The GABA Receptors, Third Edition

The Receptors

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The GABA Receptors

THIRD EDITION

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Preface

This volume is the third edition of a monograph series that was first published in 1983. The demand for this work is a testament to the impact of studies on γ-aminobutyric acid (GABA) receptors on the basic understanding of synaptic transmission and on defining the clinical importance of the neurotransmitter system. Chronicled in *The GABA Receptors, Third Edition*, are the advances made in understanding the molecular and pharmacological properties of $GABA_A$ and $GABA_B$ receptors since the topic was last reviewed in 1996. Particular emphasis is placed on describing the assembly, structure, and function of $GABA_B$ sites, the first heterodimeric G protein-coupled receptors identified in vivo. In addition, there are reports dealing with the subunit composition, trafficking, and pharmacological selectivity of $GABA_A$ receptors. Aside from providing insights into the fundamental properties of ligand-gated ion channels and second messenger systems, the findings detailed in this work point the way for developing novel therapeutics capable of more selectively manipulating these transmitter sites. Chapters in this volume contain descriptions of new agents, including allosteric modulators, capable of activating or inhibiting GABA receptors. Descriptions are provided of potential clinical candidates for treating disorders as diverse as insomnia and cognitive impairments. The reports contained herein also detail new evidence directly linking $GABA_A$ and $GABA_B$ receptor dysfunctions to a host of neuropsychiatric conditions, including epilepsy, anxiety disorders, affective illness, and pain syndromes. These data provide a biological framework for understanding the clinical utility of GABAergic drugs as treatments for neurological and psychiatric disorders, and for their use as hypnotics and anesthetics.

Numbered among the contributors to *The GABA Receptors, Third Edition*, are many who have worked in this area for decades. All of the senior authors have been actively engaged in studying GABA receptor systems and are recognized for making seminal contributions to the field. In addition to highlighting advances over the past 10 years, the authors provide opinions on the implications of these findings and suggestions on fruitful avenues for future research. As was the case for the previous two editions, the aim of this volume is to not only serve as an information source, but as a stimulus for further advances in the field. This offering should be of particular value to basic and clinical neuroscientists in general, and neuropharmacologists, psychiatrists, and neurologists in particular.

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1

The GABA Receptors

S. J. Enna

Summary

γ-Aminobutyric acid (GABA), an amino acid neurotransmitter, is widely distributed throughout the neuraxis. Two pharmacologically and molecularly distinct GABA receptors have been identified, $GABA_A$ and $GABA_B$. $GABA_A$ receptors are pentameric ligand-gated chloride-ion channels, whereas $GABA_B$ receptors are heterodimeric G protein-coupled sites. Although $GABA_A$ receptor subtypes can display pharmacological differences, the two molecularly distinct $GABA_B$ receptors have similar substrate specificities, limiting the ability to selectively manipulate this site. Gene deletion and point mutation studies have revealed the importance of GABA receptors in neural development and function, with subtle modifications in subunit amino acid composition having profound effects on behavioral phenotype and responses to drugs. The characterization of $GABA_A$ receptors has contributed substantially to the knowledge about allosteric regulation of ligand-gated ion channels. Such information is invaluable in defining precisely the mechanisms of action of numerous drugs, such as the benzodiazepines, and toxic agents. Research on $GABA_B$ receptors has proven the existence of dimeric metabotropic receptors and has provided the chemical tools necessary for defining such systems. The characterization of the pentameric $GABA_A$ and dimeric $GABA_B$ receptors has been crucial for understanding the neurobiological basis of some nervous system disorders. Given the importance of GABA in central nervous system function, further work on its receptors is likely to yield novel therapeutics for treating a host of neurological and psychiatric conditions.

Key Words: GABA; GABA pharmacology; $GABA_A$ receptors; $GABA_B$ receptors; GABA receptor subunits; GABA receptor function.

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1. Introduction

It has been nearly a quarter century since the publication of the first edition of *The GABA Receptors (1)*. That volume was devoted almost exclusively to reviewing studies on γ-aminobutyric acid-A ($GABA_A$) receptors, with only passing reference to the possibility of the existence of pharmacologically and molecularly distinct $GABA_B$ sites, evidence for which had only recently been reported *(2)*. The book consists of various chapters describing the biochemical and pharmacological properties of the $GABA_A$ receptor, with particular emphasis on the use of binding assays for characterizing this site and defining its relationship to the benzodiazepines.

Topics covered in the second edition, which appeared in 1996, reflected the pace of discoveries in the intervening 13 yr, with half of the chapters devoted to $GABA_A$ and half to $GABA_B$ *(3)*. By then the genes for $GABA_A$ receptor subunits were cloned, making it possible to more precisely determine the composition of the site. This in turn led to a better understanding of the way in which drugs interact with the $GABA_A$ system and pointed to the possibility of developing agents that affect only subsets of this receptor. As for the $GABA_B$ site, the second edition included a review of progress made in creating new receptor agonists and antagonists and of biochemical and electrophysiological studies showing that, unlike the ligand-gated $GABA_A$ receptor ion channel, the $GABA_B$ receptor is a G protein-coupled site. Although some reports suggested the existence of pharmacologically distinct $GABA_B$ subtypes, direct proof was lacking, as the gene for this receptor was proving difficult to clone. Predictions were made about potential advances in drug development once the $GABA_B$ receptor gene was isolated and the data on $GABA_A$ receptor subtypes fully exploited.

The offerings contained in this third edition review advances during the past decade. As with the last volume, approximately half the chapters deal with receptors for $GABA_A$ and half for $GABA_B$. Soon after publication of the second edition the genes associated with the $GABA_B$ receptor were cloned, revealing a heterodimeric G protein-coupled site *(4–6)*. As this was the first direct demonstration of dimeric seven transmembrane receptors, subsequent work was pioneering in describing how such sites are assembled and regulated. This discovery opened the way to identifying other G protein-coupled receptor dimers, indicating that the $GABA_B$ system is not unique in this regard *(7)*. There is also coverage in this latest edition of new $GABA_B$ receptor agonists and antagonists, including one that is undergoing a clinical trial (*see* Chapters 9 and 12).

As detailed in the present work, strides have also been made in defining the $GABA_A$ site. Examples include gene manipulation and deletion studies directly linking particular $GABA_A$ receptor subunits, indeed particular subunit amino acids, with the various pharmacological responses to drugs such as the benzodiazepines

(*see* Chapter 2) *(8)*. Such findings make possible the design of more selective and less toxic hypnotics, anxiolytics, anticonvulsants, and muscle relaxants.

The aim of this chapter is to present an overview of GABA receptor systems. Though the subject is covered from an historical perspective to guide those new to the field, emphasis is placed on introducing and placing in context topics described in detail elsewhere in the text. Each chapter is authored by experts in the field, many of whom have labored for decades in this area. Those familiar with previous editions of this work will be impressed by the amount of progress made since the last volume, and by the impact GABA receptor research has made in defining chemical neurotransmission in general. As is made clear in this text, the ongoing efforts for characterizing the $GABA_A$ and $GABA_B$ receptors will undoubtedly continue to yield new insights into mechanisms that regulate synaptic transmission, the biological abnormalities associated with a host of neurological and psychiatric disorders, and the development of new drugs for treating these conditions.

2. General Overview

GABA, an amino acid neurotransmitter, is widely distributed throughout the neuraxis. While GABA is found in some peripheral tissues, and there is evidence it may regulate neuronal activity in the intestines, lungs, and bladder, its predominant effects are in the central nervous system. Because activation of neuronal GABA receptors generally results in hyperpolarization, this amino acid is considered an inhibitory neurotransmitter. Given the number of GABAergic neurons in the brain, and their widespread distribution, GABA appears to be the major inhibitory neurotransmitter in the central nervous system. It is this ubiquity that has hindered drug development because nonselective GABA receptor agonists and antagonists have generalized effects on central nervous system function.

Two pharmacologically and molecularly distinct GABA receptors have been identified, $GABA_A$ and $GABA_B$ *(3,4)*. $GABA_A$ receptors are ligand-gated chloride ion channels, whereas $GABA_B$ sites are heterodimers coupled to G proteins. Although many pharmacologically distinct $GABA_A$ receptors have been identified, the two molecularly distinct $GABA_B$ sites display similar pharmacological selectivity (*see* Chapter 11). However, it is possible that allosteric modulators might be able to distinguish between $GABA_B$ receptor subtypes (*see* Chapter 9) *(9)*.

3. $GABA_A$ Receptors

3.1. Molecular Pharmacology

As is the case with some other ligand-gated ion channels, the $GABA_A$ receptor is a pentameric structure made up of molecularly distinct subunits (Fig. 1)

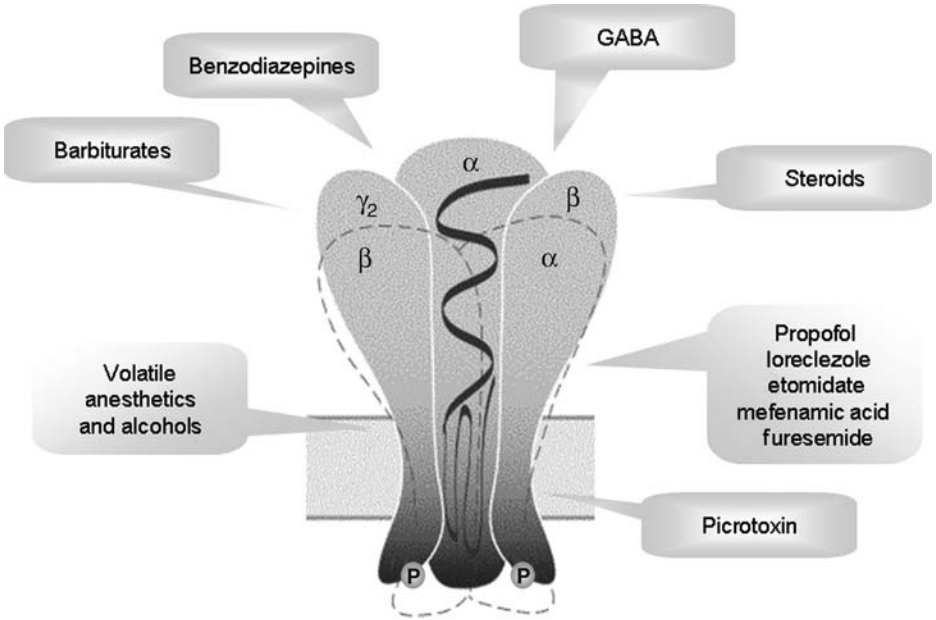


Fig. 1. Schematic representation of a $GABA_A$ receptor illustrating its pentameric structure and the different sites of action for drugs that interact with this site. The P designation represents a phosphorylation site. (Adapted from ref. *11*.)

(10,11). Generally, activation of a $GABA_A$ receptor increases the intraneuronal concentration of chloride ion, hyperpolarizing the cell (*see* Chapter 5) *(12,13)*. In select regions of the central nervous system, such as the hippocampus or dorsal root ganglia, or under certain circumstances, as during development, $GABA_A$ receptor activation causes neuronal depolarization *(12–15)*. In some circumstances this is because the intracellular concentration of chloride exceeds extracellular levels, with opening of the receptor-coupled channel resulting in a net efflux of this ion and neuronal excitation. In addition, whereas some $GABA_A$ receptors are phasically activated, others are tonically stimulated (*see* Chapters 5 and 8). The latter, such as those located in the cerebellar granule cells, can alter network excitability and can be selectively targeted by agents, such as the neurosteroids, that influence GABAergic transmission *(16–18)*.

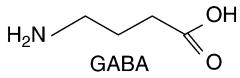
As with other ligand-gated ion channels, the sensitivity and activity of $GABA_A$ receptors are modulated by drugs acting at distinct sites on individual subunits or subunit combinations (*see* Chapter 2) *(8,19)*. Whereas GABA itself activates the receptor by attaching to the recognition site, barbiturates, benzodiazepines, alcohol, neurosteroids, and fixed and general anesthetics facilitate

$GABA_A$ receptor transmission by acting on other components of the receptor complex (Figs. 1 and 2). The same is true for antagonists, with the convulsant bicuculline being a competitive antagonist at the $GABA_A$ receptor recognition site, whereas picrotoxin attaches elsewhere on the receptor complex (Figs. 1 and 2). The benzodiazepines, such as diazepam (Fig. 2), are the best characterized of the allosteric modulators of $GABA_A$ receptor function.

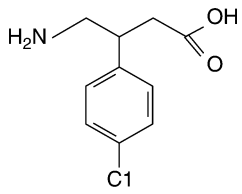
The subunits that assemble to form pentameric $GABA_A$ receptors are drawn from a pool of 19 distinct gene products (Table 1) (*see* Chapter 8). Given the variety of subunit proteins and their splice variants, the potential exists for an enormous number of molecularly distinct complexes (*see* Chapter 2) *(19,20)*. However, studies in various expression systems indicate that not all subunit combinations respond to GABA, with the estimated number of different $GABA_A$ receptors in the mammalian central nervous system believed to be less than 100, and probably as few as two dozen *(19,21,22)*.

The $GABA_A$ receptor subunits are widely and unevenly distributed throughout the brain and peripheral organs (*see* Chapter 4). Their function in peripheral tissues remains largely undefined, as GABAergic innervation is sparse outside the central nervous system. Whereas the precise stoichiometry of native $GABA_A$ receptors is unknown, subunit-labeling studies provide clues in this regard (Table 2). The largest single group of $GABA_A$ receptors appears to be made up of $\alpha_1\gamma_2$- and a β-subunit *(19)*. Indeed, it seems the majority of $GABA_A$ receptors possess either α_1- or α_2-subunit(s) in combination with a γ_2- and β-subunits(s). Subunit composition determines the biophysical and pharmacological properties of the site (*see* Chapters 2 and 5) (Table 2). For example, the α_1 or $\alpha_2\gamma_2\beta$ combination, designated as A1a- and A2a-GABA receptors, respectively, responds to benzodiazepine and nonbenzodiazepine anxiolytics and hypnotics. In contrast, receptors lacking γ-subunit, such as $\alpha_1\beta_x\delta$-, or $\alpha_1\beta_x\varepsilon$- (Table 2), or γ-subunits in combination with α_4 or α_6 are generally insensitive to the benzodiazepines and related drugs *(19)*. Trafficking and localization of $GABA_A$ receptors are determined in large measure by the subunit composition with, for example, γ_2 being important for routing receptors to synapses, whereas the δ-subunit is characteristic of $GABA_A$ receptors that accumulate at extrasynaptic sites (*see* Chapters 3–5 and 8) *(23,24)*.

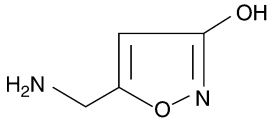
Although the vast majority of $GABA_A$ receptors are heteromers, homomeric ρ_1-receptors have been identified, as have heterodimers containing only combinations of ρ_1-, ρ_2-, and ρ_3-subunits *(25,26)*. This ρ-containing family of $GABA_A$ receptors displays a unique pharmacological profile, being sensitive to the recognition site agonist *cis*-4-aminocrotonic acid (Fig. 2) but insensitive to bicuculline, benzodiazepines and, in some cases, picrotoxin (Table 2) *(26,27)*. This substrate selectivity led initially to their designation as $GABA_C$ sites, but subsequent



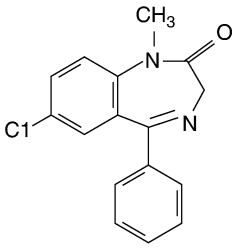
GABA



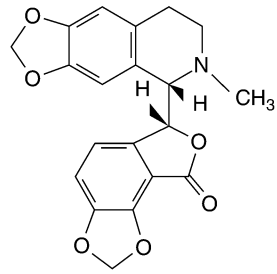
Baclofen



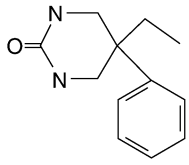
Muscimol



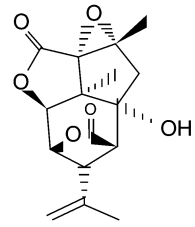
Diazepam



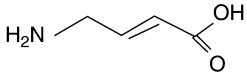
Bicuculline



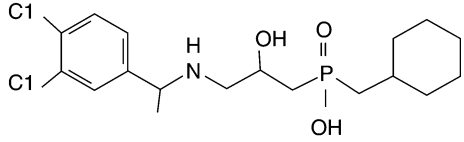
Phenobarbital



Picrotoxinin



Cis-4-aminocrotonic acid (CACA)



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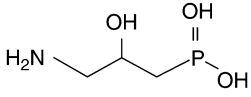

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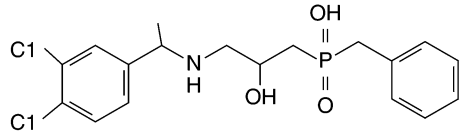

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Fig. 2. Chemical structures of various $GABA_A$ and $GABA_B$ receptor agonists and antagonists.

Table 1
GABA Receptor Subunits

Receptor subtype	Subunits	GenBank accession no.	
		Human	Mouse
$GABA_A$	α_1	NM_000806	NM_010250
	α_2	BC022488	NM_008066
	α_3	BC028315	NM_008067
	α_4	NM_000809	BC094603
	α_5	BC111979	BC062112
	α_6	BC096241	NM_008068
	β_1	BC022449	NM_008069
	β_2	NM_000813[a]	NM_008070
	β_3	BC010641[b]	NM_008071[b]
	γ_1	NM_173536	NM_010252
	γ_2	BC069348[a]	NM_177408[a]
	γ_3	NM_033223	NM_008074
	ε	NM_004961[b]	NM_017369[b]
	θ	NM_018558	AK038859
	δ	BC033801	NM_008072
	π	NM_01421	BC023693
	ρ_1	NM_002042	NM_008075
	ρ_2	–	NM_008076
	ρ_3	XM_927388	–
$GABA_B$	$GABA_{B1}$	NM_021905[c]	NM_019439
	$GABA_{B1a}$	AF099148	AF114168
	$GABA_{B2}$	NM_005458	–

[a]Transcript variant 2.
[b]Transcript variant 1.
[c]Transcript variant 4.

characterization of their subunit composition and transduction mechanism revealed them to be a $GABA_A$ receptor subtype. Thus, the molecular characterization of the $GABA_A$ receptor has made possible a more precise definition of the mechanism of action of a number of drugs, and has provided new targets for designing novel therapeutics capable of activating or inhibiting select populations of $GABA_A$ sites.

3.2. Therapeutics

Most drugs that modify $GABA_A$ receptor function enhance the activity of this neurotransmitter system. Included among this group are the benzodiazepines and barbiturates (Fig. 2), zolpidem, and propofol. By attaching to the $GABA_A$ receptor, these agents either increase the frequency (benzodiazepines) or prolong

Table 2
Subunit Composition, Nomenclature, and Pharmacological Properties of Selected $GABA_A$ Receptor Subtypes[a]

		Pharmacology selectivity[b]				
Subunits	IUPHAR nomenclature	Benzodiazepine	Barbiturates	Bicuculline	Picrotoxinin	*cis*-4-aminocrotonic acid
α_1, $\beta_x\gamma_2$	A1a	+	+	+	+	+
α_2, $\beta_x\gamma_2$	A2a	+	+	+	?	?
ρ_1[c]	AOr1	–	–	–	+	+
$\rho_1\rho_2$[c]	AOr12	–	–	–	+	+
α_1, $\beta_x\delta$	AO1	–	?	+	?	?
α_1, $\beta_x\varepsilon$	AOle	–	?	+	?	?

[a]Adapted from ref. *25*.
[b](+), Responsive; (–), nonresponsive; (?), unknown.
[c]ρ-subunit homomers or heteromers were formerly classified as $GABA_c$ receptors.

the duration (barbiturates) of chloride channel opening in response to GABA. Although flumazenil attaches to the benzodiazepine-binding component of $GABA_A$ receptors, it differs from other benzodiazepines in being a competitive antagonist at this site. With no efficacy of its own, flumazenil is used clinically to reverse the effects of benzodiazepine agonists. Each of these drugs influences only those $GABA_A$ receptors with the requisite subunit composition, making them selective in this regard.

Some agents display differential effects at various $GABA_A$ receptor subtypes. An example is 4,5,6,7-tetrahydroisoxazolol[5,4-c]-pyridin-3-ol (THIP), a direct-acting drug. THIP is a partial agonist at the $\alpha_4\beta_3\gamma_2$-$GABA_A$ site, whereas it is a full agonist at the $\alpha_4\beta_3\delta$-receptor *(28–30)*. THIP is now undergoing clinical trials as a hypnotic agent *(29,30)*. Nonselective drugs that activate $GABA_A$ receptors do so indirectly by inhibiting the metabolism (vigabatrin) or reuptake (tiagabine) of this amino acid, thereby increasing its synaptic content and prolonging its action *(31)*. It has been suggested that the anticonvulsant GABApentin may, in some way, influence GABAergic transmission *(32,33)*, although the weight of evidence suggests its primary site of action is the neuronal calcium channel *(33,34)*. Diazepam and chlordiazepoxide are prescribed for the treatment of anxiety. As is the case for all central nervous system depressants, their long-term use can result in tolerance and physical dependence (*see* Chapter 7).

Other benzodiazepines, including flurazepam, alprazolam, and traizolam, as well as zolpidem, a nonbenzodiazepine that interacts with a subgroup of benzodiazepine receptors, are routinely used as hypnotics *(35)*. Although barbiturates are also available for this purpose, the benzodiazepines and zolpidem are preferred because of their greater margin of safety. Whereas the precise mechanisms of action of chloral hydrate and paraldehyde, two older hypnotics, are still unknown, there is evidence they influence GABAergic transmission, perhaps in a manner similar to ethanol (Fig. 1).

Both the barbiturates, in particular phenobarbital, and the benzodiazepines, such as chlorazepate and clonazepam, are used for the treatment of seizures *(31)*. Gene deletion and mutation studies indicate that alterations in $GABA_A$ subunits can dramatically influence seizure threshold, suggesting such changes may be responsible for some forms of epilepsy (*see* Chapter 6). However, the utility of the benzodiazepines is limited, because tolerance develops to their anticonvulsant effects. This is not the case for phenobarbital at doses used to control generalized tonic–clonic seizures, although tolerance develops to other effects of this agent. Clonazepam is used for the management of absence and myoclonic seizures, whereas chlorazepate is used as adjunctive therapy for complex partial seizures *(31)*. Intravenous diazepam or lorazepam are treatments of choice for terminating status epilepticus, an acute medical emergency. Tiagabine and vigabatrin are also used as antiepileptics *(31)*.

Some barbiturates, such as thiopental, are used as fixed or intravenous general anesthetics. Midazolam, a benzodiazepine, is also administered for this purpose, although benzodiazepines are less complete central nervous system depressants than barbiturates. Although propofol is neither a benzodiazepine nor a barbiturate, its mechanism of action is believed to be similar to the latter (Fig. 1) *(8)*. Benzodiazepines are also used as preanesthetic medications to speed the induction rate of inhalational agents. A number of GABAergic drugs are prescribed as muscle relaxants, and for treating neuropsychiatric conditions and certain types of pain. Whereas diazepam is used as a skeletal muscle relaxant, clonazepam is useful for treating bipolar disorder and for relieving dysesthetic and paroxysmal lancinating pain. Indeed, preclinical data suggest that activation of $GABA_A$ receptors, either directly with recognition site agonists or indirectly by inhibition of GABA uptake or metabolism, yields an antinociceptive response *(36–38)*.

The most common side-effects associated with drugs that enhance $GABA_A$ receptor activity are natural extensions of their pharmacological actions. These include sedation, ataxia, and motor incoordination, reflecting a diminution of central nervous system tone. Other adverse effects are anterograde amnesia and paradoxical excitement. While barbiturate overdose can be fatal owing to medullary depression and loss of respiratory drive, benzodiazepines are safer in this regard.

4. $GABA_B$ Receptors

4.1. Molecular Pharmacology

Although baclofen (Fig. 2), a muscle relaxant, was designed as a systemically active GABA receptor agonist, early studies revealed it does not stimulate bicuculline-sensitive sites, the contemporary criteria used for identifying such agents. Subsequently it was discovered that baclofen and GABA regulate the stimulated release of various neurotransmitters through activation of a receptor that is pharmacologically distinct from the bicuculline-sensitive sites *(39)*. These receptors, termed $GABA_B$, are located both pre- and postsynaptically and, unlike the $GABA_A$ site, are coupled to G proteins *(40,41)*. As the $GABA_B$ receptor is associated with G_i and G_o its stimulation reduces the activity of adenylyl cyclase or, through coincident signaling, enhances the production of cyclic AMP *(40,41)*. The predominant response to $GABA_B$ receptor activation is an increase in potassium conductance with a consequent hyperpolarization of the neuron *(42)*. Thus, as with the $GABA_A$ system, GABA serves as an inhibitory neurotransmitter when stimulating the $GABA_B$ site. Activation of $GABA_B$ receptors also reduces neuronal calcium conductance, an effect believed to be responsible for baclofen-induced inhibition of neurotransmitter release *(43)*. In all cases it appears the $GABA_B$ receptor-mediated changes in ion channel activities are owing to liberation of G protein subunits which, in turn, directly influence channel function and the generation of second messengers *(40,41)*. Persistent activation of $GABA_B$ receptors leads to desensitization through a GRK4-dependent process (*see* Chapter 10).

As with the ionotropic $GABA_A$ receptor, the metabotropic $GABA_B$ site is localized primarily to the central nervous system. Although there are data suggesting $GABA_B$ receptors regulate the release of acetylcholine in the enteric nervous system and lungs, and substance P in the pulmonary system *(44,45)*, the predominant responses to systemically administered $GABA_B$ receptor agonists and antagonists appear to be mediated by their effects in the central nervous system.

Biochemical and molecular cloning studies demonstrate the $GABA_B$ receptor is a class III metabotropic, G protein-coupled site (*see* Chapter 11) *(46)*. These experiments reveal this receptor functions as a heterodimer, being made up of $GABA_{B(1)}$ and $GABA_{B(2)}$ subunits, also referred to as $GABA_B$R1 and $GABA_B$R2, each of which is a seven transmembrane spanning protein (Table 1) (Fig. 3). Whereas the recognition site for GABA is located on the $GABA_{B(1)}$ component, the G protein-coupled effector system is selectively associated with the $GABA_{B(2)}$ subunit *(47,48)*. This explains why neither protein is capable of forming a fully functional receptor on its own, although there is some evidence that $GABA_{B(1)}$ homodimers may display some responsiveness to GABA *(49)*. Even though a number of $GABA_B$ receptor subunit isoforms have been

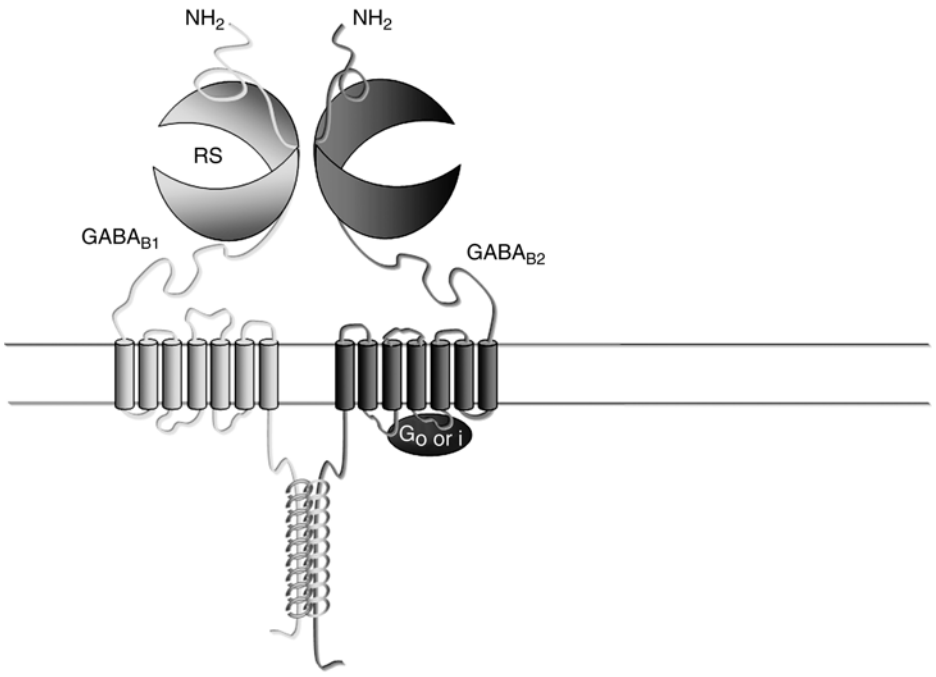

Fig. 3. Schematic representation of a $GABA_B$ receptor illustrating its dimeric structure. $GABA_{B1}$ and $GABA_{B2}$ designate the two subunits, RS the location of the recognition site on $GABA_{B1}$ and G_o or $_i$ the location of the G protein-binding site on $GABA_{B2}$. (Adapted from ref. *60*.)

identified, only $GABA_{B(1a)}$ and $GABA_{B(1b)}$ appear to be capable of forming an active site when either combines with $GABA_{B(2)}$ (*see* Chapter 8) *(5,6,41)*.

Although there is biochemical and electrophysiological evidence suggesting a multiplicity of pharmacologically distinct $GABA_B$ receptors *(50–54)*, it appears the amino acid sequence of the receptor recognition site is identical in all active forms of the $GABA_{B(1)}$ subunit *(41,55,56)*. In addition, gene deletion and mutation studies demonstrate that failure to express either $GABA_{B(1)}$ or $GABA_{B(2)}$ virtually eliminates $GABA_B$ receptor responses, supporting the conclusion that these subunits are uniquely capable of forming a fully functional receptor *(57,58)*. These findings suggest it may not be possible to synthesize $GABA_B$ receptor subtype-selective drugs, although there is evidence that allosteric agents may be capable of discriminating among different splice variants (*see* Chapters 9 and 11) *(9,59,60)*.

Numerous reports suggest a poor correlation between changes in $GABA_B$ subunit expression and receptor function *(61–63)*. For example, mild stress significantly increases $GABA_{B(1a)}$ gene expression in the spinal cord in the

Table 3
$GABA_{B1}$ Subunit Protein Partners

Partner	Function	References
CREB2/ATF4	Transcription factor	*68–70*
CHOP	Transcription factor	*71*
14-3-3	Scaffolding protein	*72*
Marlin-1	RNA-binding protein	*73*
msec7	Guanidine-nucleotide-exchange factor	*74*
Fibulin-2[a]	Extracellular matrix protein	*75*

[a]$GABA_{B1a}$ only.

CREB, cAMP response element binding; ATR4, activating transcription factor 4; CHOP, CCAT/ enhancer-binding protein (C/EBP) homologous protein.

absence of any apparent change in the responsiveness of the $GABA_B$ receptor system *(64)*. These findings indicate subunit proteins may serve functions in the cell, independent of their role as components of the $GABA_B$ receptor. Indeed, $GABA_{B(1)}$ is capable of partnering with a variety of cellular components, including transcription factors, scaffolding, RNA-binding, and extracellular matrix proteins (Table 3) (*see* Chapters 8 and 10) *(51,65–67)*. Some of these interactions are important for trafficking and anchoring the $GABA_B$ receptor heterodimer, or are involved in regulating the receptor-coupled effector system, whereas certain protein pairings could directly influence other cellular activities, such as gene transcription *(68–75)*.

A host of selective $GABA_B$ receptor agonists and antagonists have been developed (Fig. 2) (*see* Chapter 9) *(76,77)*. Included are phosphinic acid derivatives such as the agonist CGP 44532, and the antagonists CGP 54626 and CGP 55845 (Fig. 2). These and other agents were important for initially cloning the gene for the $GABA_{B(1)}$ subunit *(4)* and for characterizing the pharmacological properties of this site (*see* Chapter 12) *(78)*.

4.2. Therapeutics

Baclofen, a receptor recognition site agonist, is the only drug currently in use that directly influences $GABA_B$ receptor activity (*see* Chapter 12). Undoubtedly some of the responses to the nonselective GABAergic stimulants, such as tiagabine and vigabatrin, are the result of $GABA_B$ receptor stimulation, as these agents do not discriminate between GABA receptor subtypes.

Baclofen has for decades been used as a skeletal muscle relaxant. Although it is a primary treatment for spasticity, such as that associated with multiple sclerosis *(79)*, its effectiveness is enhanced by coadministration of diazepam or clonazepam, suggesting involvement of both $GABA_A$ and $GABA_B$ receptors in

this condition. As continuous agonist administration desensitizes the $GABA_B$ receptor, tolerance develops to baclofen, limiting its clinical utility. Baclofen, usually in combination with certain anticonvulsants, is used for the treatment of neuropathic pain (*see* Chapter 12) *(38)*. As is the case with $GABA_A$, there are a significant amount of preclinical data indicating that baclofen and other $GABA_B$ receptor agonists display antinociceptive activity in a variety of animal models. It appears this response is a result, in part, of baclofen-induced inhibition of substance P and glutamate release in the spinal cord, interrupting the transmission of the pain impulse to higher centers *(38)*.

Laboratory animal studies suggest that $GABA_B$ receptor antagonists enhance learning and memory in both rodents and primates and that baclofen decreases cognition, although these effects might vary under different conditions (*see* Chapter 12) *(80,81)*. Based on these and other data, a clinical trial was initiated with SGS 742, formerly CGP 36742, a $GABA_B$ receptor antagonist, in patients with mild cognitive impairments to assess its effect on choice reaction time, visual information processing, and working memory *(78)*.

Because $GABA_B$ receptor activation contributes to the generation of abnormal synchronous discharges characteristic of absence epilepsy, it was speculated that $GABA_B$ receptor antagonists may represent a new approach for treating this condition. Studies in laboratory animal models of absence epilepsy indicate that $GABA_B$ receptor antagonists completely suppress these discharges and their behavioral manifestations *(82)*. In fact, numerous reports suggest that modifications in $GABA_B$ receptor expression or function may be responsible for certain forms of epilepsy, and that drugs acting at this receptor may be of benefit in treating these conditions (*see* Chapters 10 and 12).

Both clinical and preclinical data suggest that activation of the $GABA_B$ system reduces the reinforcing effects of addictive substances (*see* Chapter 12) *(83–85)*. Such findings suggest the possible use of baclofen, or other $GABA_B$ receptor agonists, for treating drug abuse. Gene deletion studies have provided insights into possible clinical uses for $GABA_B$ receptor agonists and antagonists (*see* Chapter 11) *(49,57,58,86,87)*. The results confirm that $GABA_{B(1)}$ is absolutely required for formation of a functional $GABA_B$ receptor because mice lacking this subunit gene are totally unresponsive to $GABA_B$ agonists. The phenotype displayed by these animals includes a reduced seizure threshold, retarded growth, hypothermia, hyperlocomotion, hyperalgesia, memory impairment, anxiety, and decreased immobility in the forced swim test (*see* Chapter 11). Some of these findings are perplexing given the results of pharmacological studies. For example, as noted earlier, whereas $GABA_B$ agonists cause absence seizures in laboratory animals, the gene deletion studies indicate that a reduction in $GABA_B$ tone leads to seizures as well, casting doubt on the clinical utility of $GABA_B$ receptor antagonists as anticonvulsants *(88–90)*. In fact, CGP

56999A, a potent $GABA_B$ receptor antagonist, induces seizures in mice *(91)*. It is also notable that, although $GABA_B$ receptor antagonists appear to enhance cognition in laboratory animals, elimination of $GABA_B$ receptor expression compromises memory. These apparently conflicting findings may be the consequence of developmental abnormalities resulting indirectly from the gene deletion rather than being directly associated with the loss of $GABA_B$ receptors.

However, a consistency between the gene deletion and pharmacological studies was found, with respect to the forced swim, analgesia, and locomotion results. Deletion of the $GABA_{B(1)}$ gene decreases immobility in the forced swim test, a result identical to that found with $GABA_B$ receptor antagonists *(92–95)*. This suggests that $GABA_B$ receptor antagonists may display antidepressant properties. Similarly, the hyperalgesia and hyperlocomotion noted in $GABA_{B(1)}$ null mice is consistent with reports that $GABA_B$ receptor agonists increase the pain threshold and reduce locomotor activity *(36,95–97)*.

Because $GABA_B$ receptor agonists influence neurotransmitter release from neurons in various types of smooth muscle, there has been interest in testing such agents as possible treatments for asthma and disorders of the gastrointestinal system and bladder. Although the results of preclinical in vivo and in vitro studies are promising, there are few clinical data to support this hypothesis. Indeed, questions remain about the relevance of $GABA_B$ receptors located on neurotransmitter terminals in peripheral tissues, as there is little, if any, GABA innervation to these sites. This suggests peripheral $GABA_B$ receptors may be of more pharmacological than physiological importance. Side-effects associated with the use of baclofen include sedation and confusion. Other problems encountered with this drug are constipation and urinary retention, both of which are probably secondary to baclofen-induced reductions in parasympathetic drive through inhibition of acetylcholine release. Preclinical and phase I clinical trials with the $GABA_B$ receptor antagonist SGS 742M, the fumarate salt of SGS 742, indicate it is relatively free of side effects and toxicities at the doses used in humans *(78)*.

5. Conclusions

It has been nearly 60 yr since Roberts and Frankel first identified GABA in the mammalian central nervous system *(98)*. The first 15 yr were needed to prove that GABA is a neurotransmitter substance, with studies during the following decade focused on defining its distribution, synthesis, storage, release, and electrophysiological properties. In the mid-1970s attention shifted to GABA receptor sites, which remains the focus to this day. As reviewed in the three editions of this volume, research on GABA receptors has yielded a wealth of information about neurotransmission in general, and GABAergic systems in particular. Characterization of $GABA_A$ receptors contributed substantially the

knowledge about allosteric regulation of ligand-gated ion channels. The resulting information has been invaluable in defining precisely the mechanisms of action of a host of drugs that interact with such sites. Among these are the benzodiazepines and other sedative, hypnotic, and anesthetic agents.

Similarly, $GABA_B$ receptor studies have had a broad impact in the neurosciences. Although the existence of G protein-coupled receptor dimers was suspected for some time, they were difficult to demonstrate until the heterodimeric $GABA_B$ site was identified. This finding led subsequently to the discovery of a host of dimerized metabotropic sites, with significant implications regarding the design and development of drugs that interact with these complexes. The characterization of the pentameric $GABA_A$ and the dimeric $GABA_B$ receptors has also been crucial for understanding the neurobiological basis of some central nervous system disorders. Thus, gene deletion and point mutation studies have revealed the importance of GABA receptors in neural development and function. These experiments have also revealed that even subtle modifications in the amino acid composition of receptor subunits can have profound effects on the behavioral phenotype and the response to drugs. Such information has had a significant impact on the direction of research into the causes and treatment of neurological and psychiatric disorders. Given past experience it is likely that further research on GABA receptors will continue to yield information of value to neuroscientists in general, neuropharmacologists, neurologists, and psychiatrists in particular. As outlined in this chapter, and detailed in this volume, the recent studies on GABA receptors continue a tradition established during the past half a century in providing new perspectives on the functions of neurotransmitters and their receptor sites. It is hoped the information contained herein will not only broaden the perspective of those working in this field, but also provide new ideas and insights for scientists in related areas.

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