

# International Union of Pharmacology. XXXIII. Mammalian $\gamma$ -Aminobutyric Acid<sub>B</sub> Receptors: Structure and Function

N. G. BOWERY, B. BETTLER, W. FROESTL, J. P. GALLAGHER, F. MARSHALL, M. RAITERI, T. I. BONNER, AND S. J. ENNA

*Department of Pharmacology, Medical School, University of Birmingham, Edgbaston, United Kingdom (N.G.B.); Pharmacenter, University of Basel, Basel, Switzerland (B.B.); Nervous System Research, Novartis Pharma, Basel, Switzerland (W.F.); Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas (J.P.G.); Millennium Pharmaceuticals, Granta Park, Great Abington, United Kingdom (F.M.); Department of Experimental Medicine, Pharmacology and Toxicology, University of Genoa, Genoa, Italy (M.R.); Laboratory of Genetics, National Institute of Mental Health, Bethesda, Maryland (T.I.B.); and Department of Pharmacology, Toxicology and Therapeutics, Kansas University School of Medicine, Kansas City, Kansas (S.J.E.)*

This paper is available online at <http://pharmrev.aspetjournals.org>

Abstract	247
I. Introduction	248
II. $\gamma$ -Aminobutyric acid <sub>B</sub> receptor structure	248
III. $\gamma$ -Aminobutyric acid <sub>B</sub> receptor effector mechanisms	251
A. Adenylate cyclase	251
B. Ion channels	251
IV. $\gamma$ -Aminobutyric acid <sub>B</sub> receptor subtypes	252
V. $\gamma$ -Aminobutyric acid <sub>B</sub> receptor distribution	253
A. Central nervous system	253
B. Peripheral organs and tissues	253
VI. $\gamma$ -Aminobutyric acid <sub>B</sub> receptor-mediated responses	254
A. $\gamma$ -Aminobutyric acid <sub>B</sub> receptor agonists	254
1. Antispasticity	254
2. Antinociceptive	255
3. Suppression of drug craving	255
4. Miscellaneous actions	256
B. $\gamma$ -Aminobutyric acid <sub>B</sub> receptor antagonists	256
VII. Conclusions	258
References	259

**Abstract**—The  $\gamma$ -aminobutyric acid<sub>B</sub> (GABA<sub>B</sub>) receptor was first demonstrated on presynaptic terminals where it serves as an autoreceptor and also as a heteroreceptor to influence transmitter release by suppressing neuronal Ca<sup>2+</sup> conductance. Subsequent studies showed the presence of the receptor on postsynaptic neurones where activation produces an increase in membrane K<sup>+</sup> conductance and associated neuronal hyperpolarization. (–)-Baclofen is a highly selective agonist for GABA<sub>B</sub> receptors, whereas the established GABA<sub>A</sub> receptor antagonists, bicuculline and picrotoxin, do not block GABA<sub>B</sub> receptors. The receptor is G<sub>i</sub>/G<sub>o</sub> protein-coupled with mixed effects on adenylate cyclase activity. The receptor comprises

a heterodimer with similar subunits currently designated 1 and 2. These subunits are coupled via coiled-coil domains at their C termini. The evidence for splice variants is critically reviewed. Thus far, no unique pharmacological or functional properties have been assigned to either subunit or the variants. The emergence of high-affinity antagonists for GABA<sub>B</sub> receptors has enabled a synaptic role to be established. However, the antagonists have generally failed to establish the existence of pharmacologically distinct receptor types within the GABA<sub>B</sub> receptor class. The advent of GABA<sub>B1</sub> knockout mice has also failed to provide support for multiple receptor types.

## I. Introduction

The GABA<sub>B</sub><sup>1</sup> receptor was originally defined on the basis of pharmacological responses to GABA and related agonists, including baclofen (Bowery et al., 1981). In studies focusing on control of transmitter release, it was noted that a GABA receptor responsible for modulating evoked release in a variety of isolated tissue preparations differed pharmacologically from the receptor responsible for the Cl<sup>-</sup>-dependent actions of GABA. Thus, the ability of GABA to inhibit neurotransmitter release from these preparations was not blocked by bicuculline, was not mimicked by isoguvacine, and was not dependent on Cl<sup>-</sup>, all of which are characteristic of the classical GABA receptor. Most striking was the finding that baclofen ( $\beta$ -parachlorophenyl GABA), a clinically employed spasmolytic (Bein, 1972; Keberle and Faigle, 1972), mimicked, in a stereoselective manner, the effect of GABA in these systems. Furthermore, ligand-binding studies provided direct evidence of distinct attachment sites for baclofen on central neuronal membranes (Hill and Bowery, 1981). The term GABA<sub>B</sub> was coined to distinguish this site from the bicuculline-insensitive receptor, which was, in turn, designated GABA<sub>A</sub> (Hill and Bowery, 1981).

A major distinction between GABA<sub>A</sub> and GABA<sub>B</sub> receptors is that the former are ligand-gated ion channels, whereas the latter are coupled to G proteins (Wojcik and Neff, 1984; Hill, 1985; Karbon and Enna, 1985). Hence, GABA<sub>B</sub> receptors can be defined as metabotropic, whereas GABA<sub>A</sub> receptors form part of the ionotropic receptor superfamily. Characterization of the GABA<sub>B</sub> receptor has led to new insights into the structural and functional properties of seven-transmembrane receptors in general. Contained in this report is an overview of selected studies on the properties of GABA<sub>B</sub> receptors. Readers desiring additional information on particular aspects of this topic are urged to consult other sources (e.g., Enna, 1997; Enna and Bowery, 1997; Marshall et al., 1999a; Bowery and Enna, 2000; Enna, 2000). Characteristics of the GABA<sub>B</sub> receptor, which are described in detail in this review, are summarized in Table 1.

## II. $\gamma$ -Aminobutyric Acid<sub>B</sub> Receptor Structure

Functional G protein-coupled receptors are expressed in cell membranes in different ways. In some cases they may be present as a single protein, whereas in others they may form homodimers (Bouvier, 2001). The structural characterization of the metabotropic GABA<sub>B</sub> receptor revealed a third possibility. In this case, the receptor exists as a heterodimer with the subunits designated GABA<sub>B1</sub> and GABA<sub>B2</sub> (Jones et al., 1998;

Kaupmann et al., 1998a; White et al., 1998; Kuner et al., 1999; Martin et al., 1999; Ng et al., 1999). The heterodimeric nature of the GABA<sub>B</sub> receptor was not initially appreciated when the GABA<sub>B1</sub> subunit was first cloned (Kaupmann et al., 1997). Although shown to be a high molecular weight, seven-transmembrane spanning protein with homology to metabotropic glutamate receptors, the recombinant GABA<sub>B1</sub> protein exhibited binding affinities for agonists that were 1000-fold lower than those for wild-type GABA<sub>B</sub> receptors. Moreover, the coupling to presumed GABA<sub>B</sub> effector systems in heterologous cells was surprisingly inefficient (Kaupmann et al., 1997, 1998b). Subsequent studies revealed that the GABA<sub>B1</sub> protein is not transported to the plasma membrane but remains associated with the endoplasmic reticulum (Couve et al., 1998). This, and the inefficient coupling to effector systems, led to the hypothesis that a trafficking protein, such as a RAMP (receptor activity modifying protein) (McLatchie et al., 1998), might be required for the efficient functional expression of the GABA<sub>B</sub> site. Ultimately, the discovery of a second GABA<sub>B</sub> receptor subunit, GABA<sub>B2</sub>, provided the necessary explanation (see Marshall et al., 1999a). The GABA<sub>B2</sub> protein has 54% similarity and 35% homology to GABA<sub>B1</sub> and has many of the structural features of the GABA<sub>B1</sub> subunit, including a high molecular weight (110 kDa), seven-transmembrane domains, and a long extracellular chain at the N terminus. The GABA<sub>B2</sub> protein not only serves to escort GABA<sub>B1</sub> to the cell surface, it appears to be the receptor component that links to the G protein, whereas the GABA<sub>B1</sub> subunit is necessary for agonist activation (Margeta-Mitrovic et al., 2000; Calver et al., 2001; Galvez et al., 2001; Pagano et al., 2001). It appears, therefore, that the agonist binds to a component of the GABA<sub>B1</sub> subunit, producing a conformational change in the protein complex that allows GABA<sub>B2</sub> to engage and activate the G protein-coupled signaling system. In support of this model, it has been shown that GABA<sub>B2</sub> must remain linked to GABA<sub>B1</sub> after the dimer is inserted into the cell membrane to maintain receptor function (Margeta-Mitrovic et al., 2000; Calver et al., 2001; Pagano et al., 2001). Recombinant GABA<sub>B2</sub> is expressed at the cell surface in the absence of GABA<sub>B1</sub>, and early reports suggested that it could display some functionality under this condition (Kaupmann et al., 1998a; Kuner et al., 1999; Martin et al., 1999). Although it now appears unlikely that wild-type GABA<sub>B2</sub> subunits can function alone in this way (Prosser et al., 2001; Schuler et al., 2001), there is no doubt that the coupling of GABA<sub>B2</sub> with GABA<sub>B1</sub> yields a fully functional GABA<sub>B</sub> receptor, with the GABA<sub>B1</sub>, rather than the GABA<sub>B2</sub> component, displaying a high affinity for radiolabeled ligands (Kaupmann et al., 1998a; White et al., 1998). Indeed, the GABA<sub>B1</sub> isoform, when expressed as part of the heterodimer, has increased agonist affinity similar to that of the wild-type receptor (Kaupmann et al., 1998a; White et al., 1998).

<sup>1</sup> Abbreviations: GABA<sub>B</sub>,  $\gamma$ -aminobutyric acid<sub>B</sub>; 3-APPA, 3-aminopropyl-phosphinic acid; 3-APMPA, methyl homolog of 3-APPA; GHB,  $\gamma$ -hydroxybutyrate; NC-IUPHAR, International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification; CHO, Chinese hamster ovary.

TABLE 1  
GABA<sub>B</sub> receptor characteristics

Structural information	7TM heterodimer formed by combination of two subunits Subunit 1, h 961aa, AJ012185 Subunit 1, r 960aa, Y10369 Subunit 2, h 941aa, AJ012188 Subunit 2, r 941aa, AJ011318
Functional assays	Inhibition of forskolin-stimulated cAMP formation in rat cerebral cortex slices <sup>a,b</sup> Inhibition of twitch responses in rat isolated anococcygeus muscle <sup>c</sup> Neuronal hyperpolarization in rat hippocampal slice <sup>d</sup> Inhibition of depolarization-evoked neurotransmitter release from CNS synaptosomes <sup>e</sup> Effect on [ <sup>35</sup> S]GTPγS-binding in native receptor and recombinant receptor preparations <sup>f</sup>
Agonists	selective: (-)-baclofen, 3APPA, 3APMPA, CGP44532 <sup>g</sup> non-selective: GABA <sup>h</sup>
Agonist potencies	(-)-baclofen (IC <sub>50</sub> = 30 nM), 3APPA (5 nM), 3APMPA (16 nM), GABA (20 nM), CGP44532 (45 nM) <sup>i</sup>
Antagonists	selective: CGP35348, CGP36742, CGP55845A, CGP62349, SCH50911, phaclofen <sup>j</sup>
Antagonist potencies	CGP35348 (IC <sub>50</sub> = 35 μM), CGP36742 (35 μM), CGP55845A (2 nM), CGP62349 (0.5 nM), SCH50911 (11 μM) <sup>i,k</sup>
Radioligand assays	Rat brain synaptic membranes <sup>l</sup> CHO cell line expressing human GABA <sub>B(1)</sub> and GABA <sub>B(2)</sub> <sup>m</sup>
Radioligands	[ <sup>3</sup> H]GABA, <sup>l</sup> [ <sup>3</sup> H](-)-baclofen, <sup>n</sup> [ <sup>3</sup> H]CGP54626, <sup>o</sup> [ <sup>3</sup> H]CGP62349, <sup>p</sup> [ <sup>125</sup> I]CGP64213, <sup>k</sup> [ <sup>125</sup> I]CGP71872 <sup>k</sup>
Transduction mechanisms	Inhibition of adenylate cyclase <sup>h</sup> Inhibition of membrane Ca <sup>2+</sup> conductance <sup>h</sup> Increased K <sup>+</sup> conductance via G <sub>i</sub> /G <sub>o</sub> proteins <sup>h</sup> Stimulation of MAP kinase <sup>q</sup>
Receptor distribution	Pre- and postsynaptic locations on neurones of mammalian CNS and terminals of peripheral autonomic nerve fibers and sensory fibers; rat thalamic nuclei, cerebellar molecular layer, cerebral cortex, interpeduncular nucleus, superior colliculus, dorsal horn of spinal cord <sup>r,s</sup>
Tissue functions	Auto- and heteroreceptor-mediated inhibition of transmitter release Slow inhibitory postsynaptic potentials <sup>h</sup>
Phenotypes	
-GABA <sub>1</sub> knockout <sup>t,u</sup>	Loss of pre- and postsynaptic receptor function in hippocampus Lack of GABA <sub>B</sub> -binding sites throughout brain Loss of GABA <sub>B</sub> -stimulated GTPγS binding (whole brain) Survival -27 days, <sup>t</sup> >140 days <sup>u</sup> Spontaneous epileptiform seizures Hyperalgesic Hyperlocomotive Impaired learning in passive avoidance paradigm No evidence for receptor subtyping obtained

Modified with permission from the IUPHAR Compendium of Receptor Characterization and Classification (2000) IUPHAR Media, London. CNS, central nervous system; [<sup>35</sup>S]GTPγS, guanosine 5'-O-(3-[<sup>35</sup>S] thio)triphosphate; MAP, mitogen-activated protein; 7TM, seven-transmembrane.

<sup>a</sup> Xu and Wojcik, 1986.

<sup>b</sup> Hill, 1985.

<sup>c</sup> Muhyaddin et al., 1982.

<sup>d</sup> Newberry and Nicoll, 1984.

<sup>e</sup> Gemignani et al., 1994.

<sup>f</sup> Watson et al., 1996.

<sup>g</sup> Proestl et al., 1995a.

<sup>h</sup> Bowery, 1993.

<sup>i</sup> Proestl and Mickel, 1997.

<sup>j</sup> Proestl et al., 1995b.

<sup>k</sup> Kaupmann et al., 1997.

<sup>l</sup> Bowery et al., 1983.

<sup>m</sup> Hirst et al., 2000.

<sup>n</sup> Bowery et al., 1985.

<sup>o</sup> Bittiger et al., 1992.

<sup>p</sup> Bittiger et al., 1996a.

<sup>q</sup> Schulz and Holtt, 1998.

<sup>r</sup> Bowery et al., 1987.

<sup>s</sup> Chu et al., 1990.

<sup>t</sup> Prosser et al., 2001.

<sup>u</sup> Schuler et al., 2001.

Several proteins other than GABA<sub>B2</sub> have been shown to interact with GABA<sub>B1</sub> (Nehring et al., 2000; White et al., 2000; Couve et al., 2001), but none of these complexes yields a functional receptor. It is possible that the interaction of GABA<sub>B1</sub> or GABA<sub>B2</sub> with transcription factors, such as activating transcription factor-4, may serve to regulate

gene expression through a novel signal transduction pathway (Nehring et al., 2000; White et al., 2000).

The interaction of GABA<sub>B1</sub> and GABA<sub>B2</sub> within the cells appears crucial for the correct assembly of the heterodimer on the membrane surface. This has been demonstrated for both recombinant and wild-type

GABA<sub>B</sub> receptors (Marshall et al., 1999b; Filippov et al., 2000; Chronwall et al., 2001). The interaction of the C-terminal coiled-coil domains, by masking the action of the retention motif RXRR present in the C terminus of GABA<sub>B1</sub>, ensures that only correctly assembled receptor complexes traffic to the cell surface (Margeta-Mitrovic et al., 2000; Pagano et al., 2001). Expression of the coupled heterodimer in cell membranes can occur even when the GABA<sub>B1</sub> and/or GABA<sub>B2</sub> C-terminal domains are missing (Calver et al., 2001; Pagano et al., 2001), suggesting that the coiled-coil structures are not essential for heterodimerization per se. Although it has been proposed that mGlu4R can associate with GABA<sub>B1</sub> and traffic it to the cell surface (Sullivan et al., 2000), this finding could not be replicated in a subsequent study using a different experimental approach (Pagano et al., 2001). The critical importance of the GABA<sub>B1</sub> subunit is supported by the finding that tissue from mice lacking the gene for this protein fails to respond to GABA<sub>B</sub> agonists and shows a loss of detectable pre- and postsynaptic responses (Prosser et al., 2001; Schuler et al., 2001). Importantly, the GABA<sub>B2</sub> subunit is heavily down-regulated in GABA<sub>B1</sub> null-mutant mice. This requirement of GABA<sub>B1</sub> for stable GABA<sub>B2</sub> expression supports the notion that in wild-type mice virtually all GABA<sub>B2</sub> protein is associated with GABA<sub>B1</sub>, in agreement with previous biochemical studies (Benke et al., 1999). The null-mutant mice generated on the 129Sv background only survive for 3 to 4 weeks postnatally, apparently due to recurrent seizures (Prosser et al., 2001), whereas those generated on the BALB/c background survive through adulthood even though they exhibit spontaneous seizures, hyperalgesia, hyperlocomotor activity, and memory impairment (Schuler et al., 2001). The viability of BALB/c mice lacking the GABA<sub>B1</sub> subunit has allowed their characterization in GABA<sub>B</sub> receptor paradigms. GABA<sub>B</sub> agonist administration to BALB/c null-mutant mice failed to produce the typical muscle relaxation, hypothermia, or delta electroencephalogram waves observed in wild-type animals. These behavioral findings were paralleled by a loss of all biochemical and electrophysiological GABA<sub>B</sub> responses in the null-mutant mice. This demonstrates that GABA<sub>B1</sub> is an essential component of pre- and postsynaptic GABA<sub>B</sub> receptors and indicates that most, probably all, brain GABA<sub>B</sub> receptors incorporate the GABA<sub>B1</sub> subunit. Moreover, from the analysis of the GABA<sub>B1</sub> null-mutant mice it follows that GABA<sub>B2</sub> is unlikely to function as an autonomous receptor. Although these results are in line with previous work that failed to find any evidence for pharmacologically distinct GABA<sub>B</sub> receptor subtypes (Waldmeier et al., 1994), there remains the possibility that unidentified splice variants or GABA<sub>B1</sub>-associated proteins generate diversity.

Numerous splice variants of the GABA<sub>B1</sub> subunit have been identified (Kaupmann et al., 1997; Isomoto et al., 1998; Pfaff et al., 1999; Calver et al., 2000; Schwarz

et al., 2000; Wei et al., 2001a,b) with sometimes different names in rat and human. A comprehensive description of these variants is made possible by the complete sequence of the human and mouse GABA<sub>B1</sub> genes, which are contained within GenBank accession numbers AL031983 and AL078630, respectively, and the nearly complete rat gene (Pfaff et al., 1999). The 1a splice variant in all three species contains all 23 conserved exons of the gene, with the first exon being untranslated and the transmembrane domains being encoded by exons 15 to 21. It should be noted that this number of exons differs from much of the literature because Pfaff et al. (1999), apparently through assembly errors in their rat gene sequence (GenBank accession numbers AF110796 and AF110797), failed to recognize introns that split exons 7 and 11 each into two exons. The existence of these exons can be confirmed using sequence from the rat genome sequencing project (<http://www.ncbi.nlm.nih.gov/genome/seq/RnBlast.html>). In addition, Pfaff et al. (1999) did not use any 5' untranslated cDNA sequence and, thus, did not identify the first exon. The 1b splice variant initiates 5' of exon 6, thereby producing an extended exon 6, which contains a new initiation codon, giving rise to an alternative amino-terminal sequence for the 1b protein. The amino-terminal sequence unique to the 1b variant is 47 versus 162 amino acids for the sequence unique to the 1a variant. Isoform-specific antibodies have shown both variants to be expressed in rat brain with 1a predominating before birth and 1b predominating in adults (Fritschy et al., 1999). A third variant, called 1e in both rat and human, skips exon 15, which leads to premature termination prior to the first transmembrane domain. Although this isoform can heterodimerize with GABA<sub>B2</sub> subunits, it appears to be unable to activate G protein-coupled, inwardly rectifying potassium channels or to inhibit cAMP production when coexpressed with GABA<sub>B1</sub> subunits (Schwarz et al., 2000). Several variants have been observed in only one species. In humans, a variant called 1c is similar to the 1a variant but skips exon 4, resulting in the deletion of 63 amino acids. It is expressed at much higher levels in fetal brain than in adult brain (Calver et al., 2000; Martin et al., 2001). A rat variant, also called 1c, corresponds to an insertion of a 93-base exon located between exons 19 and 20, which results in the insertion of 31 amino acids into the beginning of transmembrane domain 5. Although the homologous region can be identified in the mouse gene, it is too poorly conserved to be functional due to the insertion of two bases, which disrupts the reading frame. No homologous exon is evident in the human gene. Thus, although the rat variant has been reported to be functional in vitro (Pfaff et al., 1999), it is unclear if it is functional in vivo. Rat variant 1d has a 567-base insertion corresponding to the failure to splice out intron 22. Rat variant 1f skips exon 5, resulting in the deletion of seven amino acids. Rat variant 1g has a 124-base insertion that extends the 5' end of exon



5 by using an alternative splice acceptor. This insertion shifts the reading frame and results in a severely truncated protein. Aside from variants 1a and 1b, it is presently unknown whether any of these variants act as a subunit of physiological receptors. The 1a and 1b variants are not, strictly speaking, splice variants but instead appear to be transcription start site variants that originate in high guanosine-cytosine content (~80%) regions of the gene separated by about 5 kilobases. Although such high GC content makes it difficult to make full-length cDNA, to map the transcription start sites, and could easily cause artifacts, variant-specific antibodies have provided critical evidence that both proteins are physiologically expressed at significant levels. Thus, it is appropriate to use the IUPHAR nomenclature reserved for significant splice variants, i.e., GABA<sub>B1(a)</sub> and GABA<sub>B1(b)</sub>, for these two variants.

Partial cDNAs corresponding to two potential splice variants of the human GABA<sub>B2</sub> subunit, called 2b and 2c, which delete 81 and 78 bases of the carboxyl-terminal encoding portion of the cDNA have been reported (Clark et al., 2000). Subsequent analysis of the human GABA<sub>B2</sub> gene (Martin et al., 2001) has demonstrated that neither of the deleted regions correspond to an independent exon but instead reside within the last exon of the gene. There are no appropriate splice donor or acceptor consensus sequences that might act as alternative splice sites. The lack of such sites and the presence of short (4–5 bases) repeated sequences at the ends of the deletion regions suggest that they are polymerase chain reaction artifacts. Thus, there is currently no good evidence for splice variants of the GABA<sub>B2</sub> subunit.

Although there is a 1:1 stoichiometry between GABA<sub>B1</sub> and GABA<sub>B2</sub> subunits in the functional receptor, production of the subunits appears to be regulated, at least in part, independent of one another (McCarson and Enna, 1999). Thus, whereas expression of GABA<sub>B1</sub> and GABA<sub>B2</sub> mRNA increases in rat dorsal spinal cord following 24 h of hind paw inflammation, the increase in GABA<sub>B2</sub> mRNA is significantly greater than for GABA<sub>B1</sub>. This supports the notion that GABA<sub>B</sub> receptor subunits may serve a variety of functions in the cell and could indicate that other, as yet unidentified, proteins may form functional heterodimers with GABA<sub>B1</sub> subunits to form a functional receptor.

### III. $\gamma$ -Aminobutyric Acid<sub>B</sub> Receptor Effector Mechanisms

Effector mechanisms associated with neural GABA<sub>B</sub> receptors are the adenylate cyclase system and Ca<sup>2+</sup> and K<sup>+</sup> ion channels (Hill et al., 1984; Karbon et al., 1984; Hill, 1985; Inoue et al., 1985; Andrade et al., 1986; Xu and Wojcik, 1986; Dolphin et al., 1990; Bindokas and Ishida, 1991; Gage, 1992). GABA<sub>B</sub> receptor activation is mediated by G proteins that are members of the pertussis toxin-sensitive family G<sub>i</sub> $\alpha$ /G<sub>o</sub> $\alpha$ , in particular G<sub>i2</sub> $\alpha$

(Odagaki et al., 2000; Odagaki and Koyama, 2001). However, some pertussis toxin-insensitive effects of baclofen have been noted in, for example, the magnocellular neurons of the paraventricular and supraoptic nuclei of the rat (Noguchi and Yamashita, 1999; Cui et al., 2000). In particular, it has been reported that presynaptic, compared with postsynaptic, GABA<sub>B</sub> receptor mechanisms are insensitive to pertussis toxin (Harrison et al., 1990). It has also been found that whereas exposure of spinal cord membranes to baclofen results in an increase in guanosine 5'-3-O-(thio)triphosphate binding in young rats, no such response can be obtained in membranes from animals older than 21 days. This would suggest that there may be a developmental change in the coupling of GABA<sub>B</sub> receptors and G proteins in the cord (Moran et al., 2001).

#### A. Adenylate Cyclase

GABA<sub>B</sub> agonists inhibit basal and forskolin-stimulated neuronal adenylate cyclase in brain slices (Xu and Wojcik, 1986; Knight and Bowery, 1996) through a G protein-dependent mechanism that results in a reduced level of intracellular cAMP. Activation of the GABA<sub>B</sub> receptor can also enhance cAMP formation in response to G<sub>s</sub>-coupled receptor agonists, such as isoprenaline, in brain slices but not in isolated neuronal membranes, suggesting it entails activation of cytoplasmic cyclases (Enna, 2000). The physiological relevance of these effects on cAMP production has been confirmed by in vivo microdialysis experiments in the cerebral cortex of freely moving rats (Hashimoto and Kuriyama, 1997). Both baclofen and GABA reduced the increase in cAMP generated by an infusion of forskolin, and this was blocked by CGP54626, a selective GABA<sub>B</sub> receptor antagonist, substantiating the role of GABA<sub>B</sub> receptors in this response. Baclofen was also able to potentiate the increase in cAMP produced by isoprenaline in this in vivo preparation.

A direct GABA<sub>B</sub>-mediated increase in basal adenylate cyclase activity has been detected in membranes of rat olfactory bulb (Olianas and Onali, 1999). Interestingly, this effect is blocked by pertussis toxin, suggesting an involvement of G<sub>i</sub>/G<sub>o</sub> rather than G<sub>s</sub> protein.

#### B. Ion Channels

When activated, GABA<sub>B</sub> receptors decrease Ca<sup>2+</sup> and increase K<sup>+</sup> conductance in neuronal membranes. The effect on Ca<sup>2+</sup> conductance appears to be primarily associated with presynaptic P/Q- and N-type currents (Santos et al., 1995; Lambert and Wilson, 1996; Chen and van den Pol, 1998; Takahashi et al., 1998; Bussieres and El Manira, 1999; Barral et al., 2000), although facilitation of an L-type current in non-mammalian retina has also been described (Shen and Slaughter, 1999).

Modulation of K<sup>+</sup> conductance appears to be linked primarily with postsynaptic GABA<sub>B</sub> sites and with perhaps multiple types of K<sup>+</sup> channels (Wagner and Dekin,

1993, 1997; Lüscher et al., 1997; Harayama et al., 1998). Whereas a  $K^+$ (A) current is thought to be coupled to GABA<sub>B</sub> receptors on presynaptic terminals in hippocampal cultures, changes in membrane  $K^+$  flux appear to be due to postsynaptic GABA<sub>B</sub> receptor activation (Saint et al., 1990).

Although suppression of  $Ca^{2+}$  influx is probably the most frequently observed response associated with presynaptic GABA<sub>B</sub> receptors (Doze et al., 1995; Wu and Saggau, 1995; Isaacson, 1997, 1998; Isaacson and Hille, 1997), a process independent of  $Ca^{2+}$  or  $K^+$  channels but perhaps linked with protein kinase C activation, has been reported in rodent CA1 hippocampal pyramidal cells (Jarolimek and Misgeld, 1997). This had been demonstrated previously but was then only apparent in rat hippocampal slices obtained in early postnatal life (Tremblay et al., 1995).

Low threshold  $Ca^{2+}$  T-currents, which are inactivated at normal resting membrane potentials, may also be involved in the response to GABA<sub>B</sub> receptor activation, at least within the thalamus (Scott et al., 1990). This postsynaptic hyperpolarization of long duration, which initiates  $Ca^{2+}$  spiking activity in thalamocortical cells, could contribute to the generation of spike and wave discharges associated with absence seizures (Crunelli and Leresche, 1991).

#### IV. $\gamma$ -Aminobutyric Acid<sub>B</sub> Receptor Subtypes

Although GABA<sub>B</sub> receptors appear structurally heterogeneous in the sense that several splice variants exist for the two subunits known, evidence for functionally distinct receptor subtypes is limited. Transmitter release studies with native and rat brain GABA<sub>B</sub> receptors suggest pharmacological differences between autoreceptors and heteroreceptors and even within heteroreceptors (Bonanno and Raiteri, 1993a; Banerjee and Snead, 1995; Teoh et al., 1996; Bonanno et al., 1997; Ong et al., 1998b; Phelan, 1999). Similarly, the dual action of GABA<sub>B</sub> agonists on adenylate cyclase in brain slices would support the concept of receptor subtypes (Cunningham and Enna, 1996). The existence of pharmaco-

logically distinct subtypes of a native receptor has traditionally been considered likely when the affinities of one antagonist for the hypothetical subtypes differed by at least one order of magnitude. In the case of GABA<sub>B</sub> receptors sited on terminals releasing GABA, glutamate, cholecystokinin, or somatostatin, not only do the antagonist affinities differ in some cases by more than two orders of magnitude but the orders of potency of some antagonists differ between receptors. Qualitatively similar results were obtained when GABA<sub>B</sub> receptor antagonists were tested on nerve endings isolated from human cerebrocortex (Fassio et al., 1994; Bonanno et al., 1996, 1997, 1999; Raiteri et al., 1996). The results with rat and human nerve endings are summarized in Table 2. Pharmacological differences also seem to exist between GABA<sub>B</sub> autoreceptors inhibiting GABA release in rat cerebral cortex and spinal cord (Raiteri et al., 1989; Bonanno and Raiteri, 1992, 1993b; Bonanno et al., 1998). Thus, the evidence for pharmacologically distinct subtypes of the GABA<sub>B</sub> receptor derived from release studies appears to at least equate with some other receptor systems, which can boast the chrism of molecular biology. Also it would be quite surprising if the GABA<sub>B</sub> receptor was the only example of a metabotropic receptor without subtypes. Comparative data obtained with wild-type GABA<sub>B</sub> receptors and recombinant GABA<sub>B1(b)/B2</sub> receptors expressed in CHO cells indicate that the recombinant receptor, unlike the wild-type, is insensitive to the antagonists, phaclofen, saclofen, and CGP35348 (Wood et al., 2000). However, a comparison of GABA<sub>B</sub> receptors containing different isoforms of GABA<sub>B1</sub> in combination with GABA<sub>B2</sub> in CHO cells indicate that these heterodimers are pharmacologically indistinguishable (Kaupmann et al., 1998a; Green et al., 2000).

In a recent report, Ng et al. (2001) presented data suggesting gabapentin, an anticonvulsant/analgesic agent, is a selective agonist at the GABA<sub>B1(a)/GABA<sub>B2</sub></sub> site, compared with the GABA<sub>B1(b)/B2</sub> receptors, expressed in oocytes. A similar conclusion was drawn from studies on wild-type GABA<sub>B</sub> receptors in mL-tsA58

TABLE 2  
GABA<sub>B</sub> receptor subtypes  $IC_{50}$  values of antagonists ( $\mu$ M). The drugs were tested against (-)-baclofen.

Drug	Neurotransmitter Released			
	GABA	GLU	SRIF	CCK
Rat cortex				
Phaclofen	79.2	>300	62.6	66.1
CGP35348	>300	4.2	3.6	3.5
CGP52432	0.08	9.3	3.4	0.11
CGP47656	3.1	(Ago)	Ago	>300
CGP36742	>100	>100	0.14	>100
Human cortex				
Phaclofen	≈100	>300	N.D.	N.D.
CGP35348	>100	≈10	24.4	13.9
CGP52432	≤1	>1; < 30	0.06	0.08
CGP47656	<10	N.D.	Ago	Ago
CGP36742	>100	>100	≈5	>100

GLU, glutamate; SRIF, somatotropin release-inhibiting factor; Ago, against; (Ago) partial agonist; CCK, cholecystokinin; N.D., not determined.

cells (Bertrand et al., 2001). Questions remain, however, whether this selectivity is generally detectable when testing wild-type receptors in nontransformed mammalian central nervous system tissue. In fact, the possible relationship between gabapentin and GABA<sub>B</sub> receptors has been examined independently by Lanneau et al. (2001). These authors believe that gabapentin is not a GABA<sub>B</sub> receptor agonist. In another study the antihyperalgesic effects of the GABA<sub>B</sub> agonists, baclofen and CGP35024, but not those produced by gabapentin, were blocked by CGP56433A, a GABA<sub>B</sub> receptor antagonist (Patel et al., 2001). Thus, the extent to which the actions of gabapentin are mediated in vivo by effects on GABA<sub>B</sub> receptors, remains to be conclusively demonstrated. Nevertheless, the possibility that under certain conditions gabapentin can activate a particular form of the GABA<sub>B</sub> receptor is an interesting observation.

Electrophysiological studies in mammalian brain suggest subtle distinctions between pre- and postsynaptic receptors (Colmers and Williams, 1988; Dutar and Nicoll, 1988b; Harrison et al., 1990; Thompson and Gähwiler, 1992; Deisz et al., 1997; Chan et al., 1998; Yamada et al., 1999). For example, the GABA<sub>B</sub> receptor agonist CGP44533 failed to induce an increase in postsynaptic membrane conductance whereas (–)-baclofen and CGP35024 did (Yamada et al., 1999) and, on comparing the effects of six GABA<sub>B</sub> receptor antagonists, it was found that 5- to 10-fold higher concentrations were required to block presynaptic as opposed to postsynaptic receptors in the rat hippocampus (Pozza et al., 1999). However, in general, the receptor ligands currently available do not reliably distinguish between potential subtypes. Unfortunately, studies in GABA<sub>B1</sub> null-mice have also failed to provide any positive evidence for subtyping of the GABA<sub>B</sub> receptors (Prosser et al., 2001; Schuler et al., 2001).

## V. $\gamma$ -Aminobutyric Acid<sub>B</sub> Receptor Distribution

### A. Central Nervous System

Within the mammalian brain, the highest density of GABA<sub>B</sub> binding sites is in the thalamic nuclei, the molecular layer of the cerebellum, the cerebral cortex, the interpeduncular nucleus, and the dorsal horn of the spinal cord (Bowery et al., 1987; Chu et al., 1990). In situ hybridization studies of mRNA for the GABA<sub>B1(a)</sub> and GABA<sub>B1(b)</sub> splice variants reveal they are distributed differentially in brain (Liang et al., 2000). Studies with rat and human cerebellum and spinal cord indicate that GABA<sub>B1(a)</sub> is associated with presynaptic receptors, whereas GABA<sub>B1b</sub> is located predominantly at postsynaptic sites, at least in cerebellum (Kaupmann et al., 1998b; Billinton et al., 1999; Bischoff et al., 1999; Princivalle et al., 2000; Towers et al., 2000). Elsewhere in the brain, however, the GABA<sub>B1(b)</sub> protein is in presynaptic terminals and the GABA<sub>B1(a)</sub> at postsynaptic sites (Benke et al., 1999; Princivalle et al., 2001). In the dorsal

horn of the rat spinal cord, the density of GABA<sub>B1(a)</sub> is low, whereas in the dorsal root ganglia, which contain cell bodies of the primary afferent fibers, >90% of the GABA<sub>B1</sub> subunit mRNA is GABA<sub>B1(a)</sub>, with GABA<sub>B1(b)</sub> comprising less than 10% of the total GABA<sub>B1</sub> mRNA (Towers et al., 2000). Immunocytochemical studies provide support for this in revealing that the level of GABA<sub>B1(a)</sub> protein appears to be higher than of GABA<sub>B1(b)</sub> in the dorsal horn of the rat spinal cord (A. P. Princivalle and N. G. Bowery, unpublished observation). Similarly, in rat and human cerebellum, GABA<sub>B1(a)</sub> mRNA is detected over the granule cells, which send their excitatory fibers into the molecular layer to innervate the Purkinje cell dendrites (Kaupmann et al., 1998b; Billinton et al., 1999; Bischoff et al., 1999). Presumably, the GABA<sub>B</sub> receptors on the granule cell terminals modulate the output of the excitatory transmitter. In contrast, GABA<sub>B1(b)</sub> mRNA is associated with the Purkinje cell bodies, which express GABA<sub>B</sub> receptors on their dendrites in the molecular layer postsynaptic to the GABA-ergic stellate cells. However, the contrary arrangement has also been observed elsewhere in the brain. For example, GABA<sub>B1(a)</sub> subunits appear to be postsynaptic on cell bodies in the thalamocortical circuits (Princivalle et al., 2001). Thus, it would seem that a functional role, or cellular location, cannot be generally assigned to specific GABA<sub>B</sub> receptor subunit splice variants (Poorkhalkali et al., 2000; Princivalle et al., 2001).

The regional distribution of individual GABA<sub>B1</sub> and GABA<sub>B2</sub> protein subunits is similar to that of the wild-type receptor, but in some brain areas such as the caudate-putamen, GABA<sub>B2</sub> is not detectable, even though GABA<sub>B1</sub> and the native receptor are present (Durkin et al., 1999; Margeta-Mitrovic et al., 1999; Clark et al., 2000). In addition, there appears to be very little GABA<sub>B2</sub> mRNA, relative to GABA<sub>B1</sub> mRNA, in the hypothalamus (Jones et al., 1998; Clark et al., 2000). These findings, along with those suggesting that GABA<sub>B1</sub> and GABA<sub>B2</sub> subunit expression is not regulated in tandem (McCarson and Enna, 1999), support the existence of additional, as yet unidentified, GABA<sub>B</sub> receptor subunits.

### B. Peripheral Organs and Tissues

Functional GABA<sub>B</sub> receptors are not restricted to the central nervous system. Thus, GABA has been known for some time to play an important role in modulating autonomic inputs to the intestine, and GABA<sub>B</sub> receptors are capable of mediating responses in other organs (Ong and Kerr, 1990). Moreover, GABA<sub>B</sub> receptor agonists inhibit relaxation of the lower esophageal sphincter in dogs, ferrets, and humans, and attenuate esophageal reflux by an inhibitory action on the vagus nerve (Blackshaw et al., 1999, 2000; Lehmann et al., 1999, 2000; Lidums et al., 2000; Smid and Blackshaw, 2000).



Studies monitoring functional GABA<sub>B</sub> responses suggest their presence in peripheral organs (see Bowery, 1993). More recently, Northern blot analysis and receptor protein immunoblotting has provided direct evidence for GABA<sub>B1</sub> isoforms and GABA<sub>B</sub> receptors throughout the periphery of the rat (Castelli et al., 1999; Calver et al., 2000). However, the GABA<sub>B2</sub> subunit was not always present with GABA<sub>B1</sub>, such as in uterus and spleen (Calver et al., 2000).

Western blotting revealed the presence of GABA<sub>B1</sub> and GABA<sub>B2</sub> proteins in rat heart myocytes, supporting the observation that baclofen influences inwardly rectifying K<sup>+</sup> currents in these cells (Lorente et al., 2000). Moreover, photoaffinity-labeling studies suggest that GABA<sub>B1(a)</sub> and GABA<sub>B1(b)</sub> are differentially distributed in the periphery as well as in the central nervous system (Belley et al., 1999). Thus, GABA<sub>B1(a)</sub> is present in the adrenals, pituitary, spleen, and prostate, whereas GABA<sub>B1(b)</sub>, but not GABA<sub>B1(a)</sub>, is found in the rat kidney and liver.

## VI. $\gamma$ -Aminobutyric Acid<sub>B</sub> Receptor-Mediated Responses

### A. $\gamma$ -Aminobutyric Acid<sub>B</sub> Receptor Agonists

The observation that  $\beta$ -[4-chlorophenyl] GABA (baclofen; Fig. 1), is a stereospecifically active agonist at the GABA<sub>B</sub> receptor (Bowery et al., 1980, 1981) provided part of the original evidence for the existence of a distinct receptor. Since then 3-aminopropyl-phosphinic acid (3-APPA, CGP27492; Bittiger et al., 1988) and its methyl homolog (3-APMPA, CGP35024 identical with SK&F 97541; Froestl et al., 1992, 1993; Howson et al., 1993) have emerged and are reported to be 3- to 7-fold more potent than the active isomer of baclofen [IC<sub>50</sub> values, i.e., inhibition of binding of [<sup>3</sup>H]CGP27492 to GABA<sub>B</sub> receptors on rat cerebral cortex membranes: baclofen: 107 nM, (*R*)-(-)-baclofen: 32 nM, 3-APPA (CGP27492): 5 nM, 3-APMPA (CGP35024): 16 nM]. The latter compounds are also available as tritiated radioligands (Bittiger et al., 1988; Hall et al., 1995). Other

methyl phosphinic acid-based agonists have been produced (Froestl et al., 1995a), such as CGP44532 (IC<sub>50</sub> = 45 nM) and its (*R*)-(+)-enantiomer CGP44533 (IC<sub>50</sub> = 152 nM; racemate CGP34938: IC<sub>50</sub> = 77 nM; Fig. 1), which differ only by a factor of 3 in binding to GABA<sub>B</sub> receptors and show comparative activities in biochemical paradigms (Ong et al., 2001) but show significant differences in electrophysiological experiments (Yamada et al., 1999).

Interestingly, ethyl (and higher homolog) phosphinic acids derivatives (e.g., CGP36216, IC<sub>50</sub> = 2  $\mu$ M; Fig. 2) show effects of GABA<sub>B</sub> receptor antagonists (Froestl et al., 1995b). The difluoromethyl phosphinic acid derivative CGP47656 (IC<sub>50</sub> = 89 nM; Fig. 1) with a substituent, the size of which is between a methyl and an ethyl group, showed properties of a partial GABA<sub>B</sub> receptor agonist (Froestl et al., 1995a). GABA<sub>B</sub> receptor agonists display a number of pharmacological effects, including central muscle relaxation, antitussive action, bronchiolar relaxation, inhibition of urinary bladder contraction, an increase in gastrointestinal motility, epileptogenesis, suppression of cocaine, nicotine, and opioid self-administration, antinociception, yawning, hypotension, brown fat thermogenesis, cognitive impairment, inhibition of gastric acid secretion, and inhibition of hormone release.

*1. Antispasticity.* The centrally mediated muscle relaxant effect of baclofen is the most widely exploited clinical response to this agent. This action appears due to a baclofen-induced reduction in neurotransmitter release onto motoneurons in the ventral horn of the spinal

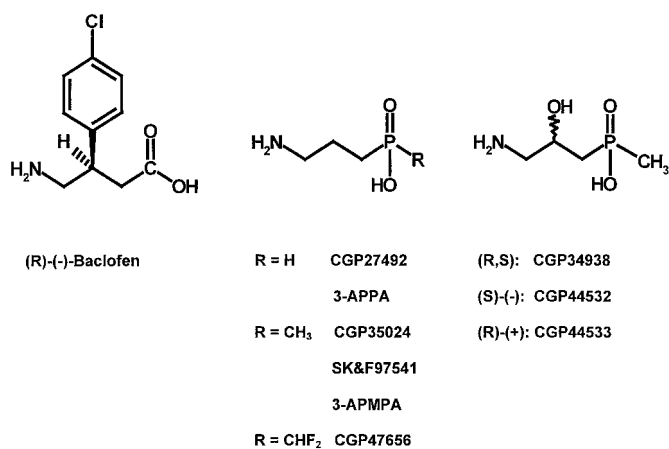


FIG 1. Structures of high-affinity GABA<sub>B</sub> receptor agonists.

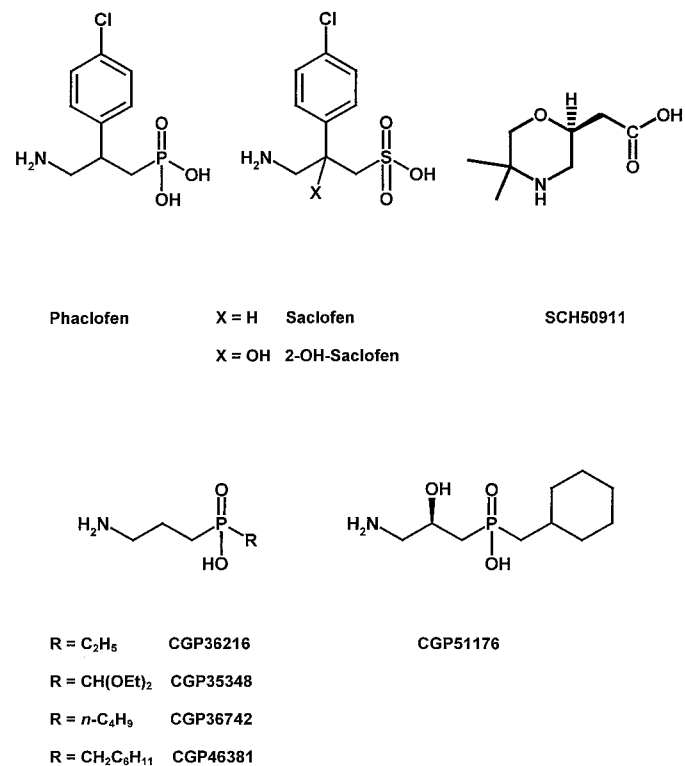


FIG 2. Structures of low-affinity GABA<sub>B</sub> receptor antagonists.



cord. There is also a suggestion that the antispastic effect is due to post- rather than presynaptic action on motoneurons (Orsnes et al., 2000a). Regardless of the site of action, the efficacy of baclofen in alleviating spasticity has made it a drug of choice for this condition, although side effects, principally sedation, limit its utility. Baclofen is effective in treating spasticity associated with tardive dystonia, brain and spinal cord injury, cerebral palsy, tetanus, multiple sclerosis, and stiff-man syndrome (Ochs et al., 1989, 1999; Penn et al., 1989; Penn and Mangieri, 1993; Becker et al., 1995, 1997, 2000; Campbell et al., 1995; Seitz et al., 1995; Albright et al., 1996; Azouvi et al., 1996; Dressnandt and Conrad, 1996; Ford et al., 1996; Paret et al., 1996; Armstrong et al., 1997; Dressler et al., 1997; Dressnandt et al., 1997; François et al., 1997, 2001; Meythaler et al., 1997; Gerszten et al., 1998; Auer et al., 1999; Orsnes et al., 2000b; Trampitsch et al., 2000; Krach, 2001).

2. *Antinociceptive.* Even though baclofen is used to treat migraine headache, musculoskeletal pain, and the pain associated with trigeminal neuralgia, stroke, and spinal cord injury, its general effectiveness as an analgesic is limited (Fromm, 1994; Taira et al., 1995; Hansson and Kinnman, 1996; Loubser and Akman, 1996; Hering-Hanit, 1999; Idänpään-Heikkilä and Guilbaud, 1999; Becker et al., 2000). Although the reason for this is unknown, it may be due to a rapid desensitization of GABA<sub>B</sub> receptors.

Laboratory animal studies have established the antinociceptive action of GABA<sub>B</sub> receptor agonists and suggest it is mediated by effects in both spinal cord and brain (Cutting and Jordan, 1975; Levy and Proudfit, 1979; Liebman and Pastor, 1980; Kendall et al., 1982; Sawynok and Dickson, 1985; Vaught et al., 1985; Serrano et al., 1992; Hammond and Washington, 1993; Smith et al., 1994; Dirig and Yaksh, 1995; Thomas et al., 1995; McCarson and Enna, 1996; Thomas et al., 1996; Wiesenfeld-Hallin et al., 1997; Cui et al., 1998; Przesmycki et al., 1998). Further evidence supporting the analgesic potential of GABA<sub>B</sub> agonists is provided by the finding that tiagabine, a GABA uptake inhibitor, displays antinociceptive activity in rodents that is blocked by CGP35348, a GABA<sub>B</sub> receptor antagonist (Ipponi et al., 1999). Inasmuch as intrathecal administration of a GABA-producing neuronal cell line permanently reverses neuropathic pain, it has been suggested that altered spinal GABA levels may contribute to the induction phase of chronic pain (Eaton et al., 1999).

In the dorsal horn of the rat spinal cord, GABA<sub>B</sub> receptors are located on small diameter afferent fiber terminals, with activation of these sites decreasing the evoked release of sensory transmitters, such as substance P and glutamate (Price et al., 1987; Malcangio and Bowery, 1996; Ataka et al., 2000; Iyadomi et al., 2000; Riley et al., 2001). It has also been suggested that modulation of voltage-dependent, nifedipine-sensitive calcium channels in dorsal horn neurons may contribute

to the antinociceptive effects of GABA<sub>B</sub> agonists (Voisin and Nagy, 2001).

Baclofen and CGP35024 reversed neuropathic mechanical hyperalgesia following single s.c. or intrathecal administration but did not affect inflammatory mechanical hyperalgesia (Patel et al., 2001). GABA<sub>B</sub> receptor agonists, such as baclofen and CGP44532, inhibit the formalin-induced increase of the expression of neurokinin-1 receptor mRNA (Enna et al., 1998).

The hot-plate, tail-flick as well as the paw pressure techniques were used to characterize acute pain behaviors in GABA<sub>B1</sub> null-mutant mice (Schuler et al., 2001). The tail-flick is a reflex response to a noxious thermal stimulus applied to the tail and is generally taken to represent a spinal reflex response, whereas the hot-plate response to a noxious thermal stimulus to the plantar surface of the paws is thought to involve supraspinal sites. In these nociceptive tests, GABA<sub>B1</sub> null-mutant mice showed pronounced hyperalgesia to noxious heat in the hot-plate and tail-flick tests and reduced paw withdrawal thresholds to mechanical pressure. From these data it is likely that GABA<sub>B</sub>-mediated effects do indeed exert a tonic control of nociceptive processes in the naive animal. The sites for this action are expected to be both spinal and supraspinal, although further experiments are needed to confirm this.

3. *Suppression of Drug Craving.* Preliminary data suggest that baclofen reduces the craving for cocaine in humans (Ling et al., 1998). In rats, baclofen suppresses cocaine self-administration at doses that do not affect responding for food reinforcement (Roberts and Andrews, 1997; Shoaib et al., 1998; Campbell et al., 1999; Munzar et al., 2000). Moreover, the selective GABA<sub>B</sub> receptor agonist CGP44532 mimics this action of baclofen without disrupting the response for food (Brebner et al., 1999, 2000a,b). Similar results are obtained whether laboratory animals are administered the GABA<sub>B</sub> agonist either systemically or directly into select brain regions (Corrigall et al., 2000).

The importance of these observations in the possible treatment of drug abuse is reinforced by the finding that baclofen reduces craving for a host of addictive substances, including heroin, alcohol, and nicotine (Xi and Stein, 1999; Corrigall et al., 2000; Lobina et al., 2000). Thus, elevation of endogenous GABA levels in the mesolimbic area by administration of vigabatrin, an inhibitor of GABA metabolism, or the GABA uptake inhibitor NO-711, attenuates heroin and cocaine self-administration in rats and prevents cocaine-induced increases in dopamine in this brain region (Ashby et al., 1999; Xi and Stein, 2000). It has also been reported that gabapentin, a putative GABA<sub>B</sub> receptor agonist (Bertrand et al., 2001; Ng et al., 2001), reduces the craving for cocaine in dependent adults (Myrick et al., 2001).

There is preclinical and preliminary clinical evidence that baclofen is effective in reducing alcohol craving and intake (Addolorato et al., 2000; Colombo et al., 2000).

Baclofen blocks the rapid tolerance to ethanol, an effect that can be blocked by GABA<sub>B</sub> antagonists such as CGP36742 and CGP56433A (Zaleski et al., 2001).

4. *Miscellaneous Actions.* GABA<sub>B</sub> receptors in the hypothalamus and nucleus tractus solitarius modulate sympathetic nerve activity, resulting in an elevation in blood pressure (Takenaka et al., 1995). GABA<sub>B</sub> receptor activation in the hypothalamus also leads to an increase in metabolic rate and brown fat thermogenesis (Addae et al., 1986). In addition to the above, other centrally mediated effects of GABA<sub>B</sub> agonists include alterations in epileptogenesis, cognition (Tang and Hasselmo, 1996), yawning (Doger et al., 1989), and micturition (Kontani et al., 1988).

Baclofen has been shown to have anti-bronchoconstrictor activity through activation of presynaptic receptors on parasympathetic nerve terminals (Chapman et al., 1991). In addition, through an action on sensory nerves in the lung, baclofen is reported to inhibit non-adrenergic, noncholinergic bronchoconstriction (Belvisi et al., 1989).

Baclofen has also displayed antitussive activity in humans and laboratory animals (Bolser et al., 1993, 1994; Dicipinigitis and Dobkin, 1997). This effect is mediated through both a direct action on peripheral nerves in the lung as well as receptors in the brain stem controlling the cough reflex. Through a similar mechanism, possibly involving GABA-ergic inputs from the nucleus raphe magnus (Oshima et al., 1998), baclofen is effective in the management of intractable hiccoughs (Guelaud et al., 1995; Nickerson et al., 1997; Kumar and Dromerick, 1998; Marino, 1998). Baclofen has also been reported to inhibit the growth of mammary cancer cells in mice and humans, and there appears to be a correlation between glandular GABA levels and mammary pathology (Opolski et al., 2000).

### B. $\gamma$ -Aminobutyric Acid<sub>B</sub> Receptor Antagonists

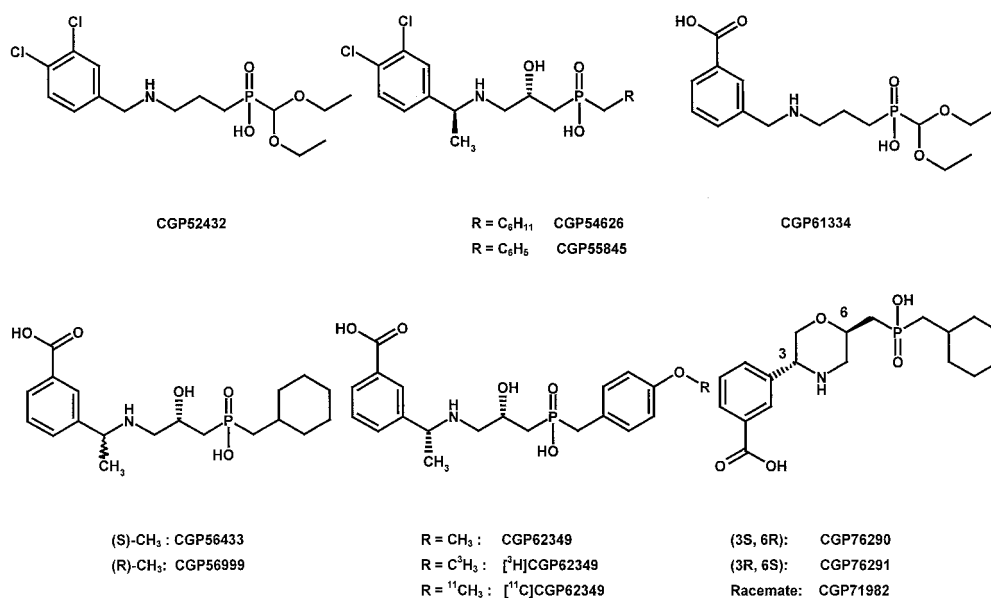
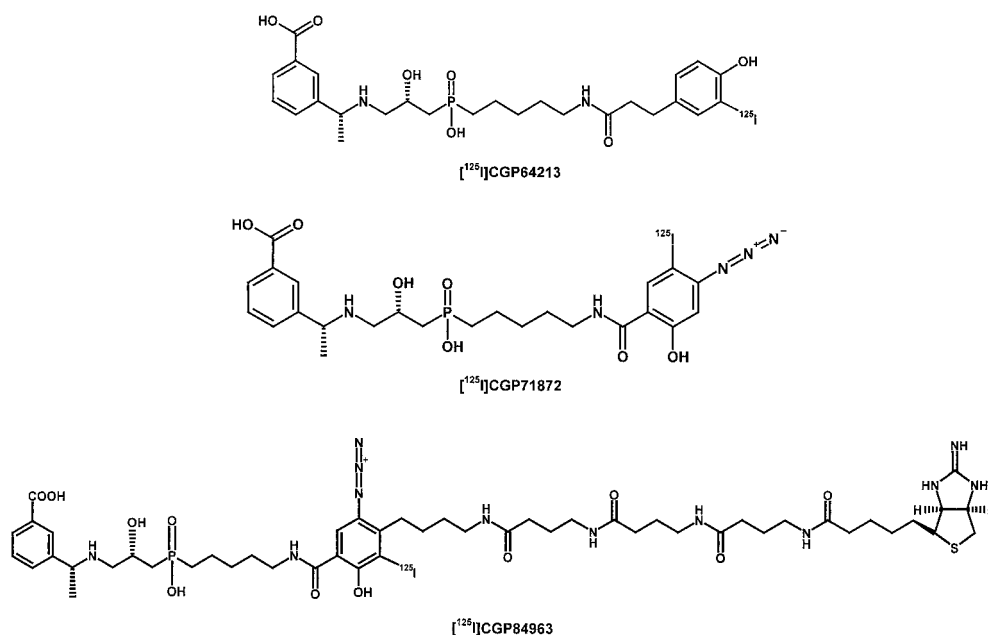
The design and development of selective, high-affinity GABA<sub>B</sub> receptor antagonists have been important in establishing the significance and isolation of the GABA<sub>B</sub> receptor genes. Kerr, Ong and their colleagues (1987, 1988; Fig. 2) described phaclofen, saclofen, and 2-hydroxysaclofen, the first selective antagonists. Although these agents have low affinities (IC<sub>50</sub> values, i.e., inhibition of binding of [<sup>3</sup>H]CGP27492 to GABA<sub>B</sub> receptors on rat cerebral cortex membranes: 130, 26, and 11  $\mu$ M, respectively) for GABA<sub>B</sub> binding sites in rat brain membranes, as the first antagonists they were important tools for defining the pharmacological and physiological relevance of GABA<sub>B</sub> receptors (Dutar and Nicoll, 1988a; Karlsson et al., 1988).

Subsequent discoveries of antagonists were derived largely by a group at Novartis in Basel, Switzerland (Froestl and Mickel, 1997). They developed the first GABA<sub>B</sub> receptor antagonist able to cross the blood-brain barrier, CGP35348, and the first orally active agents,

CGP36742 (Olpe et al., 1990, 1993a) and CGP51176 (Froestl et al., 1995b). However, these compounds, and others in this chemical series, have affinities for the GABA<sub>B</sub> receptor in the same range as 2-hydroxysaclofen (IC<sub>50</sub> values: 27, 38, and 6  $\mu$ M, respectively). The same is true for SCH 50911 (IC<sub>50</sub> = 3  $\mu$ M; Fig. 2), a chemically distinct agent that is effective following systemic administration but which has a relatively low affinity for the receptor (Bolser et al., 1995; Frydenvang et al., 1997). The most crucial breakthrough in the discovery of antagonists came with the development of compounds with affinities about 10,000 times higher than previous antagonists. This major advance stemmed from the attachment of 3,4-dichlorobenzyl or 3-carboxybenzyl substituents to the existing molecules. This produced a profusion of compounds with affinities in the low nanomolar range (Froestl et al., 1996; Froestl and Mickel, 1997). Numerous investigations have been carried out with CGP52432 (IC<sub>50</sub> = 55 nM), CGP54626A (IC<sub>50</sub> = 4 nM), CGP55845A (IC<sub>50</sub> = 6 nM), CGP56433A (IC<sub>50</sub> = 80 nM), CGP56999A (IC<sub>50</sub> = 2 nM), CGP61334 (IC<sub>50</sub> = 36 nM), and CGP62349 (IC<sub>50</sub> = 2 nM; Fig. 3). Several compounds are also available as radioligands, such as [<sup>3</sup>H]CGP54626 (Bittiger et al., 1992; Green et al., 2000), [<sup>3</sup>H]CGP56999, and [<sup>3</sup>H]CGP62349 (Bittiger et al., 1996a; Ambardekar et al., 1999; Keir et al., 1999; Sloviter et al., 1999; Billinton et al., 2000). From the latter compound a radioligand containing the positron-emitting isotope <sup>11</sup>C was prepared as a potential positron emission tomography ligand (Todde et al., 2000).

Introducing the phosphinic acid moiety into the Schering compound SCH 50911 led to a new class of very potent GABA<sub>B</sub> receptor antagonists, such as CGP76290A (Ong et al., 1998a; IC<sub>50</sub> = 2 nM, enantiomer CGP76291: IC<sub>50</sub> = 69 nM, racemate CGP71982, IC<sub>50</sub> = 8 nM; Fig. 3).

Finally, two iodinated high-affinity antagonists, i.e., [<sup>125</sup>I]CGP64213 (IC<sub>50</sub> = 1.6 nM, i.e., inhibition of binding of [<sup>125</sup>I]CGP64213 to GABA<sub>B</sub> receptors on rat cerebral cortex membranes) and [<sup>125</sup>I]CGP71872 (IC<sub>50</sub> = 2.4 nM), a photoaffinity ligand, both with high specific radioactivities of >2000 Ci/mmol were developed, which were used for the elucidation of the structure of GABA<sub>B1</sub> (Kaupmann et al., 1997; Belley et al., 1999; Calon et al., 2000; Froestl et al., 2001; Fig. 4). The ligand [<sup>125</sup>I]CGP84963 (IC<sub>50</sub> = 6 nM, i.e., inhibition of binding of [<sup>125</sup>I]CGP64213 to GABA<sub>B</sub> receptors on rat cerebral cortex membranes; Fig. 4) combines in one molecule a GABA<sub>B</sub> receptor-binding part, an azidosalicylic acid as a photoaffinity moiety separated by a spacer of three GABA molecules from 2-iminobiotin, which binds to avidin in a reversible, pH dependent fashion. This compound was prepared to facilitate isolation and purification of the extracellular N-terminal GABA<sub>B1</sub> receptor fragment for crystallization and X-ray studies of the GABA<sub>B1</sub> binding site (Froestl et al., 1999).

FIG 3. Structures of high-affinity GABA<sub>B</sub> receptor antagonists.FIG 4. Structures of high-affinity <sup>125</sup>I-labeled GABA<sub>B</sub> receptor antagonist.

Although GABA<sub>B</sub> receptor antagonists have yet to be studied in humans, results of animal studies suggest that they may have clinical utility. Thus, GABA<sub>B</sub> receptor antagonists suppress absence seizures in a variety of animal models (Marescaux et al., 1992). When administered either systemically or directly into the thalamus, GABA<sub>B</sub> receptor antagonists prevent spike and wave discharges in the electroencephalograms of genetic absence rats. Similar results are obtained with the lethargic mouse and in rats injected with  $\gamma$ -hydroxybutyrate (GHB), which produces absence-like seizure activity (Hosford et al., 1992; Snead, 1992). In all cases, GABA<sub>B</sub> receptor antagonists dose dependently reduce seizure activity. In the genetic absence rats, the spontaneous

seizures are blocked by bilateral administration of pertussis toxin, supporting the involvement of G<sub>i</sub>/G<sub>o</sub> coupling in generating and maintaining the seizures (Bowery et al., 1999). These results suggest that GABA<sub>B</sub> receptor activation may contribute to the absence syndrome, possibly through Ca<sup>2+</sup> spike generation in the thalamus (Crunelli and Leresche, 1991; Charpier et al., 1999).

At high doses, GABA<sub>B</sub> receptor antagonists induce convulsions in rats (Vergnes et al., 1997). Although the mechanism(s) underlying this action is unknown, the response is blocked by GABA<sub>B</sub> receptor agonists. Importantly, not all GABA<sub>B</sub> receptor antagonists cause seizures. For example, SCH 50911 fails to cause convul-



sions at doses 10- to 100-fold higher than those that completely block seizures in the genetic absence rat (Richards and Bowery, 1996).

The GHB-induced absence-like seizures in rats appear due, at least in part, to a weak partial agonist action at GABA<sub>B</sub> receptors (Bernasconi et al., 1999; Lingenhoehl et al., 1999). However, GHB also acts through sites distinct from GABA<sub>B</sub> receptors (Snead, 2000).

Several GABA<sub>B</sub> receptor antagonists have been found to improve cognitive performance in a variety of animal paradigms, such as the low-affinity compounds, CGP35348 (Bianchi and Panerai, 1993; Castellano et al., 1993; Saha et al., 1993; Stäubli et al., 1999) and CGP36742 (Carletti et al., 1993; Mondadori et al., 1993, 1994, 1996a,b; Nakagawa and Takashima, 1997; Yu et al., 1997; Bonanno et al., 1999; Genkova-Papazova et al., 2000; Farr et al., 2000; Pittaluga et al., 2001), or the high-affinity compounds CGP55845A, CGP56433A, CGP61334, CGP62349, and CGP71872 (Getova et al., 1997; Getova and Bowery, 1998). Olpe et al. (1993b) observed a very pronounced facilitation of long-term potentiation in vivo with doses of 100 mg/kg i.v. CGP35348 on eliciting long-term potentiation by nonprimed tetanic stimulation in the CA1 region of the hippocampus of rats. Brucato et al. (1996) reported a suppression of long-term potentiation with CGP46381 using  $\theta$ -like stimulus trains to the dentate gyrus. However, the latter GABA<sub>B</sub> receptor antagonist did not show effects on working memory in the radial maze in rats (Brucato et al., 1996) nor did it improve learning and memory in mice in a step-down passive avoidance paradigm (C. Mondadori and W. Froestl, unpublished observations).

Perhaps not surprisingly, therefore, GABA<sub>B</sub> agonists impair learning behavior in animal models (Soubrie et al., 1976; Swartzwelder et al., 1987; Nabeshima et al., 1988a,b; Sidel et al., 1988; Castellano et al., 1989; Sharma and Kulkarni, 1990 and 1993; Castellano and McGaugh, 1991; Saha et al., 1993; DeSousa et al., 1994; Stackman and Walsh, 1994; Nakagawa et al., 1995; McNamara and Skelton, 1996; Tang and Hasselmo, 1996; Arolfo et al., 1998). This induced amnesia appears to be mediated via G protein-linked receptors because the impairment produced by baclofen in mice can be blocked by pertussis toxin administered intracerebroventricularly (Galeotti et al., 1998). Baclofen has occasionally produced memory deficits in patients (Sandyk and Gillman, 1985).

GABA<sub>B</sub> receptor antagonists improve cognitive performance in a variety of animal models (Carletti et al., 1993; Mondadori et al., 1993; Brucato et al., 1996; Getova et al., 1997; Nakagawa and Takashima, 1997; Yu et al., 1997; Getova and Bowery, 1998; Stäubli et al., 1999; Genkova-Papazova et al., 2000; Farr et al., 2000). Conversely, GABA<sub>B</sub> receptor agonists impair learning, an action that is blocked by pertussis toxin, supporting the involvement of G<sub>i</sub>/G<sub>o</sub> in the action of these agents (Nakagawa et al., 1995; McNamara and Skelton, 1996; Tang

and Hasselmo, 1996; Arolfo et al., 1998; Galeotti et al., 1998). In studies with mice lacking the GABA<sub>B1</sub> receptor subunit, a clear impairment of passive avoidance performance was observed, which was related to gene dosage (Schuler et al., 2001). These passive avoidance deficits are a reflection of impaired memory processes further linking GABA<sub>B</sub> receptors to memory performance. Reports suggest that both GABA<sub>B</sub> receptor antagonists and agonists are neuroprotective. Although baclofen is neuroprotective in a gerbil cerebral ischemia model, very high doses are required (Lal et al., 1995). Moreover, baclofen attenuates the neurotoxic effect of quinolinic acid on CA1 cells in rat hippocampus (Beskid et al., 1999). In contrast, studies with mouse cultured striatal neurons reveal that GABA<sub>B</sub> receptor activation enhances the neurotoxic effects of *N*-methyl-D-aspartate, reinforcing the concept that GABA<sub>B</sub> antagonists are more likely to be neuroprotective than agonists (Lafon-Cazal et al., 1999). In support of this, low doses of GABA<sub>B</sub> receptor antagonists increase levels of nerve growth factor and brain-derived neurotrophic factor in rat brain hippocampus, neocortex, and spinal cord, which could attenuate neurodegenerative processes (Heese et al., 2000).

The potential significance GABA<sub>B</sub> receptor mechanisms in depression was first suggested by Lloyd and colleagues (Pilc and Lloyd, 1984; Lloyd et al., 1985, 1989), but this was challenged by other groups. More recently, however, further suggestions that GABA<sub>B</sub> antagonists, e.g. CGP36742, are effective in animal models of depression have emerged (Nakagawa et al., 1999). Clear antidepressant effects were seen after 4 weeks of oral treatment with CGP51176 in the chronic mild stress model (Bittiger et al., 1996b). This might be supported in due course by the observations of Heese et al. (2000) who showed that GABA<sub>B</sub> antagonists produce a rapid increase in nerve growth factor and brain-derived neurotrophic factor levels. Interestingly, antidepressants have been shown to produce the same increase in those growth factors but only after 2 to 3 weeks (Nibuya et al., 1995; Duman et al., 1997). Could there be a link between these phenomena?

## VII. Conclusions

The G protein-coupled GABA<sub>B</sub> receptor was first described over 20 years ago but only recently has the site been cloned and with this has come the identification of its unique heterodimeric structure. Even though much is known about the formation and characteristics of this receptor, many important questions remain. Thus, it is crucial to determine whether GABA<sub>B</sub> receptor subtypes exist that can be exploited pharmacologically, to determine whether other proteins can link with GABA<sub>B1</sub> to form a functional receptor, to establish whether GABA<sub>B</sub> receptor subunits serve other functions in the cell, and to assess the clinical value of GABA<sub>B</sub> receptor agonists



and antagonists. Given the pace of discovery in this field, answers to these questions will be forthcoming. These results will not only have significant implications with regard to understanding the GABA<sub>B</sub> receptor system in particular but may allow novel drugs acting at this receptor to be developed.

It is evident that the presence of two distinct proteins (or a protein and an essential accessory protein), coupled to G proteins, forming a receptor for GABA, poses certain problems for nomenclature. The subcommittee considers that there is little evidence for distinct functional types of the receptor, but this is a rare, and usually short-lived, situation in pharmacology. The present proposition is to continue to call the receptor the GABA<sub>B</sub> receptor, and when distinct splice variants are studied, i.e. GABA<sub>B1(a)/2(a)</sub>, using the NC-IUPHAR designation for splice variants. This nomenclature is provisional and may be changed when there is evidence of distinct functional GABA<sub>B</sub> receptor types or if NC-IUPHAR issues general guidelines for ligand-gated ion channels, which modify the GABA<sub>A/B</sub> terminology.

## References

- Addae JL, Rothwell NJ, Stock MJ, and Stone TW (1986) Activation of thermogenesis of brown fat in rats by baclofen. *Neuropharmacology* **25**:627–631.
- Addolorato G, Caputo F, Capristo E, Colombo G, Gessa GL, and Gasbarrini G (2000) Ability of baclofen in reducing alcohol craving and intake: II—preliminary clinical evidence. *Alcohol Clin Exp Res* **24**:67–71.
- Albright AL, Barry MJ, Fasick P, Barron W, and Shultz B (1996) Continuous intrathecal baclofen infusion for symptomatic generalized dystonia. *Neurosurgery* **38**:934–939.
- Ambardekar AV, Ilinsky IA, Froestl W, Bowery NG, and Kultas-Ilinsky K (1999) Distribution and properties of GABA<sub>B</sub> antagonist [<sup>3</sup>H]CGP62349 binding in the Rhesus monkey thalamus and basal ganglia and the influence of lesions in the reticular thalamic nucleus. *Neuroscience* **93**:1339–1347.
- Andrade R, Malenka RC, and Nicoll RA (1986) A G protein couples serotonin and GABA<sub>B</sub> receptors to the same channels in hippocampus. *Science (Wash DC)* **234**:1261–1265.
- Armstrong RW, Steinbok P, Cochrane DD, Kube SD, Fife SE, and Farrell K (1997) Intrathecally administered baclofen for treatment of children with spasticity of cerebral origin. *J Neurosurg* **87**:409–414.
- Arolo MP, Zanutto MA, and Ramirez OA (1998) Baclofen infused in rat hippocampal formation impairs spatial learning. *Hippocampus* **8**:109–113.
- Ashby CR, Rohatgi R, Ngosuan J, Borda T, Gerasimov MR, Morgan AE, Kushner S, Brodie JD, and Dewey SL (1999) Implication of the GABA<sub>B</sub> receptor in gamma vinyl-GABA's inhibition of cocaine-induced increases in nucleus accumbens dopamine. *Synapse* **31**:151–153.
- Ataka T, Kumamoto E, Shimoi K, and Yoshimura M (2000) Baclofen inhibits more effectively C-afferent than A delta-afferent glutamatergic transmission in substantia gelatinosa neurons of adult rat spinal cord slices. *Pain* **86**:273–282.
- Auer C, Siebner HR, Dressnandt J, and Conrad B (1999) Intrathecal baclofen increases corticospinal output to hand muscles in multiple sclerosis. *Neurology* **52**:1298–1299.
- Azouvi P, Mane M, Thiebaut JB, Denys P, Remy-Neris O, and Bussel B (1996) Intrathecal baclofen administration for control of severe spinal spasticity: functional improvement and long-term follow-up. *Arch Phys Med Rehab* **77**:35–39.
- Banerjee PK and Snead OC (1995) Presynaptic gamma-hydroxybutyric acid (GHB) and gamma-aminobutyric acid<sub>B</sub> (GABA<sub>B</sub>) receptor-mediated release of GABA and glutamate (Glu) in rat thalamic ventrobasal nucleus (VB): a possible mechanism for the generation of absence-like seizures induced by GHB. *J Pharmacol Exp Ther* **273**:1534–1543.
- Barral J, Toro S, Galarraga E, and Bargas J (2000) GABAergic presynaptic inhibition of rat neostriatal afferents is mediated by Q-type Ca<sup>2+</sup> channels. *Neurosci Lett* **283**:33–36.
- Becker R, Alberti O, and Bauer BL (1997) Continuous intrathecal baclofen infusion in severe spasticity after traumatic or hypoxic brain injury. *J Neurol* **244**:160–166.
- Becker R, Benes L, Sure U, Hellwig D, and Bertalanffy H (2000) Intrathecal baclofen alleviates autonomic dysfunction in severe brain injury. *J Clin Neurosci* **7**:316–319.
- Becker WJ, Harris CJ, Long ML, Ablett DP, Klein GM, and DeForge DA (1995) Long-term intrathecal baclofen therapy in patients with intractable spasticity. *Can J Neurol Sci* **22**:208–217.
- Bein HJ (1972) Pharmacological differentiations of muscle relaxants, in *Spasticity: A Topical Survey* (Birkmayer W ed) pp 76–89, Hans Huber, Vienna.
- Belley M, Sullivan R, Reeves A, Evans J, O'Neill G, and Ng GYK (1999) Synthesis of the nanomolar photaffinity GABA<sub>B</sub> receptor ligand CGP 71872 reveals diversity in the tissue distribution of GABA<sub>B</sub> receptor forms. *Bioorg Med Chem* **7**:2697–2704.
- Belvisi MG, Ichinose M, and Barnes PJ (1989) Modulation of non-adrenergic, non-cholinergic neural bronchoconstriction in guinea-pig airways via GABA<sub>B</sub> receptors. *Br J Pharmacol* **97**:1225–1231.
- Benke D, Honer M, Michel C, Bettler B, and Mohler H (1999) Gamma-aminobutyric acid type B receptor splice variant proteins GBR1a and GBR1b are both associated with GBR2 *in situ* and display differential regional and subcellular distribution. *J Biol Chem* **274**:27323–27330.
- Bernasconi R, Mathivet P, Bischoff S, and Marescaux C (1999) Gamma-hydroxybutyric acid: an endogenous neuromodulator with abuse potential. *Trends Pharmacol Sci* **20**:135–141.
- Bertrand S, Ng GYK, Purisai MG, Wolfe SE, Severid MW, Nouel D, Robitaille R, Low MJ, O'Neill GP, Metters K, et al. (2001) The anticonvulsant, antihyperalgesic agent gabapentin is an agonist at brain gamma-aminobutyric acid type B receptors negatively coupled to voltage-dependent calcium channels. *J Pharmacol Exp Ther* **298**:15–24.
- Beskid M, Rozycka Z, and Taraszewska A (1999) Quinolinic acid and GABA-B receptor ligand: effect on pyramidal neurons of the CA1 sector of rat's dorsal hippocampus following peripheral administration. *Folia Neuropathol* **37**:99–106.
- Bianchi M and Panerai AE (1993) Reversal of scopolamine-induced amnesia by the GABA<sub>B</sub> receptor antagonist CGP 35348 in the mouse. *Cognit Brain Res* **1**:135–136.
- Billinton A, Stean TO, Bowery NG, and Upton N (2000) GABA<sub>B1</sub> splice variant mRNAs are differentially affected by electroshock induced seizure in rats. *Neuro-report* **11**:3817–3822.
- Billinton A, Upton N, and Bowery NG (1999) GABA<sub>B</sub> receptor isoforms GBR1a and GBR1b, appear to be associated with pre- and post-synaptic elements respectively in rat and human cerebellum. *Br J Pharmacol* **126**:1387–1392.
- Bindokas VP and Ishida AT (1991) (–)-Baclofen and gamma-aminobutyric acid inhibit calcium currents in isolated retinal ganglion cells. *Proc Natl Acad Sci USA* **88**:10759–10763.
- Bischoff S, Leonhard S, Reymann N, Schuler V, Shigemoto R, Kaupmann K, and Bettler B (1999) Spatial distribution of GABA<sub>B1</sub> receptor mRNA and binding sites in the rat brain. *J Comp Neurol* **412**:1–16.
- Bittiger H, Bellouin C, Froestl W, Heid J, Schmutz M, and Stampf P (1996a) [<sup>3</sup>H]CGP 62349: a new potent GABA<sub>B</sub> receptor antagonist radioligand. *Pharmacol Rev Commun* **8**:97–98.
- Bittiger H, Froestl W, Gentsch C, Jaekel J, Mickel SJ, Mondadori C, Olpe HR, and Schmutz M (1996b) GABA<sub>B</sub> receptor antagonists: potential therapeutic applications, in *GABA: Receptors, Transporters and Metabolism* (Tanaka C and Bowery NG eds) pp 297–305, Birkhaeuser Verlag, Basel.
- Bittiger H, Reymann N, Froestl W, and Mickel SJ (1992) <sup>3</sup>H-CGP54626: a potent antagonist radioligand for GABA<sub>B</sub> receptors. *Pharmacol Commun* **2**:23.
- Bittiger H, Reymann N, Hall R, and Kane P (1988) CGP27492, a new potent and selective radioligand for GABA<sub>B</sub> receptors (Abstract). *Eur J Neurosci* **16** (Suppl): 10.
- Blackshaw LA, Smid SD, O'Donnell TA, and Dent J (2000) GABA<sub>B</sub> receptor-mediated effects on vagal pathways to the lower oesophageal sphincter and heart. *Br J Pharmacol* **130**:279–288.
- Blackshaw LA, Staunton E, Lehmann A, and Dent J (1999) Inhibition of transient lower esophageal sphincter relaxations and reflux in ferrets by GABA receptor agonists. *Am J Physiol* **277**:G867–G874.
- Bolser DC, Aziz SM, DeGennaro FC, Kreutner W, Egan RW, Siegel MI, and Chapman RW (1993) Antitussive effects of GABA<sub>B</sub> agonists in the cat and guinea-pig. *Br J Pharmacol* **110**:491–495.
- Bolser DC, Blythin DJ, Chapman RW, Egan RW, Hey JA, Rizzo C, Kuo SC, and Kreutner W (1995) The pharmacology of SCH 50911: a novel, orally-active GABA-B receptor antagonist. *J Pharmacol Exp Ther* **274**:1393–1398.
- Bolser DC, DeGennaro FC, O'Reilly S, Chapman RW, Kreutner W, Egan RW, and Hey JA (1994) Peripheral and central sites of action of GABA-B agonists to inhibit the cough reflex in the cat and guinea pig. *Br J Pharmacol* **113**:1344–1348.
- Bonanno G, Carita F, Cavazzani P, Munari C, and Raiteri M (1999) Selective block of rat and human neocortex GABA<sub>B</sub> receptors regulating somatostatin release by a GABA<sub>B</sub> antagonist endowed with cognition enhancing activity. *Neuropharmacology* **38**:1789–1795.
- Bonanno G, Fassio A, Sala R, Schmid G, and Raiteri M (1998) GABA<sub>B</sub> receptors as potential targets for drugs able to prevent excitatory amino acid transmission in the spinal cord. *Eur J Pharmacol* **362**:143–148.
- Bonanno G, Fassio A, Schmid G, Severi P, Sala R, and Raiteri M (1997) Pharmacologically distinct GABA<sub>B</sub> receptors that mediate inhibition of GABA and glutamate release in human neocortex. *Br J Pharmacol* **120**:60–64.
- Bonanno G, Gemignani A, Schmid G, Severi P, Cavazzani P, and Raiteri M (1996) Human brain somatostatin release from isolated cortical nerve endings and its modulation through GABA<sub>B</sub> receptors. *Br J Pharmacol* **118**:1441–1446.
- Bonanno G and Raiteri M (1992) Functional evidence for multiple GABA<sub>B</sub> receptor subtypes in the rat cerebral cortex. *J Pharmacol Exp Ther* **262**:114–118.
- Bonanno G and Raiteri M (1993a) Multiple GABA<sub>B</sub> receptors. *Trends Pharmacol Sci* **14**:259–261.
- Bonanno G and Raiteri M (1993b) Gamma-aminobutyric (GABA) autoreceptors in rat cerebral cortex and spinal cord represent pharmacologically distinct subtypes of the GABA<sub>B</sub> receptor. *J Pharmacol Exp Ther* **265**:765–770.
- Bouvier M (2001) Oligomerization of G-protein-coupled transmitter receptors. *Nat Rev Neurosci* **2**:274–286.
- Bowery NG (1993) GABA<sub>B</sub> receptor pharmacology. *Annu Rev Pharmacol Toxicol* **33**:109–147.
- Bowery NG, Doble A, Hill DR, Hudson AL, Shaw JS, Turnbull MJ, and Warrington R (1981) Bicuculline-insensitive GABA receptors on peripheral autonomic nerve terminals. *Eur J Pharmacol* **71**:53–70.
- Bowery NG and Enna SJ (2000) Gamma-aminobutyric acid (B) receptors: first of the functional metabotropic heterodimers. *J Pharmacol Exp Ther* **292**:2–7.
- Bowery NG, Hill DR, and Hudson AL (1983) Characteristics of GABA<sub>B</sub> receptor sites on rat whole brain synaptic membranes. *Br J Pharmacol* **78**:191–206.

- Bowery NG, Hill DR, and Hudson AL (1985) [<sup>3</sup>H](-)-Baclofen: an improved ligand for GABA<sub>B</sub> sites. *Neuropharmacology* **24**:207–210.
- Bowery NG, Hill DR, Hudson AL, Doble A, Middlemiss DN, Shaw J, and Turnbull M (1980) (-)-Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature (Lond)* **283**:92–94.
- Bowery NG, Hudson AL, and Price GW (1987) GABA<sub>A</sub> and GABA<sub>B</sub> receptor site distribution in the rat central nervous system. *Neuroscience* **20**:365–383.
- Bowery NG, Parry K, Boehrer A, Mathivet P, Marescaux C, and Bernasconi R (1999) Pertussis toxin decreases absence seizures and GABA<sub>B</sub> receptor binding in thalamus of a genetically prone rat (GAERS). *Neuropharmacology* **38**:1691–1697.
- Brebner K, Froestl W, Andrews M, Phelan R, and Roberts DCS (1999) The GABA<sub>B</sub> agonist CGP44532 decreases cocaine self-administration in rats: demonstration using a progressive ratio and a discrete trials procedure. *Neuropharmacology* **38**:1797–1804.
- Brebner K, Phelan R, and Roberts DCS (2000a) Effect of baclofen on cocaine self-administration in rats reinforced under fixed-ratio 1 and progressive-ratio schedules. *Psychopharmacology* **148**:314–321.
- Brebner K, Phelan R, and Roberts DCS (2000b) Intra-VTA baclofen attenuates cocaine self-administration on a progressive ratio schedule of reinforcement. *Pharmacol Biochem Behav* **66**:857–862.
- Brucato FH, Levin ED, Mott DD, Lewis DV, Wilson WA, and Swartzwelder HS (1996) Hippocampal long-term potentiation and spatial learning in the rat: effects of GABA<sub>B</sub> receptor blockade. *Neuroscience* **74**:331–339.
- Bussières N and El Manira A (1999) GABA<sub>B</sub> receptor activation inhibits N- and P/Q-type calcium channels in cultured lamprey sensory neurons. *Brain Res* **847**:175–185.
- Calon F, Morisette M, Goulet M, Grondin R, Blanchet PJ, Bedard PJ, and Di Paolo T (2000) [<sup>125</sup>I]-CGP 64213 binding to GABA<sub>B</sub> receptors in the brain of monkeys: effect of MPTP and dopaminomimetic treatments. *Exp Neurol* **163**:191–199.
- Calver AR, Medhurst AD, Robbins MJ, Charles KJ, Evans ML, Harrison DC, Stammers M, Hughes SA, Hervieu G, Couve A, et al. (2000) The expression of GABA<sub>B1</sub> and GABA<sub>B2</sub> receptor subunits in the CNS differs from that in peripheral tissues. *Neuroscience* **100**:155–170.
- Calver AR, Robbins MJ, Cosio C, Rice SQJ, Babbs AJ, Hirst WD, Boyfield I, Wood MD, Russell RB, Price GW, et al. (2001) The C-terminal domains of the GABA<sub>B</sub> receptor subunits mediate intracellular trafficking but are not required for receptor signaling. *Neuroscience* **21**:1203–1210.
- Campbell SK, Almeida GL, Penn RD, and Corcos DM (1995) The effects of intrathecally administered baclofen on function in patients with spasticity. *Phys Ther* **75**:352–362.
- Campbell UC, Lac ST, and Carroll ME (1999) Effects of baclofen on maintenance and reinstatement of intravenous cocaine self-administration in rats. *Psychopharmacology* **143**:209–214.
- Carletti R, Libri V, and Bowery NG (1993) The GABA<sub>B</sub> antagonist CGP 36742 enhances spatial learning performance and antagonises baclofen-induced amnesia in mice. *Br J Pharmacol* **109**:74P.
- Castellano C, Brioni JD, Nagahara AH, and McGaugh JL (1989) Post-training systemic and intra-amygdala administration of the GABA-B agonist baclofen impairs retention. *Behav Neural Biol* **52**:170–179.
- Castellano C, Cestari V, Cabib S, and Puglisi-Allegra S (1993) Strain-dependent effects of post-training GABA receptor agonists and antagonists on memory storage in mice. *Psychopharmacology* **111**:134–138.
- Castellano C and McGaugh JL (1991) Oxotremorine attenuates retrograde amnesia induced by post-training administration of the GABAergic agonists muscimol and baclofen. *Behav Neural Biol* **56**:25–31.
- Castelli MP, Ingianni A, Stefanini E, and Gessa GL (1999) Distribution of GABA<sub>B</sub> receptor mRNAs in the rat brain and peripheral organs. *Life Sci* **64**:1321–1328.
- Chan PKY, Leung CKS, and Yung WH (1998) Differential expression of pre- and postsynaptic GABA<sub>B</sub> receptors in rat substantia nigra pars reticulata neurones. *Eur J Pharmacol* **349**:187–197.
- Chapman RW, Danko G, Rizzo C, Egan RW, Mauser PJ, and Kreutner W (1991) Prejunctional GABA-B inhibition of cholinergic, neurally-mediated airway contractions in guinea-pigs. *Pulm Pharmacol* **4**:218–224.
- Charpier S, Leresche N, Deniau JM, Mahon S, Hughes SW, and Crunelli V (1999) On the putative contribution of GABA<sub>B</sub> receptors to the electrical events occurring during spontaneous spike and wave discharges. *Neuropharmacology* **38**:1699–1706.
- Chen G and van den Pol AN (1998) Presynaptic GABA<sub>B</sub> autoreceptor modulation of P/Q-type calcium channels and GABA release in rat suprachiasmatic nucleus neurons. *J Neurosci* **18**:1913–1922.
- Chronwall BM, Davis TD, Severidit MW, Wolfe SE, McCarron KE, Beatty DM, Low MJ, Morris SJ, and Enna SJ (2001) Constitutive expression of functional GABA<sub>B</sub> receptors in mIL-tsA58 cells requires both GABA<sub>B1</sub> and GABA<sub>B2</sub> genes. *J Neurochem* **77**:1237–1247.
- Chu DCM, Albin RL, Young AB and Penney JB (1990) Distribution and kinetics of GABA<sub>B</sub> binding sites in rat central nervous system: a quantitative autoradiographic study. *Neuroscience* **34**:341–357.
- Clark JA, Mezey E, Lam AS, and Bonner TI (2000) Distribution of the GABA<sub>B</sub> receptor subunit gb2 in rat CNS. *Brain Res* **860**:41–52.
- Colmers WF and Williams JT (1988) Pertussis toxin pretreatment discriminates between pre- and postsynaptic actions of baclofen in rat dorsal raphe nucleus in vitro. *Neurosci Lett* **93**:300–306.
- Colombo G, Agabio R, Carai MAM, Lobina C, Pani M, Reali R, Addolorato G, and Gessa GL (2000) Ability of baclofen in reducing alcohol intake and withdrawal severity: I—preclinical evidence. *Alcohol Clin Exp Res* **24**:58–66.
- Corrigall WA, Coen KM, Adamson KL, Chow BLC, and Zhang J (2000) Response of nicotine self-administration in the rat to manipulations of mu-opioid and gamma-aminobutyric acid receptors in the ventral tegmental area. *Psychopharmacology* **149**:107–114.
- Couve A, Filippov AK, Connolly CN, Bettler B, Brown DA, and Moss SJ (1998) Intracellular retention of GABA<sub>B</sub> receptors. *J Biol Chem* **273**:26361–26367.
- Couve A, Kittler JT, Uren JM, Calver AR, Pangalos MN, Walsh FS, and Moss SJ (2001) Association of GABA<sub>B</sub> receptors and members of the 14–3–3 family of signaling proteins. *Mol Cell Neurosci* **17**:317–328.
- Crunelli V and Leresche N (1991) A role for GABA<sub>B</sub> receptors in excitation and inhibition of thalamocortical cells. *Trends Neurosci* **14**:16–21.
- Cui JG, Meyerson BA, Sollevi A, and Linderth B (1998) Effect of spinal cord stimulation on tactile hypersensitivity in mono-neuropathic rats is potentiated by simultaneous GABA<sub>B</sub> and adenosine receptor activation. *Neurosci Lett* **247**:183–186.
- Cui LN, Coderre E, and Renaud LP (2000) GABA<sub>B</sub> presynaptically modulates suprachiasmatic input to hypothalamic paraventricular magnocellular neurons. *Am J Physiol Regul Integr Comp Physiol* **278**:R1210–R1216.
- Cunningham MD and Enna SJ (1996) Evidence for pharmacologically distinct GABA<sub>B</sub> receptors associated with cAMP production in rat brain. *Brain Res* **720**:220–224.
- Cutting DA and Jordan CC (1975) Alternative approaches to analgesia: baclofen as a model compound. *Br J Pharmacol* **54**:171–179.
- Deisz RA, Billard JM, and Zieglängsberger W (1997) Presynaptic and postsynaptic GABA<sub>B</sub> receptors of neocortical neurons of the rat in vitro: differences in pharmacology and ionic mechanisms. *Synapse* **25**:62–72.
- DeSousa NJ, Beninger RJ, Jhamandas K, and Boegman RJ (1994) Stimulation of GABA<sub>B</sub> receptors in the basal forebrain selectively impairs working memory of rats in the double Y-maze. *Brain Res* **641**:29–38.
- Diepningaitis PV and Dobkin JB (1997) Antitussive effect of the GABA-agonist baclofen. *Chest* **111**:996–999.
- Dirig DM and Yaksh TL (1995) Intrathecal baclofen and muscimol, but not midazolam, are antinociceptive using the rat-formalin model. *J Pharmacol Exp Ther* **275**:219–227.
- Doger E, Urba-Holmgren R, Eguibar JR, and Holmgren B (1989) GABAergic modulation of yawning behavior. *Pharmacol Biochem Behav* **34**:237–240.
- Dolphin AC, Huston E, and Scott RH (1990) GABA<sub>B</sub>-mediated inhibition of calcium currents: a possible role in presynaptic inhibition, in *GABA<sub>B</sub> Receptors in Mammalian Function* (Bowery NG, Bittiger H, and Olpe H-R eds) pp 259–271, Wiley, Chichester.
- Doze VA, Cohen GA, and Madison DV (1995) Calcium channel involvement in GABA<sub>B</sub> receptor-mediated inhibition of GABA release in area CA1 of the rat hippocampus. *J Neurophysiol* **74**:43–53.
- Dressler D, Oeljeschlager RO, and Ruther E (1997) Severe tardive dystonia: treatment with continuous intrathecal baclofen administration. *Movement Disorders* **12**:585–587.
- Dressnandt J and Conrad B (1996) Lasting reduction of severe spasticity after ending chronic treatment with intrathecal baclofen. *J Neurol Neurosurg Psychiatry* **2**:168–173.
- Dressnandt J, Konstanzer A, Weinzierl FX, Pfab R, and Klingelhöfer J (1997) Intrathecal baclofen in tetanus: four cases and a review of reported cases. *Intensive Care Med* **23**:896–902.
- Duman RS, Heninger GR, and Nestler EJ (1997) A molecular and cellular theory of depression. *Arch Gen Psychiatry* **54**:597–606.
- Durkin MM, Gunwaldsen CA, Borowsky B, Jones KA, and Branchek TA (1999) An in situ hybridization study of the distribution of the GABA<sub>B2</sub> protein mRNA in the rat CNS. *Mol Brain Res* **71**:185–200.
- Dutar P and Nicoll RA (1988a) A physiological role for GABA<sub>B</sub> receptors in the central nervous system. *Nature (Lond)* **332**:156–158.
- Dutar P and Nicoll RA (1988b) Pre- and postsynaptic GABA<sub>B</sub> receptors in the hippocampus have different pharmacological properties. *Neuron* **1**:585–591.
- Eaton MJ, Martinez MA, and Karmally S (1999) A single intrathecal injection of GABA permanently reverses neuropathic pain after nerve injury. *Brain Res* **835**:334–339.
- Enna SJ (1997) GABA<sub>B</sub> receptor agonists and antagonists: pharmacological properties and therapeutic possibilities. *Exp Opin Invest Drugs* **6**:1319–1325.
- Enna SJ (2000) GABA<sub>B</sub> receptor signaling pathways, in *Pharmacology of GABA and Glycine Neurotransmission* (Möhler H ed) pp 329–342, Springer-Verlag, Berlin.
- Enna SJ and Bowery NG (1997) *The GABA Receptors*, 2nd ed, Humana Press, Totowa, NJ.
- Enna SJ, Harstad EB, and McCarron KE (1998) Regulation of neurokinin-1 receptor expression by GABA<sub>B</sub> receptor agonists. *Life Sci* **62**:1525–1530.
- Farr SA, Uezu K, Creonte TA, Flood JF, and Morley JE (2000) Modulation of memory processing in the cingulate cortex of mice. *Pharmacol Biochem Behav* **65**:363–368.
- Fassio A, Bonanno G, Cavazzani P, and Raiteri M (1994) Characterization of the GABA autoreceptor in human neocortex as a pharmacological subtype of the GABA<sub>B</sub> receptor. *Eur J Pharmacol* **263**:311–314.
- Filippov AK, Couve A, Pangalos MN, Walsh FS, Brown DA, and Moss SJ (2000) Heteromeric assembly of GABA<sub>B1</sub>R1 and GABA<sub>B2</sub>R2 receptor subunits inhibits Ca<sup>2+</sup> current in sympathetic neurons. *J Neurosci* **20**:2867–2874.
- Ford B, Greene P, Louis ED, Petzinger G, Bressman SB, Goodman R, Brin MF, Sadiq S, and Fahn S (1996) Use of intrathecal baclofen in the treatment of patients with dystonia. *Arch Neurol* **53**:1241–1246.
- François B, Clavel M, Desachy A, Vignon P, Salle JY, and Gastinne H (1997) Continuous intrathecal baclofen in tetanus. A therapeutic alternative. *Presse Med* **26**:1045–1047.
- François B, Vacher P, Roustain J, Salle JY, Vidal J, Moreau JJ, and Vignon P (2001) Intrathecal baclofen after traumatic brain injury: early treatment using a new technique to prevent spasticity. *J Trauma Inj Infect Crit Care* **50**:158–161.
- Fritschy JM, Meskenaite V, Weinmann O, Honer M, Benke D, and Mohler H (1999) GABA<sub>B</sub>-receptor splice variants GB1a and GB1b in rat brain: developmental regulation, cellular distribution and extrasynaptic localization. *Eur J Neurosci* **11**:761–768.
- Froestl W, Bettler B, Bittiger H, Heid J, Kaupmann K, Mickel SJ, and Strub D (1999) Ligands for the isolation of GABA<sub>B</sub> receptors. *Neuropharmacology* **38**:1641–1646.
- Froestl W, Bettler B, Bittiger H, Heid J, Kaupmann K, Mickel SJ, and Strub D (2001)



- Ligands for expression cloning and isolation of GABA<sub>B</sub> receptors. *Farmacology* **56**:101–105.
- Froestl W and Mickel SJ (1997) Chemistry of GABA<sub>B</sub> modulators, in *The GABA Receptors* (Enna SJ and Bowery NG eds) pp 271–296, Humana Press Totowa, NJ.
- Froestl W, Mickel SJ, and Bittiger H (1993) Potent GABA<sub>B</sub> agonists and antagonists. *Curr Opin Therap Patents* **3**:561–567.
- Froestl W, Mickel SJ, Hall RG, von Sprecher G, Strub D, Baumann PA, Brugger F, Gentsch C, Jaekel J, Olpe H-R, Rihs G, Vassout A, Waldmeier PC and Bittiger H (1995a) Phosphinic acid analogues of GABA. 1. New potent and selective GABA<sub>B</sub> agonists. *J Med Chem* **38**:3297–3312.
- Froestl W, Mickel SJ, Schmutz M, and Bittiger H (1996) Potent, orally active GABA<sub>B</sub> receptor antagonists. *Pharmacol Rev Commun* **8**:127–133.
- Froestl W, Mickel SJ, von Sprecher G, Bittiger H, and Olpe HR (1992) Chemistry of new GABA<sub>B</sub> antagonists. *Pharmacol Commun* **2**:52–56.
- Froestl W, Mickel SJ, von Sprecher G, Diel PJ, Hall RG, Maier L, Strub D, Melillo V, Baumann PA, Bernasconi R, et al. (1995b) Phosphinic acid analogues of GABA. 2. Selective, orally active GABA<sub>B</sub> antagonists. *J Med Chem* **38**:3313–3331.
- Fromm GH (1994) Baclofen as an adjuvant analgesic. *J Pain Symptom Manage* **9**:500–509.
- Frydenvang K, Enna SJ, and Krosgaard-Larsen P (1997) (–) (R)-5,5-Dimethylmorpholinyl-2-acetic acid ethyl ester hydrochloride. *Acta Crystallogr* **C53**:1088–1091.
- Gage PW (1992) Activation and modulation of neuronal K<sup>+</sup> channels by GABA. *Trends Neurosci* **15**:46–51.
- Galeotti N, Ghelardini C, and Bartolini A (1998) Effect of picrotoxin on baclofen- and diphenhydramine-induced amnesia. *Psychopharmacology* **136**:328–334.
- Galvez T, Duthey B, Kniazeff J, Blahos J, Rovelli G, Bettler B, Prezeau L, and Pin JP (2001) Allosteric interactions between GB1 and GB2 subunits are required for optimal GABA<sub>B</sub> receptor function. *EMBO J* **20**:2152–2159.
- Gemignani A, Paudice P, Bonanno G, and Raiteri M (1994) Pharmacological discrimination between gamma-aminobutyric acid type B receptors regulating cholecystokinin and somatostatin release from rat neocortex synaptosomes. *Mol Pharmacol* **46**:558–562.
- Genkova-Papazova MG, Petkova B, Shishkova N, and Lazarova-Bakarova M (2000) The GABA-B antagonist CGP 36742 prevent PTZ-kindling-provoked amnesia in rats. *Eur Neuropsychopharmacol* **10**:273–278.
- Gerszten PC, Albright AL, and Johnstone GF (1998) Intrathecal baclofen infusion and subsequent orthopedic surgery in patients with spastic cerebral palsy. *J Neurosurg* **88**:1009–1013.
- Getova D and Bowery NG (1998) The modulatory effects of high affinity GABA<sub>B</sub> receptor antagonists in an active avoidance learning paradigm in rats. *Psychopharmacology* **137**:369–373.
- Getova D, Bowery NG, and Spassov V (1997) Effects of GABA<sub>B</sub> receptor antagonists on learning and memory retention in a rat model of absence epilepsy. *Eur J Pharmacol* **320**:9–13.
- Green A, Walls S, Wise A, Green RH, Martin AK, and Marshall FH (2000) Characterization of [<sup>3</sup>H]-CGP54626A binding to heterodimeric GABA<sub>B</sub> receptors stably expressed in mammalian cells. *Br J Pharmacol* **131**:1766–1774.
- Guelaud C, Similowski T, Bizet JL, Cabane J, Whitelaw WA, and Derenne JP (1995) Baclofen therapy for chronic hiccup. *Eur Respir J* **8**:235–237.
- Hall RG, Kane PD, Bittiger H, and Froestl W (1995) Phosphinic acid analogues of gamma-aminobutyric acid (GABA). Synthesis of a new radioligand. *J Label Compd Radiopharm* **36**:129–135.
- Hammond DL and Washington JD (1993) Antagonism of L-baclofen-induced antinociception by CGP 35348 in the spinal cord of the rat. *Eur J Pharmacol* **234**:255–262.
- Hansson P and Kinnman E (1996) Unmasking mechanisms of peripheral neuropathic pain in a clinical perspective. *Pain Rev* **3**:272–292.
- Harayama N, Shibuya I, Tanaka K, Kabashima N, Ueta Y, and Yamashita H (1998) Inhibition of N- and P/Q-type calcium channels by postsynaptic GABA<sub>B</sub> receptor activation in rat supraoptic neurones. *J Physiol* **509**:371–383.
- Harrison NL, Lambert NA, and Lovinger DM (1990) Presynaptic GABA<sub>B</sub> receptors on rat hippocampal neurons, in *GABA<sub>B</sub> Receptors in Mammalian Function* (Bowery NG, Bittiger H, and Olpe H-R eds) pp 207–221, Wiley, Chichester.
- Hashimoto T and Kuriyama K (1997) In vivo evidence that GABA<sub>B</sub> receptors are negatively coupled to adenylate cyclase in rat striatum. *J Neurochem* **69**:365–370.
- Heese K, Otten U, Mathivet P, Raiteri M, Marescaux C, and Bernasconi R (2000) GABA<sub>B</sub> receptor antagonists elevate both mRNA and protein levels of the neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) but not neurotrophin-3 (NT-3) in brain and spinal cord of rats. *Neuropharmacology* **39**:449–462.
- Hering-Hanit R (1999) Baclofen for prevention of migraine. *Cephalalgia* **19**:589–591.
- Hill DR (1985) GABA<sub>B</sub> receptor modulation of adenylate cyclase activity in rat brain slices. *Br J Pharmacol* **84**:249–257.
- Hill DR and Bowery NG (1981) <sup>3</sup>H-baclofen and <sup>3</sup>H-GABA bind to bicuculline-insensitive GABA<sub>B</sub> sites in rat brain. *Nature (Lond)* **290**:149–152.
- Hill DR, Bowery NG, and Hudson AL (1984) Inhibition of GABA<sub>B</sub> receptor binding by guanyl nucleotides. *J Neurochem* **42**:652–657.
- Hirst WD, Rice SQJ, Minton JAL, Calver AR, Pangalos MN, Jenkins O, and Price GW (2000) Characterisation of a CHO cell line stably co-expressing GABA<sub>B</sub>R1b and GABA<sub>B</sub>R2 receptors. *Br J Pharmacol* **129**:80P.
- Hosford DA, Clark S, Cao Z, Wilson WA, Lin F, Morrisett RA, and Huin A (1992) The role of GABA<sub>B</sub> receptor activation in absence seizures of lethargic (*lh/lh*) mice. *Science (Wash DC)* **257**:398–401.
- Howson W, Mistry J, Broekman M, and Hills JM (1993) Biological activity of 3-aminopropyl (methyl) phosphinic acid, a potent and selective GABA<sub>B</sub> agonist with CNS activity. *Bioorg Med Chem Lett* **3**:515–518.
- Idänpää-Heikkilä JJ and Guilbaud G (1999) Pharmacological studies on a rat model of trigeminal neuropathic pain: baclofen, but not carbamazepine, morphine or tricyclic antidepressants, attenuate the allodynia-like behaviour. *Pain* **79**:281–290.
- Inoue M, Matsuo T, and Ogata N (1985) Possible involvement of K<sup>+</sup>-conductance in the action of gamma-aminobutyric acid in the guinea-pig hippocampus. *Br J Pharmacol* **86**:515–524.
- Ipponi A, Lamberti C, Medica A, Bartolini A, and Malmberg-Aiello P (1999) Tiagabine antinociception in rodents depends on GABA<sub>B</sub> receptor activation: parallel antinociception testing and medial thalamus GABA microdialysis. *Eur J Pharmacol* **368**:205–211.
- Isaacson JS (1997) GABA receptor-mediated modulation of presynaptic currents and excitatory transmission at a fast central synapse. *Soc Neurosci Abstr* **23**:366.
- Isaacson JS (1998) GABA<sub>B</sub> receptor-mediated modulation of presynaptic currents and excitatory transmission at a fast central synapse. *J Neurophysiol* **80**:1571–1576.
- Isaacson JS and Hille B (1997) GABA<sub>B</sub>-mediated presynaptic inhibition of excitatory transmission and synaptic vesicle dynamics in cultured hippocampal neurons. *Neuron* **18**:143–152.
- Isomoto S, Kaibara M, Sakurai-Yamashita Y, Nagayama Y, Uezono Y, Yano K, and Taniyama K (1998) Cloning and tissue distribution of novel splice variants of the rat GABA<sub>B</sub> receptor. *Biochem Biophys Res Commun* **253**:10–15.
- Iyadomi M, Iyadomi I, Kumamoto E, Tomokuni K, and Yoshimura M (2000) Presynaptic inhibition by baclofen of miniature EPSCs and IPSCs in substantia gelatinosa neurons of the adult rat spinal dorsal horn. *Pain* **85**:385–393.
- Jarolimek W and Misgeld U (1997) GABA<sub>B</sub> receptor-mediated inhibition of tetrodotoxin-resistant GABA release in rodent hippocampal CA1 pyramidal cells. *J Neurosci* **17**:1025–1032.
- Jones KA, Borowsky B, Tamm JA, Craig DA, Durkin MM, Dai M, Yao WJ, Johnson M, Gunwaldsen C, Huang LY, et al. (1998) GABA<sub>B</sub> receptors function as a heteromeric assembly of the subunits GABA<sub>B</sub>R1 and GABA<sub>B</sub>R2. *Nature (Lond)* **396**:674–679.
- Karbon EW, Duman RS, and Enna SJ (1984) GABA<sub>B</sub> receptors and norepinephrine-stimulated cAMP production in rat brain cortex. *Brain Res* **306**:327–332.
- Karbon EW and Enna SJ (1985) Characterization of the relationship between gamma-aminobutyric acid B agonists and transmitter-coupled cyclic nucleotide-generating systems in rat brain. *Mol Pharmacol* **27**:53–59.
- Karlsson G, Pozza M, and Olpe HR (1988) Phaclofen: a GABA<sub>B</sub> blocker reduces long-duration inhibition in the neocortex. *Eur J Pharmacol* **148**:485–486.
- Kaupmann K, Huguel K, Heid J, Flor PJ, Bischoff S, Mickel SJ, McMaster G, Angst C, Bittiger H, Froestl W, and Bettler B (1997) Expression cloning of GABA<sub>B</sub> receptors uncovers similarity to metabotropic glutamate receptors. *Nature (Lond)* **386**:239–246.
- Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, Mosbacher J, Bischoff S, Kulik A, Shigemoto R, et al. (1998a) GABA<sub>B</sub>-receptor subtypes assemble into functional heteromeric complexes. *Nature (Lond)* **396**:683–687.
- Kaupmann K, Schuler V, Mosbacher J, Bischoff S, Bittiger H, Heid J, Froestl W, Leonhard S, Pfaff T, Karschin A and Bettler B (1998b) Human gamma-aminobutyric acid type B receptors are differentially expressed and regulate inwardly rectifying K<sup>+</sup> channels. *Proc Natl Acad Sci USA* **95**:14991–14996.
- Keberle H and Faigle JW (1972) Synthesis and structure-activity relationship of the gamma-aminobutyric acid derivatives, in *Spasticity: A Topical Survey* (Birkmayer W ed) pp 90–100, Hans Huber, Vienna.
- Keir MJ, Barakat MJ, Dev KK, Bittiger H, Bettler B, and Henley JM (1999) Characterisation and partial purification of the GABA<sub>B</sub> receptor from the rat cerebellum using the novel antagonist [<sup>3</sup>H]CGP 62349. *Mol Brain Res* **71**:279–289.
- Kendall DA, Browner M, and Enna SJ (1982) Comparison of the antinociceptive effect of gamma-aminobutyric acid (GABA) agonists: evidence for a cholinergic involvement. *J Pharmacol Exp Ther* **220**:482–487.
- Kerr DIB, Ong J, Johnston GAR, Abbenante J, and Prager RH (1988) 2-Hydroxy-saclofen: an improved antagonist at central and peripheral GABA<sub>B</sub> receptors. *Neurosci Lett* **92**:92–96.
- Kerr DIB, Ong J, Prager RH, Gynther BD, and Curtis DR (1987) Phaclofen: a peripheral and central baclofen antagonist. *Brain Res* **405**:150–154.
- Knight AR and Bowery NG (1996) The pharmacology of adenylyl cyclase modulation by GABA<sub>B</sub> receptors in rat brain slices. *Neuropharmacology* **35**:703–712.
- Kontani H, Kawabata Y, and Koshiura R (1988) The effect of baclofen on the urinary bladder contraction accompanying micturition in anesthetized rats. *Jpn J Pharmacol* **46**:7–15.
- Krach LE (2001) Pharmacotherapy of spasticity: oral medications and intrathecal baclofen. *J Child Neurol* **16**:31–36.
- Kumar A and Dromerick AW (1998) Intractable hiccups during stroke rehabilitation. *Arch Phys Med Rehabil* **79**:697–699.
- Kuner R, Köhr G, Grünewald S, Eisenhardt G, Bach A, and Kornau HC (1999) Role of heteromer formation in GABA<sub>B</sub> receptor function. *Science (Wash DC)* **283**:74–77.
- Lafon-Cazal M, Vionnois G, Kuhn R, Malitschek B, Pin JP, Shigemoto R, and Bockaert J (1999) mGluR7-like receptor and GABA<sub>B</sub> receptor activation enhance neurotoxic effects of N-methyl-D-aspartate in cultured mouse striatal GABAergic neurones. *Neuropharmacology* **38**:1631–1640.
- Lal S, Shuaib A, and Ijaz S (1995) Baclofen is cytoprotective to cerebral ischemia in gerbils. *Neurochem Res* **20**:115–119.
- Lambert NA and Wilson WA (1996) High-threshold Ca<sup>2+</sup> currents in rat hippocampal interneurons and their selective inhibition by activation of GABA<sub>B</sub> receptors. *J Physiol* **492**:115–127.
- Lanneau C, Green A, Hirst WD, Wise A, Brown J, Donnier E, Charles KJ, Wood M, Davies CH, and Pangalos MN (2001) Gabapentin is not a GABA<sub>B</sub> receptor agonist. *Neuropharmacology* **41**:965–975.
- Lehmann A, Antonsson M, Bremner-Danielsen M, Flärth M, Hansson-Bränden L, and Kärrberg L (1999) Activation of the GABA(B) receptor inhibits transient lower esophageal sphincter relaxations in dogs. *Gastroenterology* **117**:1147–1154.
- Lehmann A, Hansson-Bränden L, and Kärrberg L (2000) Effects of repeated administration of baclofen on transient lower esophageal sphincter relaxation in the dog. *Eur J Pharmacol* **403**:163–167.
- Levy RA and Proudft HK (1979) Analgesia produced by microinjection of baclofen and morphine at brain stem sites. *Eur J Pharmacol* **57**:43–55.

- Liang F, Hatanaka Y, Saito H, Yamamori T, and Hashikawa T (2000) Differential expression of gamma-aminobutyric acid type B receptor-1a and -1b mRNA variants in GABA and non-GABAergic neurons of the rat brain. *J Comp Neurol* **416**:475–495.
- Lidums I, Lehmann A, Checklin H, Dent J, and Holloway RH (2000) Control of transient lower esophageal sphincter relaxations and reflux by the GABA<sub>B</sub> agonist baclofen in normal subjects. *Gastroenterology* **118**:7–13.
- Liebmman JM and Pastor G (1980) Antinociceptive effects of baclofen and muscimol upon intraventricular administration. *Eur J Pharmacol* **61**:225–230.
- Ling W, Shoptaw S, and Majewska D (1998) Baclofen as a cocaine anti-craving medication: a preliminary clinical study. *Neuropsychopharmacology* **18**:403–404.
- Lingenhoehl K, Brom R, Heid J, Beck P, Froestl W, Kaupmann K, Bettler B, and Mosbacher J (1999) Gamma-hydroxybutyrate is a weak agonist at recombinant GABA<sub>B</sub> receptors. *Neuropharmacology* **38**:1667–1673.
- Lloyd KG, Thuret F, and Pilc A (1985) Upregulation of gamma-aminobutyric acid (GABA) B binding sites in rat frontal cortex: a common action of repeated administration of different classes of antidepressants and electroshock. *J Pharmacol Exp Ther* **235**:191–199.
- Lloyd KG, Zivkovic B, Scatton B, Morselli PL, and Bartholini G (1989) The GABAergic hypothesis of depression. *Prog Neuro-Psychopharmacol Biol Psychiatry* **13**:341–351.
- Lobina C, Pani M, Reali R, Addolarato G, and Gessa GL (2000) Ability of baclofen in reducing alcohol intake and withdrawal severity: I—preclinical evidence. *Alcohol Clin Exp Res* **24**:58–66.
- Lorente P, Lacampagne A, Pouzeratte Y, Richards S, Malitschek B, Kuhn R, Bettler B, and Vassort G (2000) Gamma-aminobutyric acid type B receptors are expressed and functional in mammalian cardiomyocytes. *Proc Natl Acad Sci USA* **97**:8664–8669.
- Loubser PG and Akman NM (1996) Effects of intrathecal baclofen on chronic spinal cord injury pain. *J Pain Symptom Manage* **12**:241–247.
- Lüscher C, Jan LY, Stoffel M, Malenka RC, and Nicoll RA (1997) G protein-coupled inwardly rectifying K<sup>+</sup> channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron* **19**:687–695.
- Malcangio M and Bowery NG (1996) GABA and its receptors in the spinal cord. *Trends Pharmacol Sci* **17**:457–462.
- Marescaux C, Vergnes M, and Bernasconi R (1992) GABA<sub>B</sub> receptor antagonists: potential new anti-absence drugs. *J Neural Transm* **35**:179–188.
- Margeta-Mitrovic M, Jan YN, and Jan LY (2000) A trafficking checkpoint controls GABA<sub>B</sub> receptor heterodimerization. *Neuron* **27**:97–106.
- Margeta-Mitrovic M, Mitrovic I, Riley RC, Jan LY, and Basbaum AI (1999) Immunohistochemical localization of GABA<sub>B</sub> receptors in the rat central nervous system. *J Comp Neurol* **405**:299–321.
- Marino RA (1998) Baclofen therapy for intractable hiccups in pancreatic carcinoma. *Am J Gastroenterol* **93**:2000.
- Marshall FH, Jones KA, Kaupmann K, and Bettler B (1999a) GABA<sub>B</sub> receptors—the first 7TM heterodimers. *Trends Pharmacol Sci* **20**:396–399.
- Marshall FH, White J, Main M, Green A, and Wise A (1999b) GABA<sub>B</sub> receptors function as heterodimers. *Biochem Soc Trans* **27**:530–535.
- Martin SC, Russek SJ, and Farb DH (1999) Molecular identification of the human GABA<sub>B</sub> R2: cell surface expression and coupling to adenylyl cyclase in the absence of GABA<sub>B</sub> R1. *Mol Cell Neurosci* **13**:180–191.
- Martin SC, Russek SJ, and Farb DH (2001) Human GABA<sub>B</sub>R genomic structure: evidence for splice variants in GABA<sub>B</sub>R1 but not GABA<sub>B</sub>R2. *Gene* **278**:63–79.
- McCarson KE and Enna SJ (1996) Relationship between GABA<sub>B</sub> receptor activation and neurokinin receptor expression in spinal cord. *Pharmacol Rev Commun* **8**:191–194.
- McCarson KE and Enna SJ (1999) Nociceptive regulation of GABA<sub>B</sub> receptor gene expression in rat spinal cord. *Neuropharmacology* **38**:1767–1773.
- McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, Solari R, Lee MG, and Foord SM (1998) RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature (Lond)* **393**:333–339.
- McNamara RK and Skelton RW (1996) Baclofen, a selective GABA<sub>B</sub> receptor agonist, dose-dependently impairs spatial learning in rats. *Pharmacol Biochem Behav* **53**:303–308.
- Meythaler JM, McCary A, and Hadley MN (1997) Prospective assessment of continuous intrathecal infusion of baclofen for spasticity caused by acquired brain injury: a preliminary report. *J Neurosurg* **87**:415–419.
- Mondadori C, Hengerer B, Ducret T, and Borkowski J (1994) Delayed emergence of effects on memory-enhancing drugs: implications for the dynamics of long-term memory. *Proc Natl Acad Sci USA* **91**:2041–2045.
- Mondadori C, Jaekel J, and Preiswerk G (1993) CGP 36742: the first orally active GABA<sub>B</sub> blocker improves the cognitive performance of mice, rats and rhesus monkeys. *Behav Neural Biol* **60**:62–68.
- Mondadori C, Moebius HJ, and Borkowski J (1996a) The GABA<sub>B</sub> receptor antagonist CGP36742 and the nootropic oxiracetam facilitate the formation of long-term memory. *Behav Brain Res* **77**:223–225.
- Mondadori C, Moebius HJ, and Zingg M (1996b) CGP36742, an orally active GABA<sub>B</sub> receptor antagonist, facilitates memory in a social recognition test in rats. *Behav Brain Res* **77**:227–229.
- Moran JM, Enna SJ, and McCarson KE (2001) Developmental regulation of GABA<sub>B</sub> receptor function in rat spinal cord. *Life Sci* **68**:2287–2295.
- Muhyaddin M, Roberts PJ, and Woodruff GN (1982) Presynaptic gamma-aminobutyric acid receptors in the rat anococcygeus muscle and their antagonism by 5-aminovaleric acid. *Br J Pharmacol* **77**:163–168.
- Munzar P, Kutkat SW, Miller CR, and Goldberg SR (2000) Failure of baclofen to modulate discriminative-stimulus effects of cocaine or methamphetamine in rats. *Eur J Pharmacol* **408**:169–174.
- Myrick H, Henderson S, Brady KT, and Malcolm R (2001) Gabapentin in the treatment of cocaine dependence: a case series. *J Clin Psychiatry* **62**:19–23.
- Nabeshima T, Noda Y, Itoh K, and Kameyama T (1988a) Role of cholinergic and GABAergic neuronal systems in cycloheximide-induced amnesia in mice. *Pharmacol Biochem Behav* **31**:405–409.
- Nabeshima T, Noda Y, and Kameyama T (1988b) GABAergic modulation of memory with regard to passive avoidance and conditioned suppression tasks in mice. *Psychopharmacology* **94**:69–73.
- Nakagawa Y, Ishibashi Y, Yoshii T, and Tagashira E (1995) Involvement of cholinergic systems in the deficit of place learning in Morris water maze task induced by baclofen in rats. *Brain Res* **683**:209–214.
- Nakagawa Y, Sasaki A, and Takashima T (1999) The GABA<sub>B</sub> receptor antagonist CGP36742 improves learned helplessness in rats. *Eur J Pharmacol* **381**:1–7.
- Nakagawa Y and Takashima T (1997) The GABA<sub>B</sub> receptor antagonist CGP36742 attenuates the baclofen- and scopolamine-induced deficit in Morris water maze task in rats. *Brain Res* **766**:101–106.
- Nehring RB, Horikawa HPM, El Far O, Kneusel M, Brandstätter JH, Stamm S, Wischmeyer E, Betz H, and Karschin A (2000) The metabotropic GABA<sub>B</sub> receptor directly interacts with the activating transcription factor 4. *J Biol Chem* **275**:35185–35191.
- Newberry NR and Nicoll RA (1984) Direct hyperpolarizing action of baclofen on hippocampal pyramidal cells. *Nature (Lond)* **308**:450–452.
- Ng GYK, Bertrand S, Sullivan R, Ethier N, Wang J, Yergey J, Belley M, Trimble L, Bateman K, Alder L, et al. (2001) Gamma-aminobutyric acid type B receptors with specific heterodimer composition and postsynaptic actions in hippocampal neurons are targets of anticonvulsant gabapentin action. *Mol Pharmacol* **59**:144–152.
- Ng GYK, Clark J, Coulombe N, Ethier N, Hebert TE, Sullivan R, Kargman S, Chateaufauf A, Tsukamoto N, McDonald T, et al. (1999) Identification of a GABA<sub>B</sub> receptor subunit, gb2, required for functional GABA<sub>B</sub> receptor activity. *J Biol Chem* **274**:7607–7610.
- Nibuya M, Morinobu S, and Duman RS (1995) Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci* **15**:7539–7547.
- Nickerson RB, Atchison JW, Van Hoose JD, and Hayes D (1997) Hiccups associated with lateral medullary syndrome. A case report. *Am J Phys Med Rehabil* **76**:144–146.
- Noguchi J and Yamashita H (1999) Baclofen inhibits postsynaptic voltage-dependent calcium currents of supraoptic nucleus neurons isolated from young rats. *Biomed Res (Tokyo)* **20**:239–247.
- Ochs G, Naumann C, Dimitrijevic M, and Sindou M (1999) Intrathecal baclofen therapy for spinal origin spasticity: spinal cord injury, spinal cord disease and multiple sclerosis. *Neuromodulation* **2**:108–119.
- Ochs G, Struppler A, Meyerson BA, Linderth G, Gybels J, Gardner BP, Teddy P, Jamous A, and Weimann P (1989) Intrathecal baclofen for long-term treatment of spasticity: a multi-centre study. *J Neural Neurosurg Psychiatry* **52**:933–939.
- Odagaki Y and Koyama T (2001) Identification of G alpha subtype(s) involved in gamma-aminobutyric acid<sub>B</sub> receptor-mediated high-affinity guanosine triphosphatase activity in rat cerebral cortical membranes. *Neurosci Lett* **297**:137–141.
- Odagaki Y, Nishi N, and Koyama T (2000) Functional coupling of GABA<sub>B</sub> receptors with G proteins that are sensitive to N-ethylmaleimide treatment, suramin and benzalkonium chloride in rat cerebral cortical membranes. *J Neural Transm* **107**:1101–1116.
- Olianas MC and Onali P (1999) GABA<sub>B</sub> receptor-mediated stimulation of adenylyl cyclase activity in membranes of rat olfactory bulb. *Br J Pharmacol* **126**:657–664.
- Olpe HR, Karlsson G, Pozza MF, Brugger F, Steinmann M, Van Riezen H, Fagg G, Hall RG, Froestl W, and Bittiger H (1990) CGP 35348: a centrally active blocker of GABA<sub>B</sub> receptors. *Eur J Pharmacol* **187**:27–38.
- Olpe HR, Steinmann MW, Ferrat T, Pozza MF, Greiner K, Brugger F, Froestl W, Mickel SJ, and Bittiger H (1993a) The actions of orally active GABA<sub>B</sub> receptor antagonists on GABAergic transmission in vivo and in vitro. *Eur J Pharmacol* **233**:179–186.
- Olpe HR, Woerner W, and Ferrat T (1993b) Stimulation parameter determine role of GABA<sub>B</sub> receptors in long-term potentiation. *Experientia (Basel)* **49**:542–546.
- Ong J, Bexis S, Marino V, Parker DAS, Kerr DIB, and Froestl W (2001) Comparative activities of the enantiomeric GABA<sub>B</sub> receptor agonists CGP 44532 and 44533 in central and peripheral tissues. *Eur J Pharmacol* **412**:27–37.
- Ong J and Kerr DIB (1990) GABA-receptors in peripheral tissues. *Life Sci* **46**:1489–1501.
- Ong J, Kerr DIB, Bittiger H, Waldmeier PC, Baumann PA, Cooke NG, Mickel SJ, and Froestl W (1998a) Morpholin-2-yl-phosphinic acids are potent GABA<sub>B</sub> receptor antagonists in rat brain. *Eur J Pharmacol* **362**:27–34.
- Ong J, Marino V, Parker DAS, and Kerr DIB (1998b) Differential effects of phosphonic analogues of GABA on GABA<sub>A</sub> autoreceptors in rat neocortical slices. *Naunyn-Schmiedeberg's Arch Pharmacol* **357**:408–412.
- Opolski A, Mazurkiewicz M, Wietrak J, Kleinrok Z, and Radzikowski C (2000) The role of GABA-ergic system in human mammary gland pathology and in growth of transplantable murine mammary cancer. *J Exp Clin Cancer Res* **19**:383–390.
- Orsnes G, Crone C, Krarup C, Petersen N, and Nielsen J (2000a) The effect of baclofen on the transmission in spinal pathways in spastic multiple sclerosis patients. *Clin Neurophysiol* **111**:1372–1379.
- Orsnes GB, Sorensen PS, Larsen TK, and Ravnborg M (2000b) Effect of baclofen on gait in spastic MS patients. *Acta Neurol Scand* **101**:244–248.
- Oshima T, Sakamoto M, Tatsuta H, and Arita H (1998) GABAergic inhibition of hiccup-like reflex induced by electrical stimulation in medulla of cats. *Neurosci Res* **30**:287–293.
- Pagano A, Rovelli G, Mosbacher J, Lohmann T, Duthey B, Stauffer D, Ristig D, Schuler V, Meigel I, Lampert C, et al. (2001) C-terminal interaction is essential for surface trafficking but not for heteromeric assembly of GABA<sub>B</sub> receptors. *J Neurosci* **21**:1189–1202.
- Paret G, Tirosh R, Ben Zeev B, Vardi A, Brandt N, and Barzilay Z (1996) Intrathecal baclofen for severe torsion dystonia in a child. *Acta Paediatr* **85**:635–637.
- Patel S, Naem S, Kesingland A, Froestl W, Capogna M, Urban L, and Fox A (2001) The effects of GABA<sub>B</sub> agonists and gabapentin on mechanical hyperalgesia in models of neuropathic and inflammatory pain in the rat. *Pain* **90**:217–226.



- Penn RD and Mangieri EA (1993) Stiff-man syndrome treated with intrathecal baclofen. *Neurology* **43**:2412.
- Penn RD, Savoy SM, Corcos D, Latash M, Gottlieb G, Parke B, and Kroin JS (1989) Intrathecal baclofen for severe spinal spasticity. *N Engl J Med* **320**:1517–1521.
- Pfaff T, Malitschek B, Kaupmann K, Prezeau L, Pin JP, Bettler B, and Karschin A (1999) Alternative splicing generates a novel isoform of the rat metabotropic GABA<sub>B</sub>R1 receptor. *Eur J Neurosci* **11**:2874–2882.
- Phelan KD (1999) N-Ethylmaleimide selectively blocks presynaptic GABA-B autoreceptor but not heteroreceptor-mediated inhibition in adult rat striatal slices. *Brain Res* **847**:308–313.
- Pilc A and Lloyd KG (1984) Chronic antidepressants and GABA "B" receptors: a GABA hypothesis of antidepressant drug action. *Life Sci* **35**:2149–2154.
- Pittaluga A, Feligioni M, Ghersi C, Gemignani A, and Raiteri M (2001) Potentiation of NMDA receptor function through somatostatin release: a possible mechanism for the cognition-enhancing activity of GABA<sub>B</sub> receptor antagonists. *Neuropharmacology* **41**:301–310.
- Poorkhalkali N, Juneblat K, Jönsson AC, Lindberg M, Karlsson O, Wallbrandt P, Ekstrand J, and Lehmann A (2000) Immunocytochemical distribution of the GABA<sub>B</sub> receptor splice variants GABA<sub>B</sub>R1a and R1b in the rat CNS and dorsal root ganglia. *Anat Embryol* **201**:1–13.
- Pozza MF, Manuel NA, Steinmann M, Froestl W, and Davies CH (1999) Comparison of antagonist potencies at pre- and post-synaptic GABA<sub>B</sub> receptors at inhibitory synapses in the CA1 region of the rat hippocampus. *Br J Pharmacol* **127**:211–219.
- Price GW, Kelly JS, and Bowery NG (1987) The location of GABA<sub>B</sub> receptor binding sites in mammalian spinal cord. *Synapse* **1**:530–538.
- Princivalle A, Spreafico R, Bowery N, and de Curtis M (2000) Layer-specific immunocytochemical localization of GABA<sub>B</sub>R1a and GABA<sub>B</sub>R1b receptors in the rat piriform cortex. *Eur J Neurosci* **12**:1516–1520.
- Princivalle AP, Pangalos MN, Bowery NG, and Spreafico R (2001) Distribution of GABA<sub>B</sub>(1a), GABA<sub>B</sub>(1b) and GABA<sub>B</sub>2 receptor protein in cerebral cortex and thalamus of adult rats. *Neuroreport* **12**:591–595.
- Prosser HM, Gill CH, Hirst WD, Grau E, Robbins M, Calver A, Soffin EM, Farmer CE, Lanneau C, Gray J, et al. (2001) Epileptogenesis and enhanced prepulse inhibition in GABA<sub>B</sub>1-deficient mice. *Mol Cell Neurosci* **10**:1–10.
- Przesmycki K, Dzieciuch JA, Czuczwar SJ, and Kleinrok Z (1998) An isobolographic analysis of drug interaction between intrathecal clonidine and baclofen in the formalin test in rats. *Neuropharmacology* **37**:207–214.
- Raiteri M, Bonanno G, Paudice P, Cavazzini P, and Schmid G (1996) Human brain cholecystokinin release of cholecystokinin-like immunoreactivity (CCK-LI) from isolated cortical nerve endings and its modulation through GABA<sub>B</sub> receptors. *J Pharmacol Exp Ther* **228**:747–751.
- Raiteri M, Pellegrini G, Cantoni C, and Bonanno G (1989) A novel type of GABA receptor in rat spinal cord. *Naunyn-Schmiedeberg's Arch Pharmacol* **340**:666–670.
- Richards DA and Bowery NG (1996) Anti-seizure effects of the GABA<sub>B</sub> antagonist, SCH-50911, in the genetic absence epilepsy rat from Strasbourg (GAERS). *Pharmacol Rev Commun* **8**:227–230.
- Riley RC, Trafton JA, Chi SI, and Basbaum AI (2001) Presynaptic regulation of spinal cord tachykinin signaling via GABA<sub>B</sub> but not GABA<sub>A</sub> receptor activation. *Neuroscience* **103**:725–737.
- Roberts DCS and Andrews MM (1997) Baclofen suppression of cocaine self-administration: demonstration using a discrete trials procedure. *Psychopharmacology* **131**:271–277.
- Saha N, Chugh Y, Sankaranaryanan A, and Sharma PL (1993) Effects of post-training administration of (–)-baclofen and chlorthalidoxepoxide on memory retention in ICRC Swiss mice: interactions with GABA<sub>A</sub> and GABA<sub>B</sub> receptor antagonists. *Pharmacol Toxicol* **72**:159–162.
- Saint DA, Thomas T, and Gage PW (1990) GABA<sub>B</sub> agonists modulate a transient potassium current in cultured mammalian hippocampal neurons. *Neurosci Lett* **118**:9–13.
- Sandyk R and Gillman MA (1985) Baclofen-induced memory impairment. *Clin Neuropharmacol* **8**:294–295.
- Santos AE, Carvalho CM, Macedo TA, and Carvalho AP (1995) Regulation of intracellular [Ca<sup>2+</sup>] and GABA release by presynaptic GABA<sub>B</sub> receptors in rat cerebrocortical synaptosomes. *Neurochem Int* **27**:397–406.
- Sawynok J and Dickson C (1985) D-Baclofen is an antagonist at baclofen receptors mediating antinociception in the spinal cord. *Pharmacology* **31**:248–259.
- Schuler V, Lüscher C, Blanchet C, Klix N, Sansig G, Klebs K, Schmutz M, Heid J, Gentry C, Urban L, et al. (2001) Epilepsy, hyperalgesia, impaired memory and loss of pre- and postsynaptic GABA<sub>B</sub> responses in mice lacking GABA<sub>B</sub>(1). *Neuron* **31**:47–58.
- Schulz S and Holt V (1998) Opioid withdrawal activates MAPkinase in locus coeruleus neurons in morphine dependent rats in vivo. *Eur J Neurosci* **10**:1196–1201.
- Schwarz DA, Barry G, Eliasof SD, Petroski RE, Conlon PJ, and Maki RA (2000) Characterization of gamma-aminobutyric acid receptor GABA<sub>B</sub>(1e), a GABA<sub>B</sub>(1) splice variant encoding a truncated receptor. *J Biol Chem* **275**:32174–32181.
- Scott RH, Wootton JF, and Dolphin AC (1990) Modulation of neuronal T-type calcium channel currents by photoactivation of intracellular guanosine 5'-O-(3-thio) triphosphate. *Neuroscience* **38**:285–294.
- Seitz RJ, Blank B, Kiwit JCW, and Benecke R (1995) Stiff-person syndrome with anti-glutamic acid decarboxylase autoantibodies: complete remission of symptoms after intrathecal baclofen administration. *J Neurol* **242**:618–622.
- Serrano I, Ruiz RM, Serrano JS, and Fernandez A (1992) GABAergic and cholinergic mediation in the antinociceptive action of homotaurine. *Gen Pharmacol* **23**:421–426.
- Sharma AC and Kulkarni SK (1990) Evidence for GABA-BZ receptor modulation in short-term memory passive avoidance task paradigm in mice. *Meth Find Exp Clin Pharmacol* **12**:175–180.
- Sharma AC and Kulkarni SK (1993) (±)Baclofen sensitive scopolamine-induced short-term memory deficits in mice. *Indian J Exp Biol* **31**:348–352.
- Shen W and Slaughter MM (1999) Metabotropic GABA receptors facilitate L-type and inhibit N-type calcium channels in single salamander retinal neurons. *J Physiol* **516**:711–718.
- Shoaib M, Swanner LS, Beyer CE, Goldberg SR, and Schindler CW (1998) The GABA<sub>B</sub> agonist baclofen modifies cocaine self-administration in rats. *Behav Pharmacol* **9**:195–206.
- Sidel ES, Tilton HA, McLamb RL, Wilson WA, and Swartzwelder HS (1988) Potential interactions between GABA<sub>B</sub> and cholinergic systems: baclofen augments scopolamine-induced performance deficits in the eight-arm radial maze. *Psychopharmacology* **96**:116–120.
- Sloviter RS, Ali-Akbarian L, Elliott RC, Bowery BJ, and Bowery NG (1999) Localization of GABA<sub>B</sub> (R1) receptors in the rat hippocampus by immunocytochemistry and high resolution autoradiography, with specific reference to its localization in identified hippocampal interneuron subpopulations. *Neuropharmacology* **38**:1707–1721.
- Smid SD and Blackshaw LA (2000) Vagal neurotransmission to the ferret lower oesophageal sphincter: inhibition via GABA<sub>B</sub> receptors. *Br J Pharmacol* **131**:624–630.
- Smith GD, Harrison SM, Birch PJ, Elliott PJ, Malcangio M, and Bowery NG (1994) Increased sensitivity to the antinociceptive activity of (±)-baclofen in an animal model of chronic neuropathic, but not chronic inflammatory hyperalgesia. *Neuropharmacology*, **33**:1103–1108.
- Snead OC (1992) Evidence for GABA<sub>B</sub>-mediated mechanisms in experimental generalized absence seizures. *Eur J Pharmacol* **213**:343–349.
- Snead OC (2000) Evidence for G protein-coupled gamma-hydroxybutyric acid receptor. *J Neurochem* **75**:1986–1996.
- Soubrie P, Simon P, and Boissier JJR (1976) Conditioned suppression: dissociation of learning in baclofen treated rats. *Experientia (Basel)* **32**:1323–1324.
- Stackman RW and Walsh TJ (1994) Baclofen produces dose-related working memory impairments after intraseptal injection. *Behav Neural Biol* **61**:181–185.
- Stäubli U, Scaffidi J, and Chun D (1999) GABA<sub>B</sub> receptor antagonism: facilitatory effects on memory parallel those on LTP induced by TBS but not HFS. *J Neurosci* **19**:4609–4615.
- Sullivan R, Chateaufneuf A, Coulombe N, Kolakowski LF, Johnson MP, Hebert TE, Ethier N, Bellef M, Metters K, Abramovitz M, et al. (2000) Coexpression of full-length gamma-aminobutyric acid<sub>B</sub> (GABA<sub>B</sub>) receptors with truncated receptors and metabotropic glutamate receptor 4 supports the GABA<sub>B</sub> heterodimer as the functional receptor. *J Pharmacol Exp Ther* **293**:460–467.
- Swartzwelder HS, Tilton HA, McLamb RL, and Wilson WA (1987) Baclofen disrupts passive avoidance retention in rats. *Psychopharmacology* **92**:398–401.
- Taira T, Kawamura H, Tanikawa T, Iseki H, Kawabatake H, and Takakura K (1995) A new approach to control central deafferentation pain: spinal intrathecal baclofen. *Stereotact Funct Neurosurg* **65**:101–105.
- Takahashi T, Kajikawa Y, and Tsujimoto T (1998) G-protein-coupled modulation of presynaptic calcium currents and transmitter release by a GABA<sub>B</sub> receptor. *J Neurosci* **18**:3138–3146.
- Takenaka K, Sasaki S, Nakamura K, Uchida A, Fujita H, Itoh H, Nakata T, Takeda K, and Nakagawa M (1995) Hypothalamic and medullary GABA<sub>A</sub> and GABA<sub>B</sub>ergic systems differently regulate sympathetic and cardiovascular systems. *Clin Exp Pharmacol Physiol Suppl* **1**:S48–S50.
- Tang AC and Hasselmo ME (1996) Effect of long term baclofen treatment on recognition memory and novelty detection. *Behav Brain Res* **74**:145–152.
- Teoh H, Malcangio M, and Bowery NG (1996) GABA, glutamate and substance P-like immunoreactivity release: effects of novel GABA<sub>B</sub> antagonists. *Br J Pharmacol* **118**:1153–1160.
- Thomas DA, McGowan MK, and Hammond DL (1995) Microinjection of baclofen in the ventromedial medulla of rats: antinociception at low doses and hyperalgesia at high doses. *J Pharmacol Exp Ther* **275**:274–284.
- Thomas DA, Navarrete IM, Graham BA, McGowan MK, and Hammond DL (1996) Antinociception produced by systemic R(+)-baclofen hydrochloride is attenuated by CGP 35348 administered to the spinal cord or ventromedial medulla of rats. *Brain Res* **718**:129–137.
- Thompson SM and Gähwiler BH (1992) Comparison of the actions of baclofen at pre- and postsynaptic receptors in the rat hippocampus in vitro. *J Physiol* **451**:329–345.
- Todde S, Moresco RM, Froestl W, Stampf P, Matarrese M, Carpinelli A, Magni F, Galli Kienle M, and Fazio F (2000) Synthesis and in vivo evaluation of [<sup>11</sup>C]CGP62349, a new GABA<sub>B</sub> receptor antagonist. *Nucl Med Biol* **27**:565–569.
- Towers S, Princivalle A, Billinton A, Edmunds M, Bettler B, Urban L, Castro-Lopes J, and Bowery NG (2000) GABA<sub>B</sub> receptor protein and mRNA distribution in rat spinal cord and dorsal root ganglia. *Eur J Neurosci* **12**:3201–3210.
- Trampitsch E, Krumpal R, Likar R, Oher M, and Gulle D (2000) Continuous intrathecal administration of baclofen in severe tetanus. *Anaesthesiol Intensivmed Notfallmed Schmerzther* **35**:532–533.
- Tremblay E, Ben-Ari Y, and Roisin MP (1995) Different GABA<sub>B</sub>-mediated effects on protein kinase C activity and immunoreactivity in neonatal and adult rat hippocampal slices. *J Neurochem* **65**:863–870.
- Vaught JL, Pelley K, Costa LG, Setler P, and Enna SJ (1985) A comparison of the antinociceptive responses to the GABA-receptor agonists THIP and baclofen. *Neuropharmacology* **24**:211–216.
- Vergnes M, Boehrer A, Simler S, Bernasconi R, and Marescaux C (1997) Opposite effects of GABA<sub>B</sub> receptor antagonists on absences and convulsive seizures. *Eur J Pharmacol* **332**:245–255.
- Voisin DL and Nagy F (2001) Sustained L-type calcium currents in dissociated deep dorsal horn neurons of the rat: characteristics and modulation. *Neuroscience* **102**:461–472.
- Wagner PG and Dekin MS (1993) GABA<sub>B</sub> receptors are coupled to a barium-insensitive outward rectifying potassium conductance in premotor respiratory neurons. *J Neurophysiol* **69**:286–289.
- Wagner PG and Dekin MS (1997) cAMP modulates an S-type K<sup>+</sup> channel coupled to GABA<sub>B</sub> receptors in mammalian respiratory neurons. *Neuroreport* **8**:1667–1670.
- Waldmeier PC, Wicki P, Feldtrauer JJ, Mickel SJ, Bittiger H, and Baumann P (1994) GABA and glutamate release affected by GABA<sub>B</sub> receptor antagonists with similar

- potency: no evidence for pharmacologically different presynaptic receptors. *Br J Pharmacol* **113**:1515–1521.
- Watson JM, Burton MJ, Price GW, Jones BJ, and Middlemiss DN (1996) GR127935 acts as a partial agonist at recombinant human 5HT<sub>1D</sub> alpha and 5HT<sub>1D</sub> beta receptors. *Eur J Pharmacol* **314**:365–372.
- Wei K, Eubanks JH, Francis J, Jia Z, and Snead OC (2001a) Cloning and tissue distribution of a novel isoform of the rat GABA<sub>B</sub>R1 receptor subunit. *Neuroreport* **12**:833–837.
- Wei K, Jia Z, Wang YT, Yang J, Liu CC, and Snead OC (2001b) Cloning and characterization of a novel variant of rat GABA<sub>B</sub>R1 with a truncated C-terminus. *Brain Res Mol Brain Res* **89**:103–110.
- White JH, McIlhinney RAJ, Wise A, Ciruela F, Chan WY, Emson PC, Billinton A, and Marshall FH (2000) The GABA<sub>B</sub> receptor interacts directly with the related transcription factors CREB2 and ATFx. *Proc Natl Acad Sci USA* **97**:13967–13972.
- White JH, Wise A, Main MJ, Green A, Fraser NJ, Disney GH, Barnes AA, Emson P, Foord SM, and Marshall FH (1998) Heterodimerization is required for the formation of a functional GABA<sub>B</sub> receptor. *Nature (Lond)* **396**:679–682.
- Wiesenfeld-Hallin Z, Aldskogius H, Grant G, Hao JX, Hökfelt T, and Xu XJ (1997) Central inhibitory dysfunctions: mechanisms and clinical implications. *Behav Brain Sci* **20**:420–425.
- Wojcik WJ and Neff NH (1984) Gamma-aminobutyric acid B receptors are negatively coupled to adenylate cyclase in brain and in the cerebellum these receptors may be associated with granule cells. *Mol Pharmacol* **25**:24–28.
- Wood MD, Murkitt KL, Rice SQ, Testa T, Punia PK, Stammers M, Jenkins O, Elshourbagy NA, Shabon U, Taylor SJ, et al. (2000) The human GABA<sub>B1b</sub> and GABA<sub>B2</sub> heterodimeric recombinant receptor shows low sensitivity to phaclofen and saclofen. *Br J Pharmacol* **131**:1050–1054.
- Wu LG and Saggau P (1995) GABA<sub>B</sub> receptor-mediated presynaptic inhibition in guinea-pig hippocampus is caused by reduction of presynaptic Ca<sup>2+</sup> influx. *J Physiol* **485**:649–657.
- Xi ZX and Stein EA (1999) Baclofen inhibits heroin self-administration behavior and mesolimbic dopamine release. *J Pharmacol Exp Ther* **290**:1369–1374.
- Xi ZX and Stein EA (2000) Increased mesolimbic GABA concentration blocks heroin self-administration in the rat. *J Pharmacol Exp Ther* **294**:613–619.
- Xu J and Wojcik WJ (1986) Gamma aminobutyric acid B receptor-mediated inhibition of adenylate cyclase in cultured cerebellar granule cells: blockade by islet-activating protein. *J Pharmacol Exp Ther* **239**:568–573.
- Yamada K, Yu B, and Gallagher JP (1999) Different subtypes of GABA<sub>B</sub> receptors are present at pre- and postsynaptic sites within the rat dorsolateral septal nucleus. *J Neurophysiol* **81**:2875–2883.
- Yu ZF, Cheng GJ, and Hu BR (1997) Mechanism of colchicine impairment on learning and memory and protective effect of CGP36742 in mice. *Brain Res* **750**:53–58.
- Zaleski MJB, Filho JRN, Lemos T, and Morato GS (2001) GABA<sub>B</sub> receptors play a role in the development of tolerance to ethanol in mice. *Psychopharmacology* **153**:415–424.