continued)

Box 6.3 (continued)

III. Safety of baclofen in the treatment of patients with AUD	
13	History of renal impairment needs to be considered before starting baclofen, as the drug is mainly excreted by the kidneys. If prescribed, the management of baclofen in patients with renal impairment requires close supervision because of the higher risk of baclofen toxicity.
14	The most frequent side effects observed among patients with AUD include sedation, fatigue, drowsiness, tiredness, somnolence, sleep disorders/insomnia, dizziness, headache, dry mouth, paresthesia, fasciculations, nausea, myalgia, and arthralgia. Most side effects occur at the beginning of baclofen treatment or if the dose is increased too rapidly.
15	Many side effects tend to be dose-related, although the contribution of other factors to the onset or severity of side effects cannot be ruled out.
16	Particular caution is needed for the combination of baclofen with other sedative medications (including alcohol) since there are additive side effects (e.g., sedation, drowsiness, and somnolence).
17	Particular caution is needed among patients with AUD and other comorbidities, such as patients with a history of epilepsy, because baclofen can lower the seizure threshold; patients with mood disorders, because baclofen can increase the risk of hypomanic and manic episodes; and patients with suicidal ideation or a history of suicide attempts, because of the risk of intentional overdose.
18	Treatment with baclofen should not be abruptly interrupted to avoid the risk of withdrawal symptoms. The daily dose should be slowly reduced (e.g., 5–10 mg/week).

This box (partially modified to include the approval in France*) was published in Lancet Psychiatry, Agabio et al. (2018), Copyright Elsevier (2018)

6.9 Conclusions

Baclofen is among the potential off-label medications available for AUD treatment. In 2018, 16 years after the first RCT (Addolorato et al. 2002), it has been approved for this new indication in France (De Beaurepaire and Jaury 2024) and included in international guidelines related to the treatment of AUD in general (Haber et al. 2021; World Health Organization 2023) and to the treatment of AUD in patients with ALD (Crabb et al. 2020; Jophlin et al. 2024). The discovery and development of new pharmacotherapies for AUD is critically needed, given that the currently available medications are limited in number, are underutilized, and not all patients respond to these medications. The experience gained in clinical practice for decades in using baclofen as a muscle relaxant agent has allowed the field to be already familiar with this medication when it started being repurposed as a potential new treatment for AUD. That said, the RCTs generated so far remain conflicting in terms of potential efficacy or lack thereof, and some cardinal questions still remain worth future investigations, including, e.g., optimal doses and possible sub-types of patients with AUD who may respond best to baclofen.

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Chapter 7
Baclofen Safety, Toxicity, Withdrawal, and Overdose

Jia W. Romito and Bryan T. Romito

Abstract Baclofen is a proven and effective agent for the management of spasticity. Its use has recently expanded to the off-label treatment of several other diseases. It is important for providers to be aware of baclofen's unique safety profile, dosing considerations, and potential complications. Both oral and intrathecal baclofen administration can be associated with life-threatening toxicity and withdrawal. The management of baclofen toxicity includes immediate discontinuation of baclofen from all sources and supportive care based on symptom management. The treatment of baclofen withdrawal includes reinitiation or supplementation of baclofen therapy along with the administration of adjunctive pharmacological agents to treat associated withdrawal symptoms. Examples of adjunctive treatment options for the management of baclofen withdrawal include benzodiazepines, cyproheptadine, propofol, dantrolene, dexmedetomidine, and tizanidine. Because both baclofen toxicity and withdrawal have the potential for rapid progression and severe clinical decompensation, providers must maintain a high index of suspicion to establish a timely diagnosis and prevent morbidity and mortality.

Keywords Baclofen · Overdose · Safety · Toxicity · Withdrawal

Baclofen is a γ-aminobutyric acid type-B ($GABA_B$) receptor agonist that is approved by the US Food and Drug Administration (FDA) for the management of spasticity. It reduces spasticity by inhibiting monosynaptic and polysynaptic reflexes at the spinal level (FLEQSUVY™ 2022; GABLOFEN® 2021; LIORESAL® INTRATHECAL 2019; LYVISPAH™ 2021; OZOBAX® DS 2023). Baclofen is

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available in both oral and intrathecal (IT) formulations. Oral baclofen, also offered in the United States under the brand names of FLEQSUVY™, OZOBAX® DS, and LYVISPAH™, is the most prescribed antispasmodic (Ertzgaard et al. 2017). Per the package inserts, it is indicated for the treatment of spasticity in the setting of multiple sclerosis and may provide benefit in patients with spinal cord injuries and other spinal cord disorders (FLEQSUVY™ 2022; LYVISPAH™ 2021; OZOBAX® DS 2023). IT baclofen is commercially available in the United States as LIORESAL® INTRATHECAL and GABLOFEN®. Per the package inserts, it is indicated for the management of severe spasticity from cerebral origin, including cerebral palsy and prior traumatic brain injury (GABLOFEN® 2021; LIORESAL® INTRATHECAL 2019) (see Chap. 4 of this volume). It is also indicated for the management of severe spasticity from spinal cord origin, including spinal cord trauma and multiple sclerosis. IT baclofen is indicated to treat spinal cord–associated spasticity only in patients who were unresponsive to oral baclofen or experienced severe central nervous system (CNS) side effects at therapeutic doses (GABLOFEN® 2021; LIORESAL® INTRATHECAL 2019). In addition to its approved indications, the use of off-label baclofen for other clinical conditions is steadily expanding. Baclofen has been used successfully in the management of alcohol use disorder (AUD) (see Chap. 6 of this volume), gastroesophageal reflux disease, muscle spasms, persistent hiccups, disorders on the autism spectrum, chronic post-traumatic stress disorder, narcolepsy, persistent speech stuttering, post-hemorrhoidectomy pain, trigeminal neuralgia, and low back pain (Bowery 2016; Romito et al. 2021). Regardless of the route of administration, it is recommended that baclofen prescribing be limited to clinicians with appropriate experience and training (Agabio et al. 2018) (see Chap. 6 of this volume).

7.1 Oral Baclofen Safety

The safety of baclofen is influenced by its degree of absorption, metabolic characteristics, and specific elimination pathway. Several pharmacokinetic properties are outlined in Table 7.1 (FLEQSUVY™ 2022; GABLOFEN® 2021; Ghanavatian and

Table 7.1 An overview of baclofen's pharmacokinetic properties

Administration route	Onset of action	Peak effect	Half-life
Oral	Rapid	45 min–2.5 h	2–6 h[a]
Intrathecal, bolus	30 min–1 h	4 h	1–5 h[b]
Intrathecal, continuous infusion	6–8 h after infusion starts	24–48 h after infusion starts	5 h[b]

FLEQSUVY™ (2022), GABLOFEN® (2021), Ghanavatian and Derian (2022), LIORESAL® INTRATHECAL (2019), LYVISPAH™ (2021), OZOBAX® DS (2023), and Romito et al. (2021)

[a]Plasma

[b]Cerebrospinal fluid

Derian 2022; LIORESAL® INTRATHECAL 2019; LYVISPAH™ 2021; OZOBAX® DS 2023; Romito et al. 2021). Oral baclofen is well absorbed and has a mean bioavailability of approximately 75–85% in healthy volunteers, depending on the dose (Agarwal et al. 2015; Schmitz et al. 2016). The plasma half-life of baclofen is variable. It ranges from 2 to 6 h at standard, therapeutic oral doses of 30–240 mg/day and can approach 35 h for doses of 450 mg/day (Boels et al. 2017; Marsot et al. 2014; Romito et al. 2021). Baclofen is primarily eliminated through the kidneys, with approximately 65–80% of the drug excreted unchanged (FLEQSUVY™ 2022; LYVISPAH™ 2021; OZOBAX® DS 2023; Romito et al. 2021; Simon et al. 2018). Its clearance is proportional to creatinine clearance (El-Husseini et al. 2011). Approximately 15% of oral baclofen undergoes hepatic metabolism via deamination to the pharmacologically inactive metabolite 3-(4-chlorophenyl)-4-hydroxybutyric acid (FLEQSUVY™ 2022; He et al. 2020; LYVISPAH™ 2021; OZOBAX® DS 2023). The low percentage of hepatic metabolism likely contributes to baclofen's safety when used for AUD, even in patients with comorbid liver disease (see Chap. 6 of this volume).

7.2 Baclofen Safety Concerns for Patients with Renal Impairment

Although there are no formal recommended dosing adjustments by the FDA in patients with decreased kidney function, there is a higher risk of baclofen toxicity in patients with renal impairment due to its metabolism and elimination pathways. There have been several case reports describing baclofen overdose in patients with kidney dysfunction (Al Marzouqi and Al Alawi 2023; Chou et al. 2006; El-Husseini et al. 2011; Mousavi et al. 2012; Patel et al. 2022; Roberts et al. 2015; Varma and Bajpai 2022).

In one case report of a dialysis-dependent patient, ingestion of 20 mg of baclofen in 24 h led to significant encephalopathy, requiring both conventional hemodialysis and continuous venovenous hemodialysis over 2 days to restore her to neurological baseline (Al Marzouqi and Al Alawi 2023). In another case report of a dialysis-dependent patient, consumption of 10 mg of baclofen led to acute encephalopathy, hyporeflexia, muscle hypotonia, and somnolence requiring emergent hemodialysis (Patel et al. 2022). Varma et al. report a case series of three patients with baseline severe chronic kidney disease who developed complications associated with baclofen administration (Varma and Bajpai 2022). The first case describes a patient with stage 4 chronic kidney disease who ingested a single dose of 20 mg of baclofen. The patient developed rapidly progressive encephalopathy and required two sessions of hemodialysis to return their level of consciousness to baseline. The second case describes a dialysis-dependent patient who ingested a single dose of 10 mg of baclofen and developed encephalopathy, requiring two sessions of hemodialysis to achieve responsiveness. The third case describes a dialysis-dependent patient who

ingested 20 mg of baclofen over 4 days. The patient developed encephalopathy and required three sessions of hemodialysis over 3 days to improve his sensorium (Varma and Bajpai 2022). Muanda et al. performed a retrospective population-based cohort study comparing the 30-day risk of encephalopathy with newly prescribed baclofen at doses ≥20 mg/day versus <20 mg/day in patients with chronic kidney disease defined by an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 but not on dialysis (Muanda et al. 2019). The authors found an increased 30-day incidence of encephalopathy in those patients with chronic kidney disease prescribed higher doses of baclofen compared with lower doses (Muanda et al. 2019). Chauvin et al. conducted a retrospective population-based cohort study to evaluate the risk of hospitalization from encephalopathy with baclofen administration (median dose 20 mg/day) in elderly patients receiving maintenance dialysis (Chauvin et al. 2020). In this study, hospitalization with encephalopathy occurred in 26 of 360 (7.2%) patients receiving baclofen, compared to under 6 of 6109 (0.09%) patients not using baclofen. It may be advisable to avoid baclofen altogether in elderly patients requiring dialysis, given the risk of encephalopathy (Chauvin et al. 2020).

Some authors have recommended avoidance of baclofen in patients with an eGFR of <30 mL/min/1.73 m^2 and a very low starting dose in patients with eGFRs between 30 and 60 mL/min/1.73 m^2 (El-Husseini et al. 2011). The authors of a multicenter, open-label, oral baclofen pharmacokinetics study recommend a dose reduction based on estimated creatinine clearance ranges (Vlavonou et al. 2014). They recommend a mean dosage reduction of 1/3 in patients with mild chronic kidney disease, 1/2 in patients with moderate chronic kidney disease, and 2/3 in patients with severe chronic kidney disease (Vlavonou et al. 2014). Baclofen should be avoided in patients with end-stage renal disease requiring hemodialysis (El-Husseini et al. 2011; Romito et al. 2021). Regardless of the dosing adjustment strategy, it is prudent for providers to closely monitor for signs of toxicity in patients with impaired renal function.

7.3 Baclofen Safety Concerns for Elderly Patients

A 2023 systematic review that included retrospective cohort studies and case reports evaluated the adverse effects of baclofen use in outpatient adults aged 50 and older (Killam-Worrall et al. 2023). The study compiled the US FDA Adverse Event Reporting System data and found baclofen was associated with a 12.1% overall incidence of adverse effects, with a 27.8% incidence of falls in older adults. Hwang et al. compared the risk of fall and fracture among older adults aged 65 and older treated with baclofen, tizanidine, or cyclobenzaprine. The study included 2205 patients administered baclofen, 1103 patients administered tizanidine, and 9708 patients administered cyclobenzaprine. Baclofen was associated with a higher risk of fall compared to tizanidine and a similar risk of fracture compared to tizanidine or cyclobenzaprine (Hwang et al. 2024). Muanda et al. performed a retrospective

population-based cohort study of adults ≥66 years comparing the 30-day risk of hospital encounter with a fall, a fracture, or hypotension with newly prescribed baclofen at doses ≥20 mg/day versus <20 mg/day in patients with chronic kidney disease defined as an eGFR <60 mL/min/1.73 m^2 but not on dialysis (Muanda et al. 2021). The authors found an increased 30-day incidence of hospitalization with a fall and hypotension in those patients with chronic kidney disease prescribed higher doses of baclofen compared with lower doses (Muanda et al. 2021). There was no difference in the incidence of hospitalization with a fracture between the two groups.

7.4 Baclofen Safety Concerns for Patients with Hepatic Impairment

Given baclofen's recent expansion as a therapeutic agent for patients with AUD (see Chap. 6 of this volume), it is important for providers to understand the safety concerns for patients with hepatic impairment. There are no formal recommended dosing adjustments by the FDA in patients with hepatic impairment. Several studies have evaluated baclofen's safety profile in this setting. Duan et al. conducted a systematic review and meta-analysis to evaluate the safety and efficacy of baclofen for patients with AUD and comorbid liver disease (Duan et al. 2023). Their review included 322 patients from five studies: two randomized controlled trials, one prospective cohort study, and two retrospective cohort studies. Baclofen was administered orally in all included studies, and the doses ranged from 30 to 150 mg/day. Safety and tolerability were assessed via a change in liver function or the development of adverse effects. The most reported adverse effects in this study were minor. The only severe adverse effect reported was the development of acute confusion, which resolved upon discontinuation of baclofen (Duan et al. 2023). In this review, baclofen administration did not have any significant effects on alanine transaminase, aspartate transaminase, γ-glutamyltransferase, total bilirubin, or albumin levels. There was no evidence that baclofen led to acute hepatic decompensation or any liver-related death events (Duan et al. 2023). Additionally, data from other large clinical trials that were not included in the 2023 review by Duan et al. also suggest that baclofen is safe in patients with concomitant hepatic disease (Addolorato et al. 2007; Barrault et al. 2017; Morley et al. 2018). Finally, Morley et al. conducted a multicenter, double-blind, placebo-controlled, randomized controlled trial of baclofen for the treatment of AUD, with or without liver disease (Morley et al. 2018). In this study, patients were randomized to placebo, baclofen 30 mg/day, or baclofen 75 mg/day for 12 weeks. There was no difference in the frequency of adverse events for patients in the alcoholic liver disease subgroup versus those in the nonalcoholic liver disease subgroup (Morley et al. 2018). Oral baclofen for AUD, in doses ranging from 30 to 150 mg/day, appears safe in patients with concomitant liver disease (Duan et al. 2023).

7.5 Intrathecal Baclofen Safety

The pharmacokinetics of baclofen are affected by the route of administration (Table 7.1). IT baclofen can achieve therapeutic drug cerebrospinal fluid concentrations while maintaining very low plasma concentrations (Romito et al. 2021). Compared to the oral route, administration of baclofen directly into the IT space allows for fewer side effects and a profound muscle relaxation effect (Ertzgaard et al. 2017). Providing baclofen through the IT route allows for administration of much lower doses compared to the oral route, on the order of mcg versus mg. Nevertheless, all patients receiving IT baclofen are at risk for complications (GABLOFEN® 2021; LIORESAL® INTRATHECAL 2019).

The IT baclofen delivery system includes a programmable pump that acts as the drug reservoir. The pump is connected to a tunneled catheter that enters the IT space, where the drug will be delivered to its intended site of action (Di Napoli et al. 2023). The pump is typically surgically implanted into the subcutaneous tissue of the anterolateral abdominal wall (Koo et al. 2023). The individual component of this system that is most susceptible to dysfunction is the catheter (Albright et al. 2003; Guzman et al. 2024; Konrad et al. 2016). Specifically, these catheters are prone to migration, laceration, and disconnection (Delhaas and Huygen 2020). Transient catheter obstruction is another known complication associated with IT drug delivery. Some providers recommend firmly securing the catheter to the supraspinous fascia and away from any bony prominences to minimize this risk (Guzman et al. 2024).

Although the use of magnetic resonance imaging is not contraindicated in patients with an IT baclofen pump, the manufacturer should be alerted before the study is performed to ensure safe practices. Exposure to an external magnetic field may result in pump rotor dysfunction, interrupting drug delivery (Miracle et al. 2011). Removal of the magnetic field should cause the device to return to normal functionality (U.S. Food and Drug Administration 2021). Magnets present in consumer electronics and other devices can impair the operation of IT baclofen pumps. Smartphones and magnetic digital tablet cases have been reported as causes of IT baclofen pump failure (Filipetto et al. 2023; Fostier et al. 2023). Pump logs can be reviewed to evaluate for the presence of motor stall alerts.

The safe administration of IT baclofen can only be achieved following a standardized screening test to assess for both therapeutic response and adverse reactions. An initial screening dose of 50 mcg is recommended by the manufacturer with a 4–8-h period of observation (Boster et al. 2016; GABLOFEN® 2021; LIORESAL® INTRATHECAL 2019). If there is a lack of both positive responses and adverse reactions, a second IT baclofen dose of 75 mcg can be administered 24 h following the first dose. With the administration of a second dose, it is recommended to monitor for 4–8 h. A third and final screening dose of 100 mcg can be administered 24 h following the second dose if there was no therapeutic response or adverse reactions thus far. It is mandatory for resuscitative equipment and appropriately trained personnel to be readily available during the screening test and all dose titrations (Boster et al. 2016; GABLOFEN® 2021; LIORESAL® INTRATHECAL 2019).

7.6 Baclofen Safety in Maternal-Fetal Medicine

There are no well-controlled studies of baclofen in pregnant patients evaluating the risk of major birth defects, maternal adverse outcomes, or miscarriages (FLEQSUVY™ 2022; GABLOFEN® 2021; LIORESAL® INTRATHECAL 2019; LYVISPAH™ 2021; OZOBAX® DS 2023). Baclofen crosses the placenta due to its relatively small size and low level of protein binding (Moran et al. 2004; Morton et al. 2009). It has the potential for a prolonged half-life in the neonate because of underdeveloped renal function and metabolic degradation processes (Moran et al. 2004). Both oral and IT baclofen should only be used in pregnant patients if the potential benefit justifies the potential risk to the fetus.

There have been several case reports of pregnancy and childbirth in patients receiving IT baclofen, and there have been no reported infant-related adverse effects (Hara et al. 2018; Romito et al. 2021). Hara et al. describe a case of pregnancy and lactation in a mother receiving IT baclofen (Hara et al. 2018). Using high-performance liquid chromatography/tandem mass spectrometry, the authors measured the concentration of baclofen in maternal milk as 0.617 ng/mL. Although the infant in this case did not ingest the milk, the pharmacological effect was believed to have been negligible because of the extremely low dose (Hara et al. 2018). Further investigation is needed to clarify the risk to infants during breastfeeding in mothers receiving IT baclofen. As with maternal baclofen use during pregnancy, breastfeeding while a patient is receiving either oral baclofen or IT baclofen should only be performed if the potential benefits outweigh the potential risks to the infant (FLEQSUVY™ 2022; GABLOFEN® 2021; LIORESAL® INTRATHECAL 2019; LYVISPAH™ 2021; OZOBAX® DS 2023).

7.7 Baclofen Overdose and Toxicity

While not routinely included on toxicology screening tests, measurements of baclofen levels can be obtained from plasma to help providers confirm the diagnosis of baclofen overdose (Franchitto et al. 2018; Nahar et al. 2016). The typical therapeutic blood plasma concentration following administration of oral baclofen is 0.08–0.4 mg/L (Schulz et al. 2012). The reported toxic concentration is 1.1–3.5 mg/L, while comatose-fatal concentrations range from 6.0 to 9.6 mg/L (Schulz et al. 2012). Despite these ranges, there is no consistent plasma level that corresponds to the development of toxicity. Similarly, improvement in symptoms does not always mirror a reduction in the measured plasma concentration (Franchitto et al. 2018). All measured plasma concentrations should be interpreted in the context of the patients' symptoms. Because of low baclofen penetration of the blood-brain barrier, the above plasma reference ranges are not reliable following IT baclofen administration (GABLOFEN® 2021; LIORESAL® INTRATHECAL 2019).

Unfortunately, there is not a definitive threshold dose above which baclofen toxicity reliably occurs. Leung et al. reported a case series of 23 patients with oral baclofen overdose (Leung et al. 2006). In their series, baclofen overdoses of less than 200 mg were not associated with severe symptoms, while overdoses of 200 mg or more were predictive of patients presenting with severe symptoms, requiring long hospital admissions and intensive care unit (ICU) admissions (Leung et al. 2006). The authors acknowledge that the 200 mg threshold predicting severe versus nonsevere toxicity was arbitrary, and all cases should be evaluated on a patient-specific basis.

Because of the limitations associated with plasma baclofen level analysis, the diagnosis of baclofen toxicity/overdose is usually made primarily based on history and physical examination. Complicating matters, there is considerable variation in the clinical presentation of baclofen toxicity. Providers need to maintain a high index of suspicion to make a timely diagnosis. Baclofen overdose should always be on the differential diagnosis for a patient presenting with hypotonia and flaccid paralysis. While most baclofen side effects are dose-related, toxicity has been reported in patients receiving low-to-moderate doses of oral baclofen (i.e., 30–140 mg/day) (Triplett et al. 2019). In general, overdose and toxicity are more likely to occur in the setting of renal insufficiency, advanced age, or coadministration with other CNS depressants, such as benzodiazepines or alcohol (Agabio et al. 2018; Dore et al. 2011; Triplett et al. 2019) (see Chap. 6 of this volume). Baclofen tolerability in the treatment of substance use disorders has been considered good (Agabio et al. 2013). Particular caution is needed in patients with AUD and other comorbidities, including epilepsy, certain mood disorders, and in those with suicidal ideation or suicide attempts (Agabio et al. 2018) (see Chap. 6 of this volume). If baclofen is prescribed to these patients, high levels of supervision are recommended to monitor for potential overdose (Dore et al. 2011; Müller et al. 2015). Baclofen toxicity can occur with oral administration; however, the most serious toxicity-related events occur following IT baclofen therapy (Romito et al. 2021). Although rare, baclofen toxicity can be fatal (Ghannoum et al. 2021). Patients presenting with hemodynamic instability, cardiac arrhythmias, and/or respiratory insufficiency should be admitted to an ICU for a high level of monitoring.

7.7.1 Signs and Symptoms of Baclofen Overdose and Toxicity

There is a wide range of signs and symptoms associated with baclofen toxicity (Table 7.2) (Duan et al. 2023; Kido and Guglin 2017; Meythaler et al. 2003; Rolland et al. 2018; Romito et al. 2021; Saulino et al. 2016). In general, safety concerns associated with baclofen administration include minor, frequently occurring side effects and severe, infrequently occurring complications (de Beaurepaire et al. 2019). Mild baclofen toxicity often presents with nonspecific signs of CNS depression. Such symptoms include confusion, depression, fatigue, headache, dizziness, and lethargy (de Beaurepaire et al. 2019; Duan et al. 2023; Rolland et al. 2018;

Table 7.2 Signs and symptoms of baclofen toxicity and withdrawal

Organ system	Baclofen toxicity	Baclofen withdrawal
General	Death, hypothermia	Death, hyperthermia, multisystem organ failure, pruritis
Cardiovascular	Autonomic dysfunction, conduction abnormalities, hypertension/hypotension, prolonged QTc interval, tachycardia/bradycardia	Acute reversible cardiomyopathy, autonomic dysfunction, cardiac arrest, hypertension/hypotension, tachycardia/bradycardia
Gastrointestinal	Abdominal pain, constipation, diarrhea, nausea, vomiting	Diarrhea, nausea, vomiting,
Musculoskeletal	Hypotonia, muscle stiffness, myalgias	Hypertonia, rhabdomyolysis
Neurological	Coma, confusion, dizziness, encephalopathy, fasciculations, fatigue, hyporeflexia, impaired memory, loss of brainstem reflexes, paresthesias, seizures, somnolence, tremor	Delirium, encephalopathy, headache, hyperreflexia, paresthesias, seizures, somnolence, tremor
Psychiatric	Agitation, anorexia, catatonia, depression, hallucinations, insomnia, mania	Anxiety, delusions, hallucinations, paranoias
Renal	Urinary retention	Acute kidney injury
Respiratory	Respiratory failure	Respiratory failure

Duan et al. (2023), Kido and Guglin (2017), Meythaler et al. (2003), Rolland et al. (2018), Romito et al. (2021), and Saulino et al. (2016)

Romito et al. 2021). Despite its $GABA_B$ receptor agonism and resultant inhibitory CNS effects, severe baclofen toxicity may present with generalized tonic-clonic or myoclonic seizures, more indicative of CNS excitation (Leung et al. 2006). Epileptogenicity may result from activation of the GABAergic and glutamatergic systems or via $GABA_B$-induced neural excitation via hyperpolarization of inhibitory interneurons (Farhat et al. 2020; Rolland et al. 2012; Wall et al. 2006). Severe baclofen toxicity can also present with metabolic encephalopathy, coma, and compete loss of brainstem reflexes, clinically mimicking brain death (Leung et al. 2006; Ostermann et al. 2000; Sullivan et al. 2012). Electroencephalogram (EEG) analysis can contribute additional information in the evaluation of suspected cases of baclofen overdose. EEG findings in patients with baclofen toxicity include status epilepticus, burst suppression, triphasic waves, and generalized slowing (Farhat et al. 2020; Kumar et al. 2010; Triplett et al. 2019).

7.7.2 Treatment of Baclofen Overdose and Toxicity

The treatment of baclofen toxicity is largely supportive and based on symptom management. Baclofen, from all sources, should be immediately discontinued. Patients receiving IT baclofen should be taken directly to a medical facility for prompt assessment and emptying of their baclofen pump reservoir (GABLOFEN® 2021; LIORESAL® INTRATHECAL 2019). Depending on the scenario, providers

can consider cerebrospinal fluid drainage via sequential lumbar punctures or lumbar drain placement (Saulino et al. 2016). Providers should administer intravenous fluids to expand circulating blood volume and vasopressors to treat hypotension. Endotracheal intubation may be needed in cases of severe baclofen toxicity associated with marked CNS depression, encephalopathy, or respiratory failure (Romito et al. 2021). Benzodiazepines should be administered to treat any associated seizures (Franchitto et al. 2018). Encephalopathic patients with baclofen toxicity may be extremely sensitive to even small doses of benzodiazepines, so a high level of monitoring is needed. It is reasonable to perform gastric lavage and activated charcoal decontamination in patients with oral baclofen overdose (Franchitto et al. 2018; Leung et al. 2006).

Baclofen's elimination is directly dependent on adequate renal function. Although baclofen can be dialyzed in cases of severe toxicity, the effectiveness of this treatment is not uniform across all patients. The "Extracorporeal Treatments in Poisoning" workgroup on the extracorporeal treatment for baclofen poisoning offers specific recommendations on the role of hemodialysis in this setting (Ghannoum et al. 2021). The workgroup recommends against the routine use of extracorporeal baclofen removal in addition to supportive care in acute poisoning (Ghannoum et al. 2021). They recommend performing intermittent hemodialysis for baclofen removal only in cases of severe toxicity in patients with renal impairment and associated coma requiring mechanical ventilation (Ghannoum et al. 2021). The workgroup recommends termination of hemodialysis when the patient's coma has improved to the point of safely permitting extubation (Ghannoum et al. 2021).

7.8 Baclofen Withdrawal

Baclofen withdrawal is a rare but potentially fatal complication of baclofen therapy. Withdrawal from baclofen can occur after both oral and IT routes of administration. Patients can develop withdrawal symptoms within hours to days following baclofen cessation. This most often occurs near the time of their oral baclofen prescription refill date, changes in their dosing regimens, or due to a malfunction in their IT baclofen pump delivery system (Romito et al. 2021; Watve et al. 2012). Performing a thorough patient history, review of medications, and physical examination are essential in diagnosing baclofen withdrawal, as symptoms are nonspecific and overlap with other life-threatening diagnoses (Mohammed and Hussain 2004; Ross et al. 2011). There is no laboratory test that reliably identifies baclofen withdrawal, as the diagnosis is based on clinical findings and patient history (Table 7.2).

7.8.1 Oral Baclofen Withdrawal

Oral baclofen withdrawal most often occurs following sudden drug cessation or large reductions in maintenance doses (Agabio et al. 2018; Alvis and Sobey 2017; Romito et al. 2021) (see Chap. 6 of this volume). Patients treated with oral baclofen should be advised to avoid abrupt drug interruption. Additionally, daily doses should be slowly reduced (e.g., 5–10 mg/week) to prevent withdrawal (Agabio et al. 2018) (see Chap. 6 of this volume). Common symptoms associated with withdrawal from oral baclofen include worsening of underlying spasticity, altered mental status, nausea, muscle rigidity, delirium, and fevers (Alvis and Sobey 2017; Khanal et al. 2022; Sanders and Ali 2021). While oral baclofen withdrawal is most often associated with the development of mild symptoms, progressive decompensation can occur without timely recognition and treatment (Alvis and Sobey 2017). There are case reports of patients developing respiratory distress requiring ICU admission and initiation of ventilatory support in the setting of withdrawal from modest oral baclofen regimens ranging from 30 to 60 mg/day (Alvis and Sobey 2017; Khanal et al. 2022). In both cases, the patients' conditions quickly improved after prompt recognition by providers and resumption of the patients' oral baclofen regimens.

7.8.2 Baclofen Withdrawal in Maternal-Fetal Medicine

There have been several case reports of newborn infants developing baclofen withdrawal following intrauterine exposure during maternal oral baclofen use (Duncan and Devlin 2013; Moran et al. 2004; Ratnayaka et al. 2001; Freeman and Delaney 2016). Maternal oral baclofen use during pregnancy has been shown to cause temporary withdrawal symptoms in newborns shortly after delivery (FLEQSUVY™ 2022; LYVISPAH™ 2021; OZOBAX® DS 2023). These symptoms can include seizures, tremors, rigidity, lethargy, respiratory distress, and severe feeding difficulties (FLEQSUVY™ 2022; Freeman and Delaney 2016; LYVISPAH™ 2021; Moran et al. 2004; OZOBAX® DS 2023; Ratnayaka et al. 2001). Conversely, maternal IT baclofen administration has not been associated with withdrawal symptoms in infants (Morton et al. 2009). This is likely because the daily doses of IT baclofen are 20–100 times lower than the daily oral doses of baclofen (Moran et al. 2004). At recommended therapeutic oral doses, baclofen is present in human milk (Morton et al. 2009). It is possible for withdrawal symptoms to occur in breastfeeding infants when maternal administration of oral baclofen is interrupted or when lactation is stopped (FLEQSUVY™ 2022; LYVISPAH™ 2021; OZOBAX® DS 2023).

7.8.3 Intrathecal Baclofen Withdrawal

There are multiple complications associated with IT baclofen therapy that can produce acute withdrawal. These include human error in pump programming or during refills; malfunction of the pump itself; migration, kinking, malposition, or occlusion of the catheter; or pump removal due to infection (Delhaas and Huygen 2020; Guzman et al. 2024; Koo et al. 2023; Romito et al. 2021). Early symptoms include worsening of spasticity, fever, pruritus, and paresthesias (GABLOFEN® 2021; LIORESAL® INTRATHECAL 2019). IT baclofen withdrawal is more likely than oral baclofen withdrawal to progress to severe, life-threatening symptoms (Romito et al. 2021). Diagnosing IT baclofen withdrawal requires a high index of suspicion, and there is no best confirmatory test. Severe baclofen withdrawal syndrome can mimic sepsis, meningitis, malignant hyperthermia, autonomic dysreflexia, serotonin syndrome, neuroleptic malignant syndrome, and other hypermetabolic states (GABLOFEN® 2021; LIORESAL® INTRATHECAL 2019; Romito et al. 2021; Satoh et al. 2022). Additionally, patients receiving IT baclofen may also be prescribed serotonergic or dopaminergic agents that can confound physical examination findings and vital sign abnormalities (Romito et al. 2021). If not treated rapidly, patients with IT baclofen withdrawal can develop seizures, rhabdomyolysis, autonomic instability, multisystem organ failure, cardiac arrest, and even death within 1–3 days (Cardoso et al. 2014; Coffey et al. 2002; Green and Nelson 1999; Reeves et al. 1998; Sampathkumar et al. 1998; Scarpino et al. 2022).

7.8.3.1 Intrathecal Baclofen Withdrawal Imaging Evaluation

Although the diagnosis of IT baclofen withdrawal based on clinical presentation alone can be challenging, some of the common device-related complications can be identified using imaging modalities. Because of its ease of performance, speed, and wide availability, a plain film radiograph may represent the first-line imaging study to evaluate for potential IT baclofen pump-catheter system dysfunction, specifically those associated with catheter discontinuities (Miracle et al. 2011). Standard plain film analysis of the IT baclofen system includes an abdominal radiograph, an anteroposterior thoracic spine radiograph, and anteroposterior/lateral lumbar spine radiographs (Coffey et al. 2002). The above plain film series will not identify catheter patency, occlusion, or leaks that occur in the absence of catheter disconnection (Miracle et al. 2011). If available, computerized tomography (CT) imaging with post-processing two-dimensional and three-dimensional reconstructions can be performed as the initial imaging study to visualize the entire drug delivery system (Delhaas and Huygen 2020). Identification of leaks and perforations in the pump and catheter tubing can be accomplished following the injection of contrast material into the IT baclofen system under fluoroscopy. The concomitant use of catheter access port myelography with CT imaging may improve diagnostic yield (Delhaas and Huygen 2020). To prevent accidental baclofen overdose during contrast

administration, it is important to first aspirate 2–3 mL of fluid from the accessory port before injection (Vender et al. 2006). If imaging studies prove to be nondiagnostic, then surgical interrogation and exploration of the pump system may be necessary to establish the diagnosis.

7.8.4 Treatment of Baclofen Withdrawal

Because of the potential for rapid decompensation, it is recommended that patients being managed for baclofen withdrawal be admitted to an ICU for close monitoring of their hemodynamics, neurological status, and respiratory function. This is especially important in cases of withdrawal from IT baclofen. Patients should receive frequent vital sign monitoring and intravenous fluids to treat dehydration, hypotension, and any associated rhabdomyolysis (Farid 2017; Romito et al. 2021; Watve et al. 2012). Vasopressors should be administered to treat refractory hypotension. Patients may require supportive treatment for any associated seizures or symptoms of delirium. Noninvasive ventilatory support or endotracheal intubation with the initiation of mechanical ventilation may be necessary in severe cases of baclofen withdrawal with respiratory failure. The severity of withdrawal symptoms does not always reliably correlate to dosing regimens (Saulino et al. 2016). Prompt resumption of baclofen and supportive care will help reduce morbidity and mortality (Coffey et al. 2002).

7.8.4.1 Baclofen

The most important step in the management of baclofen withdrawal is urgent reinitiation or supplementation of baclofen dosing. Ideally, baclofen should be administered using the same route and dose as prior to the interruption. Patients previously receiving IT baclofen should receive an IT bolus or placement of a temporary IT catheter with initiation of a continuous infusion (Saulino et al. 2016). These interventions may allow time for providers to reestablish definitive baclofen therapy. A single dose of IT baclofen can alleviate withdrawal symptoms for 6–8 h (Farid 2017; Greenberg and Hendrickson 2003). Restoring IT baclofen delivery can be associated with several challenges. Medication administration may be delayed due to the time involved with performing a diagnostic workup or coordinating a surgical team to evaluate an indwelling pump system in the operating room (Romito et al. 2021). Other patients may be unable to safely receive IT baclofen because of a severe infection that prompted the removal of their delivery system or because of profound coagulopathy. Some healthcare systems lack the resources or qualified personnel to administer IT medications. In these situations, providing oral baclofen represents the best course of action (Belisle Haley et al. 2023; Saulino et al. 2016). Unfortunately, there is no dependable oral-to-IT conversion dose. The high doses of oral baclofen needed to prevent withdrawal can induce sedation, confusion, and

somnolence (Ross et al. 2011). Dosing regimens should be frequently titrated to minimize both withdrawal symptoms and oversedation. Additionally, since many patients receiving IT baclofen have previously failed high-dose oral regimens, monotherapy with oral baclofen may be inadequate to prevent withdrawal (Schmitz et al. 2016). Adjunctive treatment with other agents may be needed to effectively manage withdrawal symptoms. These medication options are reviewed in Table 7.3 (Ackland and Fox 2005; Al-Khodairy et al. 1999; ATIVAN Injection 2021; Ativan® Tablets 2021; Bellinger et al. 2009; Coffey et al. 2002; Cyproheptadine HCl 2009; Dantrium® capsules 2012; Dantrium® Intravenous 2008; Defayette et al. 2020; Diazepam Injection 2022; DIPRIVAN® 2022; Douglas et al. 2005; Farid 2017; Gottula et al. 2020; Griffin 3rd et al. 2013; Horn and Nesbit 2004; Keating 2015; Khorasani and Peruzzi 1995; Laroche 2020; Meythaler et al. 2003; Midazolam Injection 2022; Mobolaji-Lawal et al. 2018; Morr et al. 2015; PRECEDEX™ 2022; Reeves et al. 1998; Romito et al. 2021; Ross et al. 2011; Saulino et al. 2016; Valium 2016; ZANAFLEX® 2013).

7.8.4.2 Benzodiazepines

Best practice guidelines recommend administering benzodiazepines and cyproheptadine as first-line adjunctive treatment in cases of IT baclofen withdrawal (Saulino et al. 2016). Benzodiazepines function as positive allosteric modulators on presynaptic $GABA_A$ receptors in the CNS (Griffin 3rd et al. 2013; Saulino et al. 2016). This modulation allows increased binding of the inhibitory neurotransmitter GABA to GABA type-A ($GABA_A$) receptor, thereby reducing neuronal excitability and withdrawal symptoms (Griffin 3rd et al. 2013). Benzodiazepines also help to circumvent the baclofen-mediated downregulation of $GABA_B$ receptors in patients with chronic baclofen use (Saulino et al. 2016). Benzodiazepines allow for flexibility in administration. They can be given as oral regimens, intravenous boluses, or intravenous continuous infusions. Additionally, they offer the added benefit of treatment for seizures, which can be precipitated by baclofen withdrawal. Common benzodiazepines used in this setting include diazepam, lorazepam, and midazolam, with diazepam being the most used agent (Saulino et al. 2016).

7.8.4.3 Cyproheptadine

Cyproheptadine is an oral serotonin and histamine antagonist with anticholinergic and sedative properties (Cyproheptadine HCl 2009). Per the package insert, cyproheptadine is FDA-approved for the treatment of allergic and vasomotor rhinitis, allergic conjunctivitis due to inhalant allergens and foods, mild allergic urticaria and angioedema, allergic reactions to blood or plasma, cold urticaria, dermatographism, and as adjunctive therapy for anaphylactic reactions (Cyproheptadine HCl 2009). It is thought that baclofen-mediated $GABA_B$ receptor activation may inhibit the release of serotonin in the brainstem (Meythaler et al. 2003). Withdrawal from

Table 7.3 Pharmacologic adjuncts for baclofen withdrawal treatment

Medication	Mechanism of action	Onset	Suggested dose	Half-life	Safety concerns
Diazepam	$GABA_A$ receptor agonist	IV: 0.5–3 min PO: 15–60 min	IV: 2–10 mg q4–6h PO: 5–15 mg q6h	IV: 20–50 h PO: 30–48 h	CNS depression, hypotension, risk of addiction and dependence, respiratory depression IV solution contains propylene glycol: caution for toxicity in renal impairment or with large doses
Lorazepam	$GABA_A$ receptor agonist	IV: 1–3 min PO: 20–30 min	IV: 0.5–2 mg q6h PO: 0.5–2 mg q6h	IV: 11–22 h PO: 12–14 h	CNS depression, hypotension, risk of addiction and dependence, respiratory depression IV solution contains propylene glycol: caution for toxicity in renal impairment or with large doses
Midazolam	$GABA_A$ receptor agonist	IV: 0.5–1 min	IV: 1–4 mg q6h	IV: 1.5–3 h	CNS depression, hypotension, risk of addiction and dependence, respiratory depression
Cyproheptadine	Serotonin and histamine antagonist	PO: 15–60 min	PO: 2–8 mg q6h Adult dose not to exceed 0.5 mg/kg/day	PO: 1–4 h	Has anticholinergic effects. Contraindicated in the elderly, debilitated, or patients taking MAOIs
Propofol	$GABA_A$ receptor agonist	IV: 0.5–1 min	IV: 20–200 mcg/kg/min	Context-sensitive half-life variable depending on infusion duration	CNS depression, hypotension, respiratory depression Risk of propofol infusion syndrome with prolonged administration
Dantrolene	Impairs skeletal muscle calcium release from the sarcoplasmic reticulum	IV: 1 min PO: variable	IV: divided doses up to a maximum dose of 10 mg/kg PO: 25 mg daily, with a gradual increase to 100 mg q8h over 3 weeks	IV: 4–11 h PO: 6–9 h	CNS depression, muscle weakness, respiratory depression Oral form is contraindicated in patients with liver disease

(continued)

Table 7.3 (continued)

Medication	Mechanism of action	Onset	Suggested dose	Half-life	Safety concerns
Dexmedetomidine	Alpha$_2$ receptor agonist	IV: 3–6 min	IV: 0.2–1 mcg/kg/h	IV: 2–3 h	Bradycardia, hypertension, hypotension, sinus arrest
Tizanidine	Alpha$_2$ receptor agonist	PO: 1–3 h	PO: 2–4 mg q8h Adult dose not to exceed 36 mg/day	PO: 2–3 h	Caution in patients with hepatic and renal impairment

Ackland and Fox (2005), Al-Khodairy et al. (1999), ATIVAN Injection (2021), Ativan® Tablets (2021), Bellinger et al. (2009), Coffey et al. (2002), Cyproheptadine HCl (2009), Dantrium® capsules (2012), Dantrium® Intravenous (2008), Defayette et al. (2020), Diazepam Injection (2022), DIPRIVAN® (2022), Douglas et al. (2005), Farid (2017), Gottula et al. (2020), Griffin et al. (2013), Horn and Nesbit (2004), Keating (2015), Khorasani and Peruzzi (1995), Laroche (2020), Meythaler et al. (2003), Midazolam Injection (2022), Mobolaji-Lawal et al. (2018), Morr et al. (2015), PRECEDEX™ (2022), Reeves et al. (1998), Romito et al. (2021), Ross et al. (2011), Saulino et al. (2016), Valium (2016), and ZANAFLEX® (2013)

GABA γ-aminobutyric acid, *IV* intravenous, *PO* by mouth, *q4–6h* every 4–6 h, *q8h* every 8 h, *CNS* central nervous system, *MAOI* monoamine oxidase inhibitor

chronic baclofen therapy may thus result in an acute excess of serotonin activity, presenting similarly to serotonin syndrome. In addition to baclofen and benzodiazepines, cyproheptadine has been used successfully as an off-label adjunctive treatment in cases of acute IT baclofen withdrawal (Meythaler et al. 2003; Ross et al. 2011).

7.8.4.4 Propofol

Like benzodiazepines, propofol is thought to work as a positive modulator on $GABA_A$ receptors, allowing for increased binding of the inhibitory neurotransmitter GABA to the $GABA_A$ receptor (DIPRIVAN® 2022). It is commonly used for the induction of general anesthesia or to maintain sedation during diagnostic or therapeutic procedures. Continuous propofol infusions have been used alongside other adjunctive agents in cases of baclofen withdrawal with acute hyperspasticity (Ackland and Fox 2005; Douglas et al. 2005; Ross et al. 2011). Propofol produces hypotension and respiratory depression. It should only be given by providers trained in airway management and cardiovascular resuscitation. All patients receiving propofol require a high level of monitoring and frequent assessments (DIPRIVAN® 2022).

7.8.4.5 Dantrolene

Dantrolene is a muscle relaxant that dissociates skeletal muscle excitation-contraction coupling, likely by impairing the release of calcium from the sarcoplasmic reticulum (Dantrium® Intravenous 2008). It is available in both oral and intravenous formulations. Dantrolene is approved by the FDA for the treatment of malignant hyperthermia and chronic spasticity from upper motor neuron disorders (Dantrium® capsules 2012; Dantrium® Intravenous 2008). It has been used off-label as an adjunctive treatment in baclofen withdrawal associated with severe spasticity, hyperthermia, and rhabdomyolysis (Khorasani and Peruzzi 1995).

7.8.4.6 Dexmedetomidine

Dexmedetomidine is a relatively selective, centrally acting $alpha_2$-adrenergic receptor agonist with sedative properties. It is FDA-approved for the maintenance of intravenous sedation in intubated patients following initiation of mechanical ventilation for the first 24 h and peri-procedural sedation of nonintubated patients (PRECEDEX™ injection 2022). It has been used successfully as adjunctive treatment during baclofen withdrawal in several settings (Defayette et al. 2020; Gottula et al. 2020; Laroche 2020; Mobolaji-Lawal et al. 2018; Morr et al. 2015). Patients receiving dexmedetomidine require continuous monitoring as bradycardia, hypotension, hypertension, and sinus arrest have all been reported (PRECEDEX™ injection 2022).

7.8.4.7 Tizanidine

Tizanidine is an oral, short-acting, central alpha$_2$-adrenergic receptor agonist that reduces spasticity, likely via increased presynaptic inhibition of motor neurons (ZANAFLEX® 2013). It is FDA-approved for the management of spasticity. Tizanidine has been used successfully as an adjunctive agent during baclofen withdrawal (Al-Khodairy et al. 1999; Bellinger et al. 2009; DIPRIVAN® 2022; Ross et al. 2011). Its sedative effects can be potentiated by the coadministration of other CNS depressants, such as benzodiazepines, dexmedetomidine, and propofol. Additionally, tizanidine should be used with caution in patients with renal impairment because of decreased drug clearance (ZANAFLEX® 2013).

7.9 Conclusions

The administration of both oral and IT baclofen requires diligent monitoring and awareness of patient safety considerations. Careful dose initiation and titrations are important to minimize the development of adverse effects. The risk of complications may be higher in certain populations, including patients with advanced age, renal impairment, or those taking other sedating medications. Both baclofen overdose and withdrawal represent life-threatening conditions that demand frequent monitoring, a rapid diagnosis, and timely treatment. The management of a baclofen overdose mandates immediate baclofen cessation and may necessitate hemodynamic and respiratory supportive measures. Baclofen withdrawal can mimic other serious clinical disorders and should be considered in patients who present with an increase in spasticity or hyperreflexia. The treatment of baclofen withdrawal requires prompt restoration of baclofen therapy, ideally via the same route and dose as before the interruption. Other adjunctive medications may be needed to effectively treat associated baclofen withdrawal symptoms, including benzodiazepines, cyproheptadine, propofol, dantrolene, dexmedetomidine, and tizanidine.

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Part III
Chemistry and Pharmacology of Positive Allosteric Modulators

Chapter 8
Recent Advances on the Chemistry of $GABA_B$ Receptor Allosteric Modulators

Claudia Mugnaini and Federico Corelli

Abstract Allosteric modulation of therapeutic protein targets has been proposed as a possible strategy to overcome the limitations of orthosteric ligands. However, it has been shown that many of these protein systems, including the $GABA_B$ receptor, are quite difficult to target. The development of new allosteric modulators therefore represents a major challenge. To date, no therapeutic agent that can allosterically modulate the $GABA_B$ receptor has been used clinically. Nevertheless, academic and industrial research received a remarkable boost after the discovery of the first $GABA_B$ receptor positive allosteric modulators (PAMs) in 2001–2012, leading to the identification of new compounds with promising pharmacodynamic and pharmacokinetic properties. This enabled the preclinical and clinical development of the three candidates, ASP8062, ODM-106, and ADX71441, as potential treatments for substance use disorder or essential tremor. Although the clinical trials of ODM-106 and ADX71441 have been discontinued, many hopes are pinned on ASP8062, which could become the first $GABA_B$ receptor PAM of therapeutic interest. The period 2013–2018 has thus been a harbinger of very relevant innovations, including the discovery of negative allosteric modulators of the $GABA_B$ receptor, expanding the arsenal of pharmacological tools to study the involvement of this receptor in various pathological processes.

Keywords $GABA_B$ PAMs · $GABA_B$ NAMs · SAR · Synthesis

8.1 Introduction

The metabotropic γ-aminobutyric acid (GABA) type-B ($GABA_B$) receptor was first described by Norman Bowery in 1979 (Bowery et al. 1979, 1980; Hill and Bowery 1981; see also Chap. 15 of this volume) and plays an important inhibitory role in

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neurotransmission (Kaupmann et al. 1997). The receptor was not successfully cloned until 20 years later because of the lack of high-affinity radioligands and, more importantly, because the receptor had unexpected structural features (Evenseth et al. 2020). $GABA_B$ receptor belongs to class C of G protein-coupled receptors (GPCRs) along with metabotropic glutamate (mGlu) receptors, calcium-sensing (CaS) receptor, three taste receptor type-1 (T1R) subunits, GPCR family C group 6 subtype a (GPRC6a), a promiscuous L-amino acid receptor, and several orphan receptors (Munk et al. 2016; Ellaithy et al. 2020; see also Chap. 2 of this volume).

$GABA_B$ receptor acts both pre- and postsynaptically, where it either blocks neurotransmitter release by inhibiting voltage-gated Ca^{2+} channels or induces hyperpolarization of the neuron by opening G protein-coupled inwardly rectifying potassium (GIRK) channels (Gassmann and Bettler 2012). Given its central role in neurobiology, $GABA_B$ receptor is implicated in a variety of neurological and psychiatric disorders including epilepsy, spasticity, stress, sleep disorders, neuropathic pain, depression and anxiety, and drug addiction (Shaye et al. 2021; Wang et al. 2022a, b; see also Chap. 1 of this volume). Therefore, targeting $GABA_B$ receptor activity is considered a valuable therapeutic approach for various central nervous system (CNS) disorders and cancer (Wierońska and Pilc 2019; Jiang et al. 2012; Princivalle 2022; Vlachou 2022) as well as for other conditions outside the CNS (Vlachou 2022; Healy 2016; Lehmann et al. 2016; see also Chap. 5 of this volume). Over the years, great efforts have been made to develop compounds targeting this receptor and showing pharmacokinetic and pharmacodynamic properties suitable for therapeutic use (Froestl 2010a). These studies have led to the identification of very few agonists comparable to baclofen (Fig. 8.1) in terms of potency and selectivity but also to the identification of a number of antagonists that provide essential information on the structure-activity relationship for $GABA_B$ receptor-targeted compounds and broaden the range of their potential clinical applications (Corelli and Mugnaini 2016). A recent study suggested that inhibition of $GABA_B$ receptor by the antagonist CGP52432 may have potential value for the clinical treatment of cerebral ischemia and cognitive impairment (Song et al. 2021).

Despite the large number of $GABA_B$ receptor ligands acting as either agonists or antagonists identified to date (Nieto et al. 2022), it has proven difficult to obtain compounds with favorable pharmacokinetics, desired effects, and tolerable side effects. Some of the efforts to obtain baclofen analogs actually resulted in clinically approved drugs such as pregabalin, vigabatrin, and gabapentin (Fig. 8.1) (Corelli and Mugnaini 2016), but these turned out to act through different mechanisms and bind different targets than the $GABA_B$ receptor (Mugnaini and Corelli 2016). The only approved drug that selectively acts on this receptor is baclofen, which is used as a muscle relaxant and antispasticity agent (Bowery 2016; Evenseth et al. 2020) and is currently under investigation for the treatment of alcohol use disorder (AUD) in humans (Shaye et al. 2021; see also Chap. 6 of this volume). However, baclofen has several pharmacokinetic limitations, including low blood-brain barrier (BBB) penetration, short duration of action, and rapid development of tolerance in patients (Lal et al. 2009). All of this underscores the apparent difficulty in developing selective and potent ligands (see Chap. 1 of this volume) but also the push for new ligands

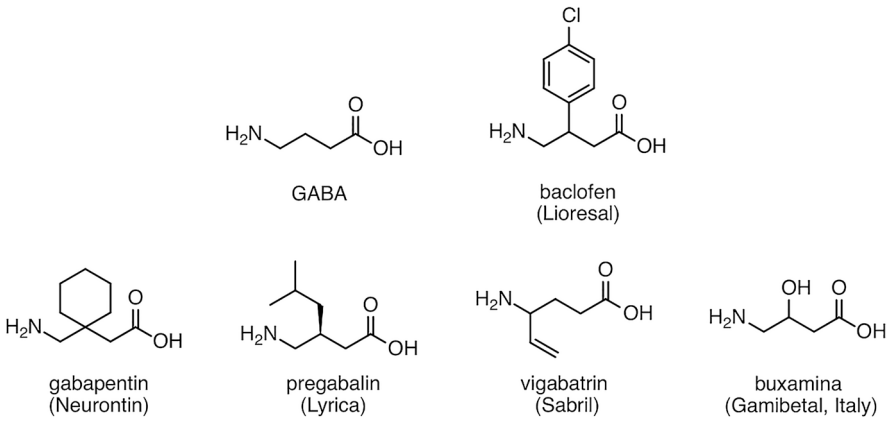


Fig. 8.1 Chemical structure of the endogenous ligand GABA and synthetic analogs for clinical use

whose chemotypes differ from known compounds acting at the $GABA_B$ receptor. New compounds acting as agonists or antagonists could potentially contribute to the understanding of therapeutic effects or become new drugs.

While the search for new $GABA_B$ receptor agonists/antagonists has lost momentum over the past 20 years, the development of allosteric modulators of this receptor has simultaneously attracted increasing interest (Kniazeff 2020; Nieto et al. 2022). Allosteric modulators are ligands that bind to allosteric sites that are topographically distinct from orthosteric sites where endogenous ligands interact. Allosteric and orthosteric ligands can occupy the same receptor simultaneously and influence their respective pharmacological properties (Urwyler 2016; see also Chap. 2 of this volume). After binding, an allosteric modulator can either increase the affinity and/or efficacy of an orthosteric ligand and thus act as a *positive allosteric modulator* (PAM) or attenuate the activity of the orthosteric ligand and thus act as a *negative allosteric modulator* (NAM). Some allosteric ligands, termed *neutral allosteric ligands* (NALs), do not affect the activity of the orthosteric ligand at equilibrium but can still occupy the allosteric site and block the access of other allosteric modulators. In addition to their modulatory effects, some allosteric ligands may have their own direct agonistic properties and activate signal transduction pathways even in the absence of the orthosteric agonist. These allosteric ligands that exhibit both modulatory and direct agonistic properties are often referred to as *PAM agonists* or *ago-PAMs* (Changeux and Christopoulos 2016; Christopoulos 2014). Synthetic PAMs represent an interesting alternative to orthosteric agonists and provide an enriched, yet challenging, landscape for novel therapeutics (Saito et al. 2024; see also Chap. 9 of this volume).

This chapter focuses mainly on allosteric modulators of the $GABA_B$ receptor developed in the last decade (2013–2023), while the compounds discussed in the earlier book (Mugnaini and Corelli 2016) are also summarized to provide a more complete overview. An attempt is made to group the known compounds according to their chemical structure. It is expected that this approach will help medicinal

chemists working in this field to explore the chemical space around the old scaffolds and eventually design new scaffolds based on established structure-activity relationship (SAR).

8.2 Positive Allosteric Modulators Discovered in 2001–2012

In 2001, a high-throughput screening process conducted by Novartis Pharma enabled the identification of compounds such as CGP7930 and its aldehyde analog CGP13501 (Fig. 8.2a), which enhanced GABA-induced signals at low micromolar concentrations without stimulating guanosine 5-O-(3-[^{35}S]thio)triphosphate (GTPγ^{35}S) binding in the absence of the physiological neurotransmitter GABA (Urwyler et al. 2001; Adams and Lawrence 2007). CGP7930 dose dependently increased both agonistic potency and maximal effect of GABA and exhibited antidepressant and anxiolytic properties in vivo. It was therefore considered the first $GABA_B$ receptor PAM of pharmacological interest. However, CGP7930 was later found to act as a partial $GABA_B$ receptor agonist and thus can be classified as a de facto ago-PAM (Binet et al. 2004). More recently, CGP7930 has been accounted as unsuitable for use as a specific $GABA_B$ receptor PAM, as it exerts multiple effects not only at the level of this receptor but also at the level of the $GABA_A$ receptor. At higher concentrations, CGP7930 also blocks GIRK channels, thereby reducing $GABA_B$ receptor signaling in human embryonic kidney 293 (HEK 293) cells (Hannan et al. 2023).

Scientists at Hoffmann-La Roche have worked up the structure of CGP7930 to novel fluorinated 2-hydroxypropionic acid derivatives and then to their lactone and lactam analogs such as *rac*-BHFF and BHFI, respectively (Fig. 8.2a) (Malherbe et al. 2007). *rac*-BHFF acted as PAM, as it increased the potency and efficacy with which GABA stimulated the binding of GTPγ^{35}S to cell membrane preparations; in particular, at 0.3 μM concentration, it increased the EC_{50} value of GABA in recombinant cells by more than 15-fold. The dextrorotatory enantiomer (+)-BHFF was significantly more potent than *rac*-BHFF, increasing this value 87-fold. However, pharmacokinetic studies in mouse plasma showed that *rac*-BHFF is quantitatively hydrolyzed to its hydroxy acid. Therefore, it was hypothesized that both compounds might be present in vivo due to rapid conversion and contribute to the observed effects in vivo (Malherbe et al. 2008). Isosteric replacement of the oxygen atom by an NH group resulted in a hydrolytically more stable lactam analog of BHFF, which is coded BHFI (Malherbe et al. 2007).

In 2002, several arylalkylamines such as fendiline and its analogs (Fig. 8.2b) were designated as new $GABA_B$ receptor PAMs (Kerr et al. 2002). Shortly thereafter, these compounds were found not to be allosteric modulators of $GABA_B$ receptor (Urwyler et al. 2004), although new findings suggested that 3-chloro-4-methoxyfendiline is a potent potentiator of pre- and postsynaptic $GABA_B$ receptor-mediated functions (Ong et al. 2005).

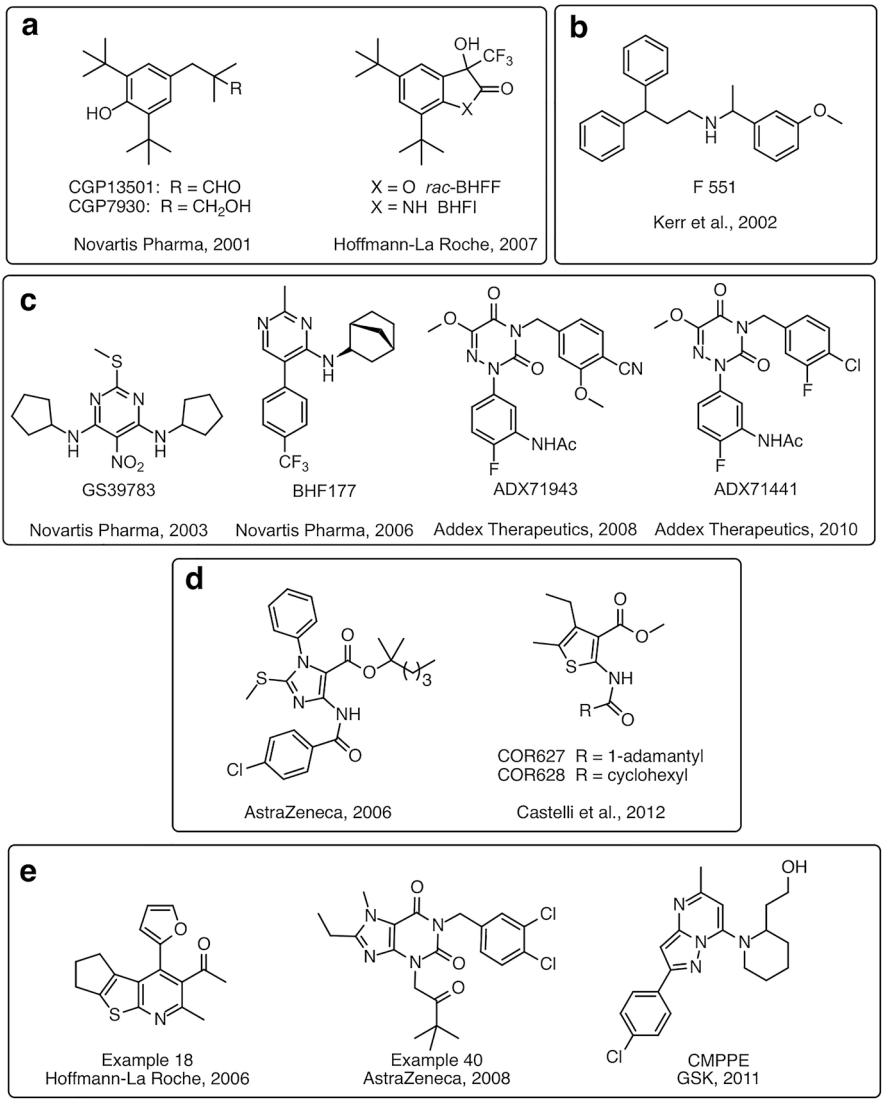


Fig. 8.2 Structure of exemplar PAMs discovered and developed in the period 2001–2012. Panel (**a**): di-(*tert*-butyl)phenols and derivatives. Panel (**b**): arylalkylamines. Panel (**c**): pyrimidines and related six-membered heterocyclic analogs. Panel (**d**): five-membered heterocyclic amides. Panel (**e**): condensed ring heterocycles

With the goal of obtaining compounds with better pharmacokinetic and drug-like properties, Novartis Pharma researchers turned to scaffolds other than the di-*tert*-butylphenol structure typical of CGP7930, opting in particular for the heterocyclic pyrimidine system. This approach led to the identification of new $GABA_B$ receptor PAMs, including the one designated GS39783 (Fig. 8.2c) (Urwyler et al. 2003). By

binding to the sixth transmembrane domain of the $GABA_{B2}$ receptor (Dupuis et al. 2006), this compound has a dual mechanism and is able to simultaneously increase the affinity and maximal efficacy of GABA by approximately eight- and twofold, respectively. In rats, it has been shown to exert anxiolytic effects (Cryan et al. 2004; Mombereau et al. 2004; Felice et al. 2016; Urwyler 2016), to decrease alcohol self-administration in alcohol-preferring animals (Orrù et al. 2005; Maccioni et al. 2012; Maccioni et al. 2015; Colombo et al. 2015; Lorrai et al. 2019; see also Chap. 12 of this volume), and to attenuate the reward-enhancing effects of psychoactive substances (Frankowska et al. 2016), particularly cocaine (Slattery et al. 2005) and nicotine (Paterson et al. 2008) (see Chap. 11 of this volume).

Another step forward was the decision to modify the structure of GS39783 to limit genotoxic effects. To this end, a series of trisubstituted pyrimidine derivatives was developed in which the nitro group was replaced by a 4-trifluoromethylphenyl group and one of the two cyclopentylamino units was removed (Fig. 8.1c) (Floersheim et al. 2006; Guery et al. 2007). Among them, BHF177 (Fig. 8.2c) 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 free from in vitro genotoxicity and exhibited anti-nicotine and anti-alcohol effects as well as anxiolytic properties in the stress-induced hyperthermia mouse assay (Paterson et al. 2008; Maccioni et al. 2009; Vlachou et al. 2011; Li et al. 2015; see also Chaps. 10, 11, and 12 of this volume). There has been increasing evidence that BHF177 is also able to modulate various signaling pathways through cross talk between $GABA_B$ and other receptors. For example, BHF177 suppresses diabetic neuropathic pain symptoms in rats by blocking the PKC/CaMKII/ERK1/2/CREB signaling pathway to activate $GABA_B$ receptors (Liu et al. 2022), while it inhibits neuron apoptosis in rats and thus protects against refractory epilepsy, by regulating the IRS-1/PI3K/Akt axis through cross talk between $GABA_B$ and insulin-like growth factor-1 receptors (Wang et al. 2022a, b).

In 2008, Addex Therapeutics patented more than 300 novel triazinedione derivatives identified through a high-throughput screening campaign of its proprietary chemical library, followed by a lead optimization process (Riguet et al. 2008). ADX71943, a potent and selective $GABA_B$ receptor PAM with a peripheral mode of action, was initially selected for preclinical development but was further characterized only as a pharmacological tool due to its insufficient safety profile (Kalinichev et al. 2014a). In a subsequent patent filed by the same researchers of Addex Therapeutics (Riguet et al. 2010), compound ADX71441, a triazinedione whose structure differs from that of ADX71943 for having a fluorine and a chlorine atom in the benzyl moiety instead of the methoxy and cyano groups, respectively (Fig. 8.2c), was selected as a lead candidate for preclinical development, thanks to its excellent preclinical efficacy and tolerability in several rodent models of pain (Kalinichev et al. 2017a; Kannampalli et al. 2017), anxiety (Kalinichev et al. 2017b), addiction (Hwa et al. 2014), and overactive bladder (Kalinichev et al. 2014b), showing also efficacy in a genetic model of Charcot-Marie-Tooth Type 1A neuropathy (Nieto et al. 2022). This PAM has been extensively studied in in vivo tests for its potential in the treatment of AUD (Hwa et al. 2014; Augier et al. 2017), and the

results of this research have recently been summarized (Maccioni and Colombo 2019; Augier 2021; see also Chap. 12 of this volume). Based on these premises, Addex received approval to initiate a phase I study with ADX71441 and entered into a strategic collaboration with Indivior in January 2018 to develop ADX71441 and discover additional $GABA_B$ receptor PAM compounds for the treatment of addiction. However, following an internal evaluation, in February 2019, Indivior decided to discontinue further evaluation of ADX71441 and focus future research on alternative $GABA_B$ receptor PAMs (Addex 2019).

While we dutifully cite the 2006 Hoffmann-La Roche patents claiming quinoline compounds as $GABA_B$ receptor PAMs (Mugnaini and Corelli 2016), the development of new allosteric agents, starting from the six-membered heterocyclic scaffolds mentioned above, essentially followed two lines, both independently pursued by different academic and industrial research groups, leading to the identification of compounds with a five-membered heterocyclic structure (Fig. 8.2d) or of bicyclic systems resulting from the fusion of five-membered rings with six-membered nitrogen rings (Fig. 8.2e).

Approximately 200 molecules characterized by a 5-membered heterocyclic scaffold functionalized with amide and ester groups were described as $GABA_B$ receptor modulators for the treatment of gastrointestinal disorders in several patents filed by AstraZeneca between 2006 and 2008. Imidazole derivatives, an example of which is shown in Fig. 8.2d, have been the most studied (Bauer et al. 2006, 2007a, b, 2008), but other heterocyclic systems such as pyrazoles (Bauer 2007), oxazoles, and thiazoles (Bauer et al. 2007c) have also received attention.

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. 8.2d), as novel $GABA_B$ receptor modulators (Castelli et al. 2012). Both compounds potentiated GABA- and baclofen-stimulated GTPγ^{35}S binding to native $GABA_B$ receptors while producing no effect when given alone and increased potency of GABA rather than its maximal efficacy. Pretreatment with per se ineffective doses of COR627 and COR628 potentiated the sedative/hypnotic effect of baclofen in mice.

Figure 8.2e shows examples of compounds with thienopyridine (Malherbe et al. 2006), imidazopyrimidine (i.e., xanthine) (Cheng et al. 2008), and pyrazolopyrimidine (Perdonà et al. 2011) structures patented by Hoffmann-La Roche, AstraZeneca, and GlaxoSmithKline, respectively, based on bicyclic systems. Most of these compounds have been presented as $GABA_B$ modulators that may be useful in the treatment of CNS disorders, gastrointestinal disorders, and regulation of food intake.

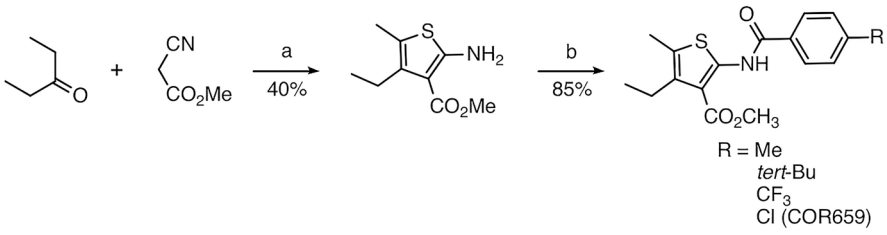
A comprehensive review of 19 patents on the various scaffolds for PAMs of $GABA_B$ receptor and the major players in this field was published by Wolfgang Froestl (2010b). This paper provides a relevant overview of various indications that could be treated with $GABA_B$ PAMs.

8.3 Positive Allosteric Modulators Discovered in 2013–2023

8.3.1 Five-Membered Heterocyclic Amides

Based on the promising results obtained in in vitro and in vivo tests with the prototypical thiophene-based PAMs COR627 and COR628 (Castelli et al. 2012), a small library of 2-(acylamino)thiophene derivatives was synthesized by systematically modifying the substituents attached to the thiophene ring of COR627, focusing on the modulation of the amide group. Acylation of methyl 2-amino-4-ethyl-5--methylthiophene-3-carboxylate (Scheme 8.1), also obtained by Gewald synthesis from methyl cyanoacetate and 3-pentanone in the presence of sulfur and a base, resulted in 35 new compounds that exhibit an interesting positive allosteric modulator profile by significantly potentiating GABA-induced GTPγ^{35}S stimulation at 2.5 and 25 μM while showing no intrinsic agonistic activity. The compounds also proved effective in vivo, potentiating baclofen-induced sedation/hypnosis in DBA mice when administered either intraperitoneally or intragastrically. Although they showed lower potency than the reference compound GS39783 in vitro, the new compounds (especially those highlighted in Scheme 8.1) exhibited higher efficacy in vivo. The combination of these compounds with a per se nonsedating dose of baclofen resulted in a shorter onset and a longer duration of loss of righting reflex in mice. The test compounds showed cytotoxic effects at concentrations comparable to or higher than those of GS39783 or BHF177 (Mugnaini et al. 2013). Other studies based on alternative approaches, such as scaffold hopping by bioisosteric replacement of the thiophene core by other heterocycles, were also reported (Mugnaini et al. 2014), but none of the new compounds proved superior to COR659 (Fig. 8.3a and Scheme 8.1).

In the following years, this new $GABA_B$ receptor PAM was the subject of several other studies aimed at characterizing its pharmacological properties in rodents, such as its ability to reduce self-administration of alcohol and palatable foods (Maccioni et al. 2017, 2019), to suppress binge-like alcohol drinking (Lorrai et al. 2022a; see also Chap. 12 of this volume), and to exert an anorectic effect in a rat model of overeating (Maccioni et al. 2023).



Scheme 8.1 Reagents and conditions: (*a*) sulfur, morpholine, EtOH, reflux; (*b*) 4-substituted benzoyl chloride, dioxane, 100 °C

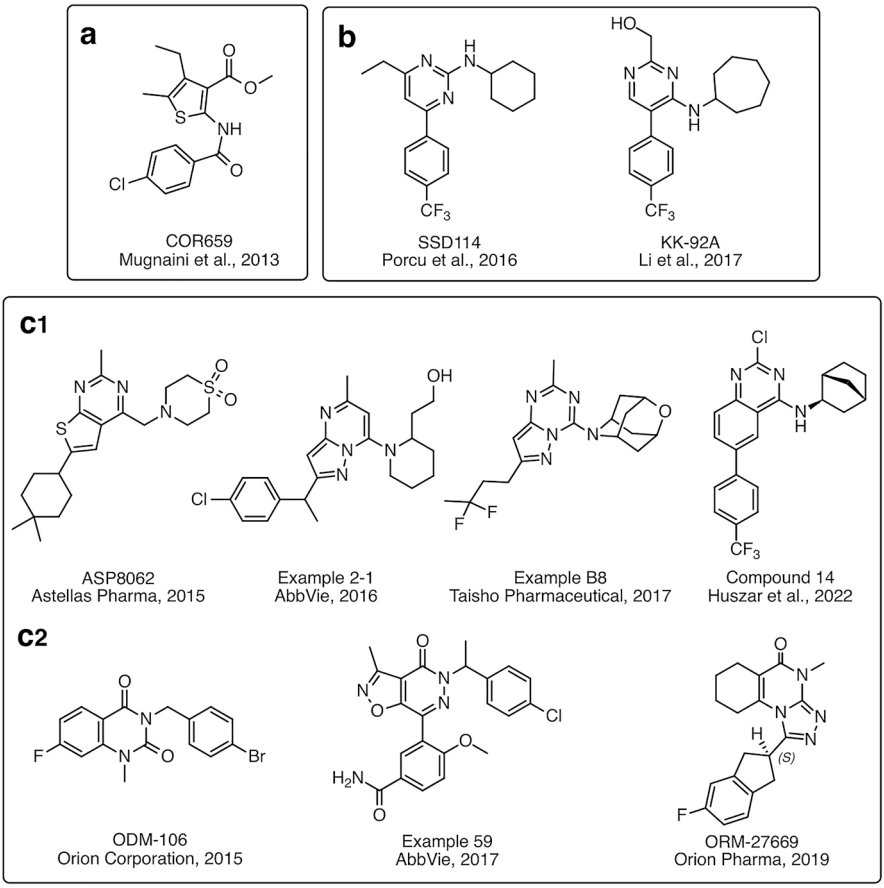


Fig. 8.3 Structure of exemplar PAMs discovered and developed in the period 2013–2023. Panel (**a**): five-membered heterocyclic amides. Panel (**b**): pyrimidines. Panel (**c**): condensed ring heterocycles

Overall, COR659 effectively reduced alcohol intake in two different rodent models of excessive alcohol consumption. However, tolerance to the suppressing effects of COR659 on alcohol intake quickly developed (Lorrai et al. 2022b). If theoretically translated to humans, these data would represent a potential limitation for the clinical use of COR659. In addition, another study was conducted to determine whether the anti-addictive properties of COR659 are transferable to drugs of abuse other than alcohol. Specifically, the effect of this compound on locomotor hyperactivity induced by cocaine, amphetamine, nicotine, and morphine was investigated in mice (Lobina et al. 2021; see also Chap. 11 of this volume). Pretreatment with COR659 reduced or even suppressed the increase in motility induced by cocaine, amphetamine, nicotine, and morphine. Since locomotor hyperactivity is a common attribute of drugs of abuse, the results of the present study provide the first evidence

for extending the preclinical anti-addiction profile of COR659 to cocaine, amphetamine, nicotine, and morphine.

It is also worth highlighting that the ability of COR659 to suppress the lever-responding for a sucrose solution in selectively bred Sardinian alcohol-preferring (sP) rats and to chocolate solution in Wistar rats was completely blocked by pretreatment with the neutral cannabinoid CB_1 receptor antagonist, AM4113 (Maccioni et al. 2017). Thus, COR659 apparently acts via a composite mechanism: positive allosteric modulation of the $GABA_B$ receptor, which is responsible for the reducing effect on alcohol self-administration, as suggested by the ability of COR659 to potentiate GABA-stimulated GTPγ^{35}S binding via the $GABA_B$ receptor, and an effect on other receptor system(s), including the cannabinoid CB_1 receptor, which mediates the reducing effect on palatable food intake. The hypothesis on the "dual" mechanism of action proposed for COR659 appears to be applicable also to many of the COR659 analogs tested (Ferlenghi et al. 2020).

Due to its intriguing in vitro and in vivo pharmacological profile, the same authors decided to further investigate COR659 by studying its in vitro metabolism in rat liver microsomes (RLM) and in vivo pharmacokinetics in either sP or Wistar rats. The initial stability studies showed that COR659 was rapidly metabolized in vitro to at least eight metabolites derived from NADPH-dependent oxidative rather than hydrolytic pathways. Two of these (M1, M2) were identified by comparing their high-resolution mass spectrometric and chromatographic properties with those of synthetic reference standards (Fig. 8.4): M1 was the product of NADPH-dependent cleavage of the ester group at C-3 of the thiophene ring, while M2 was the oxidation product of the methyl group at C-5. In rat plasma, regardless of the rat line or strain used, > 90% of COR659 was recovered intact after 24 h of incubation at 37 °C (Ferlenghi et al. 2020). This pharmacokinetic issue was addressed with the synthesis of a series of COR659 analogs in which the ester function was chemically modulated, leading to the selection of the *tert*-butyl ester derivative of COR659, which is endowed with a more favorable metabolic stability profile in vitro. Its lower potency in vivo compared to the parent compound once again confirmed the very stringent structural requirements for positive allosteric modulation of $GABA_B$ receptor within the chemical class of 2-acylaminothiophenes (Mugnaini et al. 2013).

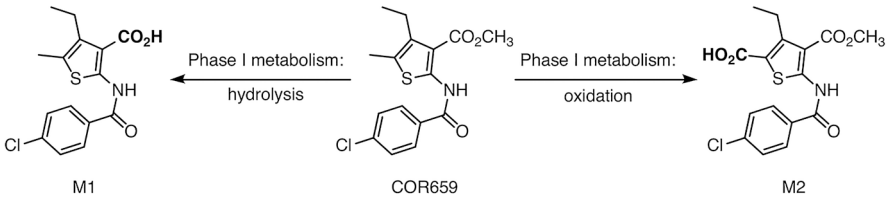
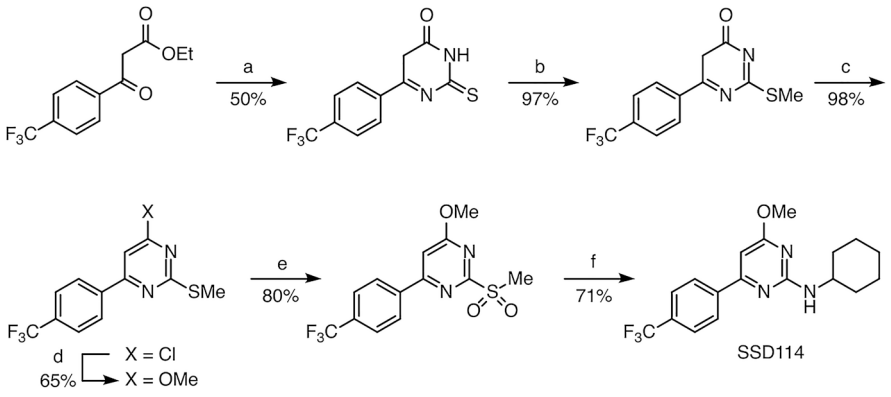


Fig. 8.4 Main metabolic pathways of COR659

8.3.2 *Pyrimidines*

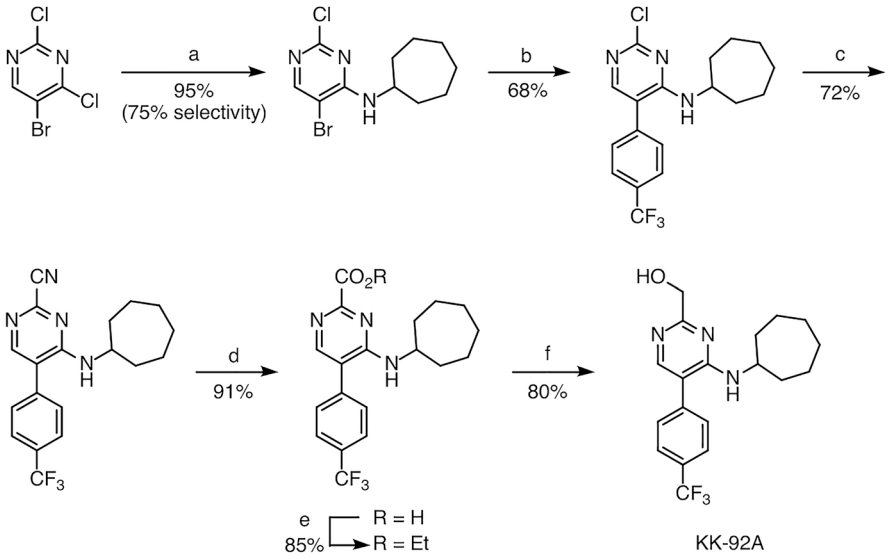
Almost simultaneously with the development of COR659, the design and synthesis of a series of substituted pyrimidines was carried out. Based on a hybridization strategy, the structural overlaps of the $GABA_B$ receptor PAMs, GS39783 and BHF177, were used as a starting point for the synthesis of 2,4,6-trisubstituted pyrimidines. Of these new compounds, *N*-cyclohexyl-4-methoxy-6-(4-(trifluoromethyl)phenyl)pyrimidin-2-amine (SSD114) (Fig. 8.3b) exhibited the pharmacological properties of a $GABA_B$ receptor PAM in vitro. Specifically, SSD114 was obtained by a six-step synthesis (Scheme 8.2) starting from the commercially available ethyl (4-trifluoromethylbenzoyl)acetate. In the first step, this β-ketoester was reacted with thiourea and sodium ethoxide in refluxing ethanol to provide the substituted thiouracil in 50% yield. Subsequent methylation at the sulfur atom by iodomethane in a basic solution of H_2O/EtOH led to the corresponding *S*-methylated compound in almost quantitative yield after crystallization from acetone. The substituted 4-pyrimidinone was quantitatively converted by reaction with phosphorous oxychloride into the corresponding 4-chloro-2-(methylthio)-6-[4-(trifluoromethyl)phenyl]pyrimidine. Replacement of the chlorine atom by nucleophilic substitution with sodium methoxide led to the 4-methoxypyrimidine derivative, which was treated with potassium monopersulfate to oxidize the thioether to sulfone in 80% yield. Finally, displacement of the sulfonyl group with cyclohexylamine in refluxing 1,4-dioxane gave SSD114 in 71% isolated yield after chromatographic purification. SSD114 enhanced $GABA_B$ receptor responses in cells and in an in vivo mouse model, whereas no intrinsic activity was observed in the same assays when SSD114 was tested alone. Although less potent than *rac*-BHFF, SSD114 is unlikely to have the side effects typical of $GABA_B$ receptor agonists and ago-allosteric agents because it has no intrinsic activity and no interaction with the orthosteric binding site of the receptor (Porcu et al. 2016).



Scheme 8.2 Reagents and conditions: (*a*) thiourea, NaOEt, EtOH, reflux; (*b*) MeI, NaOH, EtOH, rt; (*c*) $POCl_3$, 100 °C; (*d*) MeONa, MeOH, rt; (*e*) Oxone, MeOH, rt; (*f*) cyclohexylamine, 1,4-dioxane, reflux

In the search for new $GABA_B$ receptor PAMs, subtle chemical modifications of the structure of BHF177 led to the development of a 2,4,5-trisubstituted pyrimidine compound (KK-92A) (Fig. 8.3b) (Li et al. 2017). This member of the pyrimidine class shares remarkable similarities with SSD114, but the two compounds were developed independently, albeit almost simultaneously, by two different research groups. Visual inspection of the two molecules reveals that KK-92A and SSD114 have the same pyrimidine backbone substituted with three conceptually identical substituents (4-trifluorophenyl, cycloalkylamine, alkyl chain) but located at different positions on the heterocyclic ring.

The reaction of 5-bromo-2,4-dichloropyrimidine with cycloheptylamine (Scheme 8.3) resulted in the displacement of the chlorine atom at position 4 in 95% yield and 75% selectivity. The 5-bromo-4-chloro-N-cycloheptylpyrimidin-2-amine regioisomer, formed simultaneously as a side product, was effectively separated by preparative flash chromatography. Suzuki coupling with 4-(trifluoromethyl)phenylboronic acid using the [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex as catalyst in dichloromethane allowed regioselective substitution of the bromine atom to give the monochlorinated pyrimidine intermediate. Subsequently, the remaining chlorine was replaced by treatment of the original compound with potassium cyanide to afford the cyano derivative, which was in turn hydrolyzed under basic conditions to afford the corresponding carboxylic acid derivative, which was isolated in excellent yield after crystallization from ethanol. The carboxylic acid was then efficiently esterified under standard conditions, and the ethyl ester



Scheme 8.3 Reagents and conditions: (*a*) cycloheptylamine, TEA, THF, 0 °C to rt, 16 h; (*b*) 4-(trifluoromethyl)phenylboronic acid, Pd(dppf)Cl_2*DCM, Na_2CO_3, DME, 85 °C, 16 h; (*c*) KCN, DMSO, 110 °C, 16 h; (*d*) 1 M NaOH, EtOH, 110 °C, 4 h; (*e*) AcCl, EtOH, reflux, 16 h; (*f*) $NaBH_4$, *n*-BuOH/THF, 0 °C to rt

obtained was reduced with sodium borohydride in n-butanol/H_2O in the final synthetic step to afford KK-92A in 80% yield (Li et al. 2017). Among the approximately 100 analogs synthesized and studied, KK-92A showed the best PAM properties at the $GABA_B$ receptor in several cell-based functional assays, high exposure in the brain, and remarkable in vivo efficacy, especially the selective reducing effect on intravenous nicotine self-administration and cue-induced reinstatement of nicotine seeking in rats without affecting the response to food (Li et al. 2017). In an effort to extend the anti-addictive properties of this new $GABA_B$ receptor PAM to alcohol, a subsequent study demonstrated that acute treatment with KK-92A was effective in reducing operant oral self-administration and cue-induced reinstatement of alcohol-seeking behavior in sP rats (Maccioni et al. 2021). These results confirm that the ability to inhibit alcohol-motivated behaviors in rodents is common to the entire class of $GABA_B$ receptor PAMs (see Chap. 12 of this volume). However, the high potency of KK-92A in inhibiting addiction-related behaviors at doses that do not produce sedative effects or other locomotor impairment may be explained by its particular ago-allosteric profile. In vitro assays have shown that, in addition to potentiating GABA-induced cellular responses ($GABA_B$ receptor PAM activity), KK-92A also exerts a distinct, intrinsic agonistic activity that activates the $GABA_B$ receptor in the absence of GABA, similarly to its precursor BHF177 (Li et al. 2017; Maccioni et al. 2021).

8.3.3 Condensed Ring Heterocycles

In an effort to logically represent the discovery of new $GABA_B$ receptor PAMs based on their structural similarity and chronological order of appearance, the compounds with a condensed heterocyclic structure, which make up the majority of new PAMs, are divided into two subgroups: the compounds that can be interpreted as direct successors of the first generation $GABA_B$ receptor PAMs, BHF177 and CMPPE, are shown in Fig. 8.3c1, while the compounds characterized by the presence of at least one carbonyl group belonging to a cyclic amide function are shown in Fig. 8.3c2.

In a 2015 patent from Astellas Pharma, approximately 300 sulfur-containing compounds (either thieno[2,3-*d*]pyrimidines or thiazolo[5,4-*d*]pyrimidines) were described as $GABA_B$ receptor PAMs (Shiraishi et al. 2015). In vivo screening (GTPγ^{35}S assay) of the new compounds identified 37 compounds as the most promising, with potency values between 0.54 and 0.0071 μM and efficacy values in the range of 88–300%. Confirmation of PAM activity using cells (HEK 293) stably expressing the $GABA_B$ receptor led to the selection of one compound, hereafter referred to as ASP8062 (Fig. 8.3c1), to investigate its effect on improving short-term memory impairment (Y-maze test) and its analgesic activity against reserpine-induced muscle pain (Shiraishi et al. 2015) and fibromyalgia in rats (Murai et al. 2019). The remarkably good results of these studies have led to ASP8062 being taken forward in phase I studies to investigate its pharmacokinetic profile, its

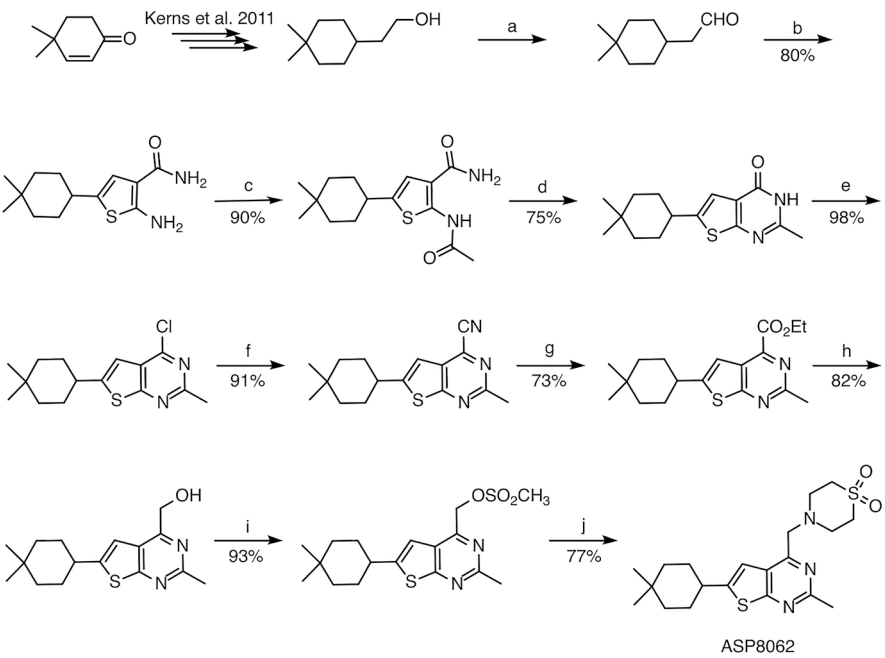
pharmacodynamic effects on the CNS, and its tolerability (Walzer et al. 2020; Walzer et al. 2021). Overall, ASP8062 has been shown to be a $GABA_B$ receptor PAM with a favorable pharmacokinetic profile, acceptable safety at all doses after single and multiple administrations, and satisfactory tolerability, with the most common adverse events such as dizziness and headache rated as mild. More recently, ASP8062 has been considered as a potential treatment for various forms of substance use disorder (SUD), particularly AUD and opioid use disorder (OUD). The results of these studies showed that ASP8062 is able to reduce alcohol self-administration and the amount consumed in both male and female rats, with the effect being stronger in male rats (Haile et al. 2021; see also Chap. 12 of this volume). ASP8062 also suppressed morphine self-administration in a nonhuman primate self-administration model without enhancing morphine-induced respiratory suppression, a known potentially lethal side effect of opioids, especially when used in combination with sedative or illicit substances (Akuzawa et al. 2023; Ito et al. 2023). Overall, these results supported the further development of ASP8062 as a potential treatment for AUD and OUD. Accordingly, ASP8062 has completed a phase II clinical trial whose primary objective was to evaluate the effects of 25 mg once daily on alcohol cue-induced craving in a human laboratory paradigm after 2 weeks of daily dosing in subjects with moderate to severe AUD. Secondary objectives included evaluating the effect of ASP8062 on reducing alcohol consumption, alcohol craving, cigarette smoking (in smokers) and nicotine consumption (in nicotine users), mood, sleep, adverse consequences of alcohol consumption, study retention, and safety and tolerability (ClinicalTrials.gov 2024).

A comparison of the physicochemical properties of ASP8062 with those of other $GABA_B$ receptor PAMs to which it is structurally more closely related, namely, CMPPE, BHF177, and KK-92A, using the SwissADME web tool (Daina et al. 2017) reveals essentially two seemingly modest but actually significant differences. ASP8062 has a calculated TPSA (topological polar surface area) value of 99.8 $Å^2$ compared to a value of $\leq$58 $Å^2$ for the other compounds: this property may have a critical impact on water solubility, intestinal absorption, and BBB penetration. ASP8062 is a crystalline substance that is practically insoluble in water and slightly soluble in ethanol. It has a basic nitrogen atom, which should enable the formation of water-soluble and physiologically acceptable salts. However, as far as is known, ASP8062 has never been administered intravenously, neither in humans nor in animals. The impracticability of this route of administration can reasonably, if not conclusively, be interpreted as a consequence of the rapid hydrolysis of ASP8062 salts in water with precipitation of the insoluble neutral substance. In fact, ASP8062 was administered intragastrically as a 5% suspension in methylcellulose in rats and monkeys, whereas in humans it was administered as a tablet together with 240 ml of water.

After a single dose of 30 mg or 70 mg ASP8062, intestinal absorption varied from very rapid to slow in different subjects, with a median time to maximum plasma concentration (T_{max}) of 4 and 2.5 h, respectively. The distribution of ASP8062 in cerebrospinal fluid (CSF) after a single dose was also rapid and similar to that in plasma but slightly delayed; median T_{max} times of 5 and 3 h were observed

in CSF after a single dose of 10 mg and 70 mg, respectively. The concentrations of ASP8062 in CSF persisted for 24 h. These data justify the daily oral dosage of 25 mg used in the clinical studies. In vitro studies in liver tissue fractions from mice, rats, rabbits, dogs, monkeys, and humans have shown that the metabolism of ASP8062 is mediated by P450 cytochrome CYP3A4 and to a lesser extent by CYP2D6. These oxidation processes produce the major metabolites AS3486189, AS3486191, and AS3486192 (structure unknown), but do not appear to significantly reduce maximum plasma concentration (C_{max}) or half-life. Most importantly, they do not lead to interaction problems when coadministered with morphine in OUD treatment, as the latter is mainly metabolized by conjugation with glucuronic acid and not by cytochrome P450 (CYP)-mediated oxidation. Taken together, these results justify the further development of ASP8062 as a potential treatment option for OUD.

The chemical synthesis of ASP8062 according to the 2015 patent (Shiraishi et al. 2015) comprises ten synthetic steps (Scheme 8.4), starting from 2-(4,4-dimethylcyclohexyl)ethanol as starting material, which in turn must be prepared in four steps from commercially available 4,4-dimethylcyclohexenone, as



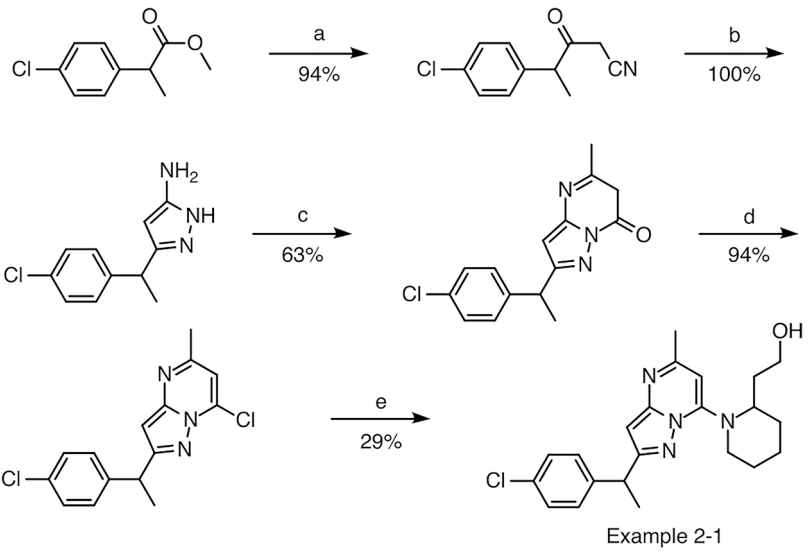
Scheme 8.4 Reagents and conditions: (*a*) sulfur trioxide/pyridine complex, DMSO, TEA, DCM, 2 h; (*b*) cyanoacetamide, sulfur, TEA, DMF, 60 °C, 12 h; (*c*) AcCl, pyridine, DCM, 0 °C to rt, 1.5 h; (*d*) 2 M NaOH, EtOH, 80 °C, 2 h, then 1 M HCl, rt; (*e*) $POCl_3$, DMF, toluene, 130 °C, 2 h; (*f*) CH_3SO_2Na, KCN, DMF, 70 °C, 15 h; (*g*) 4 M HCl/dioxane, EtOH, 80 °C, overnight; (*h*) $NaBH_4$, $CaCl_2$, THF, EtOH, rt, 4.5 h; (*i*) CH_3SO_2Cl, TEA, DCM, 0 °C, 1 h; (*j*) thiomorpholine-1,1-dioxide, TEA, DMF, rt, overnight

described in the previous literature (Kerns et al. 2011). Oxidation of the primary alcohol with the sulfur trioxide/pyridine complex and DMSO gave the corresponding aldehyde, which was used without purification in the Gewald multicomponent condensation with cyanoacetamide and sulfur to afford the 2-aminothiophene-3-carboxamide derivative in 80% overall yield. After acetylation with acetyl chloride, NaOH-catalyzed cyclodehydration led to the first bicyclic intermediate thieno[2,3-*d*]pyrimidinone (73% yield overall), which underwent several steps of functional group interconversion, namely, chlorination with $POCl_3$, replacement of chlorine by sodium methanesulfinate/potassium cyanide, conversion of the nitrile to an ester, and selective reduction of the ester to alcohol. This sequence gave (6-(4,4-dimethylcyclohexyl)-2-methylthieno[2,3-d]pyrimidin-4-yl)methanol in a very satisfactory overall yield of 53%. Conversion of this last intermediate to the more electrophilic methanesulfonate (93% yield) allowed incorporation of the thiomorpholine 1,1-dioxide moiety to afford ASP8062 in 77% yield. Although this synthesis is quite lengthy, it is still quite efficient in terms of overall yield (> 22%) and feasibility. This is obviously another strong point for the clinical development candidate ASP8062.

AbbVie has also entered the field of $GABA_B$ receptor PAMs and has published several patents since 2016 (Faghih et al. 2016) describing around 200 compounds, all based on the bicyclic pyrazolo[1,5-*a*]pyrimidine system. All of the compounds described have very close structural similarities to the CMPPE produced by GlaxoSmithKline (Fig. 8.2e), and one of them is shown as an example (Fig. 8.3c1, Example 2-1).

The only real difference from CMPPE is the distancing of the 4-chlorophenyl group from the heterocyclic core, which was realized in the AbbVie compounds: although this structural diversity looks modest, it is significant as it increases the conformational freedom of the new compounds while reducing the conjugation of the aromatic systems, which should lead to an improvement in solubility. In the GTPγ^{35}S binding assay, the new compounds showed EC_{50} values between >10 and 0.003 μM, with five compounds exhibiting the highest activity (EC_{50} < 10 nM), but no further evaluation in vitro and/or in vivo was subsequently reported. In terms of SAR, all the best $GABA_B$ receptor modulators have longer chains (ethyl, propyl, isopropyl) than the methyl attached to the benzylic carbon atom of Example 2-1.

Scheme 8.5 shows the synthesis of this last compound, which can be clearly deduced from the AbbVie patent (Faghih et al. 2016). Conversion of acetonitrile to its enolate with *n*-butyllithium and subsequent reaction with 2-(4-chlorophenyl)propanoate in tetrahydrofuran (THF) afforded the corresponding β-ketonitrile, which was reacted with hydrazine hydrate to give the intermediate 3-aminopyrazole derivative in an overall yield of about 94% over two steps. Condensation with ethyl 3-oxobutanoate in refluxing AcOH led to the bicyclic intermediate, which was converted to the chlorinated compound by treatment with phosphoryl chloride (59% overall yield). Displacement of the chlorine atom by nucleophilic substitution with 2-(2-hydroxyethyl)piperidine finally afforded the target compound in a low yield of 29%, probably due to a steric hindrance in the reaction between the two regents or difficulties in product purification or both.

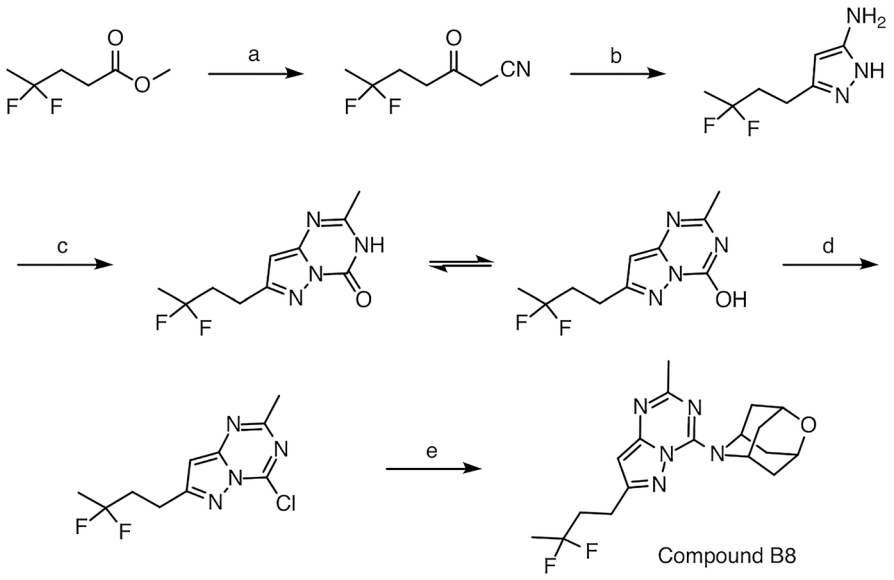


Scheme 8.5 Reagents and conditions: (*a*) ACN, *n*-BuLi, THF, −75 °C to rt, overnight; (*b*) N_2H_4, EtOH, reflux, 3 h; (*c*) ethyl 3-oxobutanoate, AcOH, reflux, 3 h; (*d*) $POCl_3$, ACN, reflux, 8 h; (*e*) 2-(2-hydroxyethyl)piperidine, DIPEA, EtOH, 50 °C, 12 h

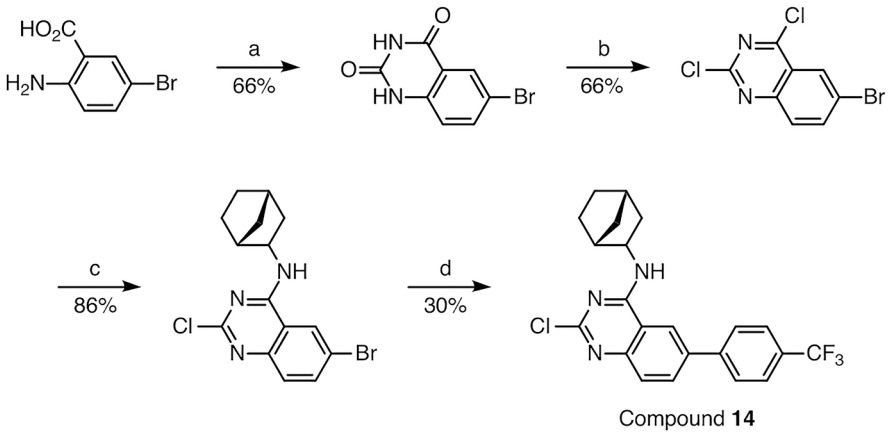
In a single patent, Taisho Pharmaceutical (Shimono et al. 2017) covered analogs of CMPPE in which the core heterocycle was replaced by pyrazolo[1,5-*a*][1,3,5]triazine. Pharmacological data have not been provided for any of the new compounds designated as $GABA_B$ receptor PAMs; moreover, the synthesis of the representative Compound B8 (Fig. 8.3c1) can only be inferred and summarized as an example in Scheme 8.6 (no yield can be reported).

The last members of the $GABA_B$ receptor PAMs to appear in the literature were quinazoline compounds substituted at position 6 with an aryl group (Huszár et al. 2022). The analysis of the structure-activity relationship has shown that the aryl group must have nonpolar and lipophilic substituents, preferably in position 4, to ensure advantages both in terms of pharmacological activity in vitro and metabolic stability. The chlorine in position 2 is preferable to the methyl in order to significantly improve the pharmacokinetic profile. Examination of the structure of compound 14 (Fig. 8.3c1), which serves as an example for the series, highlights a very close similarity to BHF177, which was in fact the model for the development of the new quinazoline compounds: surprisingly, the most recent PAMs are conceptually derived from one of the "older," albeit more important, PAMs. The synthesis of compound 14 is described in Scheme 8.7.

In the in vitro assay ($GTP\gamma^{35}S$ binding), compound 14 showed a 4.1-fold increase in the $GTP\gamma^{35}S$ binding response compared to the corresponding GABA-only response and an EC_{50} as PAM of 637 nM, values all comparable to those of BHF177. Conversely, the metabolic stability of compound 14 in mouse, rat, and human microsomes is significantly better than that of the reference compound. Other



Scheme 8.6 Reagents and conditions: (*a*) MeCN, BuLi, dry THF, −78 to −40 °C, 2 h; (*b*) N_2H_4, EtOH, 80 °C, 7 h; (*c*) ethylacetimidate hydrochloride, AcOH, MeCN, rt, 16 h; (*d*) $POCl_3$, *N*,*N*-diethylaniline, ACN, 80 °C, 4 h; (*e*) 2-oxa-6-azaadamantane, DIPEA, isopropanol, 80 °C, 1 h, then rt, 16 h



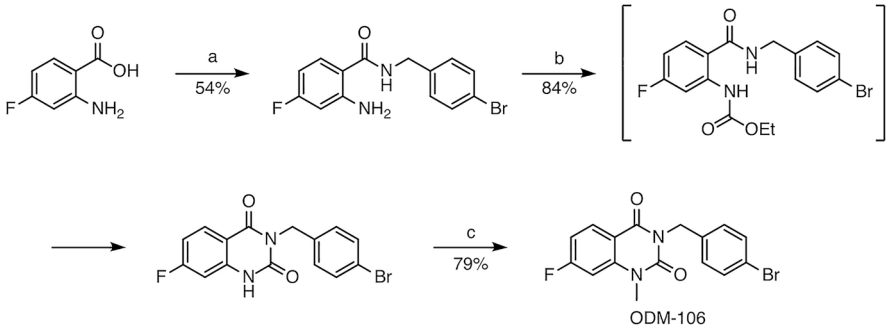
Scheme 8.7 Reagents and conditions: (*a*) urea, 150 °C, 25 h; (*b*) $POCl_3$, DIPEA, 100 °C, 16 h; (*c*) 2-aminonorbornane, K_2CO_3, MeOH, rt, 24 h; (*d*) 4-(trifluoromethyl)phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, dioxane, water, rt, 24 h

pharmacokinetic parameters ($AUC_{0–24h}$, C_{max}, T_{max}, $T_{1/2}$) also guarantee oral bioavailability and penetration into the brain. Indeed, compound 14 significantly enhanced skeletal muscle relaxation in the baclofen-induced traction test after oral administration in mice.

Despite the large number of quinazoline-2,4-dione compounds already described in both the scientific literature and patents, Orion Corporation filed a patent in 2015 investigating new pharmacologically active quinazolinedione derivatives as $GABA_B$ receptor PAMs. Some of these compounds, including ODM-106 (Fig. 8.3c2), were reported to have enhanced positive allosteric $GABA_B$ receptor modulator activity and reduced direct agonist activity (Prusis et al. 2015). From a structural perspective, these quinazolinedione compounds are no different conceptually from the xanthine derivatives reported by AstraZeneca in 2008 (Fig. 8.2e, Example 40), as they are bioisosteres obtained via a scaffold hopping approach in which the imidazole core of the xanthine system was replaced by a benzene ring.

The synthesis of ODM-106 was easily accomplished by a three-step procedure (Scheme 8.8). A solution of 2-amino-4-fluorobenzoic acid and 4-bromobenzylamine in ethyl acetate was treated overnight at room temperature with propanephosphonic acid anhydride (T3P) as coupling agent to afford 2-amino-*N*-(4-bromobenzyl)-4-fluorobenzamide in about 54% yield after crystallization from toluene. This intermediate was dissolved in THF and reacted with ethyl chloroformate in the presence of pyridine. The resulting carbamate was directly converted to 3-(4-bromobenzyl)-7-fluoroquinazoline-2,4(1*H*,3*H*)-dione in 84% yield by intramolecular *N*-acylation catalyzed by 5 M NaOH solution. Final alkylation with iodomethane in DMF using solid NaOH as base gave crude 3-(4-bromobenzyl)-7-fluoro-1-methylquinazoline-2,4(1*H*,3*H*)-dione, which was recrystallized from a mixture of acetonitrile/ethanol (95:5) to give pure ODM-106 in 79% yield.

In 2016, Orion Corporation completed an initial phase I human clinical trial to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of ODM-106 in healthy (male) volunteers (ClinicalTrials.gov NCT02393950 2023).

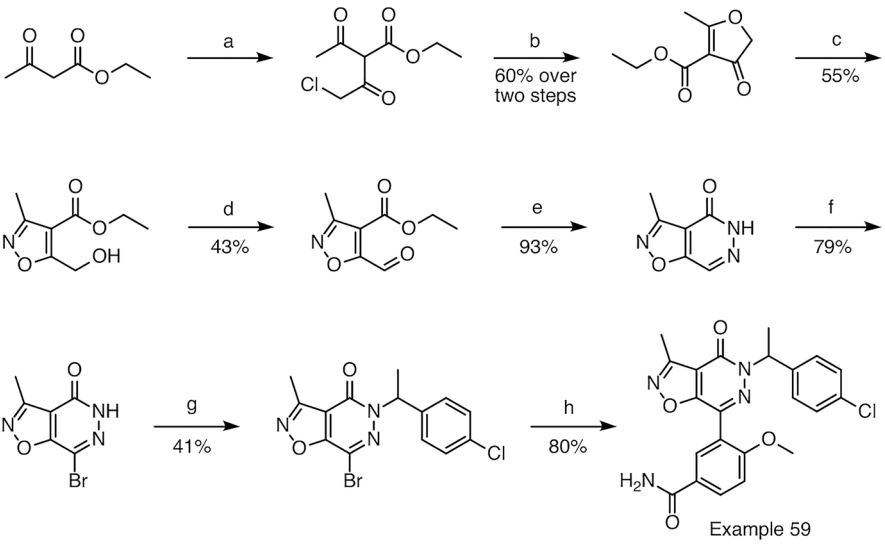


Scheme 8.8 Reagents and conditions: (*a*) 4-bromobenzylamine, T3P, TEA, EtOAc, rt, overnight; (*b*) (i) ethyl chloroformate, pyridine, dry THF, N_2 atmosphere, 0 °C to rt, 2 h; (ii) 5 M NaOH, 0 °C to 50 °C, 1 h; (*c*) NaOH, DMF, N_2 atmosphere, rt, 15 min, then iodomethane, rt, 1 h

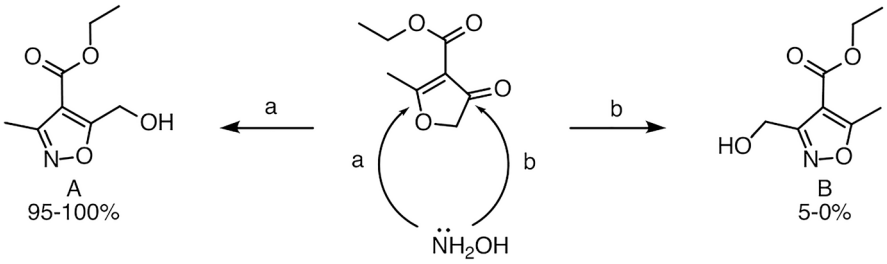
Anyhow, ODM-106 is a neutral molecule with pH-independent solubility and proved to be a virtually water-insoluble drug molecule that requires enhancement of dissolution by nanomilling techniques (Li et al. 2016). After nanonization (Oliveira Da Silva et al. 2020), ODM-106 showed good in vitro dissolution behavior and was administered to subjects as both micronized particles and nanoparticles. However, surprisingly low bioavailability was observed in in vivo studies, and inexplicably no in vitro-in vivo correlation was found (Singhal et al. 2022).

In 2017 AbbVie filed another patent application for new $GABA_B$ receptor PAMs that appear to be structurally unrelated to the previous ones and have a comparable, but not better, pharmacological profile in vitro (Dinges et al. 2017). Among the 131 compounds tested in the GTPγ^{35}S binding assay, eight of them had EC_{50} values between 0.017 and 0.056 μM, but again, no further results were reported in relation to in vitro-in vivo pharmacological studies.

The synthesis of all compounds is well described in the patent, so that it was possible to analyze the synthesis of the best compound labeled Example 59 (Fig. 8.3c2) and report it as a general example (Scheme 8.9). Acylation of the magnesium enolate of ethyl acetoacetate with chloroacetyl chloride followed by cyclization of the intermediate by intramolecular alkylation of the thermodynamically more stable enolate formed at room temperature using a weak base (TEA) yielded the 4-oxo-4,5-dihydrofuran derivative. The subsequent reaction with hydroxylamine



Scheme 8.9 Reagents and conditions: (*a*) (i) $Mg(OH)_2$, toluene, 0 °C to rt, 1 h; (ii) AcCl, ACN, −10 °C to rt, 2 h; (*b*) TEA, *tert*-butyl methyl ether, rt, overnight; (*c*) hydroxylamine hydrochloride, AcONa, dry EtOH, reflux, 1 h; (*d*) MnO_2, toluene, reflux, 6 h; (*e*) N_2H_4, EtOH, 0–5 °C, 2 h; (*f*) LiOH, Br_2, MeOH, reflux, overnight; (*g*) 1-(1-bromoethyl)-4-chlorobenzene, K_2CO_3, DMF, rt, 4 h; (*h*) 4-methoxy-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzamide, tetrakis(triphenylphoshine)-palladium(0), 2 M $NaHCO_3$, toluene, MeOH, μw irradiation, 130 °C, 30 min



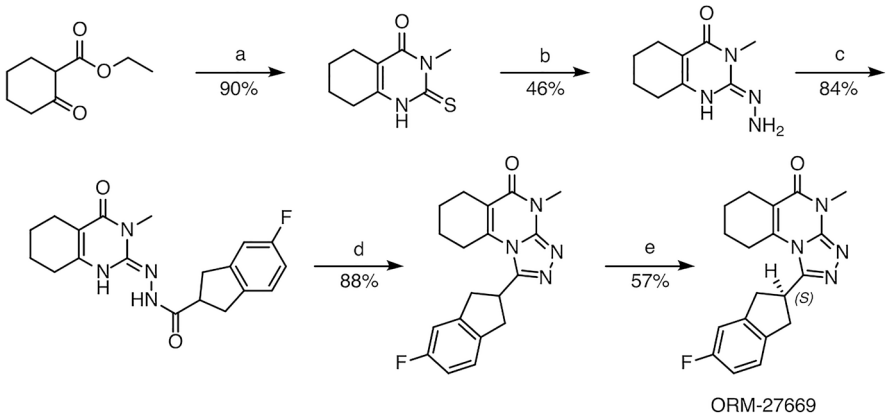
Scheme 8.10 Alternative pathways for 4-ethoxycarbonyl-3(2*H*)-furanone ring cleavage-ring closure rearrangement by reaction with hydroxylamine hydrochloride

hydrochloride in the presence of sodium acetate in refluxing EtOH led to the first isoxazole intermediate, namely, ethyl 5-(hydroxymethyl)-3-methylisoxazole-4-carboxylate, in 55% yield. This synthetic step may deserve a more detailed analysis (Scheme 8.10). Although in principle one might expect the reaction of hydroxylamine with 4-ethoxycarbonyl-3(2*H*)-furanone to give two isomeric isoxazoles A and B, it leads to the exclusive or predominant formation of product A; only a small amount of the isomeric compound B can be detected in the crude reaction mixture (Deshayes et al. 1984). The regiospecificity of the reaction can be explained by the nucleophilic addition of the N atom of the hydroxylamine to the 5-position of the furanone ring (conjugate addition), which leads to a ring cleavage-ring closure rearrangement, resulting in the formation of compound A. The presence of the electron-withdrawing 4-ethoxycarbonyl substituent enhances the electrophilic character of the C5 carbon in 3(2*H*)-furanone and favors the selective formation of the desired isomeric isoxazole. The efficiency of this reaction laid the foundation for the synthesis of these new $GABA_B$ receptor PAMs with an isoxazolopyridazine structure. The 5-(hydroxymethyl)isoxazole derivative was oxidized with manganese(IV) oxide to the aldehyde (yield: 43%), which in turn was treated with hydrazine to give the bicyclic structure in 93% yield. Bromination at position 7 with bromine in the presence of lithium hydroxide, followed by palladium-catalyzed coupling with 4-methoxy-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzamide, afforded the final compound (Example 59) in about 38% yield over two steps.

ORM-27669 has appeared in the literature in 2019 as a new $GABA_B$ receptor PAM which belongs to the subgroup of condensed ring heterocycles with a cyclic amide moiety. It shares this structural motif with the compounds listed in the lower part of Fig. 8.3c2 but also borrows some others from previously described $GABA_B$ receptor PAMs. The only source of information on ORM-27669, which is manufactured by Orion Pharma, consists of a publication (de Miguel et al. 2019) describing both the synthesis and pharmacological evaluation of the compound, in particular its ability to suppress neuroadaptations in the ventral tegmental area (VTA) and alcohol- and cocaine-induced reward-related behaviors. Against a panel of 121 receptors, ORM-27669 proved to be a highly selective $GABA_B$ receptor PAM, but with low efficacy as an agonist, while the reference compound *rac*-BHFF showed a different allosteric profile. It was a more potent PAM in the calcium-based assay and

an agonist, coupled with potent PAM activity, in the GTPγ^{35}S binding assay in rat and human recombinant receptors. Pretreatment of mice with *rac*-BHFF at nonsedating doses effectively reversed both alcohol- and cocaine-induced plasticity and attenuated intravenous cocaine self-administration and alcohol consumption. Pretreatment with ORM-27669 only reversed alcohol-induced neuroplasticity and attenuated alcohol consumption but had no effect on cocaine-induced neuroplasticity or self-administration. These results may be due to the profile of *ago*-PAM for *rac*-BHFF and pure PAM for ORM-27669.

Scheme 8.11 shows the synthetic sequence used to obtain ORM-27669 in enantiomerically pure form. The base-catalyzed condensation of ethyl cyclohexanone-2-carboxylate with *N*-methylthiourea gave 3-methyl-2-thioxo-2,3,5,6,7,8-hexahydroquinazolin-4(1*H*)-one in 90% yield, which on heating with hydrazine hydrate in EtOH afforded the hydrazone derivative in an acceptable yield of 46%. The amidation reaction with 5-fluoro-2,3-dihydro-1*H*-indene-3-carboxylic acid using T3P as coupling reagent in DCM gave the acylhydrazone intermediate, which was subsequently cyclized to the racemic final product (74% yield in two steps) by heating at 140 °C for 30 min under microwave irradiation. Finally, the homochiral ORM-27669 was obtained as a white solid by chiral HPLC separation, and its configuration was determined as (*S*) by X-ray analysis of the crystal structure. In summary, ORM-27669 could be prepared in only four synthetic steps with an overall yield of 31%, which involved basic heterocyclic chemistry and subsequent chiral separation of the racemate. Notably, ORM-27669 is the only $GABA_B$ receptor PAM that was developed as a single enantiomer. This fact supports the hypothesis that the pharmacological activity of the compound is definitely enantioselective, even though no data are available to demonstrate the difference in activity between the eutomer and the distomer.

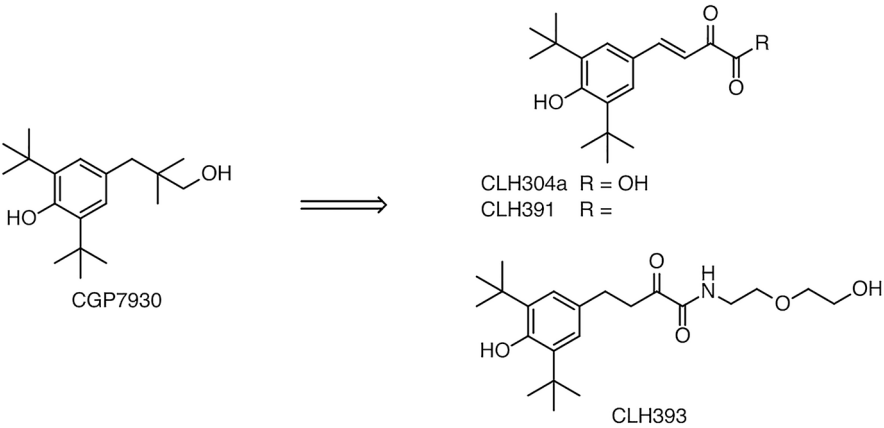


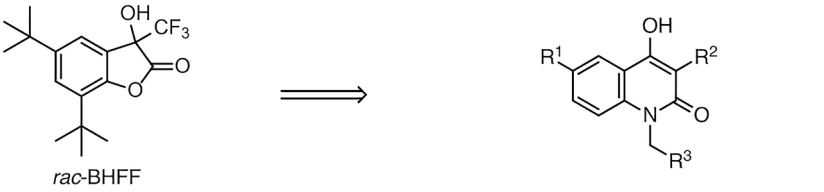
Scheme 8.11 Reagents and conditions: (*a*) *N*-methylthiourea, EtONa, EtOH, reflux, 2 h; (*b*) N_2H_4, EtOH, reflux, 4.5 h; (*c*) 5-fluoro-2,3-dihydro-1*H*-indene-2-carboxylic acid, T3P, TEA, DCM, 0 °C to rt, 4 h; (*d*) AcOH, μw, 140 °C, 30 min; (*e*) preparative chiral HPLC separation on Chiralpak IA, 0.2% DEA in EtOH/MTBE

8.4 Negative Allosteric Modulators

The first negative allosteric modulator (NAM) of $GABA_B$ receptor, namely, CLH304a (Fig. 8.5), was identified in 2014 (Chen et al. 2014) and is the result of a structural modification of the PAM CGP7930 (Mugnaini and Corelli 2016). CLH304a has been shown to decrease GABA-induced IP3 production and negatively modulate the activity of $GABA_B$ receptor as NAM via the heptahelical domain of $GABA_{B2}$ receptor subunits (Sun et al. 2016). Two amide derivatives of CLH304a, namely, CLH391 and CLH393 (Fig. 8.5), also showed comparable activity to the parent compound (Sun et al. 2016).

In the attempt to identify new $GABA_B$ receptor PAMs, the design and synthesis of new hybrid molecules obtained by combining the structures of already known





$R^1 = R^2 = R^3$ = isopropyl (COR758)
$R^1 = R^3$ = isopropyl; R^2 = *n*-butyl
$R^1 = R^3$ = isopropyl; R^2 = *tert*-butyl
$R^1 = R^2$ = *tert*-butyl; R^2 = isopropyl
R^1 = *tert*-butyl; $R^2 = R^3$ = isopropyl
R^1 = *tert*-butyl; R^2 = isopropyl; R^3 = methyl
R^1 = methoxyl; R^2 = ethyl; R^3 = methyl

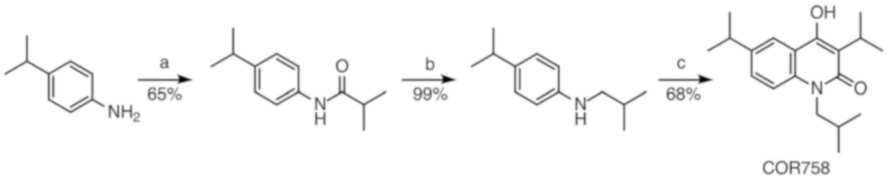
Fig. 8.5 Ideal derivation of NAMs from PAMs by crossing a thin boundary line between the two types of modulators

active compounds such as GS39783, BHF177, and *rac*-BHFF allowed the identification of several compounds belonging to different structural classes and exhibiting $GABA_B$ receptor-modulating activity (Mugnaini et al. 2020). The most promising compounds were obtained by manipulating the structure of *rac*-BHFF, a molecule in which the presence of an *O*-aryl-γ-lactone group and a chiral carbon atom bearing a particularly acidic tertiary alcohol raised some doubts about its chemical stability and developability. Replacing the benzofuran scaffold with a 2-quinolone system led to achiral, more stable, and easily accessible compounds that retain to some extent the same substitution pattern as *rac*-BHFF (Fig. 8.5). Unexpectedly, some of them significantly inhibited GABA-stimulated GTPγ^{35}S binding, revealing a functional switch with respect to the prototypical molecule.

In particular, the compound named COR758 attracted greater attention. Its synthesis is described in Scheme 8.12. The reaction of 4-isopropylaniline with isobutyryl chloride gave the amido derivative, which was reduced with lithium aluminum hydride (LAH) to the secondary amine. Heating at high temperature (270 °C) with diethyl isopropylmalonate afforded COR758 in about 44% overall yield.

COR758 inhibited GABA-stimulated GTPγ^{35}S binding but failed to displace the antagonist [^{3}H]CGP54626 from the orthosteric binding site of $GABA_B$ receptors, confirming its function as a NAM. Bioluminescence resonance energy transfer (BRET) measurements in living CHO cells stably expressing $GABA_B$ receptors showed that COR758 prevents receptor activation by GABA and dissociation of heterotrimeric G protein as well as GABA-mediated inhibition of adenylate cyclase activity. In addition, it inhibits ERK1/2 phosphorylation induced by baclofen and ago-PAM CGP7930 in CHO-$GABA_B$ cells, as well as the baclofen-induced increase in intracellular Ca^{2+} levels in HEK293, which expresses $GABA_{B1a}$ and $GABA_{B2}$ together with a chimeric Gα protein. Electrophysiological studies also showed that COR758 abolished baclofen-induced outward currents in dopamine neurons from VTA. Overall, these data suggest that COR758 acts as a NAM and negatively modulates $GABA_B$ receptor function/activity (Porcu et al. 2021).

A comparative analysis of the physicochemical properties of COR758 and the previously reported $GABA_B$ receptor NAM CLH304a revealed a slightly higher ClogP (4.20 vs. 3.53) and a lower TPSA (42.2 vs. 74.6 Å^2) for COR758, suggesting a more favorable lipophilicity profile that may facilitate permeation across the BBB when administered in vivo. It is hypothesized that the presence of an enolic OH in the structure of COR758 reduces the susceptibility to conjugation with glucuronic



Scheme 8.12 Reagents and conditions: (*a*) isobutyryl chloride, TEA, DCM, rt, 12 h; (*b*) LAH, dry THF, reflux, 5 h; (*c*) diethyl isopropylmalonate, diphenyl ether, 270 °C, 3 h

acid or sulfate compared to the phenolic OH of CLH304a, thus increasing the half-life of the compound, while the absence of the electrophilic α,β-unsaturated ketone may improve the toxicological profile. However, the potential advantages in terms of pharmacokinetic/toxicological properties of COR758 compared to CLH304a need to be confirmed in future in vivo studies.

8.5 Conclusions

Despite decades of scientific and industrial drug discovery efforts, only a few ligands targeting the $GABA_B$ receptor are known, including agonists, antagonists, and allosteric modulators. The only approved drug targeting the $GABA_B$ receptor is baclofen, which despite its multiple pharmacokinetic limitations is used as a muscle relaxant and antispastic agent.

The identification of PAMs and NAMs could potentially expand the therapeutic arsenal for the modulation of $GABA_B$ receptor. Although this receptor has long been considered an "undruggable" or "difficult to target" protein in terms of drug design (Xie et al. 2023), the recent availability of complete three-dimensional structures of the $GABA_B$ receptor makes the application of computational methods, such as structure-based drug design approaches, possible and very promising for the design of new PAMs (see Chap. 9 of this volume).

Until now, the development of allosteric modulators has had a very high attrition rate as it is challenging for reasons previously highlighted (see Chap. 1 of this volume). Furthermore, the binding modes and molecular mechanisms of allosteric modulators are still quite unclear, and multiple allosteric binding sites may exist (Shaye et al. 2020; Evenseth et al. 2020). The measurable functional effect of an allosteric modulator could also depend on the affinity of the compounds and the orthosteric ligand used, which could also complicate screening procedures due to a potentially biased signaling effect (Conn et al. 2014). The binding of an allosteric modulator may contribute to the stabilization of a receptor conformation induced by the agonist and thus to the activation of a specific signaling pathway that is unique to the particular ligand combination (Smith et al. 2018). It should also be noted that the allosteric binding site is not as highly conserved between species as the orthosteric site, so species-specific differences may influence the outcome of testing potential drug candidates in animal models (Conn et al. 2014). In addition, allosteric modulators are known to have flat or shallow SARs and can undergo a fundamental change in their mode of action (known as a "molecular switch") by even minor structural changes to the scaffold. Molecular switches and flat SARs were frequently observed in optimization processes of allosteric modulators of $GABA_B$ receptor, suggesting that structural series with this propensity should be discarded (Mugnaini and Corelli 2016).

As far as we know, no further reports on novel $GABA_B$ receptor PAMs had appeared by the end of 2023, when this chapter was finalized. ASP8062 currently appears to be at an advanced stage of testing, and overall it also appears to be the

most promising of all $GABA_B$ receptor PAMs investigated to date with regard to potential therapeutic application (Colombo 2024).

Following the comprehensive overview of the different scaffolds for PAMs of $GABA_B$ receptor published by Froestl (2010b), a recent review in this field was published in 2022 (Nieto et al. 2022). This paper also provides an overview of the current therapeutic use and potential clinical applications of $GABA_B$ receptor PAMs. The scope of potential therapeutic applications has been further expanded by a patent filed by the University of Florida Research Foundation (Martemyanov and Sutton 2023), which claims the combined use of a $GABA_B$ receptor activator (either orthosteric agonist or PAM) with an α2-adrenergic receptor (α2R) agonist for the treatment of stress-induced depression. Research on a stress-resistant antidepressant model has shown that activation of both receptors, $GABA_B$ receptor and α2R, is sufficient to alter the affective state of the model animals. Although the most thoroughly studied combination was ClonBac (a combination of clonidine and baclofen), a number of adrenergic drugs and the following $GABA_B$ PAMs were also considered: ADX71441, ASP8062, BHF177, *rac*-BHFF, CGS7930, CMPPE, COR659, GS39783, KK-92A, and ORM-27669. Although we cannot be certain that all the compounds mentioned were actually used in the experimental protocol, the mention of the above $GABA_B$ receptor PAMs suggests that they are now well established as pharmacological probes and potential drugs for clinical use.

The same cannot be said of the $GABA_B$ receptor NAMs. Although they are arousing great interest in basic scientific research, the application potential of these pharmacological agents has yet to be proven.

An innovative approach aimed at developing more selective drugs targeting the $GABA_B$ receptors that are actually involved in certain pathological conditions is attracting growing interest. The goal is to target protein-protein interactions relevant to diseases associated with specific $GABA_B$ receptors without affecting the function of uninvolved receptors. Targeting protein-protein interactions is therefore considered a promising avenue for the development of novel and highly specific therapies, even for proteins previously considered "undruggable." Initial preclinical endeavors targeting protein-protein interactions that play a role in neurological diseases are currently underway (see Chap. 1 of this volume).

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Chapter 9
$GABA_B$ Receptor Positive Allosteric Modulators: Novel Approaches for Drug Design and Discovery

Linn Samira Mari Evenseth

Abstract The $GABA_B$ receptor plays a crucial role in the central nervous system by mediating neurotransmission of GABA, the main inhibitory neurotransmitter. Dysfunction associated with the receptor function has been linked to various diseases and neuropsychiatric disorders, making it a highly relevant drug target over the past decades. Traditionally, drug discovery campaigns targeting the $GABA_B$ receptor have focused on the discovery and development of ligands targeting the binding site of the endogenous ligand (the orthosteric site). However, targeting the allosteric binding sites of the $GABA_B$ receptor has emerged as a new and promising approach for developing more selective drugs with improved therapeutic effects as multiple cryo-EM structures of the full $GABA_B$ receptor have become available and have even led to the identification of a new allosteric binding site. The recent availability of full $GABA_B$ receptor structures provides an excellent starting point for applying methods relying heavily on structure data, such as computer-aided molecular simulations and structure-based drug design, to increase our understanding of allosteric interactions as well as discovery and development of new positive allosteric modulators targeting the receptor.

Keywords G protein-coupled receptors · Allosteric binding sites · $GABA_B$ positive allosteric modulators · 3D structure determination · Computer-aided drug discovery · Structural dynamics

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9.1 Structure and Mechanism of the $GABA_B$ Receptor

The γ-aminobutyric acid (GABA) type-B ($GABA_B$) receptor is an obligate heterodimer composed of the $GABA_{B1}$ and $GABA_{B2}$ subunits. Each of the subunits consists of an extracellular Venus flytrap (VFT) domain connected to a heptahelical transmembrane (7TM) domain by a short linker (Geng et al. 2013; see also Chap. 1 of this volume) (Fig. 9.1). The extracellular VFT domains consist of an N-terminal Lobe (LB1) and a C-terminal Lobe (LB2), and the orthosteric binding site is located in the crevice between LB1 and LB2 of $GABA_{B1}$. Agonist binding in the $GABA_{B1}$ VFT domains induces large conformational changes resulting in a closed conformation of the VFT domain, while the $GABA_{B2}$ VFT domain remains in an open conformation (Geng et al. 2012). The conformational changes upon receptor activation are found to be less extensive compared to other class C members, and studies suggest that the open/inactive and closed/active conformations are the main states of the VFT domain unlike the oscillating behavior observed, e.g., metabotropic glutamate (mGlu) receptors (Geng et al. 2013; Olofsson et al. 2014; Lecat-Guillet et al. 2017; Koehl et al. 2019; Grushevskyi et al. 2019; Evenseth et al. 2020b; Mao et al. 2020).

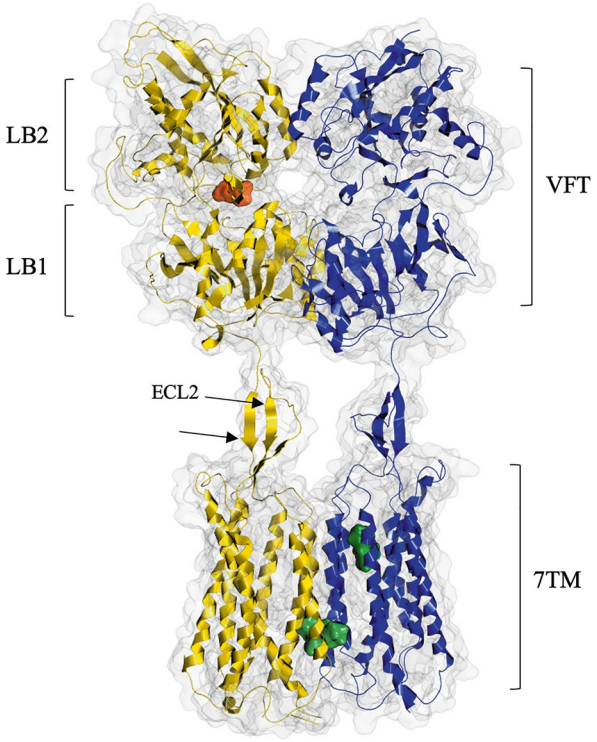

Fig. 9.1 A schematic representation of the $GABA_B$ receptor heterodimer ($GABA_{B1}$ in yellow, $GABA_{B2}$ in blue) with the Venus flytrap (VFT) domain connected by a linker to the heptahelical transmembrane domain (TM7) in the active state. The allosteric binding pockets located in the interface of the 7TMs and the 7TM of $GABA_{B2}$ are illustrated in green, and the orthosteric binding pocket located in the $GABA_{B1}$ is illustrated in orange

In the inactive $GABA_B$ receptor conformation, the 7TM domains form an interface involving TM3 ($GABA_{B1}$)-TM5 ($GABA_{B2}$) and TM5 ($GABA_{B1}$)-TM3 ($GABA_{B2}$) (Xue et al. 2019; Mao et al. 2020; Park et al. 2020). Upon receptor activation, the domains rotate to form an interface involving the TM6 of both monomers and binding of the $G_{i/o}$ promoted by intracellular loop 3 (ICL3) (Koehl et al. 2019; Xue et al. 2019; Mao et al. 2020; Kim et al. 2020). The linker connecting the VFT domain to the 7TM is believed to contribute to stabilizing the active conformation of the 7TM via extensive interactions with the extracellular loop 2 (ECL2) (Fig. 9.1) (Shaye et al. 2020; Kim et al. 2020). Structural data also suggest that the TM6/TM6 interface is difficult to stabilize without G proteins and/or a positive allosteric modulator (PAM), and all the available structures of the active receptor are in complex with a PAM stabilizing the interface (Fig. 9.1).

Early studies of the $GABA_B$ receptor have provided evidence that an allosteric binding site is located in the 7TM of $GABA_{B2}$ (Binet et al. 2004; Pin and Prezeau 2007; Kniazeff et al. 2016) which has recently been confirmed by the release of multiple cryo-electron microscopy (cryo-EM) structures of the full receptor (Fig. 9.1). In addition, the structures show the presence of a phospholipid at a site analogous to the orthosteric site in most family A G protein-coupled receptors (GPCRs) (Mao et al. 2020; Papasergi-Scott et al. 2020; Park et al. 2020). The lipid is suggested to contribute to the structural integrity of the 7TMs and is found to be present in both the active and inactive conformation of the 7TMs (Papasergi-Scott et al. 2020; Park et al. 2020). The available structures have also revealed a second allosteric pocket located in the cavity between $GABA_{B1}$ TM6 and TM6 of $GABA_{B2}$, which has also been supported by mutational studies (Mao et al. 2020; Shaye et al. 2020).

The 7TM domain of $GABA_{B2}$ is responsible for recruiting and binding to the $G_{i/o}$ class of heterotrimeric G proteins (Galvez 2001; Margeta-Mitrovic et al. 2001; Binet et al. 2004). Nonetheless, one of the recently published cryo-EM structures displays a thermostable receptor conformation where $GABA_{B1}$ also couples to G proteins, and the authors suggest that due to steric hindrance, G proteins can only bind to one subunit at the time, favoring $GABA_{B2}$ as this was found to be the most frequent populated distribution (Mao et al. 2020).

9.2 The $GABA_B$ Receptor as a Target in Drug Discovery

The $GABA_B$ receptor and its associated signaling pathway have been implicated in various diseases and neuropsychiatric disorders, including schizophrenia, addiction, anxiety, depression, epilepsy, and cancer to mention some. The involvement of the receptor across this broad spectrum of diseases emphasizes its considerable relevance as a therapeutic target. Since the initial characterization of the receptor over 40 years ago and subsequent successful cloning of it nearly 20 years later, multiple drug discovery efforts have been pursued over the past decades to identify new $GABA_B$ ligands with favorable pharmacokinetic profiles (Bowery et al. 1979;

Bowery et al. 1980; Hill and Bowery 1981; Kaupmann et al. 1997; Pilc and Nowak 2005; Tyacke et al. 2010; Lancaster et al. 2010; Varani et al. 2014; Heaney and Kinney 2016; Fatemi et al. 2017). Numerous GABA analogs have been developed, but despite the effort, the only approved drug targeting the $GABA_B$ receptor is the agonist baclofen, a muscle relaxant and antispastic drug (Herman et al. 1992; Pilc and Nowak 2005; Bowery 2016).

9.2.1 Orthosteric Ligands

Drug discovery campaigns targeting the $GABA_B$ receptor have primarily focused on the orthosteric binding site after the discovery of baclofen, a GABA analog synthesized in 1962 (Keberle et al. 1969). Baclofen has served as a starting point for great efforts aiming at developing analogs with improved properties but is currently the only drug in clinical use targeting the receptor. However, the efforts have led to the discovery of other clinically approved drugs, pregabalin, vigabatrin, and gabapentin, exhibiting other mechanisms of action than GABA (Corelli and Mugnaini 2016). Also, a number of both high- and low-affinity $GABA_B$ receptor antagonists have been derived from GABA and baclofen, and some have been developed as radioligands (Corelli and Mugnaini 2016). The analogs have provided insight into chemical diversity and structure-activity relationships of ligands, and some have also been co-crystalized with the $GABA_{B1}$ VFT domain (Geng et al. 2013).

9.2.2 Allosteric Ligands

It is well established that allosteric ligands can modulate the affinity and/or efficacy of orthosteric ligands, and they are divided into categories based on their mode of action. PAMs enhance receptor activity, and negative allosteric modulators (NAMs) reduce the activity, both without intrinsic activity. Some modulators can also bind without affecting the action of the orthosteric ligand besides competing and/or potentially blocking the site for other modulators (neutral allosteric ligands, NALs) (Christopoulos et al. 2014). Allosteric ligands can also have intrinsic efficacy and thereby affect signaling without the presence of an orthosteric ligand due to acting as an allosteric agonist or allosteric inverse agonist (Christopoulos et al. 2014). Biased allosteric modulators (BAMs) are another interesting category of allosteric ligands that are capable of affecting strength or activation of certain pathways over others, resulting in a functional selective response (Kolb et al. 2022).

The first PAM targeting the $GABA_B$ receptor, CGP7930, was discovered in a high-throughput screening campaign over 20 years ago and exhibited a dose-dependent potency and efficacy in the presence of GABA (Fig. 9.2) (Urwyler et al. 2001). The compound was later classified as an ago-PAM and showed antidepressant and anxiolytic effects in animal models, in addition to a reduction in

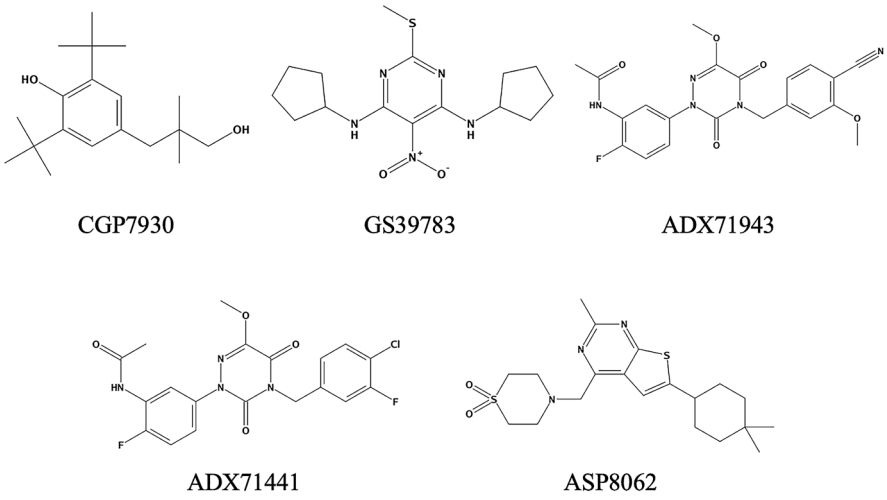

Fig. 9.2 The chemical structure of the ago-PAM CGP7930 and the PAMs GS39783, ADX71943, ADX71441, and ASP8062

addiction-related behavior toward alcohol and cocaine (Mugnaini and Corelli 2016; see also Chaps. 8, 10, 11, and 12 of this volume). The ago-PAM has served as a starting point for synthesizing a series of analogs, also resulting in the discovery of the first NAM targeting the $GABA_B$ receptor in 2014 (Chen et al. 2014). Some years after the discovery of CGP7930, a new series of PAMs were discovered, including GS39783, showing similar effects on affective disorders as seen for CGP7930 (Fig. 9.2) (Mugnaini and Corelli 2016; see also Chap. 8 of this volume).

In 2008, Addex Therapeutics patented over 300 compounds that were discovered by high-throughput screening and lead optimization (Mugnaini and Corelli 2016). The screening campaign resulted in 23 compounds with satisfactory in vitro activity which was further investigated for their effect on anxiety and pain. Among these, only a few hits were chosen for further development such as the PAMs ADX71943 and ADX71441, which unfortunately later showed poor safety profiles and/or no effect on anxiety models (Fig. 9.2).

Despite large efforts by pharmaceutical companies such as Roche, Addex, and AstraZeneca, there are currently no PAMs approved for clinical usage. Nevertheless, a newly discovered PAM, ASP8062, was recently investigated in a phase II clinical trial according to the US National Institutes of Health (ClinicalTrial.gov 2024) for its potential in the treatment of alcohol use disorder (Fig. 9.2) (Shiraishi et al. 2015; Haile et al. 2021; see also Chap. 12 of this volume) and is thereby one of the most promising compounds among the currently known allosteric modulators (see Chap. 8 of this volume).

9.3 The Allosteric Binding Site as a Drug Target

Drug discovery campaigns targeting GPCRs have traditionally focused on discovery of ligands targeting the orthosteric binding site. While this strategy has been highly successful yielding numerous approved drugs, including 27 out of the 100 bestselling drugs in 2020, the conserved nature of the orthosteric site between closely related receptor subtypes makes it highly challenging to obtain selective compounds (Evenseth et al. 2020a; Persechino et al. 2022).

Recent advancements in methods for three-dimensional (3D) structure determination of GPCRs in complex with different allosteric ligands have led to the identification of new allosteric binding sites, in addition to atomic details of ligand interactions. This has significantly increased our understanding of the molecular mechanism of allosteric modulation, and targeting the allosteric site(s) has in recent years emerged as a new and promising approach for developing more selective compounds with less off-target effects (Persechino et al. 2022). Among the benefits of targeting allosteric pockets is that they show lower evolutionary conservation and thereby have a higher potential for target selectivity, as well as selective cooperativity between the allosteric site(s) and the orthosteric and/or the effector proteins coupling site (Kenakin and Miller 2010; Wootten et al. 2013). Furthermore, pure PAMs and NAMs only exhibit activity in the presence of the endogenous orthosteric ligands and thereby preserve the spatial and temporal aspects for signaling in tissues where the ligand is exerting its effects (Wootten et al. 2013; Urwyler 2016). Interestingly, upon saturation of an allosteric ligand, additional effect of further orthosteric stimulation cannot be observed due to the cooperativity between the sites, thereby protecting patients from overdosing (Wootten et al. 2013).

Up until recently, only the 3D structures of the extracellular VFT domains hosting the orthosteric binding site have been publicly available, thus limiting our molecular understanding of allosteric modulation and application of structure-based drug discovery targeting other sites. Traditionally, in silico investigations including computer-aided drug design (CADD) targeting the allosteric binding pocket of the receptor have been confined to homology models—a 3D construct of the receptor based on its amino acid sequence (Sliwoski et al. 2014; Freyd et al. 2017). However, multiple cryo-EM structures of the full $GABA_B$ receptor in different functional states including the active and inactive conformation have been published in the past few years (Mao et al. 2020; Papasergi-Scott et al. 2020; Shaye et al. 2020). The structures present new opportunities for applying computer-aided approaches to increase our molecular understanding of receptor dynamics and further application of computational drug discovery methods focusing on the allosteric binding site.

9.4 Novel Approaches for Designing Positive Allosteric Modulators

High-throughput screening (HTS) campaigns are widely applied for the discovery of compounds with activity toward a validated therapeutic target and allow screening of large compound libraries. When a hit is discovered, it is further optimized and tested in relevant assays for characterizing pharmacological properties. The number of compounds that can be screened in an HTS has drastically increased in the last 15 years due to new technologies, automatization, computing power, and data management among other factors. Also, ligand binding assays only capable of screening a small number of compounds have been replaced with ultra-HTS methods capable of screening up to millions of compounds per week (Aherne et al. 2002). However, a considerable amount of time and money are associated with each step of the screening process—starting from the identification of a drug target to the discovery and optimization of hits to leads and finally marketing, which in the end normally takes 12–15 years and is estimated to cost billions of dollars (Mohs and Greig 2017).

CADD has emerged as a widely adopted strategy to address the challenges inherent in traditional drug discovery methods. By facilitating more efficient resource allocation toward experiments, CADD helps reduce both the time and expenses associated with drug discovery and development. Within CADD, structure-based drug design (SBDD) stands out as a particularly effective and robust approach for drug discovery and design. Nonetheless, SBDD heavily relies on the availability of 3D structures of the target proteins.

Despite the recent advancements in methods for solving 3D structures, also ensuring publication of full $GABA_B$ receptor structures, the number of publicly available protein structures remains a small fraction of all known protein sequences. To overcome this limitation, significant effort has been dedicated to predicting 3D structures from sequence data using computational techniques. Notably, cutting-edge machine learning methods such as Alphafold have shown promising results in predicting accurate 3D structures for a wide range of proteins and are aiming for nearly the entire human proteome (Tunyasuvunakool et al. 2021).

The currently available allosteric modulators targeting the $GABA_B$ receptor were all initially discovered through HTS campaigns and subsequent synthetic optimization, including deriving analogs from active compounds found during the process (Mugnaini and Corelli 2016; Urwyler et al. 2001, 2005). Computational methods have also been applied in the past to predict and characterize the 3D structure of the 7TM domain based on sequence data (Freyd et al. 2017). Nonetheless, as structural details regarding the $GABA_B$ 7TMs and allosteric binding sites were only recently publicly available, this can explain why there are currently no published studies describing discovery of PAMs targeting the receptor through CADD efforts.

As previously described, multiple 3D structures of the $GABA_B$ receptor representing inactive, intermediate, and active states have been published in recent years (Kim et al. 2020; Mao et al. 2020; Shaye et al. 2020). These structures show binding of a PAM in the 7TM of $GABA_{B2}$, as expected based on experimental studies, but

have also revealed a new allosteric binding site located in the intersubunit interface in the 7TM domains (Kim et al. 2020; Mao et al. 2020; Shaye et al. 2020). These structures provide an excellent opportunity to apply both conventional and state-of-the-art CADD methods as new approaches for discovering PAMs targeting the $GABA_B$ receptor. The final section of this chapter is dedicated to describing cutting-edge methods in CADD that have successfully been applied to identifying novel allosteric modulators of various GPCRs.

9.4.1 Computer-Aided Methods in Structural Biology and Drug Design

Recent advancements in determining the 3D structure of GPCRs have led to the discovery and characterization of previously unknown allosteric binding sites, as seen for the recently published structures of the full $GABA_B$ receptor. The growing availability of 3D structures together with an increased understanding of the regulatory mechanisms governing these specific allosteric sites has sparked a new interest in employing methods within SBDD for targeting these sites. In addition, the structures have served as excellent starting points for computer-based simulations to investigate receptor activation and conformational dynamics.

9.4.1.1 Virtual Screening

Virtual screening (VS) refers to computer-based methods aiming to identify molecules from virtual chemical libraries with a predicted complementarity and/or activity toward therapeutically relevant targets (Fig. 9.3). The approach complements experimental HTS as large and chemically diverse virtual libraries can be screened to identify potential drug candidates, thereby allowing researchers to prioritize compounds for further experimental testing and characterization (Fig. 9.3) (Shoichet 2004).

VS protocols encompass a wide array of computational techniques and algorithms but can roughly be categorized into two main categories: ligand-based drug design (LBDD) and SBDD (Sliwoski et al. 2014). LBDD is based on the basic principle that similar compounds have similar activity, and methods within this category utilize information on both active and inactive compounds to find new compounds with activity toward a target of interest (Sliwoski et al. 2014). SBDD methods rely on 3D structural information of a drug target and aim to identify compounds with complementary properties to a target binding pocket(s) (Sliwoski et al. 2014). Docking calculations are the predominant method within this category and are an approach where a compound is placed in the binding site of a target to predict its conformation, orientation, and binding energy (Sliwoski et al. 2014). A comprehensive study reviewing published VS protocols by Ripphausen et al. reported that

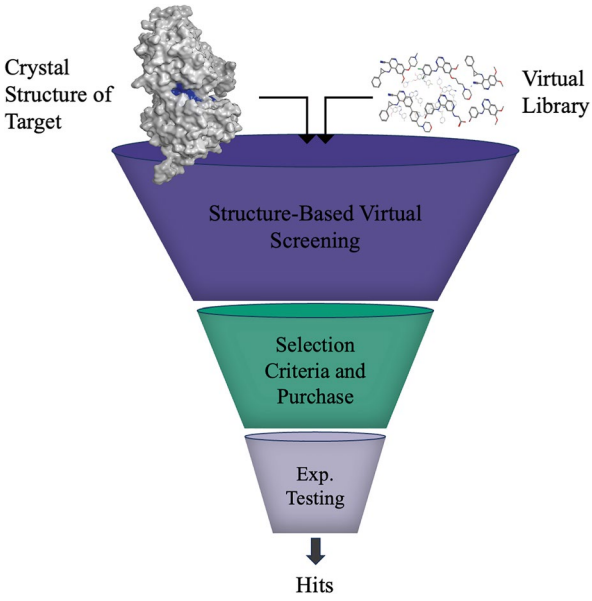

Fig. 9.3 A simplified representation of a structure-based virtual screening workflow, followed by evaluation and selection of compounds for experimental evaluation

structure-based methods were more commonly applied compared to ligand-based methods, despite that the latter methods identified compounds with higher potency (Ripphausen et al. 2010).

Multiple successful VS campaigns have been published, targeting a broad range of GPCRs, and have led to the discovery of novel ligands with activities down in the nanomolar range. Many of these studies also highlight how CADD approaches provide multiple benefits over conventional medicinal chemistry approaches such as identifying new chemotypes and higher hit rates to mention some (Maia et al. 2020).

9.4.1.2 From the Orthosteric to the Allosteric Binding Site(s)

A recently published review by Chen et al. summarizing the prospective applications of molecular docking targeting GPCRs supported by robust experimental validations reports that only 4 out of 72 studies targeted the allosteric binding site(s) (Chen et al. 2024). The first drug candidates originating from a structure-based VS protocol that reached the last stage of clinical trials highlight the crucial role of SBDD to efficiently expedite the identification of active compounds and further optimization for clinical development. The novel compound, found to be a highly potent agonist of the serotonin receptor $5\text{-}HT_{1A}$, was discovered by docking studies where a virtual library with 40,000 compounds was placed and scored in a homology model of the target (Becker et al. 2006). In total, 78 compounds were tested in a binding assay whereas 16 showed activity, yielding an impressive hit rate of 21% (Becker et al. 2006). The discovery timeline was surprisingly rapid and reached

clinical trial within 2 years after discovery, but the clinical development was later terminated due to low efficacy (Chen et al. 2024).

The pioneering study by Lane et al. in 2013 represented the first published paper exploring the allosteric binding site of a GPCR by structure-based methods (Lane et al. 2013). The study was conducted using two optimized crystal structure-based models of the human dopamine D_3 receptor, a structure with an empty binding pocket and a structure in complex with dopamine. The crystal structures were optimized by applying ligand-guided receptor optimization (LiBERO) where new conformations are generated by introducing minor variations in the protein backbone before sampling side chains with an active ligand in the pocket (Lane et al. 2013). The dataset of active compounds consisted of 28 antagonists with affinities <10 nM, and each conformer of the binding pocket was evaluated in a docking calculation by their ability to separate the active ligands from 300 decoys (assumed nonbinding compounds). A virtual library of 4.1 million compounds was further docked in the pocket of the best-performing conformers, ranked based on docking score, and further evaluated. A total of 50 compounds were further tested in experimental assays, leading to the identification of 8 novel ligands binding to the allosteric pocket, whereas the most potent ligands were found to function as NAMs (Lane et al. 2013).

There are multiple benefits of targeting the allosteric binding site such as more specific binding and thereby potentially less off-target effects as previously mentioned. A study conducted by Korczynska et al. (2018) significantly emphasizes this benefit through the discovery of a PAM binding to the M_2 muscarinic acetylcholine (mACh) receptor. The PAM enhanced the binding of the antagonist N-methyl scopolamine (NMS) and the drug scopolamine while sparing the endogenous agonist acetylcholine (Korczynska et al. 2018). The novel compound also showed a slower disassociation rate of NMS from the M_2 mACh receptor, which was not observed for any of the other receptor subtypes. The initial compound was found by docking a library of 4.6 million compounds against an experimentally solved 3D structure of the target, followed by a structure-based optimization (Korczynska et al. 2018).

Most of the available 3D structures of GPCR have been resolved in complex with orthosteric ligands, enabling the discovery/development of agonists and antagonists through structure-based modeling approaches. The recent advancements in methods such as X-ray crystallography and cryo-EM have facilitated the determination of numerous high-resolution GPCRs in complex with allosteric modulators. Prior to this structural information, these sites of action have been largely unidentified and have consequently restricted the application of structure-based VS and design toward allosteric sites as highlighted in a review by Chen et al. (2024). However, the $mGlu_5$ receptor, which also belongs to class C GPCR, was successfully used in a VS campaign shortly after it was published (Kampen et al. 2022). Kampen et al. applied a docking screening targeting the identified allosteric pocket and docked 1 chemical library containing 1.6 million fragments and another library containing 4.6 lead-like molecules (Kampen et al. 2022). In total, 59 molecules were selected from each library based on factors such as the predicted docking score, visual inspection, and chemical diversity and were further tested experimentally. The study resulted in a

hit rate of 9% with affinities in the micromolar range, and the compounds with the highest affinities were reported to be NAMs (Kampen et al. 2022).

The research mentioned above clearly demonstrates how SBDD and access to high-resolution structures of GPCRs can accelerate drug discovery and drug design targeting allosteric binding sites. In addition, increased computational power, including advanced hardware technology with faster processors, increased memory capacity, and parallel computing techniques, has enabled computers to handle more complex calculations faster. These advancements boost CADD methodologies such as high-throughput protein docking screenings. Improved algorithms, integration of machine learning, and artificial intelligence have also efficiently improved the accuracy of structure-based techniques making them essential tools in GPCR drug discovery and design.

9.4.2 Computer-Based Simulations for Understanding $GABA_B$ Receptor Conformational Dynamics

GPCRs are highly dynamic proteins and are believed to adopt multiple conformations independent of the presence of ligands. This has also been observed for members of class C GPCRs. A recent study applied single-molecule Förster resonance energy transfer (smFRET) to shed light on the dynamic behavior of $mGlu_2$ and $mGlu_3$ receptors and observed that their VFT domains underwent oscillations between open/inactive and closed/active conformations even in the absence of ligands. Furthermore, upon ligand binding, the equilibrium of these conformations shifted based on the specific function of the ligand (Olofsson et al. 2014; Grushevskyi et al. 2019). While this mechanism is believed to extend to all class C members, including the $GABA_B$ receptor, confirmation is pending. On the contrary, structural analyses of $GABA_B$ VFT domains, along with FRET and bioluminescence resonance energy transfer (BRET) studies, have outlined the association of the open/inactive conformation with antagonist binding and the closed/active conformation with agonist binding (Geng et al. 2013; Lecat-Guillet et al. 2017). This was also observed in a study applying computer-based simulations to mimic the structural dynamics of the $GABA_B$ receptor. Here, it was found that the energy barrier for the $GABA_B$ VFT domain to go from an open/inactive to a closed/active state was likely too high for the receptor to undergo this conformational transition in the absence of a ligand (Evenseth et al. 2020b).

For SBDD and VS screening protocols, the relevant conformation of a target must be applied for an accurate result, e.g., the active/closed conformation of the $GABA_B$ VFT domain should be used for the discovery of agonists, and the open/inactive VFT domain conformation should be used to identify orthosteric antagonists. However, knowledge regarding conformational dynamics of class C GPCR and the sequential mechanism of activation is still limited, in addition to conformational details about intermediate states. However, advancements in computational

power together with the increasing access to structure data have boosted the application of computer-based molecular dynamics and enhanced sampling for simulating and further analyzing the dynamic behavior of GPCRs.

9.4.2.1 Molecular Dynamics and Enhanced Sampling Methods

Recent studies have successfully applied computer-based simulations to investigate the conformational dynamics of GPCRs. As previously described, these receptors exhibit considerable flexibility, adopting diverse conformations influenced by their interactions with ligands, transducers, and changes caused by mutations. Interestingly, it has been demonstrated that agonists alone may not be sufficient for receptor activation, despite favoring the active conformation. Moreover, transducers such as G proteins have also been described as playing a role in further biasing receptor conformations toward the active state, underscoring the significance of understanding receptor dynamics when applying CADD methods for discovering novel PAMs binding to the $GABA_B$ receptor (Abrol et al. 2022).

Molecular dynamics (MD) simulations are among the most applied methods for studying receptor dynamics as the methodology aims to mimic the movements of molecules. MD allows proteins to be embedded in their native environment before solving Newton's equations of motion, considering the forces between all interaction atoms, and producing a trajectory of receptor "snapshots" representing a realistic movement of the protein (Sliwoski et al. 2014).

One of the first MD simulations was published in 1977 showing a in vacuo 9.2 picosecond (ps) simulation of a bovine pancreatic trypsin inhibitor consisting of 58 amino acids (McCammon et al. 1977). Today, improvements in algorithms and hardware, among other factors, have allowed us to access the millisecond scale by running ultra-long MD simulations. Hence, the method is frequently applied to investigate GPCR dynamics and ligand binding. Dror et al. published an excellent study in 2013 where they applied MD simulations to identify the location of an allosteric binding site and relevant receptor interactions for multiple allosteric modulators of the M_2 mACh receptor (Dror et al. 2013). The study also revealed mechanisms contributing to PAM and NAM modulation of classical ligand binding and provided key insight for the rational design of allosteric modulators targeting this receptor (Dror et al. 2013).

However, simulating physiological processes such as large conformational changes associated with GPCR activation and ligand binding can take several milliseconds to seconds and beyond and is technologically still out of reach for traditional MD simulations (Henzler-Wildman and Kern 2007). In addition, as classical MD simulations do not apply external energy, transitions from conformation A to conformation B might not be observed due to high energy barrier(s) and too short simulation time. To overcome these limitations, multiple methods based on classical MD have been developed to simplify the calculations and/or to accelerate the dynamic process by applying a bias such as an external force to characterize the free energy landscape along functionally interesting coordinates (Henzler-Wildman and

Kern 2007; Abrol et al. 2022). There are multiple flavors of enhanced sampling methods developed to probe the GPCR activation landscape such as metadynamics, adaptive-biased MD, accelerated MD, Markov state models, and MD (Abrol et al. 2022).

A recently published paper by Yang et al. applied MD and metadynamics simulations to predict and investigate the mechanism by which G proteins induce conformational changes in the 7TM of the $GABA_B$ receptor to form an intermediate pre-activated state (Yang et al. 2022). Metadynamics was employed to trace the free energy profile between conformational states where the energy barriers were too high to overcome using conventional MD within a reasonable timeframe. In essence, this works by adding a history-dependent bias potential in the form of Gaussians to appropriately chosen reaction coordinates, called collective variables (CV), to encourage the system to visit new regions and escape local energy minima. The sum of added Gaussians is further used to reconstruct the free energy (Abrol et al. 2022; Yang et al. 2022).

The structures used in the simulations were constructed based on the recently released cryo-EM structures of the $GABA_B$ receptor and provide a detailed and fresh insight into mechanistic details regarding the receptor activation. To mention some of the highlights, one simulation was carried out using the active structure of the receptor but without the PAM bound in the interface of the dimers and showed that the TM6-TM6 interface remained stable. Further, the study also investigated the role of the phospholipids found in most of the cryo-EM structures of the $GABA_B$ receptor and concluded that they are more likely structural components rather than allosteric modulators. Lastly, the authors also proposed a mechanism for $GABA_B$ receptor G protein activation beyond agonist binding inducing closing of the $GABA_{B1}$ VFT domain followed by rearrangement of the linker and thereby formation of the TM6-TM6 interface as described previously; the G protein engagement initiates conformational changes in the 7TM domain of $GABA_{B2}$ through interactions with the transmembrane helix 3, causing further 7TM rearrangements allowing the G_α to interact with the ICL1 before opening up and exchanging GDP for GTP and further intracellular signaling (Yang et al. 2022).

The study underscores the pivotal role of novel sampling methods in understanding mechanistic details of receptor activation as a complement to experimental studies, in addition to providing an excellent starting point for computer-aided methods aiming for discovery of novel PAMs targeting the $GABA_B$ receptor.

9.5 Conclusions

Traditionally, drug discovery efforts targeting the $GABA_B$ receptor have relied on time-consuming and costly stepwise synthesis of potential candidates, followed by screening assays. Also, after the discovery of the agonist baclofen, the majority of research efforts have been directed toward investigating the orthosteric binding site of the receptor.

In the last years, there has been a shift toward more efficient CADD strategies. Until recently, the lack of full 3D structures of the $GABA_B$ receptor has limited the scope of SBDD efforts to the orthosteric site. However, the new structures together with increased computer power and improved algorithms provide an excellent opportunity for applying novel computer-aided methods to investigate $GABA_B$ receptor dynamics, as well as the discovery and development of new PAM targeting the allosteric binding sites.

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Chapter 10
Recent Advances on $GABA_B$ Receptor Positive Allosteric Modulators as Potential Pharmacotherapies for Neuropsychiatric Disorders

Styliani Vlachou

Abstract The $GABA_B$ receptors have long been implicated in the pathophysiology of neuropsychiatric disorders such as schizophrenia, bipolar disorder, major depression, anxiety, epilepsy, chronic pain, autism, and substance use disorders, among others. In this direction, a number of $GABA_B$ receptor agonists were developed, one of which, baclofen, has been approved for clinical use for the treatment of muscle spasms related to multiple sclerosis and spinal cord injuries. Other $GABA_B$ receptor agonists were also developed and extensively examined at a preclinical level for their effects in a number of neuropsychiatric conditions. However, all $GABA_B$ receptor agonists exhibit moderate or severe side effects, including, but not restricted to, drowsiness, dizziness, weakness, and tolerance. In the last couple of decades, a number of $GABA_B$ receptor positive allosteric modulators (PAMs) were developed; these are molecules that bind onto a different (i.e., allosteric) site on the $GABA_B$ receptor to the one where GABA or $GABA_B$ receptor agonists bind, and promote the release of GABA, but lack the intrinsic properties of $GABA_B$ receptor agonists; thus, they show a better pharmacological profile, and they exhibit less side effects than the agonists in behavioral studies. This chapter aims to capture the history and recent advances on the role of $GABA_B$ PAMs as potential pharmacotherapies for neuropsychiatric disorders.

Keywords $GABA_B$ receptor positive allosteric modulators · Animal models · Neuropsychiatric disorders · Therapeutic effects

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10.1 Introduction

γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain, binding to three different types (type-A, type-B, and type-C) of receptors, namely, $GABA_A$, $GABA_B$, and $GABA_C$. The $GABA_B$ receptors are the only metabotropic G protein-coupled receptor (GPCR) type of receptors of the three, and they belong to class C of GPCRs (see Chap. 1 of this volume). The $GABA_B$ receptors are widely expressed in the central nervous system (CNS), and the $GABA_B$ receptor system seems to play an important role in the regulation of neuronal excitability, thus affecting various aspects of behavior. In that regard, the $GABA_B$ receptors have been extensively studied for their role in the pathophysiology of neuropsychiatric disorders such as schizophrenia, bipolar disorder, major depression, anxiety, autism, and alcohol (AUD) and substance (SUD) use disorders, among others (Bowery 2006; Cathomas et al. 2015; Colombo 2024; Fatemi et al. 2017; Felice et al. 2022; Huang et al. 2023; Jacobson et al. 2018; Leggio and Litten 2021; Ong and Kerr 2005; Pizzo et al. 2018; Vlachou 2021).

$GABA_B$ receptors are heterodimers consisting of two similar but distinct subunits, the B_1 and B_2, both of which need to be present and bonded together for the receptor to be functionable (Bettler et al. 2004; Emson 2007). Both subunits are built from an extracellular Venus flytrap (VFT) domain, a heptahelical transmembrane (7TM) domain, and an intracellular tail (Pin and Bettler 2016). The VFT domain of the B_1 subunit binds GABA, orthosteric receptor agonists such as baclofen, and antagonists, such as phaclofen, saclofen, or SCH50911. The B_2 subunit is responsible for G protein activation (Evenseth et al. 2020; Shaye et al. 2021).

After all the years of research on the role of $GABA_B$ receptors in a variety of neuropsychiatric and other conditions, the only drug approved for clinical use as a muscle relaxant has been baclofen (see Chap. 3 of this volume). However, for at least a couple of decades and since the discovery of the $GABA_B$ receptor by Norman G. Bowery over 40 years ago (Bowery et al. 1980, 1979; see also Chap. 15 of this volume), there have been extensive efforts to develop, assess, and hopefully approve for clinical use an advanced group of ligands to the $GABA_B$ receptors, the $GABA_B$ receptor positive allosteric modulators (PAMs), which appear to have an improved pharmacological profile, combined with less side effects than the $GABA_B$ receptor agonists in animal studies (Chen et al. 2006) (Fig. 10.1). The first $GABA_B$ PAMs developed and characterized were CGP7930 and GS39783, followed by *rac*-BHFF, BHF177, CMPPE, COR627 and COR628, KK-92A, ADX71441, ADX71943, ORM-27669, and ASP8062 (see Chap. 8 of this volume).

One would wonder what makes the $GABA_B$ PAMs so special and why the focus of research groups and/or pharmaceutical companies has been on developing $GABA_B$ PAMs rather than agonists in the last 20 years or so. As mentioned above, the $GABA_B$ PAMs bind to a site of the $GABA_B$ receptor different from (i.e., allosteric to) the orthosteric site where the $GABA_B$ receptor agonists, including GABA itself, bind. Through this binding, $GABA_B$ PAMs increase the effects of GABA by modulating GABA release, without any intrinsic agonistic activity. This means that

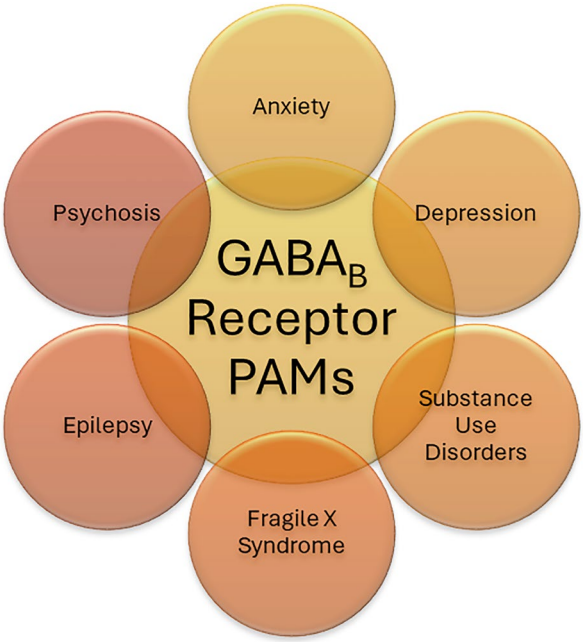

Fig. 10.1 The most common neuropsychiatric disorders that $GABA_B$ receptor positive allosteric modulators (PAMs) may have a therapeutic effect on

these compounds produce less severe and/or fewer side effects compared to the agonists or the antagonists (Vlachou 2022).

Importantly, the exact binding modes and molecular mechanisms of $GABA_B$ PAMs are still unknown. It may be the case that multiple allosteric binding sites exist affecting the selectivity and effects of the various $GABA_B$ PAMs (Evenseth et al. 2020). For example, most recent studies suggest that the main binding site of $GABA_B$ PAMs like *rac*-BHFF and GS39783 can be found between the transmembrane domains of the $GABA_{B1}$ and $GABA_{B2}$ site (Kim et al. 2020; Krámos et al. 2024; Shaye et al. 2020; see also Chap. 2 of this volume). These recent advances may extensively affect the development of new $GABA_B$ PAM ligands.

Following up from the very informative Chaps. 8 and 9 in this volume, on the recent advances on the chemistry of $GABA_B$ PAMs and the novel approaches for drug design and discovery of new $GABA_B$ PAMs, respectively, this chapter focuses on the role of the $GABA_B$ PAMs on neuropsychiatric disorders through preclinical studies of respective animal models.

10.2 Stress and Anxiety-Like Symptomatology

The effects of $GABA_B$ PAMs on anxiety-like behaviors in rodents have been examined extensively through the use of different animal behavioral models of anxiety-like behavior, including the elevated plus maze (EPM), the elevated zero maze

(EZM), the stress-induced hyperthermia (SIH), the light-dark box (LDB), the marble burying (MB), and the Vogel conflict. As one of the first $GABA_B$ PAMs developed (Urwyler et al. 2003) and later further assessed biochemically and at receptor subunit level (Dupuis et al. 2006; Gjoni et al. 2006), GS39783 has been shown to have anxiolytic-like effects in a number of tests, after both acute and chronic administration (Cryan et al. 2004; Mombereau et al. 2004). More specifically, acute treatment of $GABA_{B1}$ knockout (KO) mice with GS39783 and the benzodiazepine chlordiazepoxide in LDB showed that GS39783 (0.3–30 mg/kg, i.g.) and chlordiazepoxide (10 mg/kg, i.g.) increased the number of transitions between light and dark compartments during the test and the time spent in the light compartment, while it failed to influence the latency to enter the dark compartment (Fig. 10.2). It is important to note that $GABA_{B1}$ KO mice had previously displayed marked increases in anxiety-related behaviors in the light-dark box paradigm compared with wild-type (WT) $GABA_{B1}$ or heterozygous $GABA_{B1}$ mice, with 30% more freezing behavior when placed in the light side of the apparatus. Importantly, in these tests, GS39783 did not show the typical side-effect profile of baclofen or other $GABA_B$ receptor agonists, as it did not have an effect on locomotion, cognition, temperature, or sedation/narcosis.

In more recent studies, the precise neural circuits underlying the anxiolytic effects of GS39783 were investigated (Pizzo et al. 2018). In the study by Pizzo and colleagues (Pizzo et al. 2018), mice were exposed to basal or mild stress conditions by being placed on the open arm of an EPM. The type of condition mice were exposed to (basal *versus* mild stress) differentially affected the c-Fos expression triggered by GS39783 administration. Mice acutely treated with GS39783 and exposed to basal conditions showed increased c-Fos expression in the amygdala nuclei, cortical areas, and the periaqueductal gray area, but decreased c-Fos expression in the dorsal raphe nuclei. On the other hand, mice acutely treated with GS39783 and exposed to mild stress conditions showed reversed c-Fos expression in the granular cell layer of the dentate gyrus, no increase in c-Fos expression in the amygdala, and no reduction of c-Fos expression in the dorsal raphe nuclei. These findings indicate that the abovementioned neural circuits—the dentate gyrus of the hippocampus, the amygdala, and the dorsal raphe nuclei—are involved in the effects of GS39783 on c-Fos expression, albeit in a different manner during basal *versus* during mild stress conditions (Pizzo et al. 2018). Most recently, Bicakci and colleagues aimed to examine the factors playing a role in the sometimes contradictory findings of studies investigating the effects of $GABA_B$ PAMs on anxiety-like responses in rodents (Bicakci et al. 2022). This study hypothesized that the anxiolytic-like effects of such compounds depend on the individual basal anxiety and/or the anxiogenic properties of the used tests. For this purpose, they assessed GS39783 effects on the anxiety-like behavior of mice with different stress response levels in a LDB (Bicakci et al. 2022). Their findings suggested that anxiety tests should be designed to capture individual basal anxiety and/or stress responsiveness except for individual compound effects (Bicakci et al. 2022).

Taken together, these studies on the effects of GS39783 on anxiety-like responses appeared to be positive toward the further development of $GABA_B$ PAMs for their

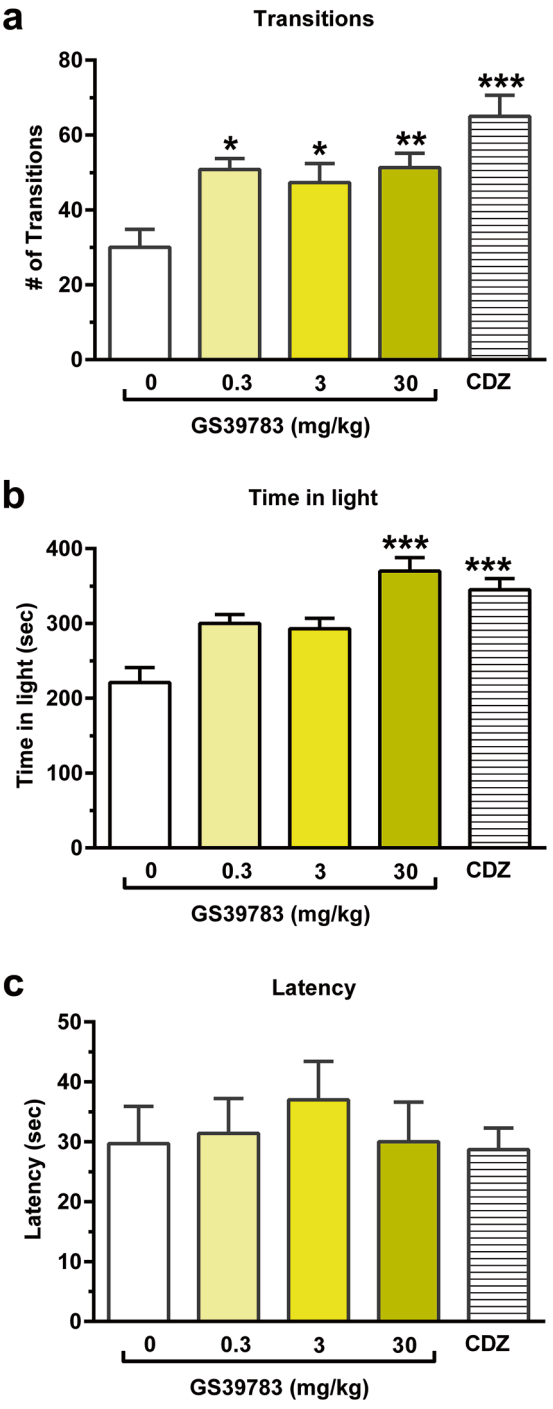

Fig. 10.2 Anxiolytic-like effects of acute treatment with the GABA$_B$ receptor positive allosteric modulator GS39783 in the light-dark box test in GABA$_{B1}$ knockout mice, which were previously displaying a heightened anxiety-like behavior in the light-dark box test. Effects of acute GS39783 treatment (doses: 0, 0.3, 3, or 30 mg/kg, i.g.) and chlordiazepoxide (CDZ, 10 mg/kg, i.g.) on (**a**) the number of transitions between light and dark compartments during the test, (**b**) the time spent in the light compartment, and (**c**) the latency to enter the dark compartment. $n = 10$ per treatment group. All bars represent mean values, with vertical lines indicating one SEM. *, **, ***Groups that differed significantly compared to vehicle-treated mice ($P < 0.05$, <0.01, and < 0.001, respectively). (Adapted from Mombereau et al. (2004) with permission from Springer Nature)

therapeutic potential on stress and anxiety. Importantly, in recent years, Pin, Rondard, and colleagues (Lecat-Guillet et al. 2017; Scholler et al. 2017) developed time-resolved fluorescence resonance energy transfer (FRET) sensors, an innovative tool allowing the more detailed screening and identification of new $GABA_B$ PAMs, among other compounds targeting other receptors, with better pharmacological and side-effect profile. Through the FRET screening, it was revealed that GS39783 has low intrinsic agonist properties, which would be highly anticipated for a $GABA_B$ PAM with low side-effect profile, while CGP7930 and *rac*-BHFF display agonist properties (Lecat-Guillet et al. 2017; see also Chap. 2 of this volume).

Similarly to GS39783, CGP7930 has also shown anxiolytic-like effects in the EZM in rats (Frankowska et al. 2007), while it also showed anxiolytic-like effects, albeit moderate, in the SIH, EZM, and staircase tests in mice (Jacobson and Cryan 2008). Importantly, there were no effects of CGP7930 in the EPM in mice (Jacobson and Cryan 2008). Of particular significance and in agreement with other $GABA_B$ PAM effects, CGP7930 showed way fewer side effects to $GABA_B$ receptor agonists in the above studies. In *in vivo* studies (Koek et al. 2010), CGP7930 enhanced the loss of righting reflex (LORR) induced by baclofen in mice, while it was less effective in enhancing LORR induced by γ-hydroxybutyrate (GHB), which also shows $GABA_B$ receptor agonist properties (see Chap. 13 of this volume). In the same study (Koek et al. 2010), the hypothermic effects of baclofen were also enhanced by CGP7930 only at doses that produced hypothermia when given alone. Importantly, the CGP7930-induced hypothermia was not attenuated by the $GABA_B$ receptor antagonist, CGP35348, at doses that blocked baclofen-induced hypothermia and was not increased by the nitric-oxide synthase inhibitor N(ω)-nitro-L-arginine methyl ester, at doses that increased the hypothermic effects of baclofen and GHB (Koek et al. 2010). Overall, findings from this study indicate that CGP7930 acts as a PAM at $GABA_B$ receptors *in vivo* mediating LORR, but not hypothermia. A further confirmation of the *in vivo* effects of CGP7930 comes from another study of the same research group, this time in pigeons (Koek et al. 2012).

Another one of the first few $GABA_B$ PAMs which made it to behavioral testing was *rac*-BHFF (Malherbe et al. 2008). In the study by Malherbe and colleagues (Malherbe et al. 2008), *rac*-BHFF dose-dependently increased LORR induced by baclofen. Further, when tested alone, *rac*-BHFF had no effect neither on LORR nor on spontaneous locomotor activity, but it exhibited anxiolytic-like activity in the SIH model in mice (Malherbe et al. 2008). These findings suggested the further investigation of *rac*-BHFF and/or its analogs/derivatives for the modulation of anxiety-like responses in rodents and their potential future development for the improvement of anxiety symptomatology in humans.

Since the synthesis and pharmacological assessment of the novel $GABA_B$ PAM BHF177, extensive work was undertaken by the research group of Athina Markou to assess the effects of this $GABA_B$ PAM in anxiety-like responses (Li et al. 2013, 2015), among other behaviors and conditions. In their initial approach (Li et al. 2013), they aimed to assess the effects of BHF177 as compared to baclofen in anxiety-like behavior, learning, and memory in mice. This study showed that

BHF177, similarly to baclofen, had no effect on anxiety-like behavior in the EPM, LDB, and Vogel conflict tests. Importantly, mice treated with BHF177 exhibited no sedation-like effects, while pro-convulsion properties were exhibited only in mice, but not in rats, indicating that this effect may be species-specific (Li et al. 2013). Overall, this study showed that BHF177 has no anxiolytic-like profile and only minimal sedative properties. In continuation of this work, BHF177 effects on anxiety-like responses were further assessed through conditioned and unconditioned physiological responses to threat in the light-enhanced startle (LES), SIH, and fear-potentiated startle (FPS) procedures in rats (Li et al. 2015). BHF177 had no effect on LES at any of the three doses tested, while it did not produce a sedative effect either. Interesting, however, when rats were grouped by high and low LES responses, BHF177 had anxiolytic-like effects on LES in high, but not low, LES-responding rats (Li et al. 2015). BHF177 also blocked SIH, but had no effect on conditioned fear responses in the FPS test, similarly to GS39783 previously, which had also shown no effect on conditioned fear responses in mice (Sweeney et al. 2013). These results indicated that BHF177 may specifically attenuate unconditioned anxiety in individuals with a heightened anxiety profile, with fewer sedative effects than orthosteric receptor agonists. The study suggested that BHF177 or other $GABA_B$ PAMs may be promising compounds for alleviating increased anxiety seen in various psychiatric disorders with a superior side-effect profile compared to $GABA_B$ receptor agonists (Li et al. 2015). These results overall enhanced the belief (and direction) toward further exploring the potential therapeutic effects of $GABA_B$ PAMs in stress-related states and anxiety disorders.

One of the most recently developed $GABA_B$ PAMs is ADX71441, a novel PAM which has shown promising results in preclinical models of anxiety, psychosis, pain, Fragile X syndrome (FXS), overactive bladder, and AUD (Augier 2021; Colombo 2024; Hwa et al. 2014; Kalinichev et al. 2017; Kannampalli et al. 2017; see also Chap. 12 of this volume). In one of these studies, among other effects, ADX71441 had an anxiolytic-like profile in the mouse MB test, when administered in the 3 mg/kg dose, while the same dose also showed an anxiolytic effect in the EPM test in mice and rats (Kalinichev et al. 2017).

Importantly, a very recent study by Yu and colleagues (Yu et al. 2022) further implicated $GABA_B$ receptors in the regulation of anxiety-related behaviors, such as social avoidance and avoidance of bright spaces. In this study, activation of dorsal raphe nuclei 5-HT∩vGluT3 neurons projecting to basal amygdala (BA) parvalbumin inhibited serotonin (5-HT) release via $GABA_B$ receptors on serotonergic terminals in BA, inducing social avoidance and avoidance of bright spaces (Yu et al. 2022). Considering these types of neurons respond to anxiogenic stimuli, it would be interesting to investigate the role of $GABA_B$ PAMs or antagonists in these processes.

Overall, the results from the above studies suggest that $GABA_B$ PAMs show an anxiolytic-like profile in studies with rodents, although these depend on the exact molecule tested as well as the test used, while in some cases, some side effects, such as hypothermia and motor impairment, also occur. Thus, $GABA_B$ PAMs decrease innate anxiety, but findings have not been consistent between tests and compounds.

More studies should focus on the development of novel $GABA_B$ PAMs for anxiety, as it appears that, although promising, in the last few years, efforts in that direction are somehow stalled.

10.3 Affective Disorders and Depression-Like Symptomatology

Some of the earlier studies would have been investigating the effects of all $GABA_B$ receptor agonists, antagonists, and PAMs on depression-like symptomatology, such as anhedonia, a common and pronounced symptom in both major depressive disorder and withdrawal from various drugs of abuse (Jacobson et al. 2018; Pilc and Nowak 2005). Early evidence from preclinical studies would have shown that alteration of the function of the $GABA_B$ receptors can contribute to affective disorder symptomatology and would have specifically pointed out the possibility of using $GABA_B$ receptor antagonists or negative allosteric modulators (NAMs) for the treatment of depression symptomatology.

In the preclinical level, numerous studies have shown that $GABA_B$ receptor agonists and antagonists reduce depression-like symptomatology in animal models, although with some controversy (Cryan and Kaupmann 2005; Cryan and Slattery 2010; Frankowska et al. 2007; Mombereau et al. 2004; Vlachou et al. 2011b). In one of these studies, acute administration of the $GABA_B$ receptor agonist SKF97541 or the $GABA_B$ PAM GS39783 induced antidepressant-like effects in the FST in rats (Frankowska et al. 2007). However, other studies have shown no antidepressant-like effects with $GABA_B$ PAMs. Slattery and colleagues did not show antidepressant-like activity in the modified FST in rats after acute administration of GS39783 (Slattery et al. 2005), while Mombereau and colleagues also found no effect of GS39783 in $GABA_{B1}$ KO mice on the FST (Mombereau et al. 2004).

Interestingly and in accordance with the inconsistent findings presented above, when investigating the anhedonia experienced during withdrawal from nicotine in the intracranial self-stimulation (ICSS) procedure in rats, both $GABA_B$ receptor activation, with the $GABA_B$ receptor agonist CGP44532 and the $GABA_B$ PAM BHF177, and blockade, with the $GABA_B$ receptor antagonist CGP56433A, elevated ICSS thresholds in all groups, resulting in exacerbated effects of nicotine withdrawal in the nicotine-treated groups (Vlachou et al. 2011b). These effects in the same direction of $GABA_B$ receptor activation and blockade on the anhedonic depression-like aspects of nicotine withdrawal were surprising and perhaps reflect differential efficacy of these compounds at presynaptic hetero- and autoreceptors, as well as postsynaptic $GABA_B$ receptors (Vlachou et al. 2011b). The exact mechanism showing (or not showing) antidepressant-like effects of agonists and PAMs or antagonists is unknown.

Many of the controversial findings seem to stem from the role that $GABA_B$ receptors play in the CNS depending on their location on the neuronal cells. To be

more specific, $GABA_B$ receptors can be found on both presynaptic and postsynaptic membranes. When presynaptic receptors are activated, they inhibit either the release of GABA (in the case of autoreceptors on GABA neuronal terminals) or the release of other neurotransmitters and peptides (in the case of heteroreceptors on different neurotransmitter neuronal terminals). When postsynaptic receptors are activated, they in turn activate K^+ channels and induce slow inhibitory postsynaptic potentials. Thus, depending on the symptomatology investigated, the brain locations involved, as well as the selectivity of the compounds used and their binding and other molecular properties, the outcome of these studies can vary a lot. Except for these factors, the animal strain and sex, as well as the animal model protocol used, seem to play an important role in these differential findings.

Irrespective of the earlier controversial findings, overall, more recent focus on the proteins that affect $GABA_B$ receptor activity suggests a possible therapeutic role of $GABA_B$ receptor compounds in depression, especially when the focus switches to novel $GABA_B$ PAMs and NAMs. Most importantly, advancements in diagnostic and therapeutic procedures, such as the use of transcranial magnetic stimulation in major and treatment-resistant depression patients (Kinjo et al. 2021), have shown deficits in $GABA_B$ receptor-mediated cortical inhibition (Lissemore et al. 2018; Premoli et al. 2014a, b; Premoli et al. 2017; Veronezi et al. 2016) affected by antidepressant medication. Further advances in the use of innovative and advanced techniques are expected to help elucidate the role of $GABA_B$ receptors in affective disorders and whether $GABA_B$ PAMs can play a therapeutic role in them.

10.4 Schizophrenia and Psychosis-Like Symptomatology

Changes in GABAergic and glutamatergic function, or expression, in the hippocampus have been proposed as a key factor in the pathophysiology of psychosis (Dubovyk and Manahan-Vaughan 2019). More specifically, decreased $GABA_B$ receptor function is suggested as one of the factors mediating some symptoms of schizophrenia. The interest in the possible role of $GABA_B$ PAMs in psychosis treatment became stronger about two decades ago, when the first few studies on the effects of $GABA_B$ receptor agonists, antagonists, and PAMs in psychosis-like symptomatology in rodents were conducted. In one of these studies by Wierońska and colleagues (Wierońska et al. 2011), the $GABA_B$ receptor agonist CGP44532 and the $GABA_B$ PAM GS39783 reversed some behavioral changes related to positive syndromes of psychosis in mice. More specifically, the effects of GS39783 and other $GABA_B$ receptor compounds were investigated in MK-801- and amphetamine-induced hyperactivity tests. The anti-hallucinogenic-like effect of the compounds was screened in the head-twitch model, with head twitches induced by DOI. Further, the effect of GS39783 and CGP44532 on DOI-induced frequency of spontaneous excitatory postsynaptic currents (EPSCs) in slices from mouse brain frontal cortices was investigated, as well as the anti-cataleptic properties of the compounds (Wierońska et al. 2011). Of particular interest to this chapter, GS39783 exhibited

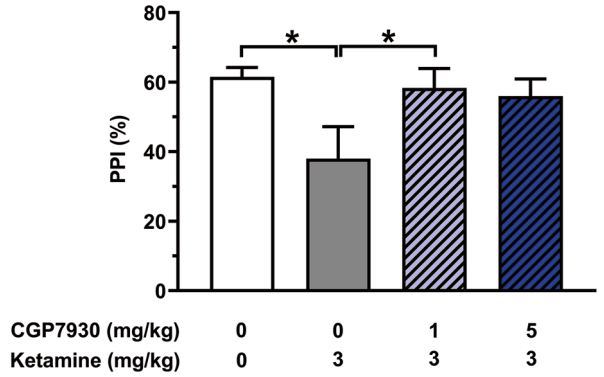

Fig. 10.3 Effect of CGP7930 on integrated prepulse inhibition (PPI) with and without ketamine injection to male Long-Evans hooded rats. CGP7930 at dose of 1 or 5 mg/kg, i.p. antagonized the ketamine-induced PPI deficit. *$P < 0.05$, significantly different between groups, post hoc Newman-Keuls test following one-way repeated measures ANOVA. (Adapted from Ma and Stan Leung (2017) with permission from Springer Nature)

antipsychotic-like effects both in the MK-801- and the amphetamine-induced hyperactivity test, as well as in the head-twitch model in mice. GS39783 also decreased the DOI-induced increased frequency of spontaneous EPSCs, while it also inhibited haloperidol-induced catalepsy and EPSCs (Wierońska et al. 2011).

Except for GS39783, CGP7930 and its effects on psychosis-like symptomatology have also been assessed. In a more recent study, the antipsychotic-like properties of CGP7930 on ketamine-induced psychosis-relevant behaviors and hippocampal electrical activity in rats were examined (Ma and Stan Leung 2017). Intraperitoneally administered CGP7930 prevented the ketamine-induced deficit of prepulse inhibition (PPI), which is the ability of a preceding low-intensity sensory signal in attenuating a startle response (Fig. 10.3). CGP7930 also prevented the decrease in gating of hippocampal auditory evoked potentials and the increase in hippocampal gamma (65–100 Hz) waves induced by ketamine (Ma and Stan Leung 2017). Ketamine-induced behavioral hyperlocomotion was also suppressed by CGP7930, while CGP7930 per se, without ketamine, did not significantly affect integrated PPI, locomotion, gating of hippocampal auditory evoked potentials, or gamma waves. Further, CGP7930 increased heterosynaptically mediated paired pulse depression in the hippocampus, a measure of $GABA_B$ receptor function *in vivo* (Fig. 10.4). Finally, unilateral intracerebroventricular (i.c.v.) infusion of CGP7930 prevented the ketamine-induced decrease in gating of hippocampal auditory evoked potentials (Ma and Stan Leung 2017). All of these findings indicate that not only CGP7930 seems to have an antipsychotic-like effect in animal models of psychosis, but that these effects may be largely mediated by the hippocampus (Grüter et al. 2015; Stan Leung and Ma 2018).

In very recent times, except for the investigation of pure psychosis-like behaviors in animal models of psychosis, there has also been an interest in further understanding drug-induced psychosis in humans through the use of relevant animal models,

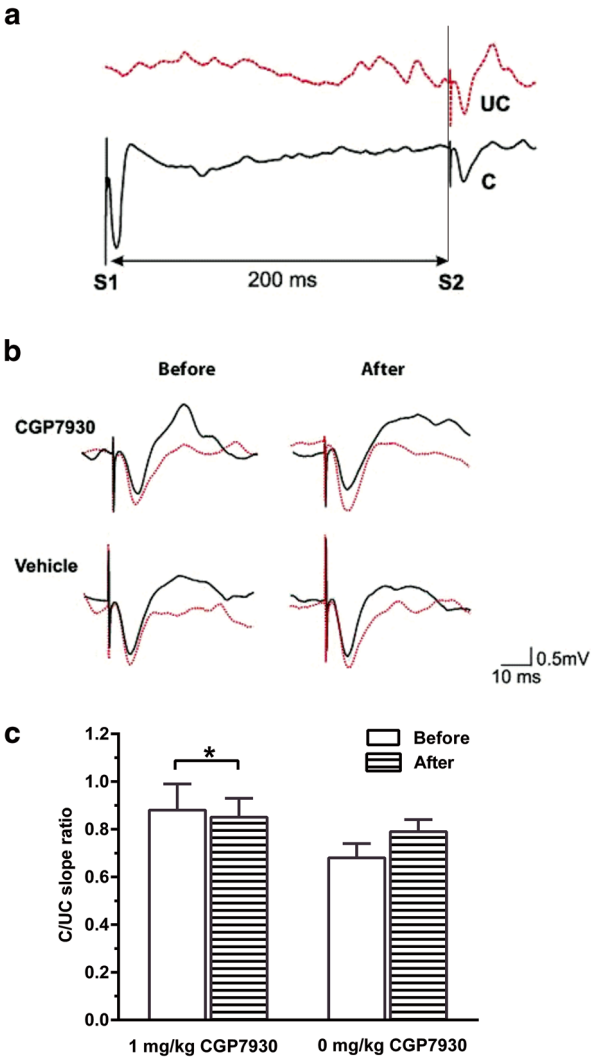

Fig. 10.4 Effect of the $GABA_B$ receptor positive allosteric modulator CGP7930 (1 mg/kg, i.p.) on paired pulse depression of hippocampal electrically evoked responses of male Long-Evans hooded rats. (**a**) Representative traces demonstrating the conditioned response (C) and the unconditioned response (UC); S1 and S2 were commissural and association stimuli, respectively, that evoked response at the CA1 recording electrode. (**b**) Representative raw traces demonstrating the unconditioned response (dotted trace) and conditioned response (solid trace) before and after CGP7930 or vehicle injections. (**c**) Mean and standard error of the ratio of the slope of C to slope of UC (C/UC slope ratio). $^*P < 0.05$, significantly different before and after injection, using post hoc Newman-Keuls test following two-way repeated measures ANOVA. (Adapted from Ma and Stan Leung (2017) with permission from Springer Nature)

such as the methamphetamine-induced locomotor sensitization psychosis model in rodents. In this effect, the role of $GABA_B$ receptors has been investigated, and some very recent promising findings further implicate the $GABA_{B1}$ receptor in the medial prefrontal cortex in particular as a mediator of these changes (Ni et al. 2022).

Although there are promising results from earlier studies both on the role of the $GABA_B$ receptors and the effects of baclofen as well as $GABA_B$ PAMs on psychosis-like symptoms in animal models of psychosis, it appears that studies in this area are limited or progress in a very slow pace. Other than GS39783 and CGP7930, not many other $GABA_B$ PAMs have been tested for their effects in psychosis-like symptomatology. Thus, future efforts should aim to lean toward this direction, especially as newer $GABA_B$ PAMs seem to be more promising pharmacological agents with more specificity and less side effects.

10.5 Other Neuropsychiatric Disorders

10.5.1 Anticonvulsant Activity and Epilepsy-Like Symptomatology

$GABA_B$ PAMs have also been assessed as potential therapeutic treatments for epilepsy through animal models of convulsant epilepsy-like activity (Mareš 2012; Mareš et al. 2013). In one of these studies, the possible anticonvulsant effects of the $GABA_B$ PAM CGP7930 were studied in cortical epileptic afterdischarges in rat pups 12, 18, and 25 days old (Mareš et al. 2013). In this study, CGP7930 decreased duration of afterdischarges only in the 12-day-old rats. In the same study (Mareš et al. 2013), CGP7930 effects were also assessed in sensorimotor performance, as well as the open field test and the EPM. Results showed that CGP7930 only moderately affected sensorimotor performance and slightly changed spontaneous behavior in the open field, while it did not alter behavior in the EPM. Altogether, these findings reluctantly suggest that CGP7930 could potentially be used as an anticonvulsant agent, although the age of the individuals should be taken into account.

Another study using GS39783 indicated that it showed a good antiepileptic effect, although it refers to GS39783 as a receptor agonist (Werner and Coveñas 2019). Further, administering GS39783 to female Mecp2(+/−) mice at doses producing no effect in WT mice strongly potentiated their basal rates of spontaneous cortical discharge activity (Zhang et al. 2016). Interestingly, there is no study having assessed anticonvulsant activity of ADX71441 in animal models of epilepsy *per se*. However, early characterization of ADX71441 *in vivo* and *in vitro* showed that it dose-dependently reduced time on the rotarod test in rats indicating muscle-relaxant properties (Kalinichev et al. 2017).

Most recently, the effects of the $GABA_B$ PAM BHF177 on refractory epilepsy (RE) were investigated (Wang et al. 2022). In this study, Wang and colleagues first established an RE model by treating animals with lithium-pilocarpine. They then recorded the seizure rate of animals and assessed their spatial learning in the Morris

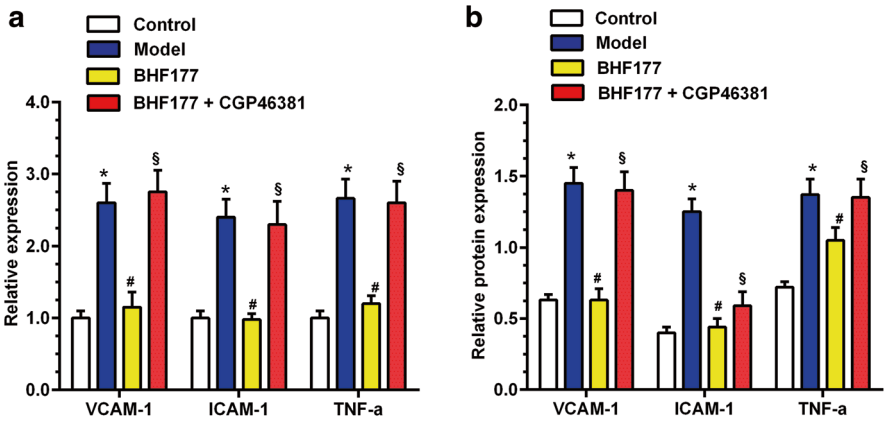

Fig. 10.5 The effects of administration of the $GABA_B$ receptor positive allosteric modulator BHF177 alone or combined with the $GABA_B$ receptor antagonist CGP46381 on expression of inflammatory related factors in hippocampal tissues of the model of refractory epilepsy (RE) rats. (**a**) The mRNA expression of inflammatory related factors in hippocampal tissues of RE rats in each group detected by RT-qPCR. (**b**) The expression levels of inflammation related proteins in hippocampal tissues of RE rats in each group detected by Western blot. $*p < 0.05$ (*vs.* control group); $\# p < 0.05$ (*vs.* model group); $\S\ p < 0.05$ (vs. BHF177 group); measurement data are shown as mean ± standard deviation; *p* value was determined by one-way ANOVA with post hoc Tukey's test; $n = 10$ per group. (Adapted from Wang et al. (2022) with permission from John Wiley and Sons)

water maze test (Wang et al. 2022). Rats treated with BHF177 showed less intense seizures, an effect that was inhibited by the administration of the $GABA_B$ receptor antagonist CGP46381 (Fig. 10.5). Interestingly, immunohistochemistry analysis showed that rats treated with BHF177 exhibited diminished P-glycoprotein (P-gp) expression in the hippocampus of RE rats. Further, BHF177 activated the $GABA_B$ receptors, leading to an upregulated expression of insulin receptor substrate 1 (IRS-1) and phosphoinositide 3-kinase (PI3K), as well as antiapoptotic factors (Bcl-2 and mTOR), along with suppression of the apoptosis factors Bax and cleaved caspase-3 in hippocampal tissues (Wang et al. 2022). Furthermore, $GABA_B$ receptor activation by BHF177 alleviated the inflammatory response in the hippocampal tissues of RE rats, as evidenced by reduced VCAM-1, ICAM-1, and tumor necrosis factor-α levels (Wang et al. 2022). Finally, primary cultured rat hippocampal neurons were treated with BHF177 and the IRS-1 selective inhibitor NT157. BHF177 inhibited hippocampal apoptosis in rat hippocampal neurons by regulating the IRS-1/PI3K/Akt axis through an interaction between $GABA_B$ and insulin-like growth factor-1 receptors (Wang et al. 2022). Altogether, these findings suggest that BHF177 inhibited neuron apoptosis and protected against RE through the IRS-1/PI3K/Akt axis (Fig. 10.6). These effects may present a new pathway for the treatment of RE.

Overall, results from research studies on the effects of $GABA_B$ PAMs in epilepsy-like symptomatology seem promising. However, more studies need to be conducted, using more and newer $GABA_B$ PAMs, to further elucidate these effects at behavioral, neurochemical, and molecular level.

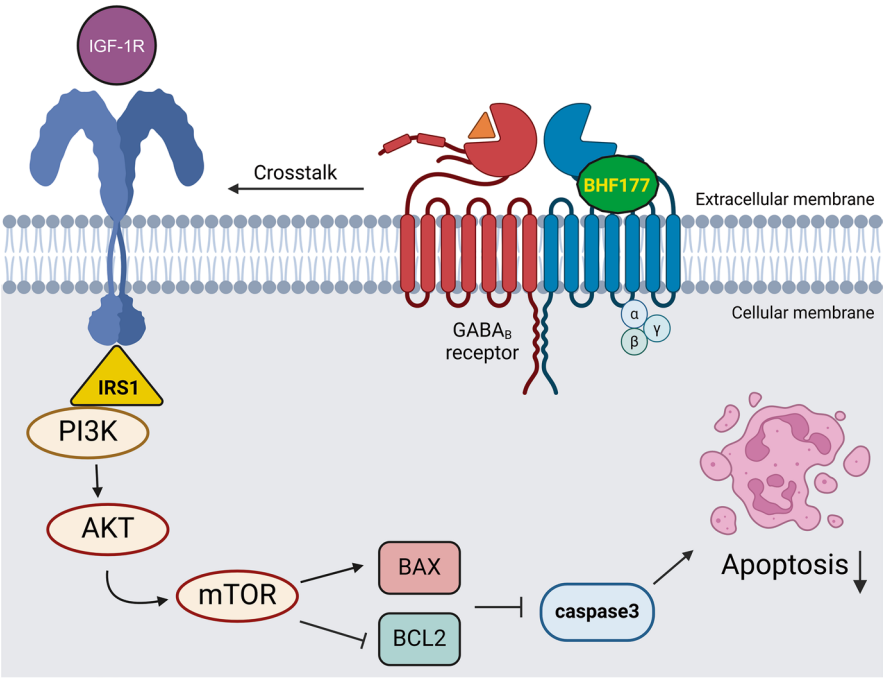

Fig. 10.6 The $GABA_B$ receptor positive allosteric modulator BHF177 exerts antiepileptic role through its significant inhibitory role on hippocampal neuronal apoptosis through the activation of $GABA_B$ receptor, which then upregulates IRS-1 by the cross talk between $GABA_B$ receptor and IGF-1 receptor, leading to the subsequent activation of PI3K/Akt pathway. (Adapted from Wang et al. (2022) with permission from John Wiley and Sons)

10.5.2 Fragile X Syndrome

FXS is considered the most common cause of inherited mental retardation—it is a common syndrome within the autism disorder spectrum and thus shows many common autism spectrum disorder features, together with other medical and psychiatric symptoms (Vlachou 2021). It is caused by the expansion of a CGG triplet repeat in the 5′ untranslated region of the FMR1 gene, which induces silencing of gene expression and the absence of the mRNA-binding protein fragile X mental retardation protein (FMRP) (Pacey et al. 2011). One of the hypotheses of FXS suggests that in the absence of FMRP, the protein translation downstream of the group I metabotropic glutamate (mGlu) receptors does not function normally, leading to enhanced mGlu receptor signaling; this contributes to many of the phenotypes associated with FXS. The mGlu receptor theory also suggests that reducing group I mGlu ($mGlu_1$ and $mGlu_5$ in particular) receptor signaling should alleviate some of the symptoms of FXS (Pacey et al. 2011). In more recent times, there has also been evidence of changes in the $GABA_B$ receptor function in the FXS that are intertwined with the overall balance between the glutamatergic and GABAergic systems. More

specifically, it is suggested that the lack of FMRP leads to atypical synaptic plasticity, potentially triggered by a homeostatic disturbance between excitatory (i.e., glutamatergic) and inhibitory (i.e., GABAergic) network functioning at the level of the synapse (Pacey et al. 2009; Zeidler et al. 2018; Zupan and Toth 2008), as in the case of different forms of epilepsy.

Based on this theory, a number of clinical trials with the $GABA_B$ receptor agonists baclofen and arbaclofen were conducted (Hopkins 2011), while, importantly, a corresponding animal model of the Fragile X Mental Retardation 1 (*Fmr1*) KO (*Fmr1*-KO) mouse was also developed (Kazdoba et al. 2014). Although not without its problems as an animal model (Crawley 2012), *Fmr1*-KO mice share several phenotypes with FXS patients including cognitive deficits, repetitive behaviors, altered spine morphology, hyperactivity, sensory hypersensitivity, and macroorchidism (Bakker and Oostra 2003).

Very few studies have been conducted with $GABA_B$ PAMs. In these studies, except for $GABA_B$ receptor agonists, both CGP7930 (Zhang et al. 2015) and GS39783 (Pacey et al. 2011) stimulated FMRP expression and corrected exacerbated protein synthesis and multiple phenotypes in *Fmr1*-KO mice (Henderson et al. 2012; Silverman et al. 2015). Tolerance developed by both agonists and PAMs in some of these studies (Pacey et al. 2011), a side effect most commonly seen with $GABA_B$ receptor agonists (Vlachou et al. 2011a).

10.6 Conclusions

Overall, findings from the above studies in animal models show a strong potential for $GABA_B$ PAMs to be developed and/or further tested, and ultimately approved, for the treatment of anxiety, depression, psychosis, and other psychiatric disorders, as well as neurological conditions such as epilepsy and FXS, among others.

Although hundreds of $GABA_B$ PAM compounds have been chemically synthesized over the years [e.g., the efforts of Katarzyna Kaczanowska and MG Finn at The Scripps Research Institute La Jolla (Li et al. 2017); see also Chap. 8 of this volume], very few of them have made it to pharmacological profiling and behavioral testing and even less have been tested extensively in animal models of psychiatric disorders or approved for testing in clinical settings.

As an example, one of the most recently developed $GABA_B$ PAMs has been KK-92A, which has exhibited positive results in suppressing alcohol and nicotine self-administration and cue-induced reinstatement in animals (Li et al. 2017; Maccioni et al. 2021; Maccioni et al. 2023); see also Chap. 12 of this volume). To the best of my knowledge, although there are extensive studies of some of the earlier synthesized $GABA_B$ PAMs in various psychiatric and neurodevelopmental conditions, there are no published studies to date of the potential effects of KK-92A in other neuropsychiatric disorders. Other $GABA_B$ PAMs with initial promising results in behavioral and pharmacological studies will also need to be further tested.

Importantly, although the collective outcomes of those pharmacological and behavioral studies seem to be reflected in a slow pace over the course of the last four decades, most promising have been the advances on the development of optimized $GABA_B$ PAMs. This challenging optimization has constantly been the focus of relevant studies that discover more components of the protein structure (Schwenk et al. 2016). Most recently, only in the last couple of months, Krámos and colleagues (Krámos et al. 2024) showed that they were able to develop a carboxylic acid derivative of $GABA_B$ PAMs, an advancement that offers the possibility to improve potency without drastically inflating the physicochemical properties (Krámos et al. 2024).

In this positive direction, very recent studies using the $GABA_B$ PAM ASP8062 in animal models, healthy adult volunteers or clinical studies of individuals with AUD or opioid use disorder are promising, and thus lead the way for further development and testing of $GABA_B$ PAMs in SUD and other psychiatric disorders (Akuzawa et al. 2023; Burnette et al. 2022; Colombo 2024; Haile et al. 2021; Ito et al. 2022, 2023a, b; Walzer et al. 2020, 2021; see also Chap. 12 of this volume).

Finally, optimization and other advances in this direction will play a major role in the further development of improved $GABA_B$ PAMs with promising therapeutic results and less side effects and the potential to be approved for the treatment of neuropsychiatric disorders and other conditions.

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Chapter 11
Recent Advances on $GABA_B$ Receptor Positive Allosteric Modulators as Potential Pharmacotherapies for Substance Use Disorder and Food Addiction

Karolina Wydra, Małgorzata Frankowska, and Małgorzata Filip

Abstract ϒ-aminobutyric acid (GABA) type-B ($GABA_B$) receptors and their positive allosteric modulators (PAMs) are indicated as potential pharmacological tools for use in the treatment of a series of brain disorders, including substance use disorder (SUD). This chapter describes the current state of preclinical findings on (i) the interaction of drugs of abuse and $GABA_B$ receptors in the brain and signaling pathways and (ii) the effects of $GABA_B$ PAMs in several behavioral paradigms relating to reward, motivation, and seeking behaviors. Data obtained in recent years has indicated that $GABA_B$ PAMs may exert therapeutic efficacy in the context of addiction to psychostimulants (amphetamines and cocaine), nicotine, and opioids. Several $GABA_B$ PAMs also displayed efficacy in reducing food-maintaining behaviors; however, further investigations should be conducted to assess whether this pharmacological approach represents a primary mechanism and may be of use in the treatment of food addiction or eating disorders. Behavioral profiling of $GABA_B$ PAMs indicates a resemblance to the clinically approved drug baclofen, an orthosteric $GABA_B$ receptor agonist. As $GABA_B$ PAMs display a better side-effect profile compared to $GABA_B$ receptor orthosteric agonists, they may be of significant use in targeting specific brain regions and processes associated with drug or food reward, seeking, and withdrawal.

Keywords $GABA_B$ receptor positive allosteric modulators · Food addiction · Substance use disorder · Psychostimulants · Nicotine

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11.1 Introduction

Substance use disorder (SUD) and food addiction produce a heavy burden on public health and society worldwide (Bonder and Davis 2022; UNODC World Drug Report 2023; Volkow et al. 2017a). Numerous drugs are capable of eliciting addictive behavior in humans and animals, including psychostimulants, opioids, cannabinoids, nicotine, and alcohol. Cannabis is the most commonly used drug worldwide, followed by psychostimulants (UNODC World Drug Report 2023). Indeed, the World Drug Report 2023 discloses a global total of approximately 300 million drug users (a 23% increase in drug intake over the last 10 years) with approx. 40 million people affected by SUD (a 45% rise over the last 10 years) (UNODC World Drug Report 2023). This increase has been largely driven by periods of lockdown during the recent coronavirus pandemic, boosting the use of nonmedical drugs of abuse such as cannabis, both in terms of amount and frequency of use.

SUD is a chronic brain disorder in which, following experience of the initial spectrum of emotions (well-being, pleasure, euphoria), behaviors typical of compulsive drug-seeking and drug-taking are manifested regardless of the known negative consequences, with the potential of triggering relapse accompanied by psychic, somatic, and vegetative symptoms subsequent to drug abstinence (Duresso 2021). Clinical diagnosis of SUD is based on two main classification systems: the International Classification of Diseases (ICD) 11th Revision (ICD-11) developed by the World Health Organization in 2019 (with amendments approved in 2022) and the Diagnostic and Statistical Manual of Mental Disorders (DSM) 5th Edition (DSM-5) produced by the American Psychiatric Association in 2013. DSM-5 only addresses the "Substance use disorder" category, identifying 3 levels of severity (American Psychiatric Association 2013) based on the number of recognized symptoms present from a total list of 11: 2–3 symptoms indicate mild SUD, 4–5 symptoms moderate SUD, and > 6 symptoms severe SUD. Furthermore, DSM-5 does not base SUD classification on harm to physical or mental health of either the affected person or of others. ICD-11 distinguishes between three separate forms: a) Episode of Harmful Substance Use, defined as an episode of use that has caused clinically significant harm to a person's physical or mental health or the health of other people; b) Harmful Pattern of Substance Use, defined as a pattern of repeated or continuous use that has caused clinically significant harm to a person's physical or mental health or the health of other people; and c) Substance Dependence, characterized by impaired control over substance use, increasing priority of substance use over other aspects of the person's life, and persistence of use despite harm or negative consequences. The diagnosis requires two or more of the three central features to be present in the individual at the same time and to occur repeatedly over a period of at least 12 months or continuously over a period of at least 1 month (see Table 11.1) (First et al. 2021; Volkow and Blanco 2023).

SUD was conceptualized as a multistage disorder, to include drug reinforcement, reward, motivation, seeking, and relapse as well as drug-evoked neuroplasticity (Lynch et al. 2010). The particular behavioral outcomes of SUD can be modeled using animal models or tests (see Table 11.2).

Table 11.1 Diagnostic criteria for Substance Use Disorder and Substance Dependence in Diagnostic and Statistical Manual of Mental Disorders 5th Edition (DSM-5) and International Classification of Diseases 11th Revision (First et al. 2021; Volkow and Blanco 2023; World Health Organization 2021)

DSM-5 Substance Use Disorder	*ICD 11* Substance Dependence
1. The substance is often taken in larger amounts or over a longer period than was intended 2. There is a persistent desire or unsuccessful efforts to cut down or control substance use 3. Craving, or a strong desire or urge to use the substance	1. *Episode of Harmful Psychoactive Substance Use* Impaired control over substance in terms of the onset, circumstances, or termination of use. Often but not necessarily accompanied by a subjective sensation of urge or craving to use the substance
4. A great deal of time is spent in activities necessary to obtain the substance, use the substance, or recover from its effects 5. Recurrent use of the substance failing to fulfill major role obligations at work, school, or home 6. Continued use of the substance despite having persistent or recurrent social or interpersonal problems can be exacerbated by the effects of the substance	2. *Harmful Pattern of Psychoactive Substance Use* Substance use becomes an increasing priority causing clinically significant damage to a person's physical health or mental health or has resulted in behavior leading to harm to the health of others. The pattern of use of the relevant substance is evident over a period of at least 12 months if substance use is episodic or at least 1 month if use is continuous Harm to heal this not better accounted for by another medical condition or another mental disorder, including another Disorder Due to Substance Use
7. Tolerance, as defined by either of the following (a) A need for markedly increased amounts of the substance to achieve intoxication or desired effect (b) A markedly diminished effect with continued use of the same amount of the substance 8. Withdrawal, as manifested by either of the following (a) The characteristic withdrawal syndrome for the substance (b) The substance (or a closely related one) is taken to relieve or avoid withdrawal symptoms	3. *Substance Dependence* Physiological features indicative of neuroadaptation to the substance, including (a) tolerance to the effects of the substance, (b) withdrawal symptoms following cessation or reduction in the use of that substance, and (c) repeated use of the substance or pharmacologically similar substances to prevent and alleviate withdrawal symptoms
9. Use of the substance is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance	*To some extent subsumed in criterion No. 2*
10. Recurrent use of the substance in situations in which it is physically hazardous	*No equivalent criterion*
11. Important social, occupational, or recreational activities are given up or reduced because of the use of the substance	*To some extent subsumed in criterion No. 2*

Table 11.2 Animal models are used in studies of drug abuse and addiction (Lynch et al. 2010)

Measurement	Model/effect	Description
Reward	Conditioned place preference	This model involves the daily association of drug treatment and a particular environment (compartment) from the one side and also the daily association of a drug vehicle in a different environment on the second side to an animal (typically rodents). Following these daily sessions, the animal chooses between drug and vehicle, while during a test session, the time the animal spends in each of the compartments during the session is recorded. A conditioned place preference is found if the animals spend significantly more time in the drug-paired compartment versus the vehicle-paired compartment. It is inferred that the drug has rewarding properties
	Intracranial self-stimulation	It is an operant paradigm pairing lever presses with electrical stimulation of discrete brain pathways (within the medial forebrain at the level of the lateral hypothalamus). Experimental sessions manipulate the frequency or amplitude of stimulation to engender a wide range of baseline response rates or response probabilities
Reinforcement	Drug self-administration	In this model, animals learn to operant respond (typically a lever pressing) that is reinforced by an intravenous infusion of a drug of abuse associated with the environmental stimuli (tone or light) delivered at the same time that the drug is administered. There are different (stable, variable, intermittent) schedules of reinforcement
Motivation for drug-taking	Progressive ratio schedules of drug delivery	In this model, animals are trained to self-administer drugs of abuse on a fixed ratio schedule, and then they are switched to a progressive ratio (PR) schedule of reinforcement. A PR reinforcement schedule is defined by an increasing response requirement for reinforcer delivery over successive sessions or trial-by-trial basis within a single session. This paradigm is used to determine the "motivational" state of an animal and drug "reinforcing efficacy" based on the observation that the PR breakpoint (BP) is proportional to the unit dose of the self-administered drug
Drug withdrawal	Physical, mental, and/or emotional symptoms	In a self-administration procedure, instrumental responses no longer result in the delivery of the rewarding substance It is a physiological response to the sudden quitting or slowing of the use of an abused drug previously taken and to which the body has grown dependent on. In animals, the withdrawal symptoms include enhanced activity of the autonomic nervous system, or body posture and motor abnormalities, or hyperexcitability of the central nervous system, including sensory hyperreactivity, convulsions, or anxiety
Extinction during drug withdrawal	Extinction training	*Extinction* is an active learning process in which an animal initially trained to self-administer an addictive drug for a period of time undergoes training in which the animal's instrumental responses no longer result in *reinforcement that has been maintaining a behavior*

(continued)

Table 11.2 (continued)

Measurement	Model/effect	Description
Drug-seeking and relapse	Reinstatement paradigm	A model in which animals are tested for responding on a lever previously associated with the drug and conditioned stimulus exposure. Drug-seeking behavior during reinstatement testing is triggered by a priming dose of the drug or the stimuli associated with the drug or by a stressor
Drug craving and drug-related neuroplasticity	Behavioral sensitization	The procedure is based on the potentiation of drug-induced locomotion after repeated noncontingent exposure to a constant dose of abused drugs. The locomotor hyperactivity in turn causes an increased craving for drugs and is what causes an exaggerated love of drugs. Additionally, behavioral sensitization to drugs of abuse induces long-lasting neuroplastic changes in brain structures such as reorganization of structural or signaling proteins in the synapse Behavioral sensitization is usually divided into two phases: the induction (or initiation) and the expression phase. During the induction phase, it is possible to measure the molecular and cellular modifications directly induced by drug exposure. The expression, tested with a drug challenge and after a variable withdrawal, is generally attributed to the long-term effects of the aforementioned drug-induced changes

In addition to harmful substances, addiction may also be caused by palatable foods including sweet drinks, chocolate-flavored beverages, or high-fat foods. Indeed, the concept of "food addiction" is based on the criteria used to diagnose SUD, representing a type of behavioral addiction that impacts the part of the brain responsible for rewards, with subjects experiencing similar effects to those experienced when abusing psychoactive substances. In the same way as substance abuse, food-motivated behaviors and binge eating disorders evoke "an intense desire to repeat an action that is pleasurable, perceived as improving wellbeing or capable of alleviating personal distress" (Bonder and Davis 2022).

The primary effects of drugs of abuse are achieved by targeting distinct effector mechanisms, such as neurotransmitter transporters, ion channels, and receptor proteins. However, the former all possess common features capable of triggering a potential for addiction, i.e., they increase dopamine neurotransmission within the mesocorticolimbic circuitry of the brain from the ventral tegmental area (VTA), nucleus accumbens (NAc), and prefrontal cortex (PFC). Specifically, psychostimulants either promote the release of dopamine (amphetamines block the dopamine transporter (DAT) and vesicular monoamine transporter, in turn eliciting an increase in synaptic levels of extracellular dopamine by DAT reversal and depletion of vesicular dopamine stores) or inhibit dopamine reuptake (cocaine and methylphenidate block DAT). Nicotine binds to nicotinic acetylcholine receptors (i.e., $\alpha4\beta2$) which increases neuronal activity in ventral dopaminergic neurons and leads to dopamine release or indirectly activates modulatory [i.e., glutamate or γ-aminobutyric acid

(GABA)] neurons in the VTA. Opioids (i.e., morphine, heroin, or fentanyl) bind to opioid receptors which stimulates an increase in dopamine release in the VTA. The mechanism of action of alcohol however affects multiple targets (enhancing GABA, μ opioid receptors, and cannabinoid signaling), thus indirectly increasing dopamine release in the NAc (Volkow and Blanco 2023). Drugs of abuse and food both share the involvement of similar brain circuits in exerting a rewarding effect. Accordingly, obesogenic foods enhance the activity of dopamine neurons in the VTA, resulting in the release of dopamine into the NAc (Lindgren et al. 2018; Volkow et al. 2017b).

A series of physiopathological mechanisms implicated in SUD and food addiction have been identified using neurobiological, genetic, neuropsychological, and brain imaging tools; the findings obtained have resulted in the development of promising therapeutic options for use in the treatment of some types of SUD (nicotine, opioids) and food addiction. Research is still ongoing aimed at fostering the development of appropriate medications for the treatment of other forms of addiction (psychostimulants).

Studies have also been carried out to investigate changes in the brain neurocircuitry following repeated delivery of drugs of abuse. These studies observed the ability of drugs to modify dopamine and glutamate transmission in NAc (Nestler 2001; Volkow et al. 2017b). The above neurotransmitters send axons that reach the synapse on γ-aminobutyric acid (GABAergic spiny cells in the NAc (Sesack and Pickel 1990)), and the accumbal spiny cell axons collateralize to provide GABAergic innervation of near adjacent spiny neurons (Pennartz et al. 1994). Preclinical findings reported how drugs of abuse alter GABA signaling in multiple brain regions, thus contributing, in part, to the development of drug addiction (Tang et al. 2005; Wydra et al. 2013).

GABA acts via ionotropic type-A ($GABA_A$) and type-C ($GABA_C$) and metabotropic type-B ($GABA_B$) receptors. $GABA_B$ receptors are considered both therapeutic tools for the treatment of pain, seizures, anxiety, and depression (Felice et al. 2022) and as a potential key target in SUD and food addiction. $GABA_B$ receptor activation has indeed been found to decrease the amount of dopamine released in target brain regions. Activation of $GABA_B$ receptors by orthosteric agonists induces side effects including sedation, myorelaxation, hypothermia, tolerance, and cognitive disruption. Having more physiological mechanisms of action than orthosteric agonists, $GABA_B$ positive allosteric modulators (PAMs) lack the sedative and muscle relaxant properties of full $GABA_B$ receptor agonists, providing a better therapeutic index and displaying less adverse effects. This review summarizes the current state-of-the-art based mainly on preclinical studies of drugs of abuse and food intake in the context of $GABA_B$ PAMs.

11.2 $GABA_B$ Receptors and Drugs of Abuse

The metabotropic ϒ-aminobutyric acid (GABA) type-B ($GABA_B$) receptor in a fully functional form is a heterodimer comprising the $GABA_{B1}$ and $GABA_{B2}$ subunits (see Chap. 1 of this volume). The $GABA_{B1}$ receptor subunit, present in splice

variants such as $GABA_{B1a}$ and $GABA_{B1b}$, is crucial for ligand-binding (endogenous neurotransmitter, orthosteric agonists and antagonists), while the $GABA_{B2}$ receptor subunit contains an allosteric binding site for PAMs (Pin and Prézeau 2007), which enhances the affinity of $GABA_{B1}$ receptor subunit to GABA and initiates intracellular signaling through G-proteins (Kaupmann et al. 1997; Kaupmann et al. 1998). The $GABA_{B1a}$-$GABA_{B2}$ heterodimer exists predominantly as a presynaptic receptor, whereas the $GABA_{B1b}$-$GABA_{B2}$ heterodimer is a postsynaptic receptor (Bischoff et al. 1999; Kulik et al. 2003). $GABA_B$ receptor activation leads to the functional enhancement of Gαi/o subunits followed by inhibition of adenylyl cyclase and cyclic adenosine monophosphate (cAMP)-dependent signaling (Enna 2001). Moreover, activation of presynaptic $GABA_B$ receptors leads to inhibition of voltage-gated Ca^{2+} (Ca_V) channels (Takahashi et al. 1998; Thompson and Gähwiler 1992), while stimulation of postsynaptic $GABA_B$ receptors opens G-protein-coupled inwardly rectifying potassium (GIRK) channels (Gähwiler and Brown 1985; Lüscher et al. 1997). Extensive in vitro characterization showed the different ways in which $GABA_B$ PAMs may activate intracellular signaling events, including modulation of cAMP, intracellular calcium levels, or extracellular signal regulated kinase (ERK) that correlates with the dissimilarities observed in rodent models and may be predictive of in vivo efficacy (Kniazeff et al. 2016; Sturchler et al. 2017).

$GABA_B$ receptors have been detected in various regions, including the central nervous system (CNS) and the peripheral nervous system. Peripherally, these receptors are present in diverse locations such as the autonomic ganglia, the spleen, the urinary bladder, the small intestine, the lung, the testis, the stomach, the pancreas, the kidney, the liver, the oviducts, the myocardium, and the skeletal muscles (see review by (Castelli and Gessa 2016; Filip et al. 2015)). In the CNS of both rodents and the human adult, $GABA_B$ receptors are widely distributed across numerous brain areas. The highest expression of $GABA_B$ receptors is observed in specific regions, including the hippocampus, thalamic nuclei, cerebellum, amygdala, neocortex, and habenula. Lower densities of these receptors have also been identified in subcortical areas, the hypothalamus, and the spinal cord (Bischoff et al. 1999; Bowery et al. 1987).

Numerous preclinical studies have demonstrated how drugs of abuse modify the function, expression, and intracellular signaling events of the $GABA_B$ receptor (Filip and Frankowska 2008; Filip et al. 2015; Frankowska et al. 2016; Vlachou and Markou 2010). In the context of acute drug treatment, rodents injected with amphetamine-type stimulants (amphetamine or methamphetamine) or cocaine depressed (downregulated) $GABA_B$ receptor-GIRK channels in VTA GABA neurons, with this effect lasting several days (Arora et al. 2011; Padgett et al. 2012). However, acute injection of cocaine did not induce changes in $GABA_B$ receptor mRNA or protein levels in several brain regions of male rats from 1 to 24 hours following administration (Yamaguchi et al. 2002). In contrast to the above molecular study, increases in $GABA_B$ receptor transcript or protein were found in rat hippocampus after 1–10-day washouts, but not after a 30-day washout (Li et al. 2003).

Diminished $GABA_B$ receptor-GIRK signaling in VTA dopamine neurons was observed in male mice following repeated methamphetamine treatment in a

self-administration procedure (Sharpe et al. 2014) as well as subsequent to repeated noncontingent drug injections (Munoz et al. 2016). Reduction in tegmental $GABA_B$ receptor-GIRK signaling enhanced local GABA neuron firing and affected both dopamine and glutamate release in male rats (Giorgetti et al. 2002). Suppression of $GABA_B$ receptor-GIRK signaling was also observed in layer 5/6 pyramidal neurons of the dorsal medial PFC following repeated cocaine treatment in male mice (Hearing et al. 2008). Repeated (14 days) cocaine administration also significantly increased $GABA_{B1}$ receptor mRNA levels in the NAc, CA1 field of the hippocampus, and thalamus; however, these mRNA levels returned to baseline 1 week after the final injection of repeated cocaine treatment in male rats (Yamaguchi et al. 2002). Chronic cocaine exposure also decreased Gαi and Gαo G-protein levels in VTA and NAc and reduced functional coupling of $GABA_B$ receptor to G-proteins in these brain structures in male rats (Kushner and Unterwald 2001; Xi et al. 2003). Chronic morphine treatment decreased the activity of inhibitory Gi/o G-proteins in the locus coeruleus (Selley et al. 2004).

Another neuroanatomical analysis reported how chronic infusions of morphine via osmotic pumps increased both $GABA_{B1}$ and $GABA_{B2}$ immunoreactivity in the globus pallidus and substantia nigra pars reticulata (Negrete-Díaz et al. 2019). In the PFC, cigarette smoke also increased the level of both $GABA_{B1}$ and $GABA_{B2}$ receptor RNA expression; however, chronic nicotine treatment or chronic exposure to oral nicotine decreases led to a reduction in levels of $GABA_{B1}$, but not $GABA_{B2}$, of RNA expression in the hippocampus or evoked a small decline in both receptor subunits in the PFC, respectively (Li et al. 2002; Li et al. 2004). Another report revealed that chronic nicotine exposure significantly reduced the level of G-protein coupling to $GABA_B$ receptors in rat medial PFC and NAc, with no alterations in VTA. By contrast, $GABA_B$ receptor density and affinity were not altered by nicotine exposure in any of the regions examined (Amantea et al. 2004). Accordingly, a reduced efficacy of the $GABA_B$ receptor agonist baclofen in decreasing electrically stimulated [^{3}H]dopamine release in a rat brain slice preparation of VTA dopamine neurons was observed after chronic nicotine treatment (Amantea et al. 2004). The impact of cannabinoids on $GABA_B$ receptor expression has not been conclusively demonstrated. Chronic exposure to Δ^9-tetrahydrocannabinol (Δ^9-THC), the primary psychoactive cannabinoid in cannabis, resulted in downregulation and desensitization of cannabinoid type-1 (CB_1) receptors in most brain regions, while $GABA_B$ receptor-stimulated G-protein activation remained unaffected (Selley et al. 2004; Sim et al. 1996). However, extended Δ^9-THC administration attenuated $GABA_B$ receptor-associated adenylyl cyclase inhibition in the mouse cerebellum (Selley et al. 2004).

Contrary to observations made following chronic administration of drugs of abuse, withdrawal from chronic morphine enhanced $GABA_B$ receptor coupling to GIRK channels in the VTA followed by a rise in signaling of $GABA_B$ receptors resulting in glutamate release and dopamine neuron activity in male rats (Labouèbe et al. 2007). Withdrawal data for other drugs of abuse indicated attenuation of $GABA_B$ receptor G-protein coupling in NAc after chronic amphetamine in male rats (Zhang et al. 2000) and reduction in $GABA_B$ transmission through impairment of

presynaptic GABA release, as observed at entopeduncular nucleus to lateral habenula synapses following chronic cocaine (Tan et al. 2000). Autoradiographic analysis revealed a substantial decrease in $GABA_B$ receptor binding in various brain regions following a 10-day withdrawal period in animals self-administering cocaine, but not in yoked animals that received noncontingent injections of cocaine; decreases were observed in brain regions most frequently associated with reward processes, including the PFC, NAc, amygdala, hippocampus, and VTA (Frankowska et al. 2008). Reduced GABA transmission was also detected following administration of a challenge injection of methamphetamine after a 14-day withdrawal period, resulting in a compensatory rise in mRNA levels of $GABA_{B1}$ receptor subunit in the prelimbic cortex and in mRNA levels of $GABA_{B2}$ receptor subunit in the orbitofrontal cortex (Wearne et al. 2016a, 2016b).

In line with data obtained in rodents, a separate report relating to individuals suffering from alcohol and cocaine addiction demonstrated a significant postmortem downregulation of $GABA_{B1}$ mRNA levels in the hippocampus of these patients, suggesting that downregulation of $GABA_{B1}$ receptor subunit might act as a predictor of addiction risk [cf. (Enoch et al. 2012)].

11.3 Positive Allosteric Modulators of the $GABA_B$ Receptor on Behavioral Effects of Psychostimulants

Several $GABA_B$ PAMs attenuated the stimulant effects of amphetamine, methamphetamine, and cocaine by inhibiting drug-induced motivation, drug-taking, and drug-seeking and, in some cases, by ameliorating drug withdrawal symptoms (see Fig. 11.1).

The $GABA_B$ PAM, GS39783, blocked the expression of amphetamine-conditioned place preference (CPP) without affecting locomotion in male rats (Halbout et al. 2011). Both this PAM and CGP7930, administered to male rats in two injections, abolished methamphetamine-evoked expression of CPP (Voigt et al. 2011). Additionally, CGP7930 enhanced the effect of an inactive dose of baclofen in blocking the development and expression of amphetamine sensitization in male rats (Cedillo and Miranda 2013).

Further to the effects elicited on amphetamines, $GABA_B$ PAMs also effectively reduced the addictive properties of cocaine in preclinical models. Thus, in male rats, the self-administration of intravenous cocaine under different schedules of reinforcement was reduced by both CGP7930 and GS39783 (Slattery et al. 2005; Smith et al. 2004). Of note, CGP7930 likewise reduced the threshold-lowering effect of cocaine on intracranial self-stimulation, while GS39783 failed to do so, suggesting a hedonic neutrality (Slattery et al. 2005; Smith et al. 2004). Nonsedative doses of another $GABA_B$ PAM, *rac*-BHFF (Malherbe et al. 2008), attenuated cocaine self-administration in male mice (de Miguel et al. 2019).

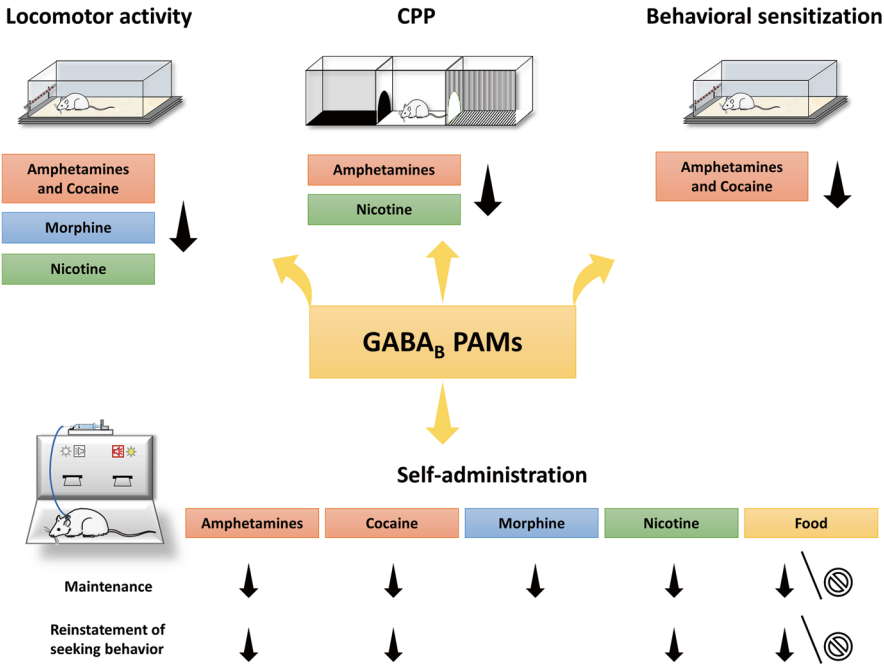

Fig. 11.1 Summary of $GABA_B$ positive allosteric modulator (PAM) effects in preclinical studies. ↓ decrease, ∅ no change, CPP conditioned place preference

An inhibitory pattern for $GABA_B$ PAMs was observed for cocaine-seeking behavior, with administration of either CGP7930 (Filip and Frankowska 2007) or GS39783 (Halbout et al. 2011) reducing cocaine priming or cue-induced reinstatement in male rats. The behavioral actions of these two $GABA_B$ PAMs were specific for cocaine reinforcement and seeking as they affected neither food response (CGP7930 and GS39783) nor discriminative stimulus effects of cocaine (CGP7930) and produced no locomotor sedation at effective (lower) anti-cocaine doses (Halbout et al. 2011). Furthermore, the effects of CGP7930 were blocked by pretreatment with a selective $GABA_B$ receptor antagonist (Filip et al. 2007), indicating selectivity for the receptor action. The same inhibitory pattern toward cue-induced reinstatement of cocaine-seeking was found for the novel $GABA_B$ PAM, CMPPE, producing no signs of sedation or loss of body weight in male rats (Vengeliene et al. 2018).

In male mice, the locomotor hyperactivity evoked by cocaine or amphetamine was attenuated by COR659 (Lobina et al. 2021), while GS39783 attenuated development of cocaine sensitization (Lhuillier et al. 2007), lending support to the control exerted by $GABA_B$ receptors over locomotor behaviors of psychostimulants.

When discussing the inhibitory effects of $GABA_B$ PAMs on psychostimulant drugs of abuse, a series of similarities and differences with orthosteric $GABA_B$ receptor agonists should be addressed. Indeed, baclofen potently reduced cocaine reward, reinforcement, motivation reinstatement, and development and expression of locomotor sensitization to amphetamine in male rats (Bartoletti et al. 2004, 2005;

Cedillo and Miranda 2013; Lotfi et al. 2022). This agonist also proved to be an effective inhibitor for methamphetamine-induced acquisition, expression, or extinction of CPP in male rats (Li et al. 2001; Voigt et al. 2011). Findings of another study reported a higher efficacy of baclofen with regard to acquisition rate and percentage of cocaine self-administration in female than in male rats (Campbell et al. 2002); however, a low dose of baclofen (1.25 mg/kg) reduced cocaine-seeking in both adult male and female animals (DePoy et al. 2016).

In the same way as baclofen, another highly selective and high affinity $GABA_B$ receptor agonist, CGP 44532, attenuated cocaine rewarding and motivational properties in male rats (Brebner et al. 1999) and reduced cocaine-primed reinstatement of cocaine-seeking in male baboons (Weerts et al. 2007). Preclinical research has shown that the $GABA_B$ receptor plays a crucial role in mediating the behavioral and molecular effects of drugs of abuse, with activation of $GABA_B$ receptors proving to be a potential anti-addictive therapeutic strategy (Roberts 2005; Vlachou and Markou 2010). In rodents, baclofen blocks cocaine-induced hyperlocomotion (Kalivas and Stewart 1991) and cocaine-conditioned hyperlocomotion (Hotsenpiller and Wolf 2003). Intra-VTA application of baclofen attenuates cocaine self-administration (Brebner et al. 2000). Furthermore, VTA neurons release dopamine in the NAc and PFC (Kalivas 1993), and baclofen antagonizes nicotine-, cocaine-, and morphine-induced dopamine release in the NAc (Fadda et al. 2003). Based on consistent preclinical observations, the activation of $GABA_B$ receptors via PAMs or orthosteric agonists may represent a potential therapeutic approach for use in the treatment of psychostimulant drug addiction. Baclofen also reduced cue-associated cocaine intake and craving in a double-blind placebo-controlled trial conducted on subjects addicted to cocaine (Vocci and Elkashef 2005; Young et al. 2014).

11.4 Positive Allosteric Modulators of the $GABA_B$ Receptor on Behavioral Effects of Opioids

A few preclinical studies have investigated $GABA_B$ PAMs in the context of opioid use disorder (OUD) (see Fig. 11.1). Accordingly, the $GABA_B$ PAM COR659 reduced morphine-induced locomotor hyperactivity in male mice (Lobina et al. 2021). Following intragastric administration to rhesus monkeys, another $GABA_B$ PAM, ASP8062, demonstrated a reduction in morphine self-administration (Akuzawa et al. 2023). These positive findings strongly indicate the potential clinical efficacy of ASP8062 for the treatment of OUD.

Based on the animal data cited above, a currently ongoing phase I clinical study is investigating the efficacy of ASP8062 on OUD. The combination of ASP8062 and morphine has not resulted in heightened respiratory depression characteristic of OUD or an increase in adverse events associated with drug abuse or withdrawal. These findings indicate a potentially low risk of increased adverse events related to drug abuse or withdrawal, as well as respiratory distress, among participants exposed to both ASP8062 and morphine (Ito et al. 2023).

11.5 Positive Allosteric Modulators of the $GABA_B$ Receptor on Behavioral Effects of Nicotine

Research studies conducted using preclinical models of nicotine use disorder have highlighted the positive modulation of the $GABA_B$ receptor as a potentially useful therapeutic approach for treatment of this disorder (see Fig. 11.1). A very early study by Mombereau and colleagues (Mombereau et al. 2007) reported that the $GABA_B$ PAM GS39783, given repeatedly in doses of 30–100 mg/kg (i.g.) during the conditioning phase, but not acutely before the test, blocked the rewarding effects of nicotine in the CPP paradigm and the accompanying molecular indicator of nicotine abuse (accumulation of DeltaFosB) in the NAc in rats. Further, the $GABA_B$ PAMs GS39783 and BHF177, as well as the BHF177 structural analog KK-92A, attenuated nicotine reinforcement and motivation in drug intravenous self-administration studies conducted under both fixed and progressive ratio schedules of reinforcement in rodents (Li et al. 2017; Sturchler et al. 2017). Interestingly, repeated (14 days) administration of BHF177 consistently decreased nicotine self-administration, showing only a small degree of tolerance over the last 7 days of treatment (Vlachou et al. 2011). In addition to inhibiting use of nicotine, BHF177 and KK-92A also reduced nicotine-seeking behavior (Li et al. 2017; Sturchler et al. 2017; Vlachou et al. 2011).

Extending the efficacy of $GABA_B$ PAMs to block the reinforcing, motivational, and rewarding properties of nicotine, both CGP7930 and GS39783 reduced hyperlocomotion-induced nicotine in male mice (Lobina et al. 2011). A recent study reported how pretreatment with COR659 reduced nicotine-induced locomotor hyperactivity in mice (Lobina et al. 2021).

To summarize, based on preclinical observations, $GABA_B$ PAMs [in the same way as $GABA_B$ receptor activation by baclofen (Lobina et al. 2011)] may represent a potential pharmacological measure capable of targeting different aspects of nicotine addiction with the aim of limiting nicotine intake and suppressing nicotine relapse. These $GABA_B$ PAMs are devoid of any food-suppressive activity (Li et al. 2017; Vlachou et al. 2011), thus representing a more specific anti-smoking therapeutic option compared to other orthosteric $GABA_B$ receptor agonists.

11.6 Positive Allosteric Modulators of the $GABA_B$ Receptor on Behavioral Effects of Food

Palatable foods such as sucrose solution or chocolate-flavored beverages represent a potent means of enhancing motivated behaviors in self-administration procedures in both satiated and food-deprived animals. This behavior therefore "resembles that observed in the context of substance abuse addictions," in reference to the impact produced on the brain and response to treatment (see Fig. 11.1).

Recent experimental evidence has indicated that $GABA_B$ PAMs might affect the seeking for and intake of highly palatable foods, with the largest body of consistent results being obtained in studies conducted using the $GABA_B$ PAM, COR659. Indeed, the latter, together with its three-substituted analog, M4, was able to reduce chocolate self-administration in male rats (Ferlenghi et al. 2020). COR659 also potently attenuated operant self-administration and reinstatement of seeking behavior for different palatable foods (sucrose solution, chocolate-flavored beverage, or calorie-rich Danish butter cookies) (Maccioni et al. 2019, 2023). The reducing effect of COR659 on alcohol and chocolate self-administration was maintained after repeated drug treatment (Maccioni et al. 2019). Additionally, COR659 suppressed reward and motivational properties of food determined by lever-responding for a sucrose solution in selectively bred Sardinian alcohol-preferring (sP) male rats ((Maccioni et al. 2019), with the results obtained further extending the anorectic profile of COR659. Another $GABA_B$ PAM, ADX71441, decreased self-administration of saccharin, a noncaloric sweetener, reducing the reinforcing properties in rats with a history of alcohol self-administration (Augier et al. 2018). Interestingly, the cannabinoid CB_1 receptor antagonist, AM4113, but not the $GABA_B$ receptor antagonist, SCH50911, fully blocked COR659-induced reduction of chocolate self-administration, indicating that (i) COR659 likely exerts its pharmacological effects via a dual mechanism of action (the $GABA_B$ PAM and CB_1 receptor inverse agonist) and that (ii) the cannabinoid CB_1 receptor controls the inhibitory effects of COR659 on consumption of highly palatable foods (Maccioni et al. 2017). Treatment with the $GABA_B$ PAM with ago-allosteric properties KK-92A (in a dose higher than that reducing alcohol self-administration) reduced sucrose self-administration in sP male rats (Maccioni et al. 2021).

In contrast to COR659 and KK-92A, GS39783 produced no effect on the self-administration of sucrose solution or food (pellets) under a stable or progressive ratio of reinforcement in male Wistar rats or rats selectively bred for alcohol preference (i.e., Indiana alcohol-preferring P, sP, and Alko Alcohol), respectively (Maccioni et al. 2012). The findings obtained also underlined how GS39783 failed to alter cue-induced reinstatement of chocolate seeking in Wistar male rats (Maccioni et al. 2017), indicating how this $GABA_B$ PAM fails to modify reinforcing, motivational, or food-seeking properties. Another study reported a reduction of food intake in the absence of impaired animal locomotor activity in male rats at high doses of both GS39783 (100 mg/kg) and a further $GABA_B$ PAM, CMPPE (30–100 mg/kg) (Perdona et al. 2011). Furthermore, BHF177 either reduced (Li et al. 2017) or minimally affected (if at all) food-maintained responding (Vlachou et al. 2011). Its structural analog, KK-92A, produced no effect on food reward or food-seeking (Li et al. 2017). An absence of effects on food (sweetened milk) reward and seeking was observed for CGP7930 in male rats (Filip and Frankowska 2007; Filip et al. 2007). Similar to data obtained in our laboratory, CGP7930 (6 mg/kg) was found to exert no effect on feeding in male rats; however, pretreatment with this $GABA_B$ PAM potentiated the hyperphagic effects of baclofen (Ebenezer 2012).

Numerous other studies have reported the finding of inhibitory effects of baclofen on food-maintained responding and seeking (Filip and Frankowska 2007; Filip

et al. 2007; Filip et al. 2015). These findings confirm the role of orthosteric $GABA_B$ receptor agonists in controlling food intake in animals. Further studies, specifically those focused on the mechanistic aspects, should be carried out to obtain a deeper insight into how $GABA_B$ PAMs affect this behavior in animals and to assess the potential of these drugs as a novel pharmacological approach for use in the treatment of eating disorders. More specifically, whether the anorectic properties of $GABA_B$ PAMs are due primarily to their agonistic activity (ago-PAMs) or should be attributed to a possible secondary (off-target) action such as that mediated, as in the case of COR659, by the CB_1 receptor should be clarified.

11.7 Conclusions

Experimental data obtained thus far tend to strongly support the anti-addiction potential of the majority of known $GABA_B$ PAMs for use in the treatment of SUD, alcohol use disorder (see Chap. 12 of this volume), and, in some cases, food addiction. However, several limitations including lack of investigation of sex differences and/or period of adolescence should be addressed.

The issue of whether $GABA_B$ PAMs might present a promising alternative approach to $GABA_B$ receptor agonists such as baclofen, the use of which is limited due to poor tolerance, toxicity issues, and important side effects (sedation, drowsiness, and sleepiness), should be investigated in future studies.

Of note, ASP8062, an orally active and brain-penetrant $GABA_B$ PAM, was recently investigated in a phase II clinical trial, showing good tolerance and no clinically relevant differences in cognition measurements, drug withdrawal, or suicidal ideation/behavior in humans (Walzer et al. 2020). The findings of this study support the future development of ASP8062 for indications in which the $GABA_B$ receptor is a target.

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Chapter 12
Recent Advances on $GABA_B$ Receptor Positive Allosteric Modulators as Potential Pharmacotherapies for Alcohol Use Disorder

Paola Maccioni and Giancarlo Colombo

Abstract To date, ten different positive allosteric modulators (PAMs) of the $GABA_B$ receptor have been tested in laboratory rodents for their suppressing effects on a series of alcohol-related behaviors. This list includes CGP7930, GS39783, BHF177, *rac*-BHFF, ADX71441, CMPPE, COR659, ASP8062, KK-92A, and ORM-27669. The large body of collected data indicates a remarkable similarity in $GABA_B$-PAM effects: all compounds reduced, or even suppressed, excessive alcohol drinking, relapse- and binge-like drinking, operant oral alcohol self-administration, reinstatement of alcohol seeking, and alcohol-induced hyperlocomotion and conditioned place preference in mice and rats exposed to validated experimental procedures that measure the reinforcing, motivational, rewarding, and stimulatory effects of alcohol. Aspects with translational value include large separation between the pharmacological and toxicological effects, limited development of tolerance, comparable efficacy in both sexes, and efficacy retained after per os administration. The current clinical testing of ASP8062 will prove to what extent these data may translate to patients with alcohol use disorder.

Keywords $GABA_B$ receptor · Positive allosteric modulators · Alcohol-related behaviors · Alcohol drinking · Alcohol self-administration · Fendiline · Mice · Rats

12.1 Introduction

Research on the ability of positive allosteric modulators (PAMs) of the γ-aminobutyric acid (GABA) type-B ($GABA_B$) receptor to suppress multiple alcohol-related behaviors in laboratory rodents stemmed from the several lines of experimental and

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clinical evidence demonstrating that the prototypic $GABA_B$ receptor agonist, baclofen, suppressed alcohol seeking and drinking in mice, rats, and baboons (see Colombo and Gessa 2018; Holtyn and Weerts 2022), as well as alcohol consumption, craving for alcohol, and signs of alcohol withdrawal syndrome in patients affected by alcohol use disorder (AUD) (see Agabio et al. 2023; see Chap. 6 of this volume for an extensive review on baclofen as a pharmacotherapy for AUD).

The discovery of modulatory binding site(s) in the structure of the $GABA_B$ receptor, together with the synthesis of the first $GABA_B$ PAMs, led several labs to investigate whether the suppressing effects of baclofen on a variety of alcohol-motivated behaviors (each modeling specific aspects of human AUD) in mice and rats extended to $GABA_B$ PAMs. Data generated to date unanimously indicate that acute or repeated treatment with all $GABA_B$ PAMs tested reduced, and in most instances even suppressed, excessive alcohol drinking, relapse- and binge-like drinking, operant oral alcohol self-administration, reinstatement of alcohol seeking, and alcohol-induced hyperlocomotion and conditioned place preference (CPP) in mice and rats.

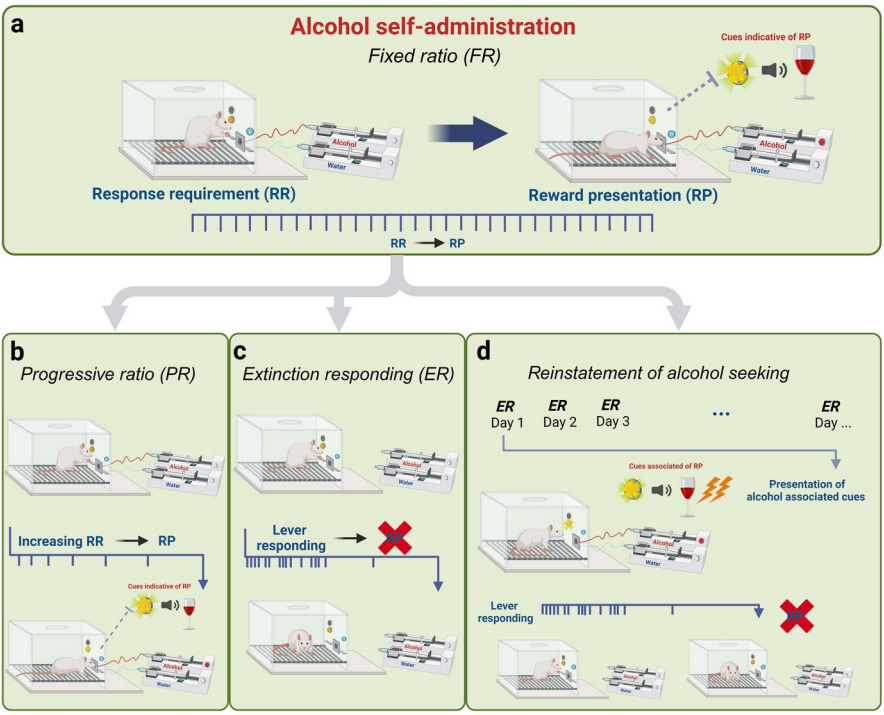
Further to their aim of reproducing baclofen effects on alcohol-related behaviors, the majority of these studies attentively investigated the breadth of separation between the pharmacological effects of $GABA_B$ PAMs and their toxicological effects. Based on their use-dependent mechanism of action (i.e., potentiation of GABA- or baclofen-induced receptor activation with no intrinsic activity), $GABA_B$ PAMs are indeed expected to display a larger separation—compared to baclofen—between the "desired," or expected, pharmacological effects and the "unwanted," off-target, toxicological effects (see Urwyler 2016; see also Chap. 2 of this volume), thus ideally overcoming a relevant shortcoming of baclofen use.

This paper devotes a separate paragraph to each distinct $GABA_B$ PAM, providing a brief description of its effects on alcohol-related behaviors in validated rodent models of AUD. This list of $GABA_B$ PAMs includes CGP7930, GS39783, BHF177, *rac*-BHFF, ADX71441, CMPPE, COR659, ASP8062, KK-92A, and ORM-27669 (see Chap. 8 of this volume for details on their synthesis and chemical structure).

12.2 Overview of the Suppressing Effects of $GABA_B$ PAMs on Alcohol-Related Behaviors

12.2.1 CGP7930

CGP7930 is the first $GABA_B$ PAM made available for in vivo studies (Urwyler et al. 2001; Carai et al. 2004). A few years after CGP7930 synthesis, the research team headed by Andrew J. Lawrence at the University of Melbourne, Australia, reported the results of a study investigating the effect of treatment with CGP7930 on operant oral alcohol self-administration (Box 12.1a) in selectively bred Indiana alcohol-preferring (P) rats (Liang et al. 2006). This was the first paper to document the reducing effect of a $GABA_B$ PAM on an alcohol-related behavior. Specifically, acute



Box 12.1 This picture depicts an operant chamber (also called Skinner box) for rodents. It features a conventional setup comprised of two retractable response levers (one dispensing the alcohol solution and one plain water), one dual liquid receptacle, two syringe pumps located outside the chamber, two stimulus lights (associated to each lever), and one tone generator. This chamber is used for a variety of operant procedures involving alcohol-seeking and alcohol-taking behaviors. Panel (**a**) oral alcohol self-administration under the fixed ratio (FR) schedule of reinforcement, in which each single drop of the alcohol solution (reinforcer) is made available once the animal has pressed the lever for an established, or indeed fixed, number of times [response requirement (RR): one to five in most studies]; animals can freely repeat this sequence of events (lever-responding, consuming alcohol, lever-responding, consuming alcohol, and so on) throughout the duration of the self-administration session; FR schedules of reinforcement provide a measure of the reinforcing properties of alcohol (see Markou et al. 1993). Panel (**b**) oral alcohol self-administration under the progressive ratio (PR) schedule of reinforcement, in which the number of lever-responses required to access each single alcohol reinforcer increases progressively over the self-administration session and up to breakpoint, i.e., the last completed ratio before lever-responding for alcohol is abandoned; breakpoint for alcohol provides a measure of the motivational properties of alcohol (see Markou et al. 1993). Panel (**c**) extinction responding (ER), in which animals are initially trained to lever-respond for alcohol under standard FR schedules of reinforcement and then exposed to an ER session during which lever-responding for alcohol is never reinforced, irrespective of the number of lever-responses; ER (i.e., the number of lever-responses performed during the ER session) provides an additional measure of the motivational properties of alcohol (Samson et al. 2003). Panel (**d**) reinstatement of alcohol-seeking behavior, in which lever-responding for alcohol is initially established under standard FR schedules of reinforcement, then extinguished over a series of consecutive ER sessions, and finally resumed—or indeed reinstated—by (i) non-contingent presentation of visual, olfactory, auditory, and/or gustatory cues previously associated to alcohol availability; (ii) exposure to stressful events (in most instances, a mild footshock delivered by the metal grid floor); or (iii) injections of given, triggering drugs; lever-responding during the reinstatement session models human loss of control over alcohol (see Martin-Fardon and Weiss 2013). (Created with BioRender.com)

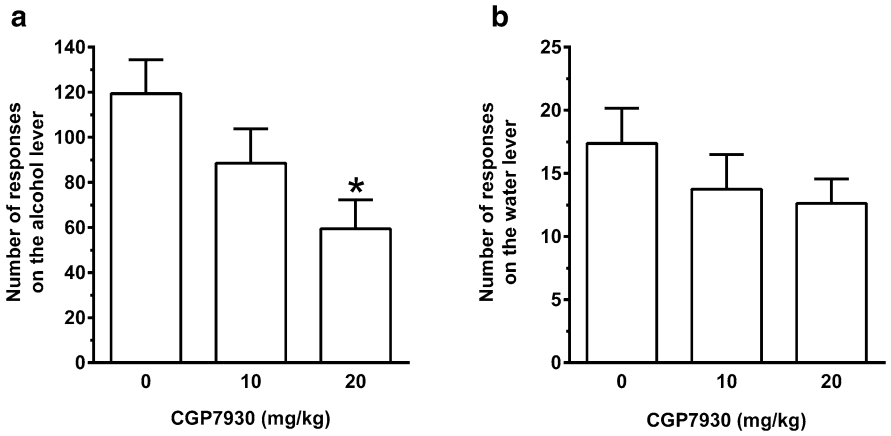


Fig. 12.1 Effect of acute, intraperitoneal (i.p.) treatment with the positive allosteric modulator of the $GABA_B$ receptor, CGP7930, on number of lever-responses for alcohol (panel **a**) and water (panel **b**) in male Indiana alcohol-preferring P rats. Rats were initially trained to lever-respond for oral alcohol (10% v/v) and water under the fixed ratio (FR) 3 schedule of reinforcement in daily 40-min self-administration sessions. Once lever-responding had stabilized, rats were tested with CGP7930 under the same FR schedule of reinforcement. CGP7930 was administered 120 min before of the self-administration session. Each bar is the mean ± SEM of $n = 8$ rats. *: $P < 0.05$ in comparison to the rat group treated with 0 mg/kg CGP7930 (Student-Newman-Keuls test). (Adapted from Liang et al. (2006) with permission from Elsevier)

and intraperitoneal (i.p.) treatment with CGP7930 (10 and 20 mg/kg) virtually halved the number of lever-responses of alcohol in male P rats trained to lever-respond for alcohol (10% v/v) under the fixed ratio (FR) 3 (FR3) schedule of reinforcement (Fig. 12.1a) (Liang et al. 2006). CGP7930 effect was selective for alcohol, as responding for a concurrently available, water-dispensing lever was unaffected by CGP7930 treatment (Fig. 12.1b), but not fully specific, as acute treatment with 20 mg/kg CGP7930 also reduced spontaneous locomotor activity (a reliable index of behavioral toxicity) (Liang et al. 2006). In terms of magnitude, the reducing effect of CGP7930 on alcohol self-administration was comparable to that produced by the acute treatment of 3 mg/kg baclofen (i.p.) (Liang et al. 2006).

Similar data were collected in a subsequent study using Sardinian alcohol-preferring (sP) rats, selectively bred—in the same way as P rats—for high alcohol preference and consumption. Acute treatment with 2.5–10 mg/kg CGP7930 (i.p.) reduced, in a dose-related fashion, number of lever-responses for alcohol and amount of self-administered alcohol in male sP rats exposed to the FR4 schedule reinforcement for alcohol (15% v/v) (Maccioni and Colombo 2019).

CGP7930 and its reducing effect on alcohol self-administration were also used in a mechanistic study aimed at investigating the neural substrate underlying the ability of $GABA_B$ receptor ligands to affect alcohol-related behaviors (Maccioni et al. 2018). This study focused on the ventral tegmental area (VTA), i.e., the area of the brain "reward" mesolimbic system in which dopamine neurons originate.

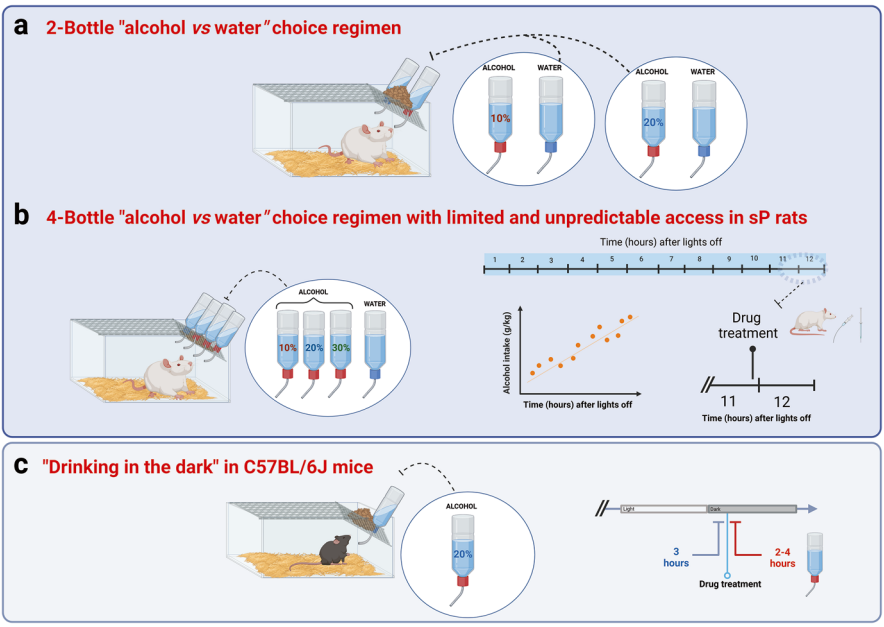
$GABA_B$ receptors are densely located in the VTA, both presynaptically on GABA and glutamate afferent neurons and postsynaptically on dopamine efferent neurons (see Castelli and Gessa 2016). It has been hypothesized that their pharmacological activation inhibits alcohol-induced stimulation of mesolimbic dopamine neurons and therefore the reinforcing, stimulating, and rewarding properties of alcohol (see Frankowska et al. 2016; Lalive and Lüscher 2016).

Specifically, male sP rats originally trained to lever-respond for alcohol (15% v/v) under the FR4 schedule of reinforcement were first implanted with a cannula aimed at the left hemisphere of the VTA and then exposed to a series of self-administration sessions occurring immediately after the intra-VTA microinjection of 5–20 μg CGP7930 (Maccioni et al. 2018). Treatment with CGP7930 was highly effective, as it halved both number of lever-responses for alcohol and amount of self-administered alcohol, with no effect on motor performance (measured by exposing the rats to the inverted screen test immediately before the self-administration session) (Maccioni et al. 2018). CGP7930 effect was also site-specific, as a 20-μg dose of CGP7930 was completely ineffective when infused directly into the deep mesencephalic nucleus (Maccioni et al. 2018).

An early study reported the ability of CGP7930 to decrease alcohol drinking in male sP rats exposed to the conventional, homecage two-bottle "alcohol (10% v/v) vs. water" choice regimen (Box 12.2a) (Orrù et al. 2005). Specifically, repeated (once daily for 5 consecutive days) and intragastric (i.g.) treatment with 25–100 mg/kg CGP7930 (i) prevented acquisition of alcohol drinking behavior in rats that had never been previously exposed to alcohol before the start of CGP7930 treatment and (ii) reduced daily alcohol intake in rats displaying a consolidated alcohol drinking behavior at the start of CGP7930 treatment (Orrù et al. 2005). Compensatory increases in daily water intake proved the selectivity for alcohol intake of CGP7930 effect (Orrù et al. 2005).

More recent studies suggested that CGP7930 may target binding sites other than the positive allosteric modulatory binding site of the $GABA_B$ receptor. CGP7930 has indeed been reported to activate the $GABA_B$ receptor also in the absence of GABA, thus displaying an ago-allosteric profile at the $GABA_B$ receptor (see Mugnaini and Corelli 2016). Additionally, CGP7930 exerted allosteric modulation (positive and negative, depending upon the concentration) of the GABA type-A ($GABA_A$) receptor (Hannan et al. 2023); these effects occurred at CGP7930 concentrations overlapping those found to modulate the $GABA_B$ receptor, leading to hypothesize a virtually equivalent contribution of ionotropic $GABA_B$ and metabotropic $GABA_B$ receptors to the neuropharmacological actions of CGP7930 (Hannan et al. 2023).

This reduced selectivity of CGP7930 apparently limits its usefulness in studies aimed at assessing the contribution of the positive allosteric modulatory binding site of the $GABA_B$ receptor to a given behavior, including—relative to the scopes of the present chapter—alcohol drinking and self-administration. It may however explain some unexpected results, including the development of partial tolerance to the reducing effect of CGP7930 on alcohol drinking (Orrù et al. 2005); the agonistic activity of CGP7930 at the $GABA_B$ receptor might indeed result in receptor



Box 12.2 This picture depicts different procedures of alcohol drinking in rodents. Specifically, panel (**a**) depicts the conventional two-bottle "alcohol vs. water" choice regimen in which animals have free access to an alcohol solution (10% or 20% v/v in most studies) and tap water. Access may be unlimited (24 h/day), limited (drinking sessions of limited periods of time), or intermittent (usually on alternate days); panel (**b**) depicts a procedure of binge-like drinking recently developed in Sardinian alcohol-preferring (sP) rats and based on daily drinking sessions with limited (1 h) and unpredictable access to water and three different alcohol concentrations (10%, 20%, and 30% v/v) (Colombo et al. 2014); panel (**c**) depicts the setup used for the so-called drinking in the dark (DID), the binge-like procedure in which C57BL/6 J mice are exposed to daily drinking sessions of 2–4 h, occurring during the dark phase of the daily light/dark cycle, and access to a single alcohol (20% v/v) bottle (see Thiele and Navarro 2014). (Created with BioRender.com)

desensitization and development of tolerance, a sequence of events not theoretically attributable to a "pure" PAM (see Urwyler 2016).

12.2.2 GS39783

GS39783 is likely the most used $GABA_B$ PAM, at least in the alcohol research field. Notably, the results of all studies focusing on GS39783 were consonant in indicating its ability to effectively reduce different alcohol-related behaviors in mice and rats. Specifically, acute and i.g. treatment with doses of GS39783 ranging from 5 to 100 mg/kg reduced, in a dose-related fashion, lever-responding for alcohol and amount of self-administered alcohol in male, alcohol-preferring P, sP, and Alko Alcohol (AA) rats exposed to the FR4 schedule reinforcement for alcohol (15% v/v)

(Maccioni et al. 2007, 2012, 2017; Lorrai et al. 2019) (Box 12.1a). When compared under identical experimental conditions, treatment with GS39783 came out to be more potent and effective in rats (P line) displaying the most robust lever-responding behavior and self-administering the largest amount of alcohol (Maccioni et al. 2012).

The reducing effect of GS39783 on alcohol self-administration was (i) reproduced, with minimal sex differences, in female sP rats exposed to the FR4 schedule of reinforcement for alcohol (15% v/v) and treated with 25–100 mg/kg GS39783 (i.g.) (Lorrai et al. 2019) and (ii) maintained unaltered throughout 10 days of repeated treatment (50 mg/kg, i.g.) in male sP rats exposed to the FR4 schedule of reinforcement for alcohol (15% v/v) (Maccioni et al. 2015).

In the above studies, no dose of GS39783 affected self-administration of alternative, nondrug reinforcers, including regular food pellets and sucrose solutions (Maccioni et al. 2007, 2012, 2015, 2017), indicative of a complete selectivity of the reducing effect of GS39783 for alcohol reinforcement.

GS39783 was also tested in a slightly different alcohol self-administration procedure, the daily sessions of which comprised an initial phase of lever-responding [up to achievement of a relatively high response requirement (RR); seeking behavior] and a subsequent, relatively long period of free access to alcohol (consummatory behavior). More specifically, male sP rats were initially trained to lever-respond under an RR55 for alcohol; achievement of RR55 gave access to alcohol (15% v/v) for 20 consecutive min. Acute treatment with GS39783 (25–100 mg/kg, i.g.) affected both "seeking" and "consummatory" components, as it effectively reduced number of rats achieving RR55 and amount of alcohol consumed by the rats that achieved RR55 (Maccioni et al. 2010a).

GS39783 was also profitably used, together with baclofen, in a "combination" experiment. Combination of a per se ineffective dose of GS39783 (5 mg/kg, i.g.) with a per se ineffective dose of baclofen (1 mg/kg, i.p.) resulted in a marked reduction in lever-responding for alcohol and amount of self-administered alcohol in male sP rats exposed to the FR4 schedule of reinforcement for alcohol (15% v/v) (Maccioni et al. 2015). These results represent a clear example of how the facilitatory ability of $GABA_B$ PAMs on $GABA_B$ receptor functionality can be reproduced in vivo. The lack of any effect of the "GS39783 + baclofen" combination on sucrose self-administration indicated that the potentiating effect of the drug association was selective for alcohol reinforcement and did not extend to sedative or motor-incoordinating, adverse-like effects (Maccioni et al. 2015).

Reduction of the motivational properties of alcohol is another consolidated aspect of the pharmacological profile of GS39783. Studies employing the progressive ratio (PR) schedule of reinforcement, in which the number of lever-responses needed to access alcohol increases progressively up to breakpoint (Box 12.1b), demonstrated indeed that acute treatment with GS39783 (25–100 mg/kg, i.g.) reduced the number of lever-responses and breakpoint for alcohol in male P, sP, and AA rats firstly trained under the FR4 schedule of reinforcement for alcohol (15% v/v) and then exposed to a conventional PR schedule of reinforcement (Maccioni et al. 2008, 2012). Similar to the results of the FR experiment, the reducing effect of GS39783 on the motivational properties of alcohol ranged from virtually no effect

in the rat line (AA rats) displaying a relatively low breakpoint value to a dose-unrelated suppression in the rat line (P rats) displaying a high breakpoint value (Maccioni et al. 2012). Selectivity of GS39783 effect for alcohol breakpoint was demonstrated by the lack of any drug effect of breakpoint for a sucrose solution (Maccioni et al. 2008).

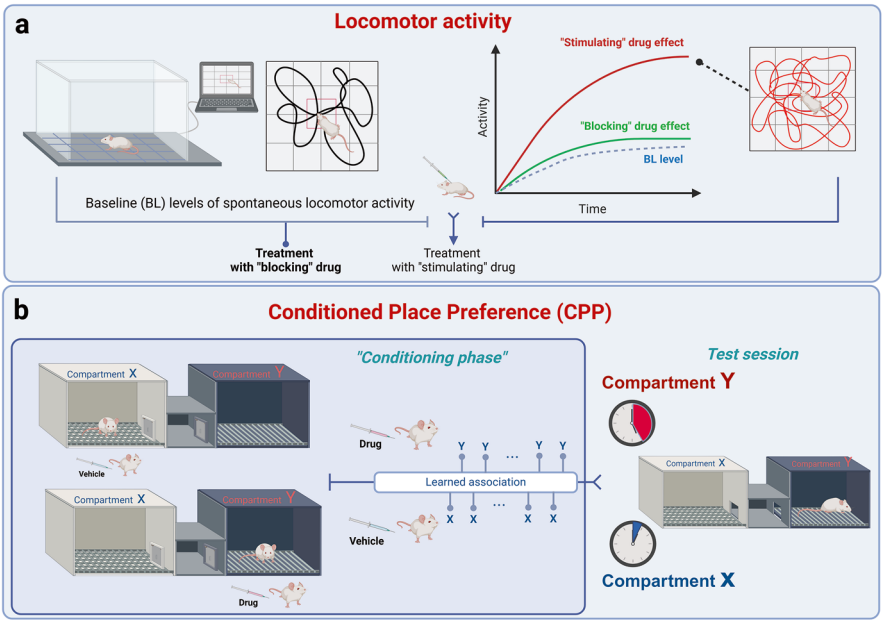
Three additional studies investigated the effect of treatment with GS39783 on different aspects of excessive alcohol drinking in rodents. The first study demonstrated that repeated (once a day for 5 consecutive days) treatment with relatively low doses of GS39783 (6.25–25 mg/kg, i.g.) prevented acquisition of alcohol drinking behavior in male sP rats exposed to the homecage two-bottle "alcohol (10% v/v) vs. water" choice regimen (Box 12.2a) (Orrù et al. 2005); higher doses of GS39783 (50 and 100 mg/kg, i.g.) were however needed to reduce daily alcohol drinking once it was already consolidated (Orrù et al. 2005). In both experiments, reduction in daily alcohol intake was associated to a compensatory increase in daily water intake, indicative of the selectivity of GS39783 effect on alcohol drinking (Orrù et al. 2005).

The other two studies employed validated models of binge drinking. In detail, acute treatment with 30 mg/kg GS39783 (i.p.) suppressed alcohol intake in C57BL/6 J mice exposed to the "drinking in the dark" (DID) procedure (Linsenbardt and Boehm 2014), an experimental condition made of daily 2–4-h drinking sessions occurring during the dark phase of the daily light/dark cycle and under which C57BL/6 J mice consume intoxicating amounts of alcohol (Box 12.2c) (see Thiele and Navarro 2014). GS39783 treatment did not alter spontaneous, homecage locomotor activity (Linsenbardt and Boehm 2014). In the second study, acute treatment with 25–100 mg/kg GS39783 (i.g.) suppressed alcohol intake in sP rats exposed to daily drinking sessions with limited (1 h) and unpredictable access to water and three different alcohol concentrations (10%, 20%, and 30% v/v) (Colombo et al. 2015); when exposed to this peculiar drinking regimen, and when the drinking session occurs at the last hours of the dark phase, sP rats consume alcohol up to intoxication (Box 12.2b) (Colombo et al. 2014). Water intake was not affected by treatment with GS39783 (Colombo et al. 2015).

Finally, acute administration of per se ineffective doses of GS39783 (1–30 mg/kg, i.p.) attenuated hyperlocomotion induced by acute alcohol treatment (2 g/kg, i.p.) in male DBA/2 J mice (Box 12.3a) (Kruse et al. 2012). These data are of relevance as they constitute the first line of experimental evidence on the ability of a $GABA_B$ PAM to block the stimulating, euphorigenic-like properties of alcohol.

12.2.3 BHF177

BHF177 is a GS39783 derivative with reduced genotoxicity (see Chap. 8 of this volume). Its pharmacological effects—related to alcohol-motivated behaviors—were highly consonant with those of the parent compound. Indeed, acute treatment with BHF177 (12.5–50 mg/kg, i.g.) reduced, in a dose-related fashion,



Box 12.3 Panel (**a**) of this picture depicts an open field arena used to assess locomotor activity in rodents; distance covered (index of horizontal activity) and the number of rearings on the hindlegs (index of vertical activity) are measured by means of infrared beams positioned around the box and recorded by computerized tracking systems. Panel (**b**) depicts a standard equipment for conditioned place preference (CPP), comprised of two contextually different compartments; rodents are initially trained to associate alcohol-induced interoceptive cues with the distinguishable, visual, and tactile stimuli of one compartment and absence of those effects (e.g., after saline injection) with the stimuli of the other comportment; after a proper number of conditioning sessions, animals are given a choice between the two compartment: preference for the alcohol-paired compartment infers that alcohol has exerted rewarding properties (see Davis 2017). (Created with BioRender.com)

lever-responding for alcohol and amount of self-administered alcohol in male sP rats exposed to the FR4 schedule of reinforcement for alcohol (15% v/v) (Box 12.1a) (Maccioni et al. 2009). Acute treatment with 50 mg/kg BHF177 (i.g.), but not lower doses, reduced breakpoint for alcohol in male sP rats initially trained to lever-respond for alcohol (15% v/v) under the FR4 schedule of reinforcement and then tested with BHF177 under a PR schedule of reinforcement (Box 12.1a) (Maccioni et al. 2009). Acute treatment with BHF177 was totally devoid of any effect on lever-responding for a sucrose solution, amount of self-administered sucrose solution, and breakpoint for the sucrose solution in male sP rats, indicative of the selectivity of BHF177 effect for alcohol reinforcing and motivational properties (Maccioni et al. 2009).

12.2.4 rac-BHFF

The first study testing *rac*-BHFF in the alcohol research field found that acute treatment with 50–200 mg/kg *rac*-BHFF (i.g.) dose-dependently suppressed lever-responding for alcohol and amount of self-administered alcohol in male sP rats exposed to the FR4 schedule of reinforcement for alcohol (15% v/v) (Box 12.1a) (Maccioni et al. 2010b). The effect of *rac*-BHFF on alcohol self-administration was selective and specific, as only treatment with 200 mg/kg *rac*-BHFF reduced lever-responding for a sucrose solution and no dose of *rac*-BHFF altered spontaneous locomotor activity in male sP rats (Maccioni et al. 2010b). Notably, and as a likely consequence of the long-lasting half-life of *rac*-BHFF (Malherbe et al. 2008), the reducing effect of *rac*-BHFF on alcohol self-administration was still detectable 24 h after treatment (Maccioni et al. 2010b).

When *rac*-BHFF (50 mg/kg, i.g.) was given repeatedly (once daily for 5 consecutive days) to male sP rats exposed to the FR4 schedule of reinforcement for alcohol (15% v/v), magnitude of its reducing effect on lever-responding for alcohol and amount of self-administered alcohol increased progressively over time, as the likely result of tissue accumulation and long-lasting bioavailability (Maccioni et al. 2015). Accordingly, alcohol self-administration was still reduced over the first 2 days of the posttreatment phase (Maccioni et al. 2015).

Similar to GS39783 (see above), acute treatment with a per se ineffective dose of *rac*-BHFF (5 mg/kg. i.g.) interacted synergistically with a per se ineffective dose of baclofen (1 mg/kg, i.p.), unraveling a marked reduction in lever-responding for alcohol and amount of self-administered alcohol in male sP rats exposed to the FR4 schedule of reinforcement for alcohol (15% v/v) (Maccioni et al. 2015). Conversely, the drug combination was totally ineffective—similarly to each drug when given alone—on sucrose self-administration (Maccioni et al. 2015).

Treatment with *rac*-BHFF yielded interesting results also when tested on excessive alcohol drinking.

Repeated (once a day for 7 consecutive days) treatment with 50–200 mg/kg *rac*-BHFF suppressed, in a dose-related fashion, daily alcohol intake in male sP rats offered alcohol under the homecage two-bottle "alcohol (10% v/v) vs. water" choice regimen (Box 12.2a) (Loi et al. 2013). Reduction in daily alcohol intake was fully compensated by an increase in daily water intake, so that daily total fluid intake was virtually unaltered by treatment with *rac*-BHFF (Loi et al. 2013). Additionally, acute treatment with 30 mg/kg *rac*-BHFF (i.p.) virtually halved binge-like, alcohol intake in C57BL/6 J mice exposed to the DID procedure (Box 12.2c) (de Miguel et al. 2019). Specificity was proven by the lack of any effect of 30 mg/kg *rac*-BHFF (i.p.) on spontaneous locomotor activity in a companion set of mice (de Miguel et al. 2019).

rac-BHFF was also found to diminish the rewarding properties of alcohol, measured in male C57BL/6 J mice exposed to a CPP test (Box 12.3b) (de Miguel et al. 2019). Specifically, injection of 30 mg/kg *rac*-BHFF prior to alcohol injection (0.5 g/kg, i.p.) in each of the four "alcohol" conditioning sessions prevented the development of alcohol-induced CPP in the final, drug-free test session (de Miguel et al. 2019).

12.2.5 ADX71441

Acute administration of ADX71441 (1–30 mg/kg, i.p.) resulted in a dose-dependent, virtually complete suppression of lever-responding for alcohol and number of earned alcohol reinforcers in male Wistar rats trained to lever-respond for alcohol (20% v/v) under the FR2 schedule of reinforcement (Box 12.1a) (Augier et al. 2017). Only the highest dose tested (30 mg/kg) affected spontaneous locomotor activity, thus limiting its specificity (Augier et al. 2017).

This initial set of data was corroborated by a series of additional, relevant results. Acute treatment with ADX71441 (1 and 3 mg/kg, i.p.) was more potent and effective in reducing alcohol self-administration under an FR3 schedule of reinforcement for alcohol (20% v/v) in male Wistar rats made alcohol-dependent by prolonged exposure to alcohol vapors than control, air-exposed rats (Augier et al. 2017); specifically, treatment with 1 mg/kg ADX71441 halved lever-responding for alcohol in alcohol-dependent rats while being ineffective in alcohol-nondependent rats (Augier et al. 2017). Additionally, acute treatment with relatively low doses of ADX71441 (3 and 10 mg/kg, i.p.) suppressed breakpoint for alcohol in male Wistar rats exposed to a PR schedule of reinforcement (Box 12.1b) (Augier et al. 2017).

At variance with data collected with most of the other $GABA_B$ PAMs, treatment with ADX71441 (1–10 mg/kg, i.p.) also suppressed self-administration of a saccharin solution in male Wistar rats exposed to the FR2 schedule of reinforcement (Augier et al. 2017), suggesting the relatively unique ability of ADX71441 to affect a broad range of reinforcers.

ADX71441 was the first $GABA_B$ PAM tested on reinstatement of alcohol-seeking behavior, a validated and widely used experimental model of human loss of control over alcohol and relapse into heavy drinking (Box 12.1d) (see Martin-Fardon and Weiss 2013). In this study, male Wistar rats were initially trained to lever-respond for alcohol (20% v/v) under the FR2 schedule of reinforcement; once established, lever-responding was first extinguished (lever-responses were not reinforced) and then reinstated by intermittent (i) exposure to a conventional stressor such as mild footshock or (ii) presentation of environmental cues previously associated with alcohol availability (Augier et al. 2017). Under both experimental circumstances, acute treatment with 3 and 10 mg/kg ADX71441 (i.p.) completely suppressed alcohol-seeking, lever-responding behavior in the final reinstatement session (Augier et al. 2017).

ADX71441 was also effective in reducing alcohol intake in two mouse models of excessive alcohol drinking. In the first study (Hwa et al. 2014), acute treatment with ADX71441 (3–30 mg/kg, i.g.) produced a dose-dependent suppression of alcohol intake in male C57BL/6 J mice exposed to the binge-like DID procedure (Box 12.2c). In the second study (Hwa et al. 2014), acute treatment with ADX71441 (3–17 mg/kg, i.g.) reduced alcohol intake in C57BL/6 J mice exposed to the intermittent (once every other day) access to two bottles containing alcohol (20% v/v) and water, respectively (Box 12.2a), a procedure known to generate remarkable escalation of alcohol drinking in rodents (see Carnicella et al. 2014).

12.2.6 CMPPE

In line with data from all other $GABA_B$ PAMs, acute treatment with CMPPE (2.5–10 mg/kg, i.p.) produced a dose-related reduction in lever-responding for alcohol and amount of self-administered alcohol in female sP rats trained to lever-respond for alcohol (15% v/v) under the FR5 schedule of reinforcement (Box 12.1a) (Maccioni et al. 2019b). Selectivity of this effect was demonstrated by the inability of the same dose range of CMPPE to alter lever-responding for a chocolate solution in a companion set of female sP rats (Maccioni et al. 2019b). Acute treatment with CMPPE (2.5–10 mg/kg, i.p.) also reduced, still in a dose-related fashion, breakpoint for alcohol in female sP rats initially trained to lever-respond for alcohol (15% v/v) under the FR5 schedule of reinforcement and then exposed to a test session under a PR schedule of reinforcement (Box 12.1b) (Maccioni et al. 2019b).

CMPPE was also profitably tested under the reinstatement procedure (Box 12.1d). Specifically, in a first study, acute treatment with CMPPE (10 and 30 mg/kg, i.p.) suppressed cue-induced reinstatement of alcohol seeking in male Wistar rats initially trained to nose-poke for alcohol (10% v/v) under the FR3 schedule of reinforcement (Vengeliene et al. 2018). In a subsequent study, acute treatment with CMPPE (10 and 30 mg/kg, i.p.) suppressed cue-induced reinstatement of alcohol seeking in female sP rats initially trained to lever-respond for alcohol (15% v/v) under an FR5 schedule of reinforcement (Fig. 12.2) (Maccioni et al. 2019b). In both cases, reinstatement of alcohol-seeking behavior was virtually completely abolished by the highest doses of CMPPE.

In addition to reinstatement of alcohol seeking, human episodes of relapse drinking can be effectively modeled by the so-called alcohol deprivation effect (ADE), i.e., the temporary, although substantial, increase in voluntary alcohol drinking occurring in rodents after a period of alcohol deprivation (mimicking human abstinence from alcohol) (Box 12.2a). Repeated (five injections occurring across the phases of alcohol deprivation and alcohol reaccess) treatment with CMPPE (10 and 30 mg/kg, i.p.) resulted in a dose-related reduction of ADE in male Wistar rats exposed to a four-bottle "alcohol (5%, 10%, and 20% v/v) vs. water" choice regimen (Vengeliene et al. 2018).

12.2.7 COR659

Acute treatment with doses of COR659 ranging between 2.5 and 10 mg/kg (i.p.) suppressed, in a dose-related fashion, lever-responding for alcohol and amount of self-administered alcohol in male sP rats exposed to the FR4 schedule of reinforcement for alcohol (15% v/v) (Box 12.1a) (Maccioni et al. 2017, 2019a; Ferlenghi et al. 2020). This effect was maintained—with relatively limited development of tolerance—after repeated treatment (2.5–10 mg/kg, i.p.; injections occurring before ten consecutive daily self-administration sessions) (Maccioni et al. 2019a). Notably,

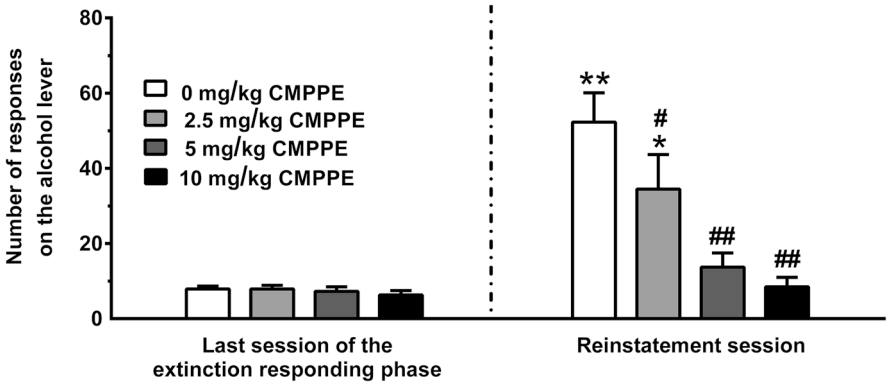


Fig. 12.2 Effect of acute, intraperitoneal (i.p.) treatment with the positive allosteric modulator of the GABA$_B$ receptor, CMPPE, on cue-induced reinstatement of alcohol-seeking behavior in female Sardinian alcohol-preferring (sP) rats. Rats were initially trained to lever-respond for oral alcohol (15% v/v) and water under the fixed ratio (FR) 5 and FR1 schedules of reinforcement, respectively, in daily 30-min self-administration sessions. Once lever-responding had stabilized, rats were exposed to an extinction responding (ER) phase during which lever-responding was unreinforced. The reinstatement session occurred once each single rat had achieved the extinction criterion (≤12 responses on the alcohol lever per session for 2 consecutive sessions). In the reinstatement session, unreinforced lever-responding was resumed by five repeated presentations of a stimulus complex—comprised of auditory (tone), visual (turning on of the stimulus lights), and gustatory (0.1 ml alcohol solution in the liquid receptacle)—previously associated with alcohol availability. Reinstatement sessions lasted 60 min. CMPPE was administered 30 min before start of the reinstatement session. Each bar is the mean ± SEM of n = 10–11 rats. * is $P < 0.001$ and ** is $P < 0.0001$ in comparison with the same rat group in the last session of the extinction responding phase (Tukey's test); # is $P < 0.05$ and ## is $P < 0.001$ in comparison with the vehicle-treated rat group in the reinstatement session (Tukey's test). (Adapted from Maccioni et al. (2019b), with permission from Elsevier)

COR659 doses suppressing alcohol self-administration were far lower than those affecting spontaneous locomotor activity (Maccioni et al. 2017).

Acute treatment with COR659 (2.5–10 mg/kg, i.p.) (i) markedly reduced breakpoint for alcohol in male sP rats initially trained to lever-respond for alcohol (15% v/v) under an FR4 schedule of reinforcement and then exposed to a test session under a PR schedule of reinforcement (Box 12.1b) (Maccioni et al. 2017) and (ii) completely abolished cue-induced reinstatement of alcohol seeking in male sP rats previously trained to lever-respond for alcohol (15% v/v) under an FR4 schedule of reinforcement (Box 12.1d) (Maccioni et al. 2019a).

COR659 was also effective in experimental procedures of excessive alcohol drinking. Specifically, acute treatment with COR659 (2.5–10 mg/kg, i.p.) reduced alcohol intake in male sP rats exposed to the homecage two-bottle "alcohol (10% v/v) vs. water" choice regimen (Box 12.2a) (Ferlenghi et al. 2020). However, when given repeatedly (5–20 mg/kg, i.p.; injections occurring before seven consecutive daily drinking sessions), tolerance to the suppressing effect of COR659 on voluntary intake of alcohol developed relatively rapidly (Lorrai et al. 2022b). Finally,

acute treatment with COR659 suppressed binge-like drinking: (i) injection of 2.5–40 mg/kg COR659 (i.p.) markedly decreased alcohol intake in male sP rats exposed to the experimental procedure comprised of daily drinking sessions with limited (1 h) and unpredictable access to water and three different alcohol concentrations (10%, 20%, and 30% v/v) and known to produce, at least in this rat line, escalations in alcohol drinking up to intoxication (Box 12.2b); (ii) injection of 10–40 mg/kg COR659 (i.p.) suppressed alcohol intake in male C57BL/6 J mice exposed to the DID procedure (Box 12.2c) (Lorrai et al. 2022a). A subsequent experiment found that tolerance to the suppressing effect of COR659 on alcohol drinking under the DID model developed rapidly when COR659 (10–40 mg/kg, i.p.) was administered repeatedly (i.e., before seven consecutive daily drinking sessions) (Lorrai et al. 2022b).

The results of a series of additional experiments revealed that COR659 displays a unique pharmacological profile, made of at least two distinct mechanisms of action: positive allosteric modulation of the $GABA_B$ receptor and antagonism/inverse agonism at cannabinoid type-1 (CB_1) receptor (Ferlenghi et al. 2020). At a behavioral level, this dual mechanism of action was confirmed by (i) the ability of pretreatment with the $GABA_B$ receptor antagonist, SCH50911, to partially block the suppressing effect of COR659 on alcohol self-administration in sP rats and (ii) the ability of pretreatment with the neutral cannabinoid CB_1 receptor antagonist, AM4113, to fully block the reducing effect of COR659 on chocolate self-administration in Wistar rats (Maccioni et al. 2017). This off-target mechanism of action may help to explain why tolerance developed to the reducing effect of COR659 on alcohol drinking, a phenomenon not expected when testing a PAM (see above). Conversely, and taking into account the well-known ability of the prototypic cannabinoid CB_1 receptor antagonist/inverse agonist, rimonabant, to suppress alcohol intake in mice and rats, it is likely that the cannabinoid CB_1 receptor component of the complex mechanism of action of COR659 contributed to the reducing effect of COR659 on alcohol drinking and was the molecular substrate involved in the development of tolerance to the reducing effects of COR659 on alcohol drinking (Lorrai et al. 2022b).

12.2.8 ASP8062

A recent experimental study tested ASP8062 on alcohol self-administration in rats (Haile et al. 2021). Specifically, ASP8062 was administered i.g., at doses ranging between 1 and 10 mg/kg, before four consecutive self-administration sessions to both female and male Sprague-Dawley rats trained to lever-respond for alcohol (10% v/v) under the FR2 schedule of reinforcement (Box 12.1a). Treatment with ASP8062 suppressed lever-responding for alcohol and number of earned alcohol reinforcers, with male rats resulting to be more sensitive than female rats to the suppressing effect of ASP8062 on alcohol reinforcement (Fig. 12.3). No dose of ASP8062 affected spontaneous locomotor activity in both female and male rats.

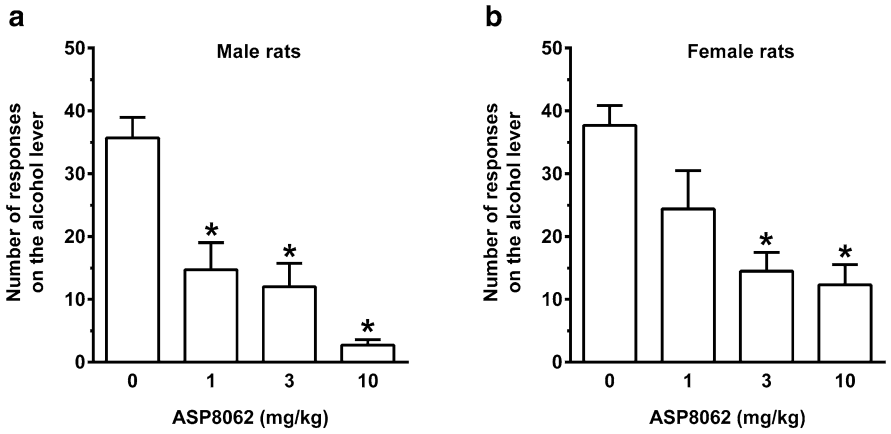


Fig. 12.3 Effect of repeated, intragastric (i.g.) treatment with the positive allosteric modulator of the $GABA_B$ receptor, ASP8062, on number of lever-responses for alcohol in male (panel a) and female (panel b) Sprague-Dawley rats. Rats were initially trained to lever-respond for oral alcohol (10% v/v) and water under the fixed ratio (FR) 2 schedule of reinforcement in daily 60-min self-administration sessions. Once lever-responding had stabilized, rats were tested with ASP8062 under the same FR schedule of reinforcement. ASP8062 was administered 30 min before start of consecutive self-administration sessions. Data depicted here refer only to day 4 of treatment. Each bar is the mean ± SEM of $n = 10$ rats. *: $P < 0.001$ in comparison with the rat group treated with 0 mg/kg ASP8062 (Dunnett's test). (Adapted from Haile et al. (2021) with permission from Springer Nature)

Based on these promising results, ASP8062 has recently reached clinical testing. More specifically, an initial phase I study reported safety and tolerability of oral ASP8062, even when administered in combination with alcohol (Ito et al. 2022). A subsequent trial has investigated the effect of oral ASP8062 on alcohol craving and consumption in subjects with moderate-to-severe AUD (ClinicalTrial.gov 2024); when available, data from this study will constitute the first line of clinical evidence on the therapeutic potential of $GABA_B$ PAMs in the AUD research field (see Burnette et al. 2022).

12.2.9 KK-92A

KK-92A is one of the $GABA_B$ PAMs most extensively evaluated, at least based on the number of different experimental procedures, for its suppressing effects on alcohol-related behaviors. Acute treatment with KK-92A (5–20 mg/kg, i.p.) dose-dependently suppressed lever-responding for alcohol and amount of self-administered alcohol in female sP rats exposed to the FR5 schedule of reinforcement for alcohol (15% v/v) (Box 12.1a) (Maccioni et al. 2021). When given repeatedly (once daily for 10 consecutive days), partial tolerance developed to the suppressing effect of KK-92A on alcohol reinforcement in female sP rats (Maccioni et al. 2022);

the agonistic component of the ago-allosteric profile of KK-92A (Li et al. 2017) likely produced some degree of receptor desensitization, thus being responsible for the observed development of partial tolerance (Maccioni et al. 2022).

The suppressing effect of KK-92A was not fully selective for alcohol, as acute treatment with KK-92A (5–20 mg/kg, i.p.) also affected—although less efficaciously—lever-responding for a sucrose solution in female sP rats exposed to the FR5 schedule of reinforcement (Maccioni et al. 2021). Again, the agonistic activity of KK-92A might be the key element in producing an effect repeatedly observed with baclofen (see Colombo and Gessa 2018).

The FR schedule of reinforcement was used to generate two additional sets of data. First, combination of a per se ineffective dose of KK-92A (2.5 mg/kg, i.p.) with a per se ineffective dose of baclofen (1 mg/kg, i.p.) synergistically reduced number of lever-responses for alcohol and amount of self-administered alcohol in female sP rats lever-responding for alcohol (15% v/v) (Maccioni et al. 2021), replicating the potentiating effect on baclofen action previously observed with GS39783 and COR659 (see above). Second, acute and i.g. administration of KK-92A (20 and 40 mg/kg) reduced lever-responding for alcohol and amount of self-administered alcohol in female sP rats tested under the FR5 schedule for alcohol (15% v/v) reinforcement (Maccioni et al. 2023), indicating that the ability of KK-92A to reduce alcohol self-administration is maintained also after per os treatment.

Acute treatment with KK-92A (5–20 mg/kg, i.p.) suppressed, in a dose-related fashion, lever-responding and breakpoint for alcohol in female sP rats initially trained to self-administer alcohol (15% v/v) under the FR5 schedule of reinforcement and then exposed, in the test session, to a conventional PR schedule of reinforcement (Fig. 12.4a; Box 12.1) (Maccioni et al. 2021). The suppressing effect of KK-92A on the motivational properties of alcohol was then extended to an alternative experimental procedure named extinction responding (ER) and characterized by the absence of any alcohol reinforcement whatever the number of lever-responses (Box 12.1c). Specifically, acute treatment with KK-92A (5–20 mg/kg, i.p.) reduced ER for alcohol in female sP rats initially trained to self-administer alcohol (15% v/v) under the FR5 schedule of reinforcement and then exposed, in the test session, to alcohol-seeking non-reinforced lever-pressing (Fig. 12.4b) (Maccioni et al. 2023).

In line with data previously collected with ADX71441, CMPPE, and COR659 (see above), treatment with KK-92A was also extremely effective in suppressing reinstatement of alcohol-seeking behavior (Box 12.1d). Indeed, acute treatment with 5–20 mg/kg KK-92A (i.p.) completely abolished cue-induced reinstatement of alcohol seeking in female sP rats initially trained to lever-respond for alcohol (15% v/v) under the FR5 schedule of reinforcement, ultimately leading to a virtually complete extinguishing of alcohol-seeking, lever-pressing behavior, and finally exposed animals to a session of cue-induced reinstatement (Maccioni et al. 2021).

Repeated (once daily for 7 consecutive days) treatment with KK-92A (5–20 mg/kg, i.p.) markedly reduced, with limited development of tolerance, excessive alcohol drinking in male sP rats exposed to daily 1-h drinking sessions under the homecage two-bottle "alcohol (10% v/v) vs. water" choice regimen (Box 12.2a) (Maccioni et al. 2023).

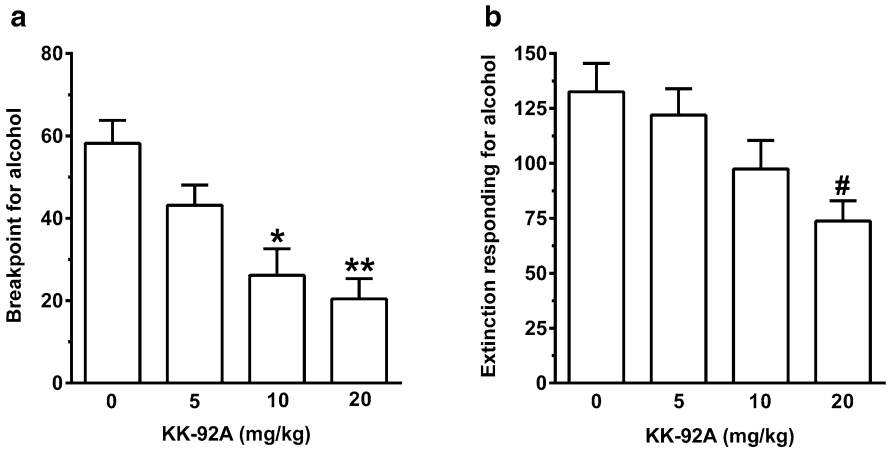


Fig. 12.4 Effect of acute, intraperitoneal (i.p.) treatment with the positive allosteric modulator of the GABA$_B$ receptor, KK-92A, on breakpoint for alcohol (panel **a**) and extinction responding (ER; panel **b**) in female Sardinian alcohol-preferring (sP) rats. In both studies, rats were initially trained to lever-respond for oral alcohol (15% v/v) and water under the fixed ratio (FR) 5 and FR1 schedules of reinforcement, respectively, in daily 30-min self-administration sessions. Once lever-responding had stabilized, rats of the "breakpoint" study were exposed to a single, 60-min self-administration session under a conventional progressive ratio (PR) schedule of reinforcement; breakpoint was defined as the lowest response requirement not achieved. Rats of the "ER" study were exposed to a single, 60-min ER session in which lever-responding was not reinforced. In both studies, KK-92A was administered 30 min before start of the test session. In panel a, each bar is the mean ± SEM of $n = 12$ rats; *: $P < 0.001$ and **: $P < 0.0001$ in comparison to the rat group treated with 0 mg/kg KK-92A (Tukey's test). In panel b, each bar is the mean ± SEM of $n = 16$ rats; #: $P < 0.005$ in comparison with the rat group treated with 0 mg/kg KK-92A (Dunn's test). (Panel **a**: adapted from Maccioni et al. (2021). Panel **b**: adapted from Maccioni et al. (2023), with permission from Elsevier)

Two final, and somewhat ancillary, experiments demonstrated that none of the i.p. administered doses of KK-92A found to suppress alcohol-related behaviors altered (i) alcohol palatability in male sP rats (Maccioni et al. 2023) and (ii) spontaneous locomotor activity (Maccioni et al. 2021) in female sP rats, thus providing evidence that the reducing effects of KK-92A were not due to an increase in taste aversiveness of alcohol solutions or sedative and motor-incapacitating effects, respectively.

12.2.10 ORM-27669

To date, the latest synthesized GABA$_B$ PAM, ORM-27669, has been tested solely in DID and CPP procedures. Specifically, acute treatment with 100 mg/kg ORM-27669 (i.p.) suppressed alcohol intake in male C57BL/6 J mice exposed to the binge-like DID procedure (Box 12.2c) (de Miguel et al. 2019). The same ORM-27669 dose,

given prior to alcohol injection (0.5 g/kg, i.p.) in each "alcohol" conditioning session, prevented the development of alcohol-induced CPP in the final, drug-free test session in male C57BL/6 J mice (Box 12.3b) (de Miguel et al. 2019). Specificity of these reducing effects of ORM-27669 on alcohol drinking and alcohol rewarding properties was demonstrated by the lack of any effect of acute treatment with 100 mg/kg ORM-27669 (i.p.) on spontaneous locomotor activity in male C57BL/6 J mice (de Miguel et al. 2019).

12.3 The Putative $GABA_B$ PAM, Fendiline, and Alcohol Self-Administration

Fendiline is a human-approved, L-type Ca^{2+} channel blocker. Approximately 20 years ago, an intriguing debate arose on the ability of fendiline to act as a $GABA_B$ PAM in addition to blocking the L-type Ca^{2+} channels. Fendiline application to rat neocortical slices enhanced baclofen-induced hyperpolarization, an effect blocked by SCH50911 (Kerr et al. 2002). Similarly, fendiline application induced a leftward shift of baclofen concentration-response curve in rat midbrain slices (Chen et al. 2005). A subsequent study led by Stephan Urwyler (the chemist of Novartis, Basel, Switzerland, who headed the synthesis of CGP7930 and GS39783) challenged these data reporting the complete inability of fendiline to enhance (i) $GABA_B$ receptor-mediated guanosine 5-O-(3-[^{35}S]thio)triphosphate (GTPγ^{35}S) binding (an assay that evaluates functionality of the receptor by measuring its activation via the G-protein) in membrane preparations from CHO cells and rat brain cortex as well as (ii) affinity of GABA for $GABA_B$ receptors in a competition, radioligand binding assay using rat brain cortex (Urwyler et al. 2004).

Two more recent behavioral studies reported that fendiline exerted anti-addictive, $GABA_B$ PAM-like effects, revamping some interest on this compound. Specifically, (i) repeated treatment with fendiline prevented and abolished (once established) methamphetamine-induced CPP in rats (Voigt et al. 2014) and (ii) acute treatment with fendiline attenuated cue- and cocaine-induced reinstatement of cocaine seeking in rats (Cunningham et al. 2015). Unfortunately, no pharmacological blockade experiment investigated which component of the dual mechanism of action of fendiline (i.e., blockade of the L-type Ca^{2+} channels and positive allosteric modulation of the $GABA_B$ receptor) was actually responsible for these effects.

Nevertheless, intrigued by these anti-addictive effects of fendiline clearly evocative of those of $GABA_B$ PAMs (see Chap. 11 of this volume), we recently investigated fendiline effect on operant oral alcohol self-administration (Box 12.1a). Specifically, female sP rats were initially trained to lever-respond for alcohol (15% v/v) under the FR5 schedule of reinforcement in daily 30-min self-administration sessions. Once lever-responding had stabilized, rats were exposed to a test session preceded by acute treatment with 0, 1.7, 3, and 5.6 mg/kg fendiline (i.p.). Fendiline dose range was identical to that tested in the "methamphetamine" (Voigt et al. 2014) and "cocaine" (Cunningham et al. 2015) studies. Selectivity was investigated

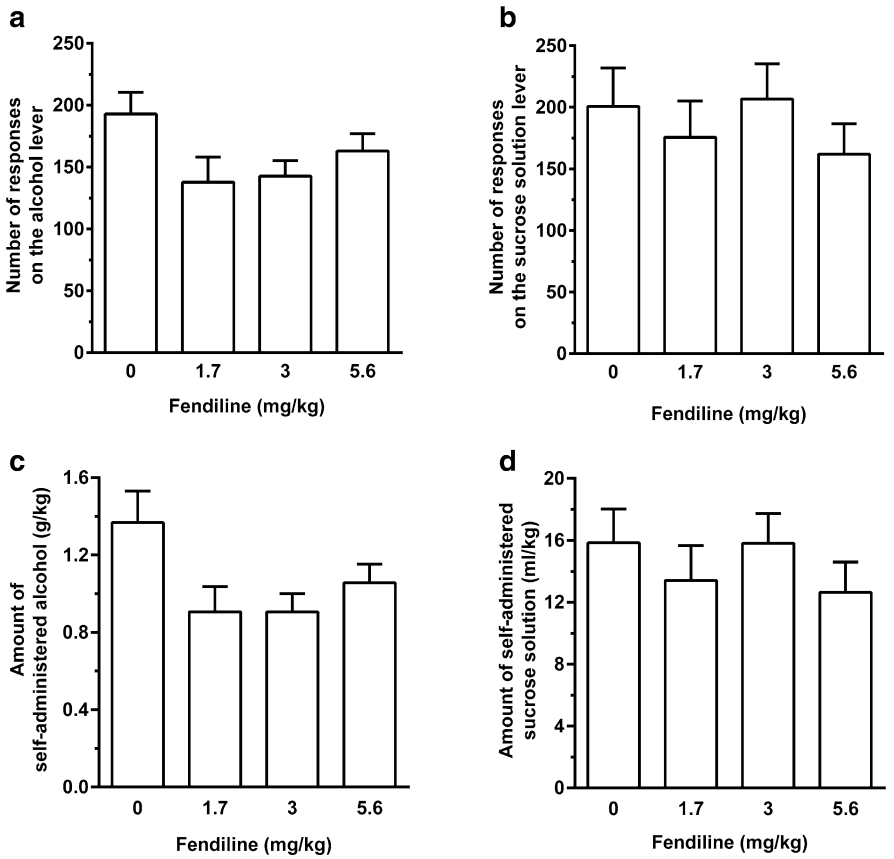


Fig. 12.5 Effect of acute, intraperitoneal (i.p.) treatment with the L-type Ca^{2+} channel blocker and putative positive allosteric modulator of the $GABA_B$ receptor, fendiline, on number of lever-responses for alcohol (panel **a**), amount of self-administered alcohol (panel **c**), number of lever-responses for a sucrose solution (panel **b**), and amount of self-administered sucrose solution (panel **d**) in female Sardinian alcohol-preferring (sP) rats. Rats were initially trained to lever-respond for oral alcohol (15% v/v) [or sucrose solution (0.3% w/v)] and water under the fixed ratio (FR) 5 and FR1 schedules of reinforcement, respectively, in daily 30-min self-administration sessions. Once lever-responding had stabilized, rats were tested with fendiline under the same FR schedule of reinforcement. Fendiline was administered 20 min before start of the self-administration session. Each bar is the mean ± SEM of n = 11–12 rats. Analysis of variance (ANOVA) for number of lever-responses for alcohol: $F(3,47) = 7.40$, $P > 0.05$; ANOVA for amount of self-administered alcohol: $F(3,47) = 7.70$, $P > 0.05$; ANOVA for number of lever-responses of the sucrose solution: $F(3,40) = 0.55$, $P > 0.05$; ANOVA for amount of self-administered sucrose solution: $F(3,40) = 0.63$, $P > 0.05$

testing the same fendiline doses on self-administration of a sucrose solution (0.3% w/v) in an independent set of female sP rats exposed to the FR5 schedule of reinforcement. Basal levels of lever-responding for the sucrose solution equated those sustained by alcohol.

As depicted in Fig. 12.5, no dose of fendiline altered either number of lever-responses for alcohol and amount of self-administered alcohol or number of

lever-responses for sucrose solution and amount of self-administered sucrose solution. Testing higher doses of fendiline was not advisable, as they were found to produce motor incoordination in rats (Voigt et al. 2014). The lack of any fendiline effect on alcohol self-administration prevented subsequent, pharmacologically blockade experiments from being conducted with $GABA_B$ receptor antagonists.

12.4 Conclusions

To summarize, all $GABA_B$ PAMs tested to date have invariably been reported to reduce, or even suppress, multiple alcohol-motivated behaviors, including excessive alcohol drinking, binge-like alcohol drinking, relapse-like alcohol drinking, operant oral alcohol self-administration, alcohol seeking, cue- and stress-induced reinstatement of alcohol seeking, and alcohol-induced hyperlocomotion and CPP, in mice and rats.

These data possess remarkable translational value, synthesized in the following bullet points:

- Data were generated using validated, experimental procedures that effectively model single aspects of human AUD (see Bell et al. 2017).
- The reducing, or suppressing, effects of $GABA_B$ PAMs on alcohol-related behaviors occurred at doses largely lower than those producing sedation and motor incoordination. When calculated (Maccioni et al. 2017, 2021), the resulting Therapeutic Index was remarkably high, suggestive of a large separation between the pharmacological and toxicological effects of $GABA_B$ PAMs.
- Development of tolerance to the reducing effects of repeatedly administered $GABA_B$ PAMs on alcohol drinking and alcohol self-administration was relatively limited, in line with the expected, minimal propensity of PAMs to produce receptor desensitization.
- $GABA_B$ PAMs came out to be more potent and effective in those experimental conditions under which rats displayed strong reinforcing and motivational properties of alcohol and self-administered large amounts of alcohol.
- No major sex differences were observed in the (still relatively few) studies comparing the effects of $GABA_B$ PAMs on alcohol-related behaviors in female and male rats.
- Virtually all $GABA_B$ PAMs maintain their ability to affect alcohol-related behaviors when given per os.

The recent transition of ASP8062 to clinical testing will soon reveal whether these promising preclinical data replicate in humans and whether $GABA_B$ PAMs may represent a feasible option among pharmacotherapies for AUD.

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Part IV
Miscellanea

Chapter 13
γ-Hydroxybutyric Acid: A $GABA_B$/GHB Receptor Agonist with a Unique Neuropharmacological and Therapeutic Profile

Francesco Bavato

Abstract γ-Hydroxybutyric acid (GHB) is a $GABA_B$/GHB receptor agonist which naturally occurs in the human brain. As a pharmaceutical agent, GHB was first synthesized by Henri Laborit in 1960 and was soon recognized to have unique pharmacological and therapeutic properties. While physiological GHB concentrations seem to be insufficient to stimulate $GABA_B$ receptor, the stimulation of this receptor appears to be responsible for most of its psychotropic effects when exogenously administered. The complex pattern of neuropharmacological, behavioral, and clinical effects induced by GHB administration is a paradigmatic example of the crucial role of the $GABA_B$ receptor in modulating brain functioning and human behavior. This chapter will briefly introduce the neuropharmacological properties of GHB and give an insight into current human research addressing both neurobiological mechanisms of GHB and its clinical application. GHB represents both a powerful pharmacological model and a promising therapeutic approach to manipulate $GABA_B$/GHB receptor activity in humans. Safety aspects and addictive liability are discussed as limiting factors for future human research.

Keywords γ-Hydroxybutyric acid · Sodium oxybate · GHB · $GABA_B$ receptor · Slow-wave sleep · Narcolepsy · Parkinson's disease · Fibromyalgia · Depression · Liquid ecstasy

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13.1 History

γ-Hydroxybutyric acid (GHB) is an endogenous short-chain fatty acid with a potent depressant effect on the central nervous system (CNS). Henri Laborit first synthesized exogenous, orally active GHB in 1960 as a γ-aminobutyric acid (GABA) receptor agonist (Laborit 1964; Laborit et al. 1960), aiming for sedative effects to facilitate anesthesia induction (Langlois et al. 1960). Although its use in anesthesia was soon found to be limited due to insufficient pain relief, risk of delirium, and epileptic seizures (Kam and Yoong 1998), early observations of heterogeneous effects of GHB prompted a broad spectrum of preclinical and clinical trials assessing the compound's therapeutic potential across various medical fields, including obstetrics, anesthesia, neurology, and psychiatry.

GHB was initially used as a tranquilizer across psychiatric disorders but, when administered intravenously, soon yielded more specific antidepressant and anxiolytic effects (Tanaka et al. 1966), improving clinical outcomes in disorders ranging from schizophrenia to depression and anxiety (Danon-Boileau et al. 1962; De Couedic and Voisse 1964; Rinaldi et al. 1967). Despite its unique profile offering a great therapeutic potential, the clinical use of GHB was displaced by the increasingly prescribed tricyclic antidepressants.

Unlike other available GABAergic ligands, such as barbiturates or benzodiazepines, GHB has been suggested to foster sleep patterns closely mimicking those observed in natural deep sleep phases (Mamelak et al. 1973). Consequently, GHB was postulated to be advantageous not merely for the facilitation of sleep onset but also for enhancing the overall quality of sleep, which, in turn, lead to the discovery of its therapeutic potential in narcolepsy (Broughton and Mamelak 1979). Furthermore, following the identification of GHB's capacity to stimulate growth hormone release (Takahara et al. 1977), the compound gained popularity as a dietary supplement and was extensively adopted by bodybuilders for its anabolic effects.

However, the widespread and unregulated use of GHB led to an uptick in reported intoxications (Chin et al. 1992; Dyer 1991) and heightened concerns over its potential misuse as a sedative in sexual assault cases (Bismuth et al. 1997). Following these concerns, the US Food and Drug Administration prohibited the prescription-free sales of the substance, and, in 2000, the US Government classified GHB as a Schedule I narcotic.

Despite its controversial reputation, the early 2000s saw a resurgence in the clinical research of GHB, leading to its adoption as a first-choice treatment for excessive daytime sleepiness and cataplexy in narcolepsy type 1 (Black and Houghton 2006). Its sodium salt, sodium oxybate, is marketed as Xyrem® in regions including the USA, Canada, the European Union, and Switzerland. Additionally, GHB finds use as an intravenous anesthetic in Germany, marketed as Somsanit® and as Alcover® in Italy and Austria for treating alcohol withdrawal symptoms (Addolorato et al. 2009; Leone et al. 2006), and off-label for opioid withdrawal treatment (Gallimberti et al. 1994). Further current therapeutic indications include fibromyalgia (Spaeth et al.

2012), binge-eating disorder (BED) (McElroy et al. 2011), Parkinson's disease (Büchele et al. 2018), and cluster headache (Hidalgo et al. 2013).

Currently, GHB continues to be used in non-medical context, primarily for its euphoric effects, especially within chemsex communities for sexual enhancement (Bosch and Seifritz 2016). Despite its association with sexual assault, its predominant illicit use continues to be as an aphrodisiac and mild anxiolytic in self-administration (Bosch and Seifritz 2016).

13.2 Pharmacology

13.2.1 Pharmacokinetics

GHB can be externally administered either directly or as one of its precursors, γ-butyrolactone (GBL) or 1,4-butanediol (1,4-BD) (Bosch and Seifritz 2016) (Fig. 13.1).

Following oral administration, GHB, GBL, or 1,4-BD are absorbed quickly and nonlinearly with a ceiling effect for higher therapeutic doses. The bioavailability

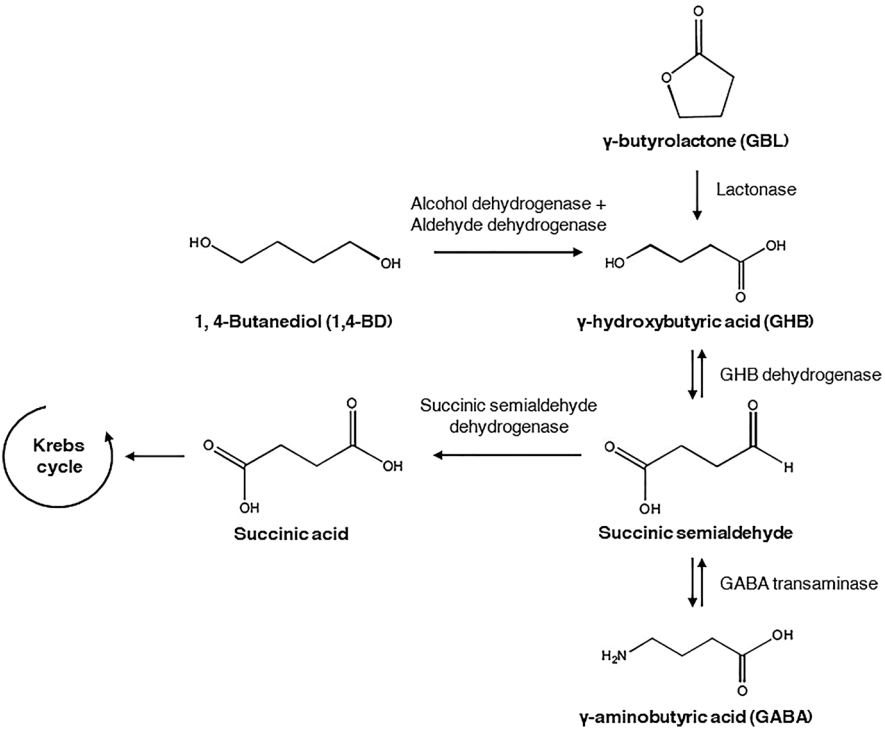

Fig. 13.1 Graphic representation of the metabolic pathway of GHB

reaches approximately 25% of the oral dose (Vree et al. 1978) due to a significant first-pass effect. Peak plasma concentrations of GHB are typically observed within 25–60 min following ingestion (Brailsford et al. 2012; Brenneisen et al. 2004; Liechti et al. 2016; Thai et al. 2007). The distribution of GHB adheres to a two-compartment model, characterized by rapid dissemination throughout the body and minimal binding to plasma proteins.

In the liver, GHB is metabolized to succinic semialdehyde by GHB-dehydrogenase. Succinic semialdehyde is then metabolized to succinic acid by succinic semialdehyde dehydrogenase and enters the Krebs cycle or acts as a precursor for GABA synthesis.

GHB's elimination exhibits first-order kinetics at low doses and shifts to nonlinear or zero-order kinetics at higher doses, attributed to the kidneys' saturable reabsorption process (Morse et al. 2012a, b; Wang et al. 2007). Typically, GHB is eliminated from the body within 4–8 h after consumption (Ferrara et al. 1992; Hoes et al. 1980).

The swift metabolism of GHB presents two practical issues. Firstly, it complicates its detection in clinical and forensic settings due to a limited urine detection time. However, recent advancements in analytical methods that can identify GHB metabolites conjugated with amino acids in urine up to 28 h post-consumption might enhance exogenous GHB detectability in clinical and forensic toxicology (Steuer et al. 2023). Secondly, the rapid clearance of GHB from the body limits its therapeutic use, requiring multiple doses for continued efficacy. Nonetheless, recent results of clinical trials foster optimism for the development and market introduction of long-acting GHB formulations (Kushida et al. 2022).

13.2.2 Pharmacodynamics

Endogenous GHB is a naturally occurring neurotransmitter, with highest concentrations in the hypothalamus and the basal ganglia (Bessman and Fishbein 1963; Snead III and Morley 1981) and in the brown fat tissue and kidneys of mammals (Nelson et al. 1981).

Despite its mechanism of action not being fully elucidated, GHB is considered a weakly binding partial agonist at the GABA type-B ($GABA_B$) receptor (Engberg and Nissbrandt 1993) and an agonist at the GHB-specific binding site (Benavides et al. 1982). The existence of the latter was first suggested after evidence of high-affinity binding of the radioligand [^{3}H]GHB in a site that was not displaceable by GABA or baclofen (Benavides et al. 1982). Robust evidence in favor of a separate binding site was also provided by studies in $GABA_B$ receptor knockout (KO) mice, which display unchanged high-affinity GHB-specific binding sites compared to (wild-type) WT mice (Kaupmann et al. 2003). This GHB-specific binding site is considered presynaptic, G protein-coupled, and it may primarily act by inhibiting the release of GABA (Snead 3rd and Gibson 2005). It was also found to be specific for the CNS and most abundant in the hippocampus, frontal, temporal, insular,

cingulate, and entorhinal cortex, as well as in the striatum (Castelli et al. 2000). However, the molecular identification of the GHB-specific binding site remains elusive, as the cloned proteins described as GHB receptors did not appear to correlate with high-affinity binding (Andriamampandry et al. 2007). Moreover, the GABA type-A ($GABA_A$) receptor subunit α4 was shown to be at least partially involved in GHB high-affinity binding, thus sparking discussion on $GABA_A$ receptor involvement in this mechanism (Absalom et al. 2012).

The effects of GHB on $GABA_B$ receptors are bidirectional, dose- and localization-dependent, and rely on both direct and indirect mechanisms (Cruz et al. 2004). Endogenous GHB reaches approximately 0.1% of GABA's cerebral concentrations and seems to be insufficient to stimulate $GABA_B$ receptor on its own (Maitre 1997) but might inhibit GABAergic transmission via GHB-specific negative feedback. Exogenous GHB reaches higher concentrations and may act on $GABA_B$ receptor both directly (as a weak partial receptor agonist), indirectly (through the metabolic conversion of GHB to GABA), and via feedback control of GABA release (Kamal et al. 2016; Snead 3rd and Gibson 2005). Pretreatment with specific $GABA_B$ receptor antagonists in mice models prevented both GHB and its precursor 1,4-BD to elicit sedative/hypnotic effects (Carai et al. 2001, 2002). Same physiological effects were also absent in $GABA_B$ receptor −/− mice treated with GHB (Kaupmann et al. 2003). On the contrary, antagonism of the GHB-binding site with 6,7,8,9-tetrahydro-5-hydroxy-5H-benzo-cyclohept-6-ylideneacetic (NCS-382) potentiated, instead of antagonizing, GHB-induced sedative effects (Carai et al. 2001). Therefore, despite some data demonstrating that GHB may be biologically active at its own binding site (Colombo et al. 1995), most of GHB's clinical effects are considered to be mediated by modulation of the $GABA_B$ receptor (Castelli et al. 2004). Importantly, the indirect activation of the $GABA_B$ receptor through its conversion to GABA is discussed as a potential core mechanism of action of exogenous GHB (Snead 3rd and Gibson 2005). This pathway could explain the surprisingly high concentration of GHB required to produce $GABA_B$ receptor-mediated effects.

The putative involvement of $GABA_A$ receptor in the mediation of the pharmacological effects of GHB is also debated. The conversion of GHB into GABA and the GHB-induced stimulation of GABA release in specific brain areas might ultimately result in activation of the $GABA_A$ receptor (Enna 2012). However, the direct involvement of the $GABA_A$ receptor in the effects mediated by GHB is still unclear, as the higher heterogeneity of this receptor class (i.e., large number of subtypes and subunit combinations) and the lack of recombinant functional studies limit the investigations in this regard (Absalom et al. 2012).

Overall, the effects of GHB on GABAergic transmission are bidirectional and may lead to opposite effects: a stimulating effect on one side and an inhibitory effect on the other side (Kamal et al. 2016). These effects show a dose- and localization-dependent pattern, which might be dependent on region- and cell-specific interaction of the $GABA_B$ receptor with different G protein-coupled inwardly rectifying potassium (GIRK) channel subunits (Labouèbe et al. 2007). The difference in the coupling between $GABA_B$ receptor and GIRK subunits also provides a mechanism for the modulation effects of GHB on the neuronal mesolimbic dopamine system.

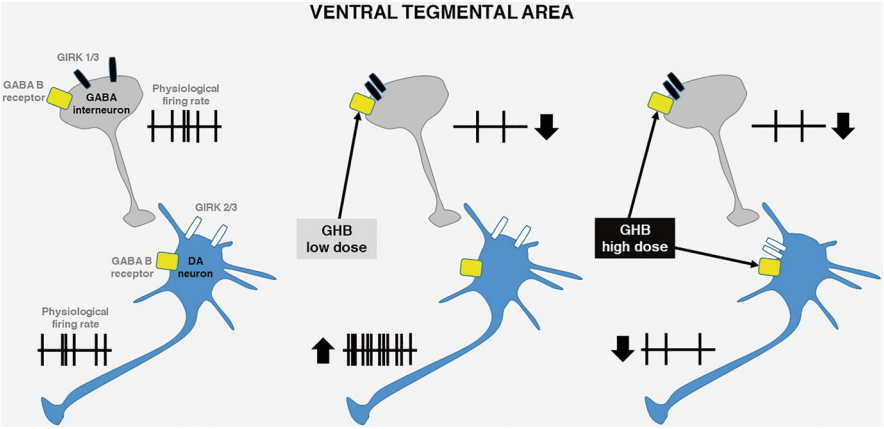

Fig. 13.2 Graphical representation of the differential effects of low and high doses of GHB on GABAergic interneurons and dopaminergic neurons in the ventral tegmental area. (Figure adapted from Crunelli et al. (2006))

Due to the higher efficiency of the GIRK coupling to the $GABA_B$ receptor in the presynaptic GABAergic interneurons in the ventral tegmental area (VTA), these neurons are more sensitive to GHB and can be targeted even at low doses of GHB (Cruz et al. 2004). The GHB activation of GIRK channels on the VTA interneurons suppresses their spontaneous activity and leads to disinhibition of the dopamine neurons by increasing their firing rate and enhancing dopamine output. This effect might be responsible for the strong addictive potential of low doses of GHB, as described in the comprehensive review by Kamal et al. (2016) (Fig. 13.2).

Following higher exogenous doses of GHB, however, endogenous GHB is downregulated, and thus GABA disinhibition occurs (Carter et al. 2009; Engberg and Nissbrandt 1993). This mechanism together with direct stimulation of postsynaptic $GABA_B$ receptor and the conversion of GHB into GABA is considered to mediate the sedative and sleep-promoting proprieties of moderate-to-high doses of GHB (Snead 3rd and Gibson 2005). Notably, while the sedative proprieties of GHB resemble the effects of the $GABA_B$ receptor agonist baclofen, GHB shows a higher addictive liability than baclofen. Here, the higher affinity of baclofen for the $GABA_B$ receptor in dopaminergic neurons at therapeutic doses compared to GHB would already inhibit them and thus decrease dopamine release (Cruz et al. 2004).

GHB interacts with a variety of other neurotransmitter and neuromodulator systems, affecting the release of glutamate, serotonin, norepinephrine, and acetylcholine in a biphasic and dose-dependent matter (Andresen et al. 2011; Castelli et al. 2003; Drasbek et al. 2006; Hechler et al. 1991; Szabo et al. 2004). The release of monoaminergic neurotransmitters is initially inhibited, leading to neuronal accumulation, followed by stimulation of their release and thus temporarily increasing their output (Hechler et al. 1991; Maitre 1997; Pardi and Black 2006). Exogenously applied GHB has been suggested to modulate glutamate levels and glutamate-dependent synaptic plasticity in a concentration-dependent manner. Nocturnal administration of GHB

increases the glutamate concentration in the anterior cingulate cortex in the following morning (Dornbierer et al. 2023), potentially explaining GHB's post-acute vigilance-enhancing properties. Moreover, additional effects by GHB on different neurotransmitter system have been reported: (i) increase in serotonin synthesis (Gobaille et al. 2002; Szabo et al. 2004); (ii) modulation of the cholinergic system (Volpi et al. 2000); and (iii) activation of the opioid system, as demonstrated by blockade of GHB effects by the opioid-receptor antagonist naloxone (Feigenbaum and Howard 1996; Feigenbaum and Simantov 1996; Hechler et al. 1991). GHB also acutely increases progesterone secretion, without changing the plasma levels of kisspeptin, oxytocin, and testosterone (Bavato et al. 2023b; Bosch et al. 2015).

Finally, findings from animal studies suggest that GHB might offer neuroprotection against transient global lesions (Kemmel et al. 2010; Ottani et al. 2003; Wendt et al. 2014) and decrease cellular energy requirements by lowering oxygen demand in brain cells (Kamal et al. 2016; Mamelak 1989).

13.3 Subjective Effects and Non-medical Use

GHB ingestion leads to a wide range of effects that differ between different doses (for an extensive overview see Bosch and Seifritz 2016). Low doses (<30 mg/kg) induce feelings of disinhibition, euphoria, and relaxation (Bosch et al. 2015), while higher doses (30–50 mg/kg) lead to increasing levels of sedation and deep sleep promotion (Barker et al. 2007; Rosen et al. 1997). Doses above 50 mg/kg lead to deep sedation up to coma with respiratory depression (Metcalf et al. 1966). Patients in emergency units with GHB-induced coma showed GHB serum levels that ranged from 72 to 430 mg/l, abruptly regaining consciousness at levels under 75–150 mg/l (Sporer et al. 2003; Van Sassenbroeck et al. 2007).

GHB's most prevalent non-medical use occurs within the club scene, where it is also known as "liquid ecstasy", "G", or "Gina", for its mixed stimulating-anxiolytic subjective effects (Galloway et al. 2000). In this context, GHB is taken at relatively low doses and has been described by users as disinhibiting, euphorigenic, and empathogenic. Additionally, GHB possesses a unique libido-enhancing effect, which, in combination with its disinhibiting effects, influence multiple aspects of sexuality (Barker et al. 2007). Users primarily seek GHB for recreational purposes, with motivations including sexual enhancement, social facilitation, and the pursuit of altered states of consciousness (Barker et al. 2007; Degenhardt et al. 2002; Miotto et al. 2001; Sumnall et al. 2008).

13.4 Functional Brain Correlates

Recent neuropsychopharmacological developments have emphasized the relevance of assessing changes in neural activity obtained from functional magnetic resonance imaging (fMRI) methods, such as cerebral resting-state functional connectivity

(rsFC). This approach offers a data-driven framework for understanding the complex actions of substances on brain networks, going beyond their receptor actions.

Menon's model of rsFC postulates three main neurocognitive networks: the default mode network (DMN), the central executive network (CEN), and the salience network (SN) (Menon 2011). Distinct activation patterns of these networks have been shown with various psychoactive substances. Sedatives, such as midazolam and propofol, have been shown to reduce DMN and DMN-CEN connectivity (Greicius et al. 2008; Jordan et al. 2013; Liang et al. 2015; Wang et al. 2021). Stimulant drugs, such as cocaine and modafinil, were reported to predominantly enhance CEN and CEN-SN connectivity (Cera et al. 2014; Esposito et al. 2013; Kufahl et al. 2005; Yoo et al. 2018).

In a recent investigation, an acute administration of GHB at moderate doses (35 mg/kg) has been demonstrated to increase the inter-network connectivity between the SN and both the DMN and the CEN (Bosch et al. 2018). A selective increase of cerebral perfusion in the right anterior insula and in the anterior cingulate cortex (ACC) was also observed in this study. Thus, the SN is considered to play a crucial role in mediating the acute effects of GHB administration. After a nocturnal GHB administration (50 mg/kg), a significant increase in functional connectivity between the SN and the right CEN was observed the following morning (Bavato et al. 2023a). This pattern suggests a shift toward a more externally focused brain state, potentially underlying GHB's ability to promote wakefulness.

In recreational users, chronic GHB consumption has been linked to reduced rsFC within the right CEN and between the right CEN and the DMN. Interestingly, multiple GHB-induced comas did not lead to further changes in rsFC (Raposo Pereira et al. 2019). However, comas induced by GHB were linked to microstructural changes in white matter, alongside increased self-reported impulsivity (Raposo Pereira et al. 2020), suggesting relevant effects of repeated GHB use on brain structure and function.

In electroencephalography (EEG) studies, GHB has been shown to increase low-frequency power during rapid-eye movement (REM) sleep, non-rapid-eye movement (NREM) sleep, and wakefulness (Dornbierer et al. 2019b; Mamelak et al. 1977; Metcalf et al. 1966; Vienne et al. 2012; von Rotz et al. 2017). In this context, source localization and functional connectivity analysis suggest that low-frequency oscillations induced by GHB originate from the posterior cingulate cortex (PCC) in the wakeful state (von Rotz et al. 2017) and from the medial prefrontal cortex, parahippocampal gyrus, and fusiform gyrus during NREM sleep (Dornbierer et al. 2019b). Results of both studies also suggested that GHB sedation might be related to increased lagged phase synchronization between PCC and other regions (Dornbierer et al. 2019b; von Rotz et al. 2017). Notably, GHB has been reported to induce sleep-typical EEG phenomena, such as an increase in delta wave activity, even in awake subjects (Jenney et al. 1962). Considering the dissociation between typical neural correlates of unconsciousness and their behavioral manifestations, GHB has been proposed as a valuable pharmacological tool for identifying biomarkers of consciousness in the presence of abnormal cortical dynamics (Frohlich et al. 2023). This unique property

of GHB might enhance our understanding of consciousness and offer a novel approach to studying its underlying neurobiological mechanisms.

13.5 Sleep-Wake Cycle Regulation

GHB has a unique spectrum of effects on vigilance and sleep-wake cycle regulation. Acute nocturnal application of GHB induces sedation and promotes sleep, including an increase in slow wave sleep (SWS) with a paradoxical rebound effect enhancing vigilance on the next morning after application (Dornbierer et al. 2019a) (Fig. 13.3).

An early investigation revealed that intravenous GHB causes alterations in consciousness closely following plasma GHB concentrations, ranging from wakefulness to deep sleep (Helrich et al. 1964). A recent study in healthy individuals showed that oral nocturnal GHB (50 mg/kg GHB given at 2:30 a.m.) increased SWS, diminished REM sleep, and increased delta-theta activity across both NREM and REM sleep stages while reducing spindle frequency activity in the more superficial N2 sleep stage. Overall, the GHB-induced changes of sleep architecture were found to resemble the EEG characteristics of recovery sleep after sleep deprivation (Dornbierer 2019).

GHB's impact on vigilance and the sleep-wake cycle involves intricate interactions with neurotransmitter systems (Dornbierer et al. 2023). Firstly, GHB is

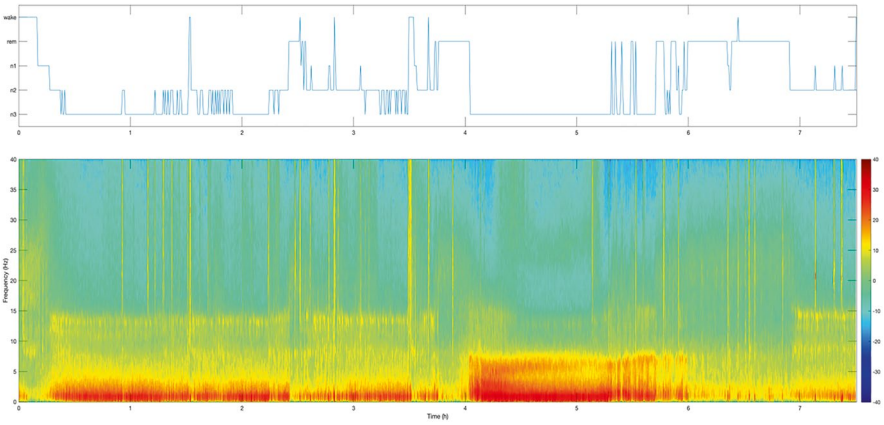

Fig. 13.3 Hypnogram (top row) and time-frequency analysis (bottom row) of a single representative healthy individual ($n = 1$) after nocturnal administration of GHB (liquid solution, oral intake, 50 mg/kg, administrated at 03:30 am). Strong slow wave activity (0.75–4.5 Hz) and sustained SWS (N3 stage) are clearly visible around 30 min after GHB administration. N1 stage 1 NREM sleep, N2 stage 2 NREM sleep, N3 stage 3 NREM sleep. (Unpublished data from Dornbierer et al. (2019a)). The study was approved by SwissMedic and Ethics Committee of the Canton of Zurich. The participant provided written informed consent according to the declaration of Helsinki

considered to elicit sleep-promoting effects via the activation of presynaptic $GABA_B$ receptor, resulting in the inhibition of Ca2+ ion influx and reduced release of wake-promoting neurotransmitters (Bettler et al. 2004), together with direct and indirect activation of the GABAergic transmission (Snead 3rd and Gibson 2005). On the other hand, GHB has been postulated to have biphasic effects on glutamatergic activity, with an acute nocturnal decrease in glutamate, followed by an increase the next day, although evidence in this regard is limited (Dornbierer et al. 2023). Moreover, preclinical studies suggest that GHB may modulate sleep behavior via the hypocretin/orexin pathway, a neuropeptide that regulates arousal, wakefulness, and appetite, but a clear understanding of this interaction is still lacking (Wu et al. 2023).

Finally, in a recent study using a somatostatin-cre mouse model, GHB was found to promote SWS by acting directly on the cortex, and this effect was prevented by chemogenetic inhibition of somatostatin-positive interneurons (Spano et al. 2024). Accordingly, the authors suggested that GHB might promote SWS by potentiating the ability of interneurons to inhibit large populations of cortical cells.

13.6 Therapeutic Use in Neurological and Musculoskeletal Disorders

GHB's potential to regulate the sleep-wake cycle and sleep architecture has been therapeutically used in a number of neurological and musculoskeletal conditions, most prominently in narcolepsy, Parkinson's disease, and fibromyalgia.

Narcolepsy is a chronic neurological disorder that significantly disrupts sleep and wakefulness. This condition is characterized by excessive daytime sleepiness (EDS), cataplexy (sudden, brief loss of muscle tone triggered by strong emotions), disrupted nighttime sleep, sleep paralysis, and hypnagogic hallucinations (Bassetti and Aldrich 1996). GHB is recognized as the first-line treatment for narcolepsy type 1, uniquely addressing the full spectrum of symptoms associated with the disorder. Nocturnal administration of GHB reduces not only the EDS but also the frequency of cataplectic episodes, normalizes REM-sleep patterns (Bogan et al. 2015; Boscolo-Berto et al. 2012; Kushida et al. 2022), and improves patients' quality of life (Weaver and Cuellar 2006). It is administered in two nightly doses, and patients typically report a clinical improvement within 2 months of daily administration (Bogan et al. 2015). A recent study on FT218, a novel long-acting formulation of GHB, which can be administered only once per night, showed a significant improvement in subjective EDS in clinical trials (Kushida et al. 2022), indicating promising potential for future therapy of narcolepsy.

Parkinson's disease (PD) is a neurodegenerative disorder, characterized by a broad spectrum of neuropsychiatric manifestations. Beyond the well-documented motor symptoms such as rigidity, tremor, and postural instability, patients frequently experience non-motor complications including chronic pain, gastrointestinal issues,

cognitive decline, and affective disorders (DeMaagd and Philip 2015). Notably, EDS and disrupted nighttime sleep architecture emerge as significant non-motor symptoms, which are often resistant to first-line treatments (Knie et al. 2011). In addressing this gap, a recent proof-of-concept clinical trial has demonstrated GHB's potential to mitigate both subjective and objective measures of EDS, alongside enhancing subjective sleep quality and the duration of SWS (Buchele et al. 2018). Despite these promising outcomes, significant side effects have been noted in a subset of PD patients, such as obstructive sleep apnea and parasomnias (Büchele et al. 2018). Some authors have further speculated that GHB could offer neuroprotective effects in PD by enhancing energy availability, modulating neurotransmission, reducing oxidative stress, and potentially slowing disease progression, alongside improving daytime sleepiness and fatigue symptoms (Mamelak 2023).

Fibromyalgia is a complex musculoskeletal disorder marked by widespread pain, fatigue, sleep disturbances, and cognitive and affective symptoms (Russell et al. 2011). GHB has been proposed as a treatment for fibromyalgia's core symptoms, including pain, excessive daytime sleepiness, and fatigue (Russell et al. 2011). However, the implementation of GHB in fibromyalgia treatment is met with concerns about tolerability. In a 14-week, phase 3, double-blind, randomized, controlled trial, 23% of patients discontinued the medication due to adverse effects, including nausea, headache, dizziness, nasopharyngitis, vomiting, sinusitis, diarrhea, anxiety, insomnia, influenza, somnolence, muscle spasms, urinary tract infections, and viral gastroenteritis (Russell et al. 2011). This wide array of potential side effects might limit the applicability of GHB for fibromyalgia.

Other conditions: GHB has shown therapeutic potential in neurological conditions beyond narcolepsy, PD, and fibromyalgia. A case study demonstrated the successful use of GHB in a patient suffering from episodic cluster headaches, possibly mediated through its sleep-stabilizing effects (Hidalgo et al. 2013). Similarly, GHB has been found to significantly improve symptoms of spasmodic dysphonia, reducing voice breaks and severity of voice tremor, especially in patients with alcohol-responsive symptoms (Rumbach et al. 2017). However, larger randomized studies are warranted to support a clinical indication in these conditions.

13.7 Therapeutic Use in Psychiatric Disorders

Beyond its early broad application as a sedative across psychiatric conditions, more recently GHB has been mainly studied for its more specific therapeutic potential in alcohol use disorder (AUD), major depressive disorder, and binge-eating disorder.

Alcohol use disorder is a prevalent psychiatric condition characterized by an impaired ability to stop or control alcohol use despite adverse social, occupational, or health consequences. Alcohol withdrawal syndrome (AWS) is a dangerous clinical condition that can develop in patients suffering from AUD within 6–24 h after the abrupt discontinuation of alcohol consumption (Mirijello et al. 2015). GHB has been explored for its therapeutic potential in AUD, both in managing AWS and

maintaining abstinence (Caputo et al. 2016; Guiraud et al. 2022). This interest arose from GHB's potential to mimicking, or substituting, alcohol effects (Chick and Nutt 2012). A Cochrane meta-analysis showed that GHB (50–100 mg/kg/day) can be used in AWS with the same efficacy as benzodiazepines and clomethiazole (Leone et al. 2006). Furthermore, GHB has shown promising efficacy in treating AUD by promoting abstinence, with nearly 50–60% of patients maintaining total alcohol abstinence after the first 3 months of treatment (Caputo et al. 2015), and a mean increase in cumulative abstinence duration after alcohol detoxification of 34 days compared to placebo (Guiraud et al. 2022). While clinical trials indicate that the risk of dependence or abuse with the medical use of GHB is relatively low, concerns, particularly in individuals with poly-drug use disorder or psychiatric comorbidities, remain present (Caputo et al. 2015; Keating 2014; Leone et al. 2006; Skala et al. 2014).

Major depressive disorder (MDD) is a highly prevalent affective disorder characterized by depressive mood, anhedonia, lack of drive and motivation, as well as a range of other symptoms including social withdrawal, loss of libido, and sleep disturbances. Driven by the monoamine hypothesis, the main classes of currently used antidepressants enhance serotonergic, dopaminergic, or noradrenergic transmission (Bosch et al. 2012). As these therapies are not successful in all patients, Bosch et al. (2012) have suggested GHB as a repurposing candidate in MDD. Although initial research highlighted the sedative, anxiolytic, and antidepressant effects of GHB (De Couedic and Voisse 1964; Rinaldi et al. 1967), these findings were overshadowed by the rise of monoaminergic substances and have not been thoroughly re-evaluated using modern clinical trial standards (Bosch et al. 2012). In particular, GHB's sleep-promoting proprieties might found relevant application in reducing main clinical features of MDD such as reduced SWS, REM sleep disruption, oversleeping, and sleep inertia (Borbély et al. 1984; Germain et al. 2004; Kupfer 1978; Steiger and Kimura 2010).

Binge eating disorder is the most prevalent eating disorder, often associated with significant psychiatric and medical comorbidities, as well as functional impairment. GHB was tested in a 10-week, randomized, placebo-controlled, flexible dose trial, on 20 outpatients with BED, leading to significant reductions in binge days, episodes, clinical severity, and body weight, with 9 patients achieving remission (McElroy et al. 2011).

13.8 Safety Profile and Addictive Potential

Post-marketing surveillance suggests that GHB exhibits a relatively low toxicity profile, with the most common adverse effects being nausea, insomnia, headaches, dizziness, and vomiting (Wang et al. 2021). However, the tolerability and impact of these side effects can vary significantly across different medical conditions, which, in turn, influences the continuation of treatment and the overall utility of GHB. For

instance, its effectiveness in treating conditions like fibromyalgia may be compromised due to its limited tolerability (Russell et al. 2011).

In clinical trials dedicated to treating AUD and narcolepsy, there have been no documented cases of death related to GHB use, indicating a safe profile under controlled conditions (Keating 2014; Skala et al. 2014; Wang et al. 2021).

GHB's addictive potential is a notable concern, with studies showing its addictive properties to be similar to benzodiazepines. Although therapeutic GHB is associated with a low addiction risk, in recreational users, the risk increases in a dose-dependent manner with chronic use, leading to tolerance and severe withdrawal symptoms upon cessation (Bosch and Seifritz 2016). Moreover, even if withdrawal symptoms in therapeutic doses tend to be less severe, steadily decreasing the GHB dose when intending to discontinue the treatment is recommended.

In Europe, non-medical GHB use remains relatively low (1–1.4%) among adults and school-aged populations (Guerreiro et al. 2011). Nevertheless, recent years have witnessed a significant increase in GHB-related medical treatments in some countries, with the Netherlands experiencing a fourfold rise in patients admitted to addiction treatment centers for GHB detoxification (Wisselink and Mol 2013). Additionally, in 2011, GHB accounted for 20% of drug medical emergencies reported in the Netherlands (Laar et al. 2012) and 7% of acute poisoning admissions in Norway (Bramness and Haugland 2011).

In addition to its addictive and acutely toxic potential in unregulated use, GHB has also been implicated in criminal activities, notably as a "date-rape drug," because of its incapacitating effects at high doses, earning it the moniker "k.o. drops" (Hall and Moore 2008). The challenge of forensic assessments in cases of GHB-facilitated sexual assaults is further complicated by its short urinary detection time (Brailsford et al. 2012; Kavanagh et al. 2001). Despite the focus on GHB in media reports, more readily available substances, such as alcohol and benzodiazepines, are more commonly utilized than GHB in sexual assaults (Hall and Moore 2008).

13.9 Conclusions

In summary, GHB is a complex compound with significant pharmacological and therapeutic value. Originally developed as an anesthetic, it now shows great promise in neurology, psychiatry, and sleep medicine, enhanced by recent advances in long-acting galenic formulations. Neuroscientific studies addressing the neuronal correlates of GHB effects provide unique insights into the role of the $GABA_B$ receptor in the human brain. While balancing its therapeutic benefits with safety and abuse risks remains critical, GHB's diverse therapeutic applications and potential in various medical fields could offer new treatment opportunities in different clinical conditions.

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Chapter 14
Naturally Occurring $GABA_B$ Receptor Ligands

Giancarlo Colombo

Abstract The present chapter provides an overview of the multiple medicinal plants whose extracts and/or active ingredients exert specific pharmacological effects via the $GABA_B$ receptor. Solid experimental data are reported for the medicinal plants, *Bupleurum falcatum*, *Withania somnifera*, *Glycyrrhiza glabra*, *Valeriana officinalis*, and *Passiflora incarnata*, with preliminary, although intriguing, data relating to several other plants. Medicinal plants may thus represent a valuable source of new $GABA_B$ receptor agonists, antagonists, and allosteric modulators. In addition to the identification of new $GABA_B$ receptor ligands, research in this field may contribute toward clarifying the pharmacological bases underlying the traditional use of these herbal remedies as well as suggest their use for new therapeutic purposes.

Keywords $GABA_B$ receptor ligands · Medicinal plants · *Bupleurum falcatum* · *Withania somnifera* · *Glycyrrhiza glabra* · *Valeriana officinalis* · *Passiflora incarnata* · Saikosaponin A · Isoliquiritigenin · Dihydrovaltrate · Ferulic acid

14.1 Introduction

Medicinal plants may represent a plentiful source of valuable new and potentially effective γ-aminobutyric acid (GABA) type-B ($GABA_B$) receptor ligands. However, still relatively few and somewhat erratic, experimental studies suggest indeed that novel $GABA_B$ receptor ligands may be identified in medicinal herbs.

The present paper briefly reviews the literature data suggesting that extracts from and active constituents of a variety of plants belonging to different traditional medicinal systems behave as $GABA_B$ receptor agonists, antagonists, or positive

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allosteric modulators (PAMs) when tested in appropriate in vitro and in vivo pharmacological assays. Naturally occurring $GABA_B$ receptor ligands might thus represent a new, effective line of drug discovery in the $GABA_B$ receptor pharmacology. Identification of a $GABA_B$ receptor ligand, or a $GABA_B$ receptor-involving mechanism of action, in specific medicinal plants may also contribute toward clarifying the pharmacological bases of their use in both traditional and modern medicine and providing potential indications for new therapeutic purposes [as it might be the case for extracts of *Bupleurum falcatum*, *Withania somnifera*, and *Glycyrrhiza glabra* in the treatment of alcohol and substance use disorder (see below)].

The present paper devotes a separate paragraph to each medicinal plant, the extract(s) and/or active component(s) of which have been found to bind to the $GABA_B$ receptor or exert $GABA_B$ receptor-mediated pharmacological effects. This list of medicinal herbs includes *Bupleurum falcatum*, *Withania somnifera*, *Glycyrrhiza glabra*, *Valeriana officinalis*, *Passiflora incarnata*, *Hypericum perforatum*, *Ginkgo biloba*, *Angelica sinensis*, *Ligusticum chuanxiong*, *Albizia lebbeck*, *Emblica officinalis*, *Nardostachys jatamansi*, *Clerodendrum mandarinorum*, and *Terminalia bellirica*.

14.2 Overview of Medicinal Plants with $GABA_B$ Receptor-Mediated Activity

14.2.1 Bupleurum falcatum

Bupleurum falcatum L. (sickle-leaved hare's ear, Chinese thoroughwax) is a perennial, flowering plant in the Apiaceae family (Fig. 14.1). It grows primarily in the temperate biome and is native to Europe and western Asia (see Kew Science 2024). Roots of *Bupleurum falcatum* are widely used in traditional Chinese, Japanese, and Korean medicines to prevent and cure a series of diseases, including psychiatric and neurological disorders (see Ashour and Wink 2011). Preparations containing roots of *Bupleurum* species have been used in China for more than 2000 years. Their use subsequently spread to the Middle East, with preparations from *Bupleurum falcatum* also being common in Iranian folk medicine (Ahmadimoghaddam et al. 2021).

Triterpene saponins named saikosaponins (SSs; "Saiko" stands for *Bupleurum falcatum* in Japanese) are the main active constituents of *Bupleurum falcatum* roots, as well as roots of several other *Bupleurum* species (see Yang et al. 2017; Li et al. 2018a). Their content totals approx. 7% of the dry weight of roots of *Bupleurum* species (see Ashour and Wink 2011). Among the several different SSs identified, SS A (SSA; Fig. 14.2) and its epimer, SS D (SSD), are considered to be the most pharmacologically active (Yang et al. 2017; Li et al. 2018b). The broad pharmacological profile of SSA and SSD includes anti-inflammatory, hepatoprotective, antineoplastic, anti-viral, and anti-addictive activities (Yuan et al. 2017; Li et al. 2018a, b).

Fig. 14.1 *Bupleurum falcatum* L. (*Photo credits*: Dr. Andrea Moro, Department of Life Sciences, University of Trieste, Trieste, Italy)

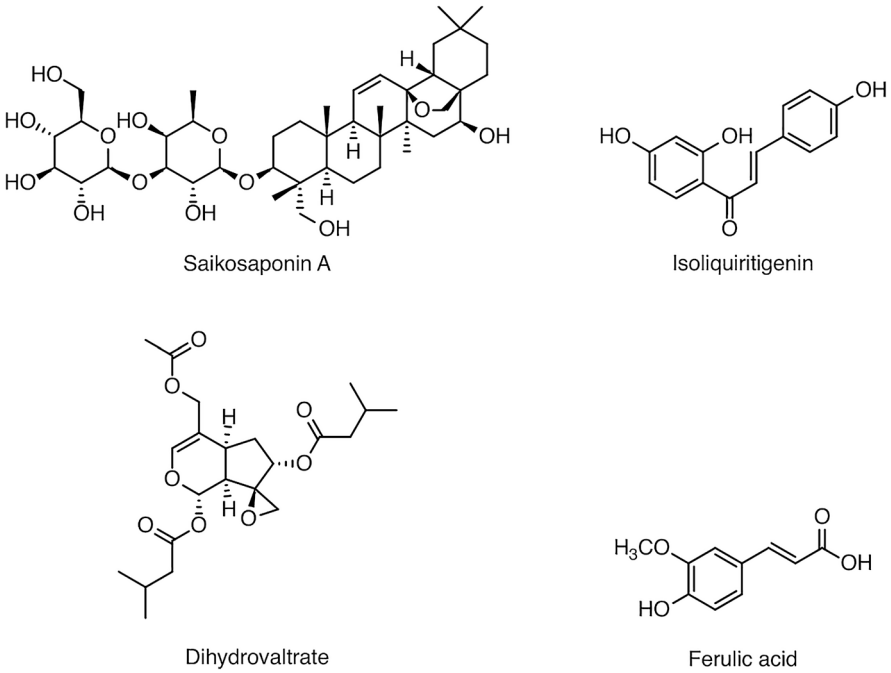

Fig. 14.2 Chemical structure of saikosaponin A (SSA), isoliquiritigenin (ISL), dihydrovaltrate (DH-VAL), and ferulic acid (FA)

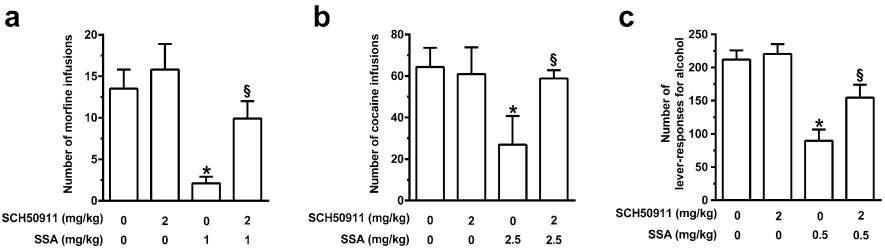

Fig. 14.3 Pharmacological blockade exerted by the $GABA_B$ receptor antagonist, SCH50911, of the suppressing effect of saikosaponin A (SSA) on morphine (**a**), cocaine (**b**), and alcohol (**c**) self-administration in rats. Sprague-Dawley rats were trained to lever-respond for intravenous morphine or cocaine under the fixed ratio (FR) 1 (FR1) schedule of reinforcement (i.e., each response on the lever resulted in infusion of 0.1 mg/kg morphine or 0.25 mg/kg cocaine, respectively); selectively bred Sardinian alcohol-preferring rats were trained to lever-respond for oral alcohol (15% v/v) under the FR4 schedule of reinforcement (i.e., 4 lever-responses were required to access 0.1 ml alcohol solution). Daily self-administration sessions lasted 3 hours in the "morphine" and "cocaine" studies and 30 min in the "alcohol" study. SSA was administered intraperitoneally 30 min prior to the start of the self-administration session. SCH50911 was administered intravenously (**a**) or intraperitoneally (**b**, **c**) immediately before SSA administration. In panel **a**, each bar is the mean ± SEM of n = 6-7 rats; *: $P < 0.01$ in comparison to vehicle-treated rats (0 mg/kg SCH50911 + 0 mg/kg SSA) (Tukey's test); §: $P < 0.05$ in comparison to rats treated with SSA alone (0 mg/kg SCH50911 + 1 mg/kg SSA) (Tukey's test). In panel **b**, each bar is the mean ± SEM of n = 6-9 rats; *: $P < 0.05$ in comparison to vehicle-treated rats (0 mg/kg SCH50911 + 0 mg/kg SSA) (LSD test); §: $P < 0.05$ in comparison to rats treated with SSA alone (0 mg/kg SCH50911 + 2.5 mg/kg SSA) (LSD test). In panel **c**, each bar is the mean ± SEM of n = 12 rats; *: $P < 0.0005$ in comparison to vehicle-treated rats (0 mg/kg SCH50911 + 0 mg/kg SSA) (Tukey's test); §: $P < 0.05$ in comparison to rats treated with SSA alone (0 mg/kg SCH50911 + 0.5 mg/kg SSA) (Tukey's test). (Adapted from Yoon et al. 2012 (panel **a**); Yoon et al. 2013 (panel **b**); and Maccioni et al. 2016 (panel **c**), with permission from Elsevier)

The anti-addictive properties of SSA are of particular relevance for the purposes of the present chapter. Recent experimental studies have demonstrated that treatment with SSA potently and selectively suppressed multiple behaviors motivated by drugs of abuse and highly palatable foods, including intravenous operant self-administration of morphine (Yoon et al. 2012) and cocaine (Yoon et al. 2013), oral operant self-administration of alcohol (Maccioni et al. 2016, 2020, 2023), operant self-administration and reinstatement of seeking behavior of chocolate (Lorrai et al. 2017; Maccioni et al. 2020) and sucrose (Maccioni et al. 2023), and overeating of butter and chocolate cookies (Maccioni et al. 2018) in rats. When investigated, these anti-addictive effects of SSA were found to be mediated by the $GABA_B$ receptor. In vivo experiments of pharmacological blockade demonstrated indeed that acute treatment with the $GABA_B$ receptor antagonist, SCH50911, (i) completely prevented the suppressing effect of SSA on morphine (Yoon et al. 2012) (Fig. 14.3a) and cocaine (Yoon et al. 2013) (Fig. 14.3b) self-administration in unselected Sprague-Dawley rats and (ii) partially prevented the suppressing effect of SSA on alcohol self-administration in selectively bred alcohol-preferring rats (Maccioni et al. 2016) (Fig. 14.3c). Additionally, the combination of a per se ineffective dose

of SSA with a per se ineffective dose of the $GABA_B$ PAM, GS39783, markedly reduced alcohol self-administration in alcohol-preferring rats (Maccioni et al. 2016); these data are suggestive of SSA behaving as a $GABA_B$ receptor agonist, with its anti-alcohol effects potentiated by positive allosteric modulation of the $GABA_B$ receptor. Altogether, these experimental results suggest that the $GABA_B$ receptor system is a key component of the neural substrate underlying the anti-addictive properties of SSA. Additionally, these data closely resemble those on the ability of the prototypic $GABA_B$ receptor agonist, baclofen, to suppress multiple rodent behaviors motivated by alcohol (see Colombo and Gessa 2018) and drugs of abuse (see Phillips and Reed 2014). As thoroughly reviewed in Chap. 6 of this volume, nowadays baclofen is a widely accepted medication used in the treatment of alcohol use disorder; ideally, this confers translational validity also to experimental data on the anti-alcohol effects of SSA (Maccioni et al. 2016, 2020, 2023) and extracts of *Bupleurum falcatum* (Maccioni et al. 2022).

14.2.2 Withania somnifera

Withania somnifera (L.) Dunal (winter cherry) is a shrub in the Solanaceae family (Fig. 14.4). It grows primarily in the subtropical biome and is native to southern Europe, Africa, southwestern Asia, and central China (see Kew Science 2024). In traditional Indian medicine, and due to its potential therapeutic effects, *Withania somnifera* is known as "Indian ginseng" (see Seenivasagam et al. 2011; Dar et al. 2015). Preparations containing *Withania somnifera* have been reported to ameliorate a variety of neurological disorders, including epilepsy, Alzheimer's disease, Parkinson's disease, cerebral ischemia, and tardive dyskinesia (see Paul et al. 2021; Tewari et al. 2022). The species name *somnifera* ("sleep-inducer" in Latin) accounts for its tranquilizing and stress-reducing properties (see Paul et al. 2021). Therapeutic properties of *Withania somnifera* include—among several others—anti-inflammatory, antidiabetic, cardioprotective, hepatoprotective, anti-osteoporotic, and antineoplastic activities (see Paul et al. 2021; Tewari et al. 2022).

Using in vitro radioligand receptor binding assays, three independent studies found that methanolic extracts of *Withania somnifera* roots displayed moderate affinity [measured as inhibitory constant (K_i), μg/ml] for the $GABA_B$ receptor (Misra et al. 1993; Ruiu et al. 2013; Orrù et al. 2014). None of these studies investigated which individual chemical(s) contained in the extract was(were) actually the receptor ligand(s).

Extracts of *Withania somnifera* roots have been reported to exert anti-addictive effects in rodents, including suppression of operant oral alcohol self-administration in rats (Peana et al. 2014) and development of conditioned place preference [CPP; index of drug-induced reward (see McKendrick and Graziane 2020)] induced by alcohol (Spina et al. 2015) and morphine (Ruiu et al. 2013) in mice. Suppression of alcohol self-administration was completely prevented by treatment with the $GABA_B$ receptor antagonist, phaclofen, suggesting that this extract effect was mediated by

Fig. 14.4 *Withania somnifera* (L.) Dunal. (*Photo credits*: Dr. Andrea Moro, Department of Life Sciences, University of Trieste, Trieste, Italy)

the $GABA_B$ receptor (Peana et al. 2014). To our knowledge, this was the only study conducted to investigate whether a pharmacological effect of a *Withania somnifera* extract was mediated by the $GABA_B$ receptor. Other receptor systems, including GABA type-A ($GABA_A$), μ- and δ-opioid, and glutamate *N*-methyl-D-aspartate (NMDA), are likely part of the neural substrate underlying the central effects of *Withania somnifera* root extracts (Misra et al. 1993; Ruiu et al. 2013; Orrù et al. 2014): the broad pharmacological effects of *Withania somnifera* root extracts, including their anti-addictive properties, might therefore be the sum of multiple actions mediated by different receptor systems.

14.2.3 Glycyrrhiza glabra

Glycyrrhiza glabra L. (licorice) is a perennial, flowering subshrub in the Fabaceae family (Fig. 14.5). It grows primarily in the temperate biome and is native to the central Mediterranean area, western Asia, and Mongolia (see Kew Science 2024). Ethnopharmacological information, as well as multiple lines of preclinical and clinical evidence, suggests that preparations based on *Glycyrrhiza glabra* exert protective effects against multiple neurological diseases, including long-term depression, cognitive deficits, anxiety, Alzheimer's disease, epilepsy, and drug addiction (see

Fig. 14.5 *Glycyrrhiza glabra* L. (*Photo credits*: Dr. Andrea Moro, Department of Life Sciences, University of Trieste, Trieste, Italy)

Sharma et al. 2023). *Glycyrrhiza glabra* and its bioactive constituents also possess anti-inflammatory, antiviral, and immunomodulatory activities (see Bisht et al. 2022).

The flavonoid, isoliquiritigenin (ISL; Fig. 14.2), is a main constituent of roots of *Glycyrrhiza glabra* (see Wahab et al. 2021). ISL has been reported to exert multiple pharmacological effects, including—among several others—antineoplastic activity (against multiple types of cancer), neuroprotection, memory enhancement, and anti-inflammation (see Peng et al. 2015; Ramalingam et al. 2018; Wang et al. 2021). A recent molecular docking study investigated the interaction between the chemical structure of ISL and the molecular structure of the $GABA_B$ receptor (Lin et al. 2021). Specifically, it was estimated that ISL establishes bonding interactions with amino acid residues Glu349, His170, and Cys129. Notably, both Glu349 and His170 are two major residues of N-terminal Lobe (LB1) domain, the portion of the $GABA_B$ receptor essential for agonist recognition and binding (e.g., Geng et al. 2013).

Three different pharmacological studies confirmed that ISL, or extracts of *Glycyrrhiza glabra* roots, behave as $GABA_B$ receptor agonists. In the first study, acute treatment with ISL completely prevented cocaine-induced dopamine release in the rat nucleus accumbens (NAc; i.e., the neurochemical correlate of the rewarding properties of cocaine) (Jang et al. 2008). Notably, this ISL effect was attenuated by pretreatment with SCH50911 (Jang et al. 2008). ISL-induced activation of $GABA_B$ receptors located either pre-synaptically (modulating neurotransmitter

release) or post-synaptically (hyperpolarizing neurons) in the ventral tegmental area is the likely mechanism underlying this ISL effect. The suppressing effect of ISL on cocaine-induced dopamine release in rat NAc was reproduced by acute administration of a methanolic extract of *Glycyrrhiza glabra* roots (Jang et al. 2008).

In the second experiment, repeated treatment with a methanolic extract of *Glycyrrhiza glabra* roots prevented locomotor sensitization induced by methamphetamine in rats (Zhao et al. 2014). Locomotor sensitization, i.e., the progressive enhancement of behavioral response to repeated psychostimulant treatment, models compulsive drug-seeking behavior (see Robinson and Berridge 1993). Pretreatment with SCH50911 completely blocked the development of extract-induced locomotor sensitization, suggesting that the active ingredient(s) of the extract likely exerted its(their) action via activation of the $GABA_B$ receptor (Zhao et al. 2014).

Repeated treatment with the same methanolic extract of *Glycyrrhiza glabra* roots also inhibited both the development and expression of methamphetamine-induced CPP in rats (Zhao et al. 2014). Contrary to locomotor sensitization, the extract effect on methamphetamine-induced CPP was not prevented by treatment with SCH50911 (Zhao et al. 2014). Use of low, and possibly ineffective, doses of SCH50911 or involvement of neural substrates other than the $GABA_B$ receptor may explain this discrepancy.

In the third experiment, the application of ISL resulted in a concentration-dependent inhibition of glutamate release in rat cerebrocortical nerve terminals (synaptosomes) (Lin et al. 2021). This effect was completely prevented by co-application of the $GABA_B$ receptor antagonist, CGP35348 (Lin et al. 2021). Inhibition of (excessive) glutamate release might be the molecular basis of the reported neuroprotective effect of ISL (and extracts of *Glycyrrhiza glabra* roots).

14.2.4 Valeriana officinalis

Valeriana officinalis L. (valerian) is a perennial, flowering plant in the Caprifoliaceae family (Fig. 14.6). It grows primarily in the temperate biome and is native to Europe and Iran (see Kew Science 2024). Preparations derived from *Valeriana officinalis* roots are among the most widely used and effective herbal sedatives and tranquilizers. Relief from anxiety, restlessness, and sleep disturbance, increase in sleep quality, and sleep promotion are indeed some of the most commonly reported effects of *Valeriana officinalis* root preparations (see Shinjyo et al. 2020; Das et al. 2021). Their use as a sleep aid is regulated in the USA by the Food and Drug Administration and in the European Union by the European Medicine Agency (see Shinjyo et al. 2020; Das et al. 2021). Root extracts contain more than 150 different compounds; the principal chemical constituents are alkaloids, organic acids, terpenes, iridoids, lignanoids, and flavonoids (see Patočka and Jakl 2010; Orhan 2021).

A wide-spectrum screening program conducted in the nineties at the US National Institute on Mental Health (NIMH) included *Valeriana officinalis* among medicinal

Fig. 14.6 *Valeriana officinalis* L. (*Photo credits*: Dr. Andrea Moro, Department of Life Sciences, University of Trieste, Trieste, Italy)

plants, the crude extracts of which had a binding affinity for the $GABA_B$ receptor with a K_i equal to or lower than 2 μg/ml (Cott 1994).

An in vitro binding assay conducted using rat brain cortical tissues found that the valepotriate, dihydrovaltrate (DH-VAL; Fig. 14.2), potentiated [^{3}H]-baclofen binding to the $GABA_B$ receptor (Mennini et al. 1993); the authors of this study hypothesized an underlying positive allosteric modulatory mechanism (notably, this hypothesis was imaginatively advanced a decade before the discovery of the positive allosteric modulatory binding site of the $GABA_B$ receptor and synthesis of the first $GABA_B$ PAMs), making DH-VAL the possible first-documented, and currently sole, naturally occurring $GABA_B$ PAM. However, no subsequent study has ever explored the contribution of this mechanism to the pharmacological activities of DH-VAL and *Valeriana officinalis* extracts.

Application of an aqueous extract of *Valeriana officinalis* roots inhibited reuptake and stimulated release of [^{3}H]-GABA in synaptosomes isolated from rat brain cortex (Santos et al. 1994). Both actions purportedly result in an increase of GABA concentration in the synaptic cleft; this extra pool of GABA may then bind also to $GABA_B$ receptors.

Finally, administration of an extract of *Valeriana officinalis* roots induced marked pulmonary vascular dilation and decrease in lobar arterial pressure in cats (Fields et al. 2003). This effect was effectively prevented by treatment with the $GABA_B$ receptor antagonist, saclofen (Fields et al. 2003). Pretreatment with the $GABA_A$

Fig. 14.7 *Passiflora incarnata* L. (*Photo credits*: *Orto e Museo Botanico*, University of Pisa, Pisa, Italy)

receptor antagonist, bicuculline, produced a comparable effect, suggesting that both GABA receptor types modulate the vasodilatory effects of *Valeriana officinalis* root extracts (Fields et al. 2003).

14.2.5 Passiflora incarnata

Passiflora incarnata L. (passionflower, maypop, wild apricot) is a climbing, flowering subshrub or tree in the Passifloraceae family (Fig. 14.7). It grows primarily in the temperate biome and is native to central and eastern USA and Bermuda (see Kew Science 2024). The most widely recognized therapeutic properties of its flowers, fruits, and aerial parts include anxiolysis, sedation, sleep promotion, anti-spasm, and analgesia (see Dhawan et al. 2004; Janda et al. 2020). Amelioration of symptoms and signs of opioid dependence has also been reported (see Dhawan et al. 2004; Janda et al. 2020).

Passiflora incarnata is one of the few medicinal plants, the crude extract of which—according to the screening program conducted several years ago at NIMH—had binding affinity for the $GABA_B$ receptor with a K_i equal to or lower than 2 μg/ml (Cott 1994).

Accordingly, a more recent binding study using rat brain tissues found that a dry extract of *Passiflora incarnata* inhibited, in a concentration-dependent manner,

binding of the potent and selective $GABA_B$ receptor antagonist, [^{3}H]-CGP54626, to $GABA_B$ receptors (Appel et al. 2011). A subsequent and complementary guanosine 5-O-(3-[^{35}S]thio)triphosphate (GTPγ^{35}S) binding assay, which evaluates the functionality of the receptor by measuring its activation via the G-protein, revealed that *Passiflora incarnata* extract behaved as a $GABA_B$ receptor antagonist (Appel et al. 2011). To our knowledge, this *Passiflora incarnata* extract together with an extract of *Albizzia lebbeck* leaves and perhaps *Hypericum perforatum* (see below) represents the sole natural $GABA_B$ receptor antagonists described to date.

Apparently, no subsequent studies have extended this finding to pharmacological assays or investigations on which individual chemical(s) contained in the extract was(were) actually the receptor ligand(s).

14.2.6 Hypericum perforatum

Hypericum perforatum L. (St. John's wort) is a perennial, flowering plant in the Hypericaceae family (Fig. 14.8). It grows primarily in the temperate biome and is native to Europe, western Asia, southeastern China, and Sudan (see Kew Science 2024). The antidepressant and mood-elevating effects of *Hypericum perforatum* have been known since the time of Hippocrates (see Zhang et al. 2020; Nobakht

Fig. 14.8 *Hypericum perforatum* L. (*Photo credits*: Dr. Andrea Moro, Department of Life Sciences, University of Trieste, Trieste, Italy)

et al. 2022). Notably, recent clinical studies have demonstrated that products containing *Hypericum perforatum* are as effective as major synthetic antidepressants (viz., tricyclic antidepressants and selective serotonin reuptake inhibitors) in the treatment of mild-to-moderately severe depressive disorders, possibly with fewer and milder side effects, making *Hypericum perforatum* one of the best-selling botanical products on the US and European markets (see Ng et al. 2017; Zirak et al. 2019).

An initial study—intended as a continuation of the above-mentioned NIMH screening program aimed at identifying the receptor system(s) mediating the pharmacological actions of a large series of medicinal plants—tested an extract of *Hypericum perforatum* in a battery of 39 different in vitro radioligand receptor binding assays (Cott 1997). To this end, a crude extract of fresh flowers and buds of *Hypericum perforatum* was used; rat cortical membranes were used as substrate. Notably, the $GABA_B$ receptor was unquestionably the receptor system for which the extract displayed the highest affinity, with a K_i value of 6 ng/ml (Cott 1997). These data were only partially confirmed by a subsequent study reporting an approximately 100-fold lower affinity of a hydromethanolic extract of *Hypericum perforatum* for $GABA_B$ receptors in rat brain synaptosomes (Gobbi et al. 1999); this moderate, rather than high, affinity for the $GABA_B$ receptor somehow conflicts with the initial idea that $GABA_B$ receptor is the primary site of action of orally administered, conventional doses of *Hypericum falcatum*-based preparations (Cott 1997).

In agreement with the above view, pharmacological studies provided conflicting results. Faced with data suggesting the involvement of the $GABA_B$ receptor in the pharmacological effects of *Hypericum perforatum*, it is worth mentioning an electrophysiological study in which an ethanolic extract of *Hypericum perforatum* stimulated neuronal excitability and synaptic transmission, as suggested by an increase in electrically evoked population spikes and field potential changes in hippocampal slices of guinea pigs (Langosch et al. 2002). This effect was reproduced by a hydrosoluble fraction of the initial extract (Langosch et al. 2002). The latter effect was further amplified by the application of the $GABA_B$ receptor antagonist, phaclofen, suggesting—according to the study authors—that the extract fraction behaved as a $GABA_B$ receptor antagonist reducing inhibitory $GABA_B$ergic inputs (Langosch et al. 2002).

By contrast, the suppressing effect of a hypercritical carbon dioxide extract of *Hypericum perforatum* on voluntary alcohol intake in selectively bred alcohol-preferring rats was not altered, even minimally, by pretreatment with pharmacologically active doses of the $GABA_B$ receptor antagonists, CGP36742 and phaclofen (Perfumi et al. 2002). Similarly, the vasodepressor effect of an extract of *Hypericum perforatum* flowers in a feline pulmonary vascular bed was totally unaffected by pretreatment with the $GABA_B$ receptor antagonist, saclofen (Hoover et al. 2004). The large variety of raw material, extraction procedures, content of active ingredients, pharmacological effects, and related underlying substrates may explain the discrepancies between the results of these studies.

14.2.7 Ginkgo biloba

Ginkgo biloba L. (maidenhair tree) is a large tree in the Ginkgoaceae family (Fig. 14.9). It grows primarily in the temperate biome and is native to southeastern China (see Kew Science 2024). In terms of ethnopharmacology, *Ginkgo biloba* has been used in China for more than 2000 years; its use subsequently spread worldwide making *Ginkgo biloba* one of the most extensively used herbal remedies (see Liu et al. 2022; Noor-E-Tabassum et al. 2022). Therapeutic applications include—among several others—anticancer, anti-dementia, anti-inflammation, hepatoprotection, antihypertension, and neuroprotection (see Liu et al. 2022; Noor-E-Tabassum et al. 2022).

Fig. 14.9 *Ginkgo biloba* L. (*Photo credits*: Dr. Andrea Moro, Department of Life Sciences, University of Trieste, Trieste, Italy; *Orto e Museo Botanico*, University of Pisa, Pisa, Italy (top-left picture))

Ginkgo biloba is the third and last medicinal plant an extract of which emerged from the NIMH screening program for its ability to bind the $GABA_B$ receptor with a K_i equal to or lower than 2 μg/ml (Cott 1994). This initial finding has apparently not been followed by any other study.

14.2.8 Angelica sinensis *and* Ligusticum chuanxiong

Ferulic acid (FA; Fig. 14.2) is a major component of the rhizomes of Chinese medicinal plants, *Angelica sinensis* (Oliv.) Diels (family: Apiaceae; female ginseng) (Fig. 14.10, left) and *Ligusticum chuanxiong* Hort (also known as *Ligusticum striatum*; family: Umbelliferae) (Fig. 14.10, right). Both *Angelica sinensis* and *Ligusticum chuanxiong* have long been used in traditional Chinese medicine to treat a variety of cardiovascular and cerebrovascular diseases, including stroke (see Han et al. 2021; Li et al. 2022). FA is also a major active constituent of *Cimicifuga heracleifolia* Komarov (family: Ranunculaceae) (Sakai et al. 1999) and *Centaurium erythraea* Rafn (family: Gentianaceae) (Banjanac et al. 2017), two medicinal plants used in China and Europe, respectively.

It has repeatedly been reported that FA exerts neuroprotective effects, including protection from ischemia-induced apoptosis (e.g., Cheng et al. 2008, 2010; for review, see Wang et al. 2023). More specifically, FA prevented ischemia/reperfusion-induced apoptosis in rats exposed to temporary occlusion of the right middle cerebral artery (Cheng et al. 2010). This effect was completely abolished by treatment with saclofen, suggesting a role for $GABA_B$ receptor in the neuroprotective actions of FA (Cheng et al. 2010). In agreement with these data, treatment with FA increased

Fig. 14.10 *Angelica sinensis* (left picture) and *Ligusticum chuanxiong* (right picture). (*Photo credits*: Tran Huu Dang (*Angelica sinensis*) and Alexander Greene (*Ligusticum chuanxiong*))

the expression of $GABA_{B1}$ receptor subunit in the rat ischemic cortex (Cheng et al. 2010), in line with the idea that enhancement of the $GABA_B$-mediated neurotransmission may protect against glutamate-induced excitotoxicity (e.g., Ouyang et al. 2007).

14.2.9 Albizzia lebbeck

Albizzia lebbeck (L.) Benth. (Indian siris, Indian walnut) is a flowering tree in the Fabaceae family (Fig. 14.11). It grows primarily in the seasonally dry tropical biome and is native to Indian subcontinent and Myanmar (see Kew Science 2024). In Indian Ayurvedic medicine, preparations based on *Albizzia lebbeck* are used for a series of therapeutic purposes, including treatment of asthma, inflammation, dysentery, and tuberculosis (see Verma et al. 2013).

An in vivo study found that rats pretreated with a butanolic extract of *Albizzia lebbeck* leaves displayed reduced levels of baclofen-induced hypothermia, hypotonia, and sedation, suggesting that the extract exerted an antagonistic-like action at $GABA_B$ receptor (Une et al. 2001).

Fig. 14.11 *Albizzia lebbeck* (L.) Benth. (*Photo credits*: Brisbane Botanic Gardens, Mt. Coot-tha, Brisbane City Council, Brisbane, Australia)

Fig. 14.12 *Emblica officinalis* Gaertn. (*Photo credits*: Dr. Andrea Moro, Department of Life Sciences, University of Trieste, Trieste, Italy)

14.2.10 Emblica officinalis

Emblica officinalis Gaertn., known also as *Phyllanthus emblica* L. (Indian gooseberry, Malacca tree), is a flowering tree in the Phyllanthaceae family (Fig. 14.12). It grows primarily in the wet tropical biome and is native to tropical and subtropical Asia (see Kew Science 2024). *Emblica officinalis* is another important plant in Ayurvedic medicine due to its tonic, antidiabetic, anti-inflammatory, antioxidant, and hepatoprotective properties (see Yadav et al. 2017).

An in vivo study reported that the antidepressant-like effects of an aqueous extract of *Emblica officinalis* fruits—assessed in mice exposed to the tail suspension test—were reversed by treatment with baclofen, suggesting an underlying $GABA_B$ receptor-mediated mechanism (Dhingra et al. 2012). However, the use of a baclofen dose that altered per se the basal levels of immobility time (i.e., the primary depression-like measure in the tail suspension test) renders somewhat questionable the study results.

14.2.11 Nardostachys jatamansi

Nardostachys jatamansi (D.Don) DC. (spikenard, muskroot) is a perennial, flowering plant in the Caprifoliaceae family (Fig. 14.13). It grows primarily in the temperate biome and is native to western and central China and northern Myanmar (see

Fig. 14.13 *Nardostachys jatamansi* (D.Don) DC. (*Photo credits*: Dr. Andreas Gröger, Botanischer Garten München-Nymphenburg (left picture); Dr. Franz Schlegel, Botanischer Garten München-Nymphenburg (right picture))

Kew Science 2024). *Nardostachys jatamansi* is an Ayurvedic medicine plant known for its beneficial effects on cognition, vertigo, seizures, and anxiety (see Pathak and Godela 2024).

An ethanolic extract of *Nardostachys jatamansi* rhizomes exerted antidepressant-like effects in mice exposed to the tail suspension test (Dhingra and Goyal 2008). The effect was reversed by baclofen treatment, suggesting the possible involvement of the $GABA_B$ receptor (Dhingra and Goyal 2008). The use of a relatively high, and likely sedative, dose of baclofen—which altered basal activity per se—calls for a replication of this study before drawing more definitive conclusions.

14.2.12 Clerodendrum mandarinorum

Clerodendrum mandarinorum Diels is a shrub or tree in the Lamiaceae family (Fig. 14.14). It grows primarily in the wet tropical biome and is native to southern China and Vietnam (see Kew Science 2024). *Clerodendrum mandarinorum* is a plant used in traditional Chinese medicine (Zhu et al. 1996a).

An ethanolic extract of *Clerodendrum mandarinorum* roots was found to possess an ability to bind to multiple receptor systems, including the $GABA_B$ receptors, in

Fig. 14.14 *Clerodendrum mandarinorum* Diels. (*Photo credits*: Dr. Andrea Moro, Department of Life Sciences, University of Trieste, Trieste, Italy)

Fig. 14.15 *Terminalia bellirica* (Gaertn.) Roxb. (*Photo credits*: Dr. Andrea Moro, Department of Life Sciences, University of Trieste, Trieste, Italy)

rat brain synaptosomes (Zhu et al. 1996a, b). However, none of the 14 isolated compounds displayed any affinity for the $GABA_B$ receptor (Zhu et al. 1996a, b), leaving the question as to the active ingredient(s) responsible for the $GABA_B$ receptor-binding activity of the tested extract unanswered.

14.2.13 Terminalia bellirica

Terminalia bellirica (Gaertn.) Roxb. is a flowering tree in the Combretaceae family (Fig. 14.15). It grows primarily in the wet tropical biome and is native to Indian subcontinent and southern China (see Kew Science 2024). The use of *Terminalia*

bellirica is described in different traditional medicinal systems, including Indian Ayurveda and Siddha, South Asian Unani, and traditional Chinese medicine (see Gupta et al. 2020). Its effects include anti-inflammatory, immunomodulatory, antimicrobial, hepatoprotective, antidiabetic, anti-hyperlipidemic, and anticancer activities (see Gupta et al. 2020).

A methanolic extract of dried fruits of *Terminalia bellirica* underwent a large screening of in vitro radioligand receptor bindings (Misra et al. 1994). An ethyl acetate fraction of the initial extract displayed a binding affinity for the $GABA_B$ receptor, with a K_i of approximately 14 μg/ml (Misra et al. 1994). No identification of pure active compound(s) was pursued.

14.2.14 Other Medicinal Plants

A study including Norman G. Bowery (see Chap. 15 of this volume) among its authors investigated the ability of extracts of a series of medicinal plants to bind to multiple receptor systems, including the $GABA_B$ receptor (Zhu et al. 1996a). Radioligand receptor binding assays were conducted using tissues from rat and pig brains.

The results of this study indicated that extracts of (i) *Schefflera bodinieri* leaves and roots, (ii) *Schefflera delavayi* leaves, roots, and stems, (iii) *Clerodendrum bungei* root bark, (iv) *Celastrus orbiculatus* stems, (v) *Celastrus angulatus* stems, (vi) *Periploca calophylla* stems, (vii) *Periploca forrestii* stems, (viii) *Alangium platanifolium* root barks, and (ix) *Uncaria rhynchophylla* stems had strong binding with $GABA_B$ receptors in rat brain synaptosomes (Zhu et al. 1996a).

In spite of these interesting, although preliminary results, to our knowledge no study has ever investigated the $GABA_B$ receptor-mediated effects of any preparation from these medicinal plants or has aimed at identifying the active ingredients responsible for their $GABA_B$ receptor-mediated effects.

14.3 Conclusions

The identification of naturally occurring $GABA_B$ receptor ligands appears to be a promising, although as yet unexploited, field of research. On the lighter side of this research field, one finds numerous medicinal plants, belonging to different traditional medicinal systems, which represent a source of extracts and/or active constituents capable of behaving as agonists, antagonists, and even PAMs of the $GABA_B$ receptor. These ligands apparently replicate the pharmacological properties and therapeutic potential of synthetic ligands. Instances of comparable, or even higher, potency and efficacy have emerged, including—as a paradigmatic example—the anti-addictive properties of SSA (that proved to be more potent than baclofen in its anti-alcohol effects in rats; see above).

Broadly speaking, compared to synthetic ligands, natural compounds exhibit a series of additional *pluses*, with the majority featuring remarkable therapeutic potential. The latter is also theoretically the case for naturally occurring $GABA_B$ ligands, including a frequently observed, more favorable toxicological profile (i.e., a larger separation between the "desired" pharmacological effects and adverse toxicological effects), high patient acceptance of treatment intervention, and abundance of supplies (see Wang et al. 2018). Additional beneficial features that render plant extracts more effective and safer compared to single active ingredients administered at corresponding doses include pharmacodynamic synergies, complementary (additive) mechanisms of action, and pharmacokinetic interactions resulting in increased bioavailability of the active compound(s) (see Wagner and Ulrich-Merzenich 2009).

Conversely, the darker side of this research field apparently includes a lack of systematic screening programs aimed at identifying naturally occurring $GABA_B$ receptor ligands, with the exception of the above-mentioned, outdated screening studies conducted at NIMH (Cott 1994, 1997) and the study fostered by Norman G. Bowery in the same period (Zhu et al. 1996a). Additionally, the majority of studies conducted to date have focused on composite plant extracts, without probing into the single active ingredient(s) responsible for eliciting specific pharmacological effects. To date, the list of pure compounds identified as $GABA_B$ receptor ligands is limited to the 4 molecules—namely SSA, ISL, DH-VAL, and FA—depicted in Fig. 14.2. This paucity makes it difficult to investigate the underlying mechanism of action (as mentioned above, the multiple components of a plant extract often exert distinct effects, possibly via different mechanisms of action), including the possibility of "weighting" the actual contribution of the $GABA_B$ receptor to mediation of the observed pharmacological effects.

Acknowledgments I am grateful to Professor Federico Corelli for drawing Fig. 14.2, Dr. Chae Ha Yang for allowing me to reproduce in Fig. 14.3 his fundamental data on the anti-addictive properties of saikosaponin A, Dr. Paola Maccioni for drawing Fig. 14.3, Ms. Anne Farmer for language editing of the manuscript, the several friends and colleagues (mentioned in figure captions) who provided me with pictures of the examined plants, and my daughter, Silvia, for organizing the "harvest" of these pictures.

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Chapter 15
Norman G. Bowery: A Founding Father of the $GABA_B$ Receptor Research Field: Reflections on His Contribution

Alessandra P. Principalle

Abstract This chapter is dedicated to the history of Norman George Bowery and his groundbreaking discovery of $GABA_B$ receptor. This chapter focuses on Norman's brilliant career from the beginning, toward his retirement, and to the legacy that he left in the scientific community: that is why Norman is called the "Father of $GABA_B$ receptor." Reported here are the first evidence of $GABA_B$ receptor existence spanning to the pharmacological characterization, the following development of Norman's own group on $GABA_B$ receptor, and the huge innovation that he brought to the scientific community because the $GABA_B$ receptor is a major factor in the central nervous system inhibition; therefore, when not working properly, it is involved in many diseases. The chapter also reports about the collaboration between Norman Bowery and Alessandra Principalle, who was so honored and fortunate to have the opportunity to work with such an inspirational and worldwide recognized scientist and is now the author of this work. This chapter consider a brief overview of the latest Noman's work, before moving to GlaxoSmithKline, Verona, Italy. This chapter concludes with the greatest recognition to Norman Bowery for being Norman and discovering the $GABA_B$ receptor.

Keywords Norman G. Bowery · $GABA_B$ receptor · Pharmacology · $GABA_B$ receptor localization

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15.1 Norman Bowery

Norman George Bowery (Figs. 15.1 and 15.2) was born in London in 1944. Norman began his career at the National Institute of Medical Research. He then worked at the newly opened CIBA Research Unit (a Swiss company later bought out) at Horsham in Sussex, UK. Norman left CIBA to begin a PhD with Professor David Brown, and it was this fundamental work that led to his groundbreaking discovery of the γ-aminobutyric acid (GABA) type-B ($GABA_B$) receptor. He was then invited to take the Chair of Pharmacology at the prestigious School of Pharmacy in London. He spent successful years in this position. His following appointment was at the University of Birmingham, becoming Chair of Pharmacology, and Head of Division of Neuroscience, and it was here in 1998 that I had the fantastic opportunity to start to collaborate with him. Norman came to pick me up at the airport as the friendliest professor I have ever met up to that point in my life. The first impression was then confirmed in the following years we worked together and became friends with respective families. Norman demonstrated a very happy, easy, and warm personality, always ready for a good laugh in company. When any problem arose, work or life, he was ready to discuss it and find a solution for it. That was the inspirational man for everybody who met him: collaborators, students at all levels from undergraduates to PhD. Norman was a huge lover of Italy and Italians and Italian wines; in fact, when he left the University of Birmingham, he moved to work for GlaxoSmithKline (GSK) in Verona, Italy, before going back to University of Birmingham to deliver pharmacology lectures as Honorary Professor, and he did it as a hobby. He was president of the British Pharmacological Society from 1995 to 1997 and from 1999 to 2000.

Fig. 15.1 Professor Norman George Bowery FBPhS (1944–2016) British pharmacologist

Fig. 15.2 Young Norman Bowery in the laboratory

15.2 Norman and Early Evidence of a Second Type of GABA Receptor (1970s–1982)

Norman started his work on GABA in the seventies during his time in Professor Brown's laboratory, reporting in vitro observation of depolarization of sympathetic ganglia induced by gamma-aminobutyric acid (Bowery and Brown 1972). Since then, his interest in the action of GABA and its action in different compartments of the central (CNS) and peripheral (PNS) nervous system never stopped.

He continued his studies investigating the action of three analogues, namely, 4-aminotetrolic acid (4-ATA), *trans* 4-aminocrotonic acid (4-ACA), and imidazole-4-acetic acid (IAA), capable of depolarizing the ganglia in a way similar to GABA (Bowery and Jones 1976). After these analogs, he evaluated other compounds including other amino acids (Bowery et al. 1976a) and bicyclic phosphorus esters as convulsant (Bowery et al. 1976b). Norman and colleagues also investigated several

other aspects of the GABA inhibitory system in ganglia (Bowery and Dray 1976, 1978; Neal and Bowery 1977). In 1978, Norman and his collaborators clarified aspects of isoguvacine and muscimol analogues acting on GABA receptors in rat ganglia (Bowery et al. 1978). During the following few years Norman and colleagues advanced their studies by using baclofen which identified a new type of GABA receptor (Bowery et al. 1980). In this historic paper in Nature journal, Norman and coworkers shed light on why GABA affected and reduced the release of H^3-noradrenaline in atria and H^3-acetylcholine in preganglionic terminals, and these events were not affected by bicuculline. So, their hypothesis was that there must be a different and separate type of GABA receptor present on nerve terminals. To test this hypothesis, they used three different in vitro systems with specific neurotransmitters and measured the release of evoked K^+. The first were slices from rat cerebellum, in which the release of H^3-noradrenaline was measured in three different settings incubated with (i) high KCl (25 mM), (ii) baclofen 100 μM, and (iii) GABA 100 μM. The same set was used to test the striatum and the release of H^3-dopamine and the frontal cortex and the release of H^3-serotonin. All the experiments gave the same results: a reduction in the release of the tritiated neurotransmitters (Figs. 15.3 and 15.4).

They also tested other analogues of the GABA, and different concentrations of K^+ support these findings. The huge breakthrough of a second GABA binding site, or as they call it first "a novel GABA receptor", was made… YES! Hurray! This was the birth of $GABA_B$ receptor and Norman Bowery was its absolute father. Norman's work on this fascinating and exciting novelty continued by further corroboration for the existence of the second GABA binding site. Norman with David Hill performed classical homogenized crude synaptic membranes radioligand binding experiments on rat brain first using H^3-baclofen, with different experimental conditions adding different cations to the buffer to check saturability of baclofen. They first set the best conditions for the investigation of the second GABA binding site, and then they used (−) baclofen and GABA as non-labelled displacing compounds. They showed

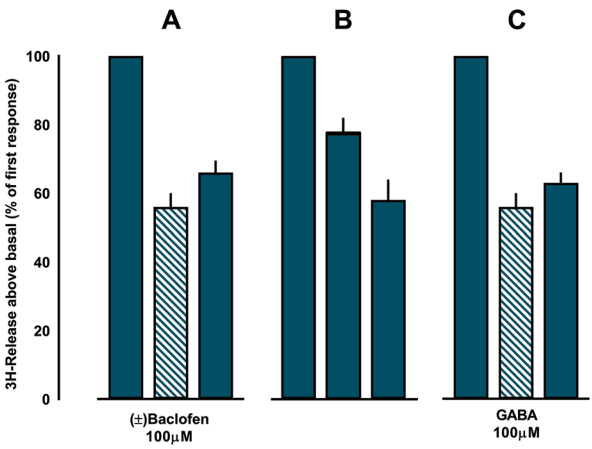

Fig. 15.3 (**a**, **c**) (±) Baclofen (100 μM) and GABA (100 μM), respectively, added 1 min before the second period of superfusion with KCl. (**b**) The responses to successive additions of KCl. (Figure adapted from Bowery et al. (1980), with permission from Springer Nature)

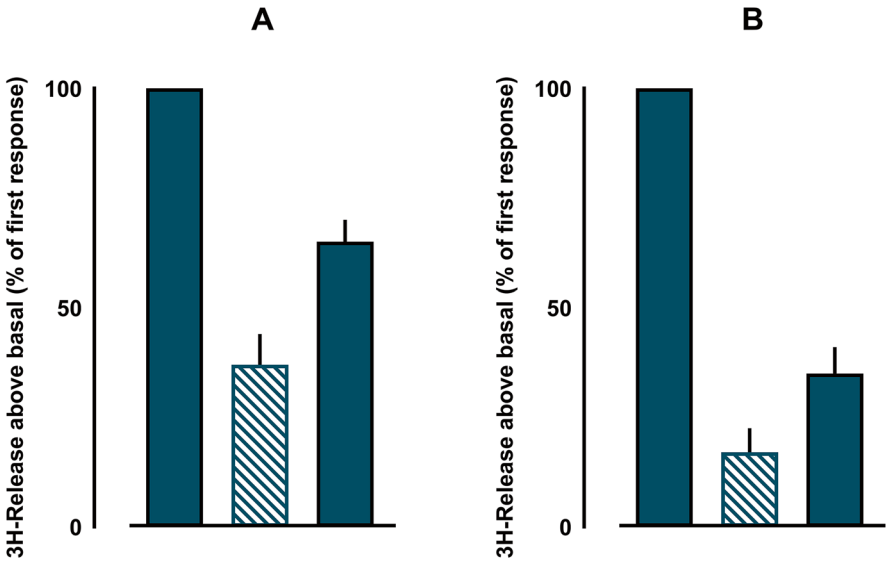

Fig. 15.4 Results obtained depression of K^+-evoked release of ^{3}H-dopamine (**a**) and ^{3}H-serotonin (**b**) by (±)baclofen. (**a**) The evoked release of ^{3}H-dopamine from striatal slices. (**b**) ^{3}H-Serotonin from cortical slices. (Figure (**a** and **b**) adapted from Bowery et al. (1980), with permission from Springer Nature)

that the displacement curves for GABA and (−) baclofen were the same in the same experimental conditions, whereas (+) baclofen did not show any displacement (Fig. 15.5). They also tested other GABA type-A ($GABA_A$) receptor agonists (isoguvacine) and antagonists (bicuculline), and they did not have any effect on the displacement of H^3-baclofen (Fig. 15.5).

Finally, to distinguish the two different binding sites, Norman and David named $GABA_A$ receptor the original binding site and $GABA_B$ receptor the newly discovered and confirmed binding site (Hill and Bowery 1981).

Norman and collaborators did it and continued their research on the $GABA_B$ receptor, and a lot of new knowledge emerged quickly (Bowery et al. 1981a; Doble et al. 1981).

Other papers were based on pharmacological techniques which led to the breakthrough of the existence of a GABA receptor which was not the ionotropic one but a metabotropic (Bowery et al. 1981b; Wilkin et al. 1981).

15.3 Norman's Studies on the "Novel GABA Receptor" (1982–1992)

Norman and various collaborators continue their studies to localize this novel GABA receptor which they named $GABA_B$ to be distinguished from the benzodiazepine-activated ionotropic receptor named $GABA_A$ (Bowery et al. 1983).

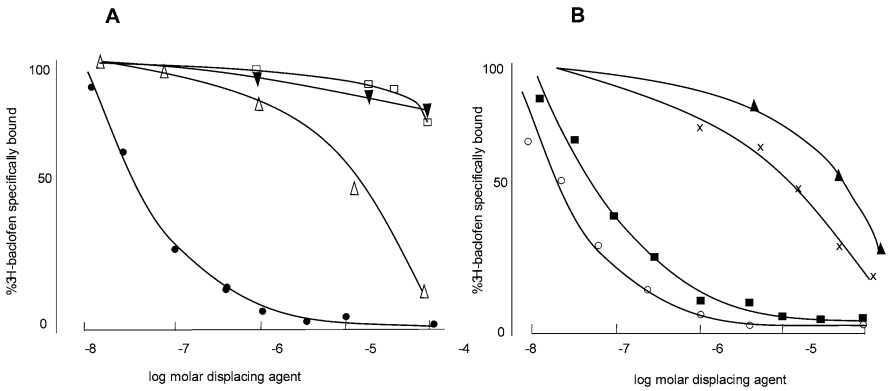

Fig. 15.5 Inhibition of (±)^{3}H-baclofen binding by GABA-related compounds. Unlabeled analogs at final concentrations between 0.01 and 100 μM were added to each incubation mixture together with ^{3}H-baclofen (20 nM final concentration). Specific binding was taken as the amount of label displaced by (±)baclofen (100 μM). The amount displaced by individual concentrations of each analog (log molar, abscissa) is expressed as a percentage of the total displaced by 100 μM (±) baclofen within the same experiment. The data have been separated for clarity. (**a**) Shows the effect of GABA (●), muscimol (Δ), isoguvacine (▼), and bicuculline methobromide (□). (**b**) Shows the effects of (−)baclofen (○), (±)baclofen (▬), 3-aminopropanesul-phonic acid (×) and (+) baclofen (▲). (Figure and legend adapted from Hill and Bowery (1981), with permission from Springer Nature)

Norman and his team went on with their studies to gather and advance knowledge about this new receptor: there were a lot of new questions opening at the horizon of this discovery, such as the localization in different nuclei and parts, the physiological pathways of CNS and PNS, and the specific cellular and subcellular localization (Bowery et al. 1984, 1987; Price et al. 1984, 1987; Bristow et al. 1986). Norman also did other studies on the heterogeneity of $GABA_B$ receptors. Sort of "a preconception" to the molecular characterization of the whole $GABA_B$ multidimer (Bowery et al. 1990), and they were also able to pharmacologically differentiate "subtypes" of $GABA_B$ receptors. Additional studies in Norman's group demonstrated that neuronal degeneration could be induced by tetanus toxin accompanied by a reduction of $GABA_A$ and not $GABA_B$ sites in rat hippocampus (Bagetta et al. 1990), a further corroboration for the two different GABA receptors. Most importantly, from the whole body of evidence emerged a new versatile drug target available (Bowery and Pratt 1992).

15.4 Norman's Pharmacological Studies on the $GABA_B$ Receptor (1993–1997)

Norman went on, with his amazing and contagious curiosity, to inspire collaborators and they tested several compounds such as pertussis toxin in CNS (Knott et al. 1993a, b) or forskolin in spinal cord (Malcangio and Bowery 1993a) and Substance

P in rat cortex (Malcangio and Bowery 1993b) and desipramine (Pratt and Bowery 1993) on the recently isolated $GABA_B$.

Then Norman and Meret Facklam isolated the $GABA_B$ receptor from pigs' brains and found the receptor had the same pharmacological characteristics as those isolated from rats' brains (Facklam and Bowery 1993). However, these $GABA_B$ receptors were present in homogenates derived from CNS or PNS and cell membrane, and up to this point, there was no evidence of the gene or protein for this pharmacologically characterized receptor. After three more years of further studies combining neurotransmitters, their actions, and responses in different compartments of the nervous system, Norman and coworkers make another step forward describing that once $GABA_B$ receptors are activated, it can determine opposite effects of up- or downregulation, therefore the activation or inhibitions of adenylyl cyclase (Malcangio et al. 1995; Knight and Bowery 1996). Furthermore, Norman with Hill and Hudson start to describe the $GABA_B$ receptors binding site in rat brain (Bowery et al. 1997).

At the end of the 1990s, the application of molecular biology techniques gave a huge burst to the pharmacology field, due to the increasing number of genes cloned for receptors, receptors subunits, and neurotransmitters, and it was the case also for the $GABA_B$ receptor.

Kaupmann et al. (1997) published a paper in Nature reporting the molecular cloning of the gene for $GABA_B$ receptor and the sequence of the protein, including a predicted structure similar to that of the metabotropic glutamate receptors. Norman and David Brown analyzed the latest scientific development concerning the $GABA_B$ (Bowery 1997; Bowery and Brown 1997).

15.5 When Norman Met Alessandra: Localization and Expression Studies of the $GABA_B$ Receptor in the Brain and Spinal Cord (1998–2003)

I met Norman in person at the very end of January 1998, as mentioned in part 15.1. However, at the annual American Society for Neuroscience (SfN) meeting, in New Orleans, LA, in 1997, I already heard scientists referring to Norman as "*$GABA_B$ man*," which says all about him. Being called "$GABA_B$ man" meant that he was internationally, worldwide, recognized as the guru and the father of the $GABA_B$ receptor.

Our collaboration and friendship started that long ago; I cannot believe it is over 25 years ago (scary), but better refocus our attention on what we did together concerning the $GABA_B$ receptor research.

In 1998, I began to collaborate with Norman in his laboratory with a fellowship from a European project, at The Medical School in Birmingham, UK. Here, we did immunohistochemistry on rat brains to initially localize the $GABA_B$ protein, finding specific patterns of expression in the piriform cortex (Princivalle et al. 2000a).

While I was working with Norman, I deepened my knowledge of all that he did scientifically and how much he had contributed to the knowledge in the field of $GABA_B$ receptor, no wonder why he was referred to as "$GABA_B$ man" (very American way) and what an honor to be there doing research with him!

The European project was based on previous evidence showing a decrease inhibition in the thalamus and somatosensory cortex of non-epileptic rat and Genetics Absence Epilepsy Rats from Strasbourg (GAERS), the rat model of *petit mal* or absence seizures. This was a validated model of human absence epilepsy based on neurological, behavioral, and pharmacological findings (Vergnes et al. 1982; Marescaux et al. 1992). So, we started investigating the localization of the $GABA_B$ protein in rat somatosensory cortex and thalamus in adult and developing non GAERS rat brains (Princivalle et al. 2000b). Then, we moved forward to investigate the semi-quantitative expression of $GABA_B$ receptor in control rats and GAERS (Princivalle et al. 2003b). At the end of 1998, Norman organized a $GABA_B$ Satellite Symposium for the 28th Annual Meeting of the SfN in Los Angeles, CA, of course who else!!! On that occasion, Kaupmann and Bettler (1998) unveiled the finer structure of the fully functional $GABA_B$ receptor, demonstrating for the first time that the $GABA_B$ receptor is a metabotropic heterodimeric receptor… no way!!! Norman and I listened to this exciting piece of news, and we both were almost incredulous. This was opening the door to a new and immense field for pharmacological research and treatment of countless diseases.

The following year, I went back again to Norman's laboratory, and we started a 3-year collaboration to finish some of the previous European projects to put together the first few papers of our collaboration. Norman offered me a position to work with him on a newer project on $GABA_B$. I must admit that the idea of working with "*$GABA_B$ man*" was overly exciting, and, of course, how could I say no to such an amazing offer? So later that year, I started this new position continuing in parallel the previous European project and other collaborations (Towers et al. 2000; Princivalle et al. 2001) and starting the new project focused on the expression of $GABA_B$ receptor in human sclerotic hippocampi from temporal lobe epilepsy (TLE)-affected patients not responding to drug therapies. There was already indirect evidence showing a decreased inhibition in animal model of pharmaco-resistant TLE associated with hippocampal sclerosis (HS). Therefore, our hypothesis was that $GABA_B$ receptor might play a role in this specific category of epileptic patients and likely to show a lower expression in the hippocampal regions of TLE-HS. From the first part of this project, we used a classical quantitative imaging technique, the autoradiographic binding, using a newly synthesized radioligand with higher affinity for the $GABA_B$ receptor and published these results demonstrating a decreased expression of the $GABA_B$ receptor in the CA3, dentate gyrus, and hilus of the hippocampal region. We found an increased expression of $GABA_B$ receptors per surviving neuron in CA3 of TLE-HS patients compared to post-mortem controls (Princivalle et al. 2002). Since with the imaging quantitative technique we investigated the protein receptor and found downregulation of the $GABA_B$ receptor, we wanted to also check if that was due to quantitative differential expression of the transcripts for the $GABA_B$ receptor. We used three different probes to check the

three different transcripts for the two splice variants called $GABA_{B1}$ subunit ($GABA_{B1a}$ and $GABA_{B1b}$) and the second subunit called $GABA_{B2}$ (Kaupmann et al. 1998). That research was done using quantitative imaging with in situ hybridization technique (Princivalle et al. 2003a). We also investigated the plasticity of $GABA_B$ receptor in heterosynaptic connections at mossy fibers (Chandler et al. 2003).

15.6 Norman's 2004–2008

I then moved to a Senior Lecturer position in Sheffield, UK, but that did not mean that our friendship and collaboration were over. In 2004, Norman moved to GSK, Verona, Italy, continuing collaborations with other groups. Norman worked on further expanding knowledge on the $GABA_B$ receptor focusing on physiological aspects and pharmacological properties, within his group (Amantea and Bowery 2004; Amantea et al. 2004; Smith et al. 2007) and in collaboration with others (Dang et al. 2004; Meza-Toledo and Bowery 2008).

Norman also wrote reviews about the diverse topics involving $GABA_B$ (Enna and Bowery 2004; Bowery 2006, 2010). His last work before passing away was a chapter in a book edited by the same editor of this book, Giancarlo Colombo, and it was a historical overview of the $GABA_B$ receptor from Norman's initial studies on modelling presynaptic inhibition, passing to the pharmacological characterization, agonist and antagonist, the physiological role, a quick consideration of the distribution in the CNS, the structure, modulation, and clinical aspects targeting the receptor with baclofen as muscle relaxant (Bowery 2016).

Part of the work I started in Norman's lab was concluded and published posthumous his death, so much so that the article was dedicated to him (Sheilabi et al. 2018), even if some of the authors never met him but knew about his greatest discovery.

15.7 Conclusions

To collaborate with the father of $GABA_B$ receptor and the most inspirational scientist I have met so far, and still, was awesome!!! One other most important quality of Norman was leaving total freedom to the collaborators in technical terms, cogitating, trying new ways for research, expanding and expressing themselves as researchers and scientists, and spreading enthusiasm around to everyone, anywhere, and in any context.

From our collaborations, papers were published, but the major lesson from Norman was to never give up: if there is a problem, we need to find the solutions. Last but not least, the way of doing research that was taught to me by Norman, which I still use with my younger collaborators and students when observing and/or interpreting data, is: "Do I see what I believe, or do I believe what I see?". This is

what every scientist must always ask themselves when they look at their data. This question represents the essence of an intellectually honest, inspirational, and great scientist like Norman Bowery.

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