

Recent advances in GABA_B receptors: from pharmacology to molecular biology

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KEY WORDS GABA; GABA-B receptors; GABA_BR2 receptors; baclofen; GABA agonists; GABA antagonists

ABSTRACT

Bicuculline-insensitive receptors for the inhibitory neurotransmitter γ -aminobutyric acid (GABA), GABA_B receptors, are a distinct subclass of receptors that mediate depression of synaptic transmission and contribute to neuronal inhibition. When activated, these receptors reduce transmission at excitatory and inhibitory synapses, as a result of an increase in K⁺ conductance, or a decrease in voltage-dependent Ca²⁺ currents. They are also linked to G-proteins, or intracellular effector systems in a very complex manner. The recent development of highly specific and potent agonists and antagonists for these receptors has led to a much better understanding of their physiology and pharmacology, including their heterogeneity, as well as their molecular biology. Over the past year, expression and cloning studies have contributed to major advances in characterizing GABA_B receptor structure, with the discovery of the amino acid sequences of GABA_BR1a/R1b splice variants and GABA_BR2 receptors. These isoforms are widely distributed throughout the nervous system, and can be functionally expressed. Importantly, GABA_BR2 receptors can form a heteromeric assembly with GABA_BR1 proteins to operate as a heterodimer that displays robust coupling to inward-rectifying K⁺ channels, as well as inhibition of forskolin-stimulated adenylate cyclase activity. Further insights underlying the mechanisms of GABA_B receptor functions can now be gained, leading ultimately to the therapeutic potential of drugs acting at these sites. It is increasingly clear that new information on GABA_B receptor molecular structure will provide a plethora of targets for pharmaceutical inter-

vention in areas such as drug addiction, nociception and absence seizures. This review summarizes the renewed efforts, and highlights the recent advances emerging in this field.

INTRODUCTION

Half a century ago, γ -aminobutyric acid (GABA) was first identified in brain extracts, independently in two laboratories by Eugene Roberts, and by Awapara and colleagues^[1,2], and was later found to be an inhibitory transmitter at the crustacean neuromuscular junction^[3] where its actions are blocked by the known convulsant picrotoxin. Despite accumulating evidence that a picrotoxin-insensitive agent was a strong candidate for an inhibitory neurotransmitter in the mammalian central nervous system, GABA did not become accepted in this inhibitory role for some twenty years after its original demonstration in the brain, due to doubts about the suitability and specificity of picrotoxin as a GABA antagonist. Indeed it was not until the isoquinoline alkaloid bicuculline was established as an antagonist that GABA became accepted as an inhibitory transmitter in the mammalian central nervous system. Interestingly, the GABA-antagonist activity of bicuculline was, in fact, first shown by Kang Tsou at the Shanghai Institute of Materia Medica in the mid 1960's, but this work was not then published, and the GABA-antagonist properties of bicuculline did not become widely known until the work of Johnston in 1970^[4]. Even then there were reports that bicuculline was not a good GABA antagonist^[5], since some depressant responses to GABA persisted in its presence. In reality, these were probably responses mediated by what became known as bicuculline-insensitive GABA receptors.

A bicuculline-insensitive action of GABA, shortening of the Ca²⁺ component of action potentials in embryonic dorsal-root ganglia, was earlier described by Dunlap and Fischbach^[6]; and subsequently seen with baclofen^[7]. Such bicuculline-insensitive receptors were eventually characterized definitively by Bowery *et al*^[8],

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Received 1999-09-13 Accepted 1999-11-04

leading to the present recognition of two GABA receptor types GABA_A and GABA_B, the latter being the bicuculline-insensitive receptors, for which baclofen (β -p-chlorophenyl-GABA) is a specific agonist. Actually, baclofen was first synthesised by H. Keberle of Ciba-Geigy in 1962^[9], and entered clinical use as an antispastic agent soon after, but its mode of action remained unknown until the seminal study by Bowery and his colleagues^[8,10], showing that baclofen depresses transmitter release as does GABA itself in the presence of bicuculline. The bicuculline-insensitive GABA receptor responsible for this action was designated the GABA_B receptor^[11], whilst the more familiar bicuculline-sensitive receptor became known as the GABA_A receptor.

Hill *et al*^[12] also provided the first evidence that GABA_B receptors are in some way related to a broad family of G-protein-coupled receptors that are characterized by their sensitivity to guanine nucleotides. This, together with cation sensitivity of their agonist binding^[13], clearly marks the GABA_B receptors as belonging to an entirely different class from that of the GABA_A receptor. The latter are now designated as 'ionotropic', being linked to integral Cl⁻ channels, whereas the GABA_B receptors are recognised as metabotropic and coupled to GTP-binding proteins which mediate their actions. GABA_B receptors can be further subdivided into presynaptic, including autoreceptors, and postsynaptic receptors, which are linked to either Ca²⁺ or K⁺ channels (Fig 1). It was Thalmann^[14,15] who originally showed that GABA_B receptor-mediated neurophysiological responses in the central nervous system are sensitive to pertussis-toxin and GTP γ S, indicative of Gi protein involvement. There have been few formal studies of G-protein involvement in GABA_B receptor function, but amiloride modulation of baclofen actions in the neocortex has been described^[16], as has the potentiation of baclofen activation by G-proteins with aluminium fluoride, and their irreversible blockade by N-ethylmaleimide^[17]; all of which are indicative of allosteric modification of GABA_B receptor-associated G-proteins which are coupled to K⁺ and Ca²⁺ channels.

GABA_B RECEPTOR LIGANDS

At first, the major difficulty in establishing the functional significance of these new GABA_B receptors was the absence of any suitable antagonist. Originally, Muhyaddin *et al*^[18] identified the GABA homologue 5-

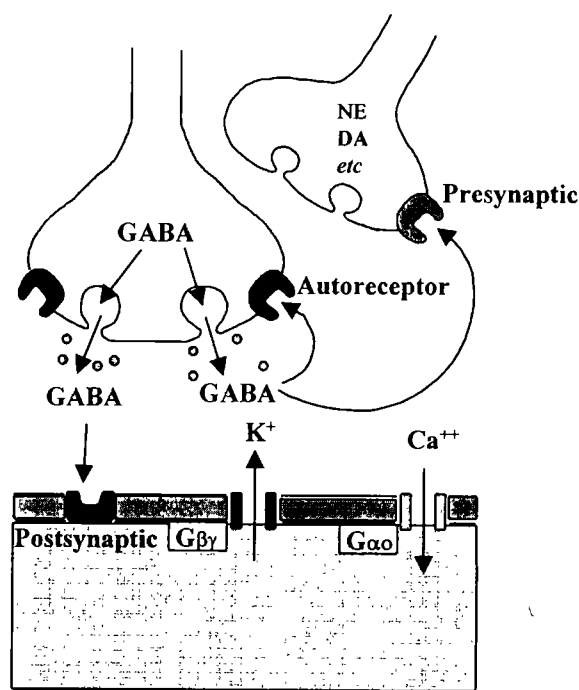


Fig 1. Schematic representation of GABA_B receptor subtypes, showing presynaptic, postsynaptic and autoreceptors regulating transmitter release, and their coupling to Ca²⁺ or K⁺ channels. The neurotransmitters involved include GABA itself, NE (norepinephrine), and DA (dopamine).

amino-valeric acid (DAVA) as a GABA_B receptor antagonist, albeit weak, at presynaptic GABA receptors on the motor nerve of the rat anococcygeus muscle which lacks GABA_A receptors, and we confirmed its GABA_B receptor antagonist action in the guinea-pig ileum^[19], as did Luzzi *et al*^[20]. Nevertheless, DAVA, in the presence of bicuculline to block GABA_A receptor-mediated actions, was successfully used in 1985 to demonstrate GABA_B receptors in the hippocampus where it antagonised the depression of CA₁ field potentials by baclofen^[21]. This was the first demonstration of a GABA_B receptor-mediated action in the central nervous system, induced by an agonist and abolished by an antagonist. The problem of finding a selective GABA_B receptor antagonist devoid of any actions at GABA_A receptors remained for a number of years, including the suggestion that the GABA analogue homotaurine (3-aminopropanesulphonic acid; 3-APS) was a more potent antagonist than DAVA^[22]; unfortunately 3-APS was also a very potent GABA_A receptor agonist. Baclofen remains the prototypical GABA_B receptor agonist, although the sulphonic acid analogue (siclofen; Fig 2) is also active^[23] as is the thienyl analogue 4-amino-3-(5-chlorothien-2-yl)-butanoic acid^[24].

The chemical structures of some of these compounds are illustrated in Fig 2.

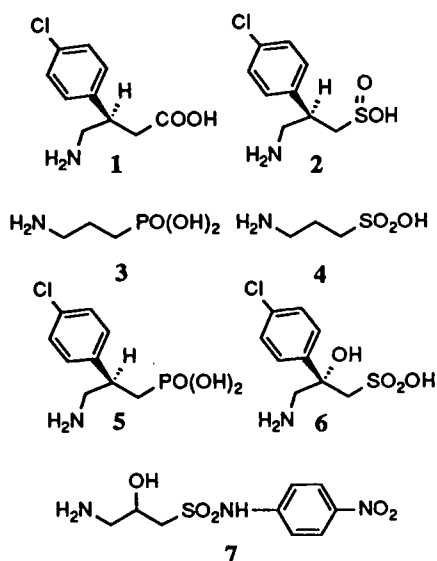


Fig 2. Chemical structures of ligands active at GABA_B receptor sites. The compounds are baclofen (1), siclofen (2), 3-aminopropylphosphonic acid (3), 3-aminopropylsulphonic acid (4), phaclofen (5), 2-hydroxysaclofen (6) and 3-amino-2-hydroxy-N-(4-nitrophenyl)-propanesulphonamide (AHPNS) (7). Baclofen and siclofen are agonists, whereas 3-aminopropylphosphonic acid is a partial agonist, whilst AHPNS is relatively inactive at GABA_B autoreceptors but active at heteroreceptors. The prototypic antagonists phaclofen and 2-hydroxysaclofen are derivatives of baclofen.

It was already known that the phosphono analogue of GABA, 3-aminopropylphosphonic acid (Fig 2), was active in binding and functional studies on peripheral GABA_B receptors^[25,26] whilst the higher homologue 4-aminobutylphosphonic acid is also a useful GABA_B receptor antagonist devoid of activity at GABA_A receptor sites^[27,28]. The phosphonic analogue of baclofen as a possible GABA_B receptor antagonist had already occurred to us, following the success of the γ -phosphono analogue of glutamic acid as a glutamate antagonist^[29]. Synthesis of phaclofen, this phosphonic analogue of baclofen (Fig 2), was achieved by our group in 1987^[30,31], and its utility as a specific GABA_B receptor antagonist was soon established (see reviews^[32-34]). Following this success, we investigated the corresponding sulphonic analogues of GABA and baclofen. In particular, 3-amino-2-hydroxy-propylsulphonic acid was more potent, and with less confounding GABA_A receptor actions than the

lead compound 3-APS. The corresponding sulphonic analogues of baclofen were then prepared, providing saclofen and 2-hydroxy-saclofen (Fig 2) with a 10 fold improvement in antagonist potency relative to the original phosphonic antagonists^[35-37]. Prominent among the advances made in GABA_B neurophysiology/pharmacology using these compounds has been the demonstration that the delayed hyperpolarizations, seen following activation of GABAergic pathways in many brain areas, are in fact GABA_B receptor-mediated and due to opening of inward-rectifier K⁺ channels, whereas GABA_B receptor-mediated presynaptic inhibition is due to reduced influx of Ca²⁺ at the terminals (see recent reviews^[38,39]). The latter action has recently been found in the crayfish opener muscle^[40], illustrating the phylogenetic antiquity of GABA_B receptors; indeed, GABA_B receptor genes have been described in the nematode worm *Caenorhabditis elegans*^[41]. Our development of phaclofen, saclofen and 2-hydroxy-saclofen thus provided suitable GABA_B receptor antagonists that have been used in a great variety of studies on these receptors throughout the 1990's, and marked the beginning of a modern era in neuropharmacological and neurophysiological studies on GABA_B receptors in this last decade.

With all the earlier phosphonic and sulphonic analogues of GABA and baclofen, their utility as GABA_B receptor antagonists was limited by low potency and poor penetration into the brain. In a study of phosphonic analogues of GABA, including 3-aminopropylphosphonic acid^[25] (Fig 2), it was pointed out that phosphinic acids would actually be closer bioisosteres of carboxylates than phosphonic and sulphonic analogues. This idea was originally explored by Dingwall *et al*^[42] who developed improved synthetic methods for the GABA analogue 3-aminopropylphosphinic acid (Fig 3) and related compounds. A sample of this analogue was prepared for us by G Hofer (The University of Sydney), which proved to be a more potent agonist at GABA_B receptors than baclofen or GABA itself in the guinea-pig ileum, as well as rat hippocampal cultured neurons, using material from Hall^[43]. However, in the rat neocortex, this compound displayed a lower affinity for GABA_B receptors than baclofen itself^[44], but this was later found to be due to uptake^[45]. Eventually, a precursor of the phosphinic acid CGP 35348 (3-aminopropyl-P-diethoxymethylphosphinic acid; Fig 3) was found to be a more potent GABA_B receptor antagonist than the phosphonic analogues^[46], as well as CGP 54626 (Fig 3) which is more potent than

CGP 35348. Within five years, this group from Ciba-Geigy (now Novartis), under W Froestl and SJ Mickel, established the ground-work for the modern GABA_B receptor antagonists, including very high affinity ligands developed for the detection of GABA_B receptor gene expression cloning. They also found that inclusion of the 2-hydroxypropyl moiety (as in our 2-hydroxysaclofen) improved the antagonist potency. More importantly, they established that α -Me-benzyl, with various substituents on the ring of a benzyl group at the γ -amino functionality, would provide antagonists of nanomolar potency among these phosphinic acids^[47]. This work constitutes one of the most elegant structure-action studies in recent medicinal chemistry (some of these structures are represented in Fig 3).

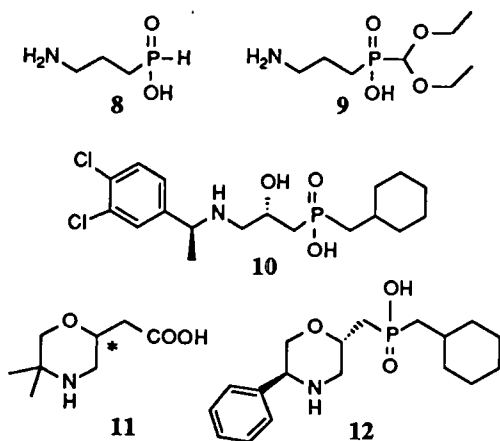


Fig 3. Phosphinic acid and morpholino-acetic acid analogues of GABA, with agonist and antagonist activities at GABA_B receptors. The GABA_B receptor agonist is 3-aminopropylphosphinic acid (8), whilst the antagonists are CGP 35348 (9), CGP 54626 (10), Sch 50911 (11) and CGP 71980 (12). CGP 35348 is the prototypical phosphinic acid antagonist, but with low potency, whilst both CGP 54626 and CGP 71980 are antagonists of high potencies. Sch 50911 is a unique antagonist, being in a locked conformation.

A further major advance in structure-action studies on antagonist ligands for GABA_B receptors came from D Blythin of Schering-Plough. By incorporating the β -hydroxy and γ -amino of the propyl skeleton into a morpholino-ring, these workers found that (+)-(*S*)-5, 5-dimethylmorpholinyl-2-acetic acid (Sch 50911; Fig 3) is a relatively potent GABA_B receptor antagonist that is active *in vivo*, and readily reversible^[48,49]. More potent phosphinic analogues of these morpholin-acetic acids such as CGP 71980 (Fig 3) were subsequently developed by

Novartis, providing us with a new series of GABA_B receptor antagonists^[50].

There has been some interest in the question of heterogeneity at GABA_B receptors, in particular the question of autoreceptors versus heteroreceptors. There is little evidence for antagonists or agonists compounds that will discriminate between these receptors, based on the large series of P-substituted phosphinic acid analogues of GABA available^[51]. Among the agonists, the phosphinic acid analogues of baclofen are paradoxically less potent than this lead itself. In practice, only the thienyl analogues of baclofen in any way approach the potency of R-(-)-baclofen, the active stereoisomer of these chiral compounds where the β carbon of the GABA backbone is chiral. The same chirality also applies for phaclofen, saclofen and 2-hydroxysaclofen, as it does for β -phenyl-GABA, the des-chloro analogue of baclofen. However, there are antagonists that will discriminate between peripheral and central GABA_B receptors. This is interesting, since GABA_B receptor-mediated depression of excitatory transmission in the enteric nervous system was among the first GABA_B receptor actions established. These compounds have been summarized elsewhere^[32-34]. Most recently, we have found a series of sulphonamide analogues of GABA and baclofen that will discriminate between GABA_B auto- and heteroreceptors. Among these 3-amino-2-hydroxy-N-(4-nitrophenyl)-propane-sulphonamide (AHPNS; Fig 2) is sufficiently potent to be used for this purpose in the central nervous system^[52].

MOLECULAR CHARACTERIZATION OF GABA_B RECEPTORS

Until recently, despite intensive efforts, the molecular identity of GABA_B receptors has remained elusive. For the past 10 years, these receptors have been the target of numerous cloning and purification attempts^[53,54,55]. Eventually, expression cloning of GABA_B receptors was made possible with the availability of high affinity GABA_B receptor antagonist ligands, CGP 64213 and CGP 71872 (Fig 4), that allowed the identification of cDNAs encoding two GABA_B receptor proteins, GABA_BR1a and GABA_BR1b of 130K and 100K, respectively^[56], illustrated in Fig 5. The GABA_BR1 gene has been mapped to human chromosome 6p21.3, in the vicinity of a susceptibility locus for idiopathic generalized epilepsies, multiple sclerosis and schizophrenia^[57,58], opening new

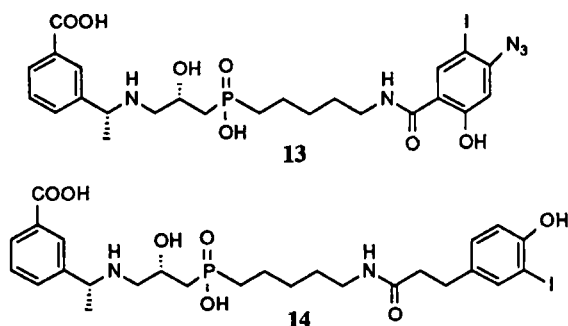


Fig 4. Chemical structures of GABA_B receptor antagonists of low nanomolar potencies, for use in the cloning of GABA_B receptor splice variants R1a and R1b. CGP 71872 (13) is a photoaffinity label, used as a radioligand, iodinated with ¹²⁵I. CGP 64213 (14) was also iodinated with ¹²⁵I, and used for autoradiography to pick up GABA_B receptor clones from a cDNA library after transfection in COS-1 cells.

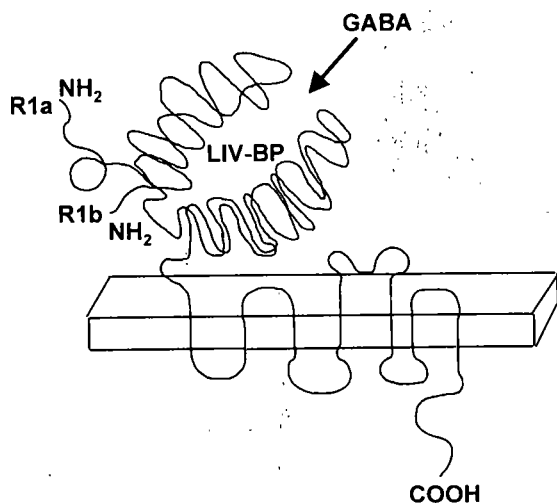


Fig 5. Molecular structure of GABA_B receptors showing the splice variants GABA_BR1a (944 amino acids) and GABA_BR1b (815 amino acids), with the N-terminal 'Venus fly-trap' domain and carboxy-terminal tail.

perspectives into investigating genetic associations between markers located in the HLA region and patients affected by these disorders.

CLONING OF GABA_BR1a AND GABA_BR1b ISOFORMS

Both splice variants (GABA_BR1a/GABA_BR1b) have essentially identical pharmacological properties, with similar ligand-binding affinities, and both are expressed

throughout the brain, where their transcript distribution qualitatively parallels that of radioligand-binding sites^[56,58-60]. Structurally, the two splice variants have a large extracellular amino terminus which differs only by the presence of extra residues at the N-terminal portion in GABA_BR1a, but otherwise have identical ligand-binding, effector domains, and intracellular carboxyl terminus. They also share extended sequence similarity with metabotropic glutamate receptors, the Ca²⁺-sensing receptor, a family of vomeronasal pheromone receptors, and periplasmic bacterial amino acid binding proteins, in having a large extracellular N-terminal domain, seven transmembrane segments, and a large cytoplasmic carboxyl-terminal tail^[56,61]. However, the intracellular loops of the GABA_B receptor are not as well conserved as in the other G-protein-coupled receptors^[62].

One of hallmark features of the receptor proteins is that the N-terminal extracellular domain of GABA_BR1b is limited to the region with structural similarity to the bacterial leucine-binding proteins (LBP)^[56]. Indeed, based on the known structure of the LBP, a three-dimensional model of the LBP-like domain of the GABA_B receptor has been constructed. Site-directed mutagenesis of the residues in the extracellular domain support a 'Venus-fly-trap' mechanism for ligand binding, suggesting that the initial step in agonist activation of the receptor results from the closure of the two lobes of the LBP-like binding domain upon binding of the agonist^[62]. This, in turn, leads to a change in conformation that is transduced to the transmembrane region to activate the associated G-protein. Cysteine residues of the LBP-like domain are not present in the GABA_B receptor, but one residue (Ser-246) emerges as likely to be involved in the binding of GABA_B receptor ligands. When compared to the classic G-protein-coupled receptors such as adrenoceptors or opioid receptors, the GABA_BR1a/b proteins are considerably larger, but are similar in size to the metabotropic glutamate receptors ranging from 872 to 1203 amino-acid residues. Interestingly, although there is some (43 - 48) % homology in related residues, the GABA_BR1 receptor, however, does not show any sequence similarity to metabotropic glutamate receptors in the region of the second intracellular loop which determines G-protein coupling^[63], suggesting that GABA_B receptors possess different signal transduction pathways. Region 2 in GABA_BR1a/1b is rich in basic residues, and is expected to be involved in G-protein interaction^[56]. Since the intracellular domains are identical in both GABA_BR1a and

1b, they are expected to be coupled through G-proteins to the same effector systems, such as adenylyl cyclase activity or Ca^{2+}/K^{+} channels.

PHARMACOLOGICAL AND FUNCTIONAL STUDIES

In ligand binding studies, the rank order of agonist and antagonist binding affinities is identical for expressed $GABA_B R1$ and native receptors, even though the affinities for agonists at the recombinant receptors were very much lower than that of native receptors, by a factor of 100 ($GABA_B R1a$) to 150 ($GABA_B R1b$) fold^[56]. This discrepancy in agonist binding affinity requires further examination, but possibly could be due to the presence of multiple $GABA_B$ receptor subtypes, originally proposed in neurochemical release studies^[64,65,66], as well as inefficient G-protein coupling or intracellular effectors in the heterologous expression system, all of which could influence agonist affinity. Unlike many G-protein-coupled receptors, $GABA_B R1a/1b$ do not desensitize after phosphorylation/dephosphorylation in heterologous expression systems^[58].

Activation of effector ion channels by recombinant $GABA_B$ receptors has proved difficult. Moreover, it has been previously shown that the binding of agonist, but not antagonist, to $GABA_B$ receptors is dependent on the status of G-protein coupling, particularly since GTP or its stable analogue Gpp(NH)p reduces the agonist affinity of native $GABA_B$ receptors by uncoupling the receptors from their G-proteins^[12]. In HEK293 cells, expressed $GABA_B R1a$ and $GABA_B R1b$ negatively couple to adenylyl cyclase, as described for native $GABA_B$ receptors^[56]. In the biochemical assay, a maximum concentration of baclofen weakly inhibits forskolin-stimulated cyclic AMP accumulation by some 30%, sensitive to the antagonist CGP 54626A, and to pertussis-toxin^[56]. Also, $GABA_B R1a$ is thought to couple to G_i proteins, since G_o proteins are absent in HEK293 cells. Yet, in cultured dorsal root ganglion neurones, $GABA_B$ receptors are coupled to voltage-sensitive Ca^{2+} channels via $G_{\alpha o}$ proteins and not $G_{\alpha i}$, as $G_{\alpha o}$ antibodies and antisense oligonucleotides complementary to mRNA sequences for $G_{\alpha o}$, respectively, reduced baclofen-inhibition of Ca^{2+} currents^[67,68]. Whether such differences are simply due to different tissue preparations, or to receptor subtypes remain to be explored, but the existence of multiple recep-

tor subtypes has not been substantiated by Kaupmann *et al*^[56], despite the identification of two receptor isoforms.

$GABA_B R1c$ AND $GABA_B R1d$ SPLICE VARIANTS

Additionally, two novel splice variants, $GABA_B R1c$ and $GABA_B R1d$ have been identified recently, when screening a rat cerebellum cDNA library, and tissue distributions of their mRNAs were examined using reverse transcription polymerase chain reaction analysis^[69]. Although they share some amino acid sequence homology with the $GABA_B R1a$ and $GABA_B R1b$ isoforms, they have structural variations in the extracellular, transmembrane and carboxyl-terminal end, with $GABA_B R1c$ and $GABA_B R1d$ having additional splices in the transmembrane and 3'-translated regions of $GABA_B R1b$, respectively. $GABA_B R1c$ is ubiquitously expressed throughout the nervous system, and $GABA_B R1d$ expressed mainly in forebrain, cerebellum, eye, kidney and urinary bladder. However, $GABA_B$ receptors in the enteric nervous system deserve more attention, since their pharmacology shows a different profile from those in the brain, using a series of novel ligands^[33,34].

COUPLING OF $GABA_B$ RECEPTORS TO K^{+} CHANNELS

Recent electrophysiological studies show that baclofen is not able to induce any current alterations in the *Xenopus* oocyte expression system when any one of $GABA_B R1b$, 1c or 1d is co-expressed with Kir3.1 and Kir3.2^[69]. This suggests that either other classes of $GABA_B$ receptor subtypes exist that can activate K^{+} channels, or that accessory proteins which are expressed endogenously in HEK293 cells but not in oocytes may be required for $GABA_B$ receptor functions^[69]. When other G-protein-coupled receptors are co-expressed with Kir3.0 channels in *Xenopus* oocytes, the agonists for each of these receptors do activate K^{+} currents. Similarly, in oocytes co-injected with rat cerebellar poly(A) + RNA and cRNAs for Kir3.1 and Kir3.2, K^{+} currents were elicited by $GABA_B$ receptor agonists such as baclofen and 3-aminopropylphosphinic acid^[70]. The $GABA_B$ receptors in the rat cerebellum can thus be functionally expressed in oocytes, and activate the cloned G-protein-gated inwardly rectifying K^{+} channels (GIRKs) comprising GIRK1 and GIRK2 as heteromultimers. In fact, ba-

clofen elicits inward K^+ currents in >65 % of the oocytes, in comparison to other studies where baclofen elicited either outward K^+ currents, or Ca^{2+} -activated Cl^- currents in <10 % of oocytes examined^[71,72].

Several studies indicate that native $GABA_B$ receptors mediate their actions through Kir3 inwardly rectifying K^+ channels; for instance in the hippocampus of Kir3.2-deficient mice, $GABA_B$ receptor-mediated K^+ -dependent inhibition is significantly impaired^[73,74]. In an *in vivo* study, intracerebroventricular injection of an antisense oligonucleotide inactivates the Kvl.1 gene that codes for a member of the Shaker-like K^+ channels with delayed rectifier properties. The latter induced a dose-dependent inhibition of baclofen and morphine antinociception in mice^[75]. This is intriguing since the morphine-induced, but not baclofen-induced, antinociception is modified by blockers of K_{ATP} current^[76]. In one of the earliest studies, employing antisense oligonucleotide technology, Holopainen and Wojcik^[77] showed that $GABA_B$ receptors belong to the superfamily of receptors having seven-transmembrane spanning regions. A 15-mer antisense oligodeoxynucleotide, that binds to mRNA encoding the amino acid sequence for the seven-transmembrane domain, prevented $GABA_B$ receptor-mediated inhibition of cyclic AMP formation.

$GABA_B$ RECEPTORS AND Ca^{2+} CHANNELS

So far, the coupling of $GABA_B$ receptor proteins to cloned Ca^{2+} channels has been unsuccessful. Nevertheless, Morris *et al*^[78] recently constructed an antisense deoxynucleotide knockdown probe for $GABA_B R1a$ and $1b$ splice variants that is complementary to 18 bp from the point at which the two sequences first become homologous. In the melanotrope cells of the pituitary intermediate lobe, chronic co-incubation with baclofen and the antisense nucleotide completely eliminated the inhibition of Ca^{2+} channels by baclofen alone, suggesting the coupling of $R1a/R1b$ to inhibition of high voltage-activated Ca^{2+} channels in these cells.

DISTRIBUTION OF $GABA_B R1a$ AND $GABA_B R1b$ TRANSCRIPTS

In general, the overall distribution of $GABA_B R1a$ and $GABA_B R1b$ transcripts in the brain parallels that of previously identified $GABA_B$ radioligand binding sites,

with abundant expression in the central nervous system^[56,59,60,79]. Thus, given its widespread localization, it is surprising that this receptor cDNA has eluded so many cloning attempts over the years. The two $GABA_B$ proteins are, however, differentially expressed in the nervous system, where the 100K ($R1b$) protein predominates over the 130K ($R1a$) protein in the cerebellum, whereas in the spinal cord, the 130K protein is more prevalent^[56]. *In situ* hybridization studies and radioligand binding data hint that $GABA_B R1a$ and $GABA_B R1b$ alone may provide the distinction between pre- and postsynaptic sites. For example, in the cerebellum, $GABA_B R1a$ mRNA expression was found to predominate in the granule cell layer, whilst $GABA_B R1b$ elements were predominantly expressed in the Purkinje cells^[80], $GABA_B R1b$ is thought to be associated with postsynaptic Kir3 channels^[58]. The high expression levels of $GABA_B R1a$ transcripts in the granule cells indicate the presence of $R1a$ proteins in the parallel fiber terminals which are excitatory to Purkinje cell dendrites in the molecular layer. On the other hand, $GABA_B R1b$ is likely to be expressed on Purkinje cells dendrites that are postsynaptic to $GABAergic$ stellate and baskets cells in the molecular layer^[58].

Using antisera against the transcripts, subcellular $GABA_B$ receptors are also localized immunohistochemically in rat retina, where presynaptic, including autoreceptors, and postsynaptic receptors may play a paracrine function^[81]; $GABA_B R1$ receptors are thus present at a variety of inhibitory and excitatory synapses. However, discrepancies exist in other brain areas where the $GABA_B$ receptor mRNA levels are either very low or absent, particularly in nuclei that contain mainly $GABAergic$ neurons^[79]. It is possible that the autoreceptors are of a different subtype, undetectable by *in situ* hybridization histochemistry using the known oligonucleotide probes. In the retina, Zhang *et al*^[82] recently demonstrated the presence of $GABA_B R1a$ transcripts in the inner nuclear and ganglion cell layers, whereas $GABA_B R1b$ transcripts were detected in the ganglion cell layer only, again suggesting a preferential targeting of the receptor variants to pre- and postsynaptic sites, with $GABA_B R1a$ and $-R1b$ localized to axonal and dendritic compartments, respectively. In dorsal root ganglia, the density of $GABA_B R1a$ proteins is high and confined to the neuronal cell bodies^[61]. The highest expressions of the $GABA_B R1$ receptor proteins are found to be at early postnatal stages, but the affinity of ligands is 10 fold lower

than in adult brain and gradually increases with aging⁽⁸³⁾. In a very recent study, GABA_BR1 immunoreactivity is detected on more than (80–90) % of neurones containing parvalbumin and choline acetyltransferase, and nitric oxide synthase in the neostriatum⁽⁸⁴⁾. GABA_BR1 clones isolated from a human cerebellum library are very similar to rat GABA_BR1, with the receptors sharing 98.6% sequence identity⁽⁸⁵⁾. Here, northern blotting shows that GABA_BR1 mRNA is widely expressed in most regions of the brain, but none was detected in the spinal cord.

IDENTIFICATION OF A GABA_BR2 RECEPTOR PROTEIN

Following the cloning of the two original GABA_B splice variants, identification of a second receptor protein, GABA_BR2, was independently reported by four separate groups who isolated the cDNA through homology screening of expressed sequence tag databases^(86–89). From this, it is known that GABA_B receptors operate as a heterodimer, with GABA_BR2 forming a heteromeric assembly with GABA_BR1 through an interaction at their intracellular carboxy-terminal tails, possibly through a conserved coiled-coil domain⁽⁸⁹⁾. The GABA_BR2 protein contains a large amino-terminal extracellular domain and corresponds in size to the GABA_BR1 receptor, displaying a sequence homology of about 35%. Co-expression of the two receptor proteins may promote expression of GABA_BR1 at the cell surface, and appears to be a prerequisite for maturation and transport of GABA_BR1 to the plasma membrane, resulting in high-affinity GABA binding and G-protein activation⁽⁸⁹⁾. Martin *et al*⁽⁹⁰⁾ demonstrated that the FLAG-tagged GABA_BR2 is localized to the plasma membrane in transfected COS cells. Indeed, Couve *et al*⁽⁹¹⁾ recently reported that recombinant GABA_BR1a receptors are retained in the endoplasmic reticulum and fail to reach the cell surface when introduced into COS cells and HEK293 cells. Perhaps, GABA_BR1 requires a trafficking factor or binding partner to reach the cell surface.

GABA_BR1 AND GABA_BR2 FUNCTION AS HETERODIMERS

GABA_BR1 and GABA_BR2 heterodimers couple to

effector systems, display inhibition of forskolin-stimulated adenylate cyclase activity, and robust coupling to inward-rectifying K⁺ channels^(86–89,92). The physical interaction between GABA_BR1 and GABA_BR2 appears to be essential for the coupling of GABA_B receptors to K⁺ channels, given that GABA_BR1a, GABA_BR1b and GABA_BR2 alone do not activate Kir3 channels efficiently, but co-expression of these receptors yield a robust coupling to the K⁺ channels. In contrast, however, GABA_BR2 by itself is sufficient to decrease forskolin-stimulated cyclic AMP production, since both GABA_BR1 and GABA_BR2 induce the response to the same extent as GABA_BR2 alone, indicating that the heteromeric assembly is not required for inhibition of adenylyl cyclase activity⁽⁸⁸⁾. The human GABA_BR2 gene, distinct from the GABA_BR1 gene, and located on chromosome 9q22.1, close to the genetic disorder hereditary sensory neuropathy gene (9q22.1–9q22.3), has recently been identified⁽⁹⁰⁾. It differs from the GABA_BR1 in having an additional 80 amino acids in the carboxy-terminal tail, and it negatively couples to adenylyl cyclase in response to agonists such as GABA, baclofen and 3-aminopropyl (methyl) phosphinic acid in CHO cells lacking GABA_BR1. Whilst this is similar to the findings of Kuner *et al*⁽⁸⁸⁾ who also reported that GABA_BR2 alone negatively couples to adenylyl cyclase in transfected cells, White *et al*⁽⁸⁹⁾, on the other hand, reported that GABA_BR2 does not couple to adenylyl cyclase. One possible explanation for this discrepancy is that differences in the levels of G protein expression between different cell lines could result in differences in effector coupling.

LOCALIZATION OF GABA_BR1 AND GABA_BR2 PROTEINS

In the striatum, GABA_BR1 but not GABA_BR2 mRNA transcripts are detected, whilst they are both co-expressed in various brain regions such as the Purkinje cell layer of the cerebellum, whereas no signal is detected in the spinal cord⁽⁹⁰⁾. Perhaps the latter is not so unexpected since a baclofen-insensitive autoreceptor has been found in the spinal cord⁽⁶⁵⁾. Assuming that neither GABA_BR1 nor GABA_BR2 is this receptor, it is possible that other GABA_BR genes have yet to be discovered. It has been shown that GABA_BR1 mRNA expression is more widespread than that of GABA_BR2 in some areas of

the brain, and has an earlier onset; such a delayed developmental expression of GABA_BR2 may contribute to a maturation of GABA_B receptors^[88]. Indeed, there are some regions, such as caudate/putamen, where GABA_BR1 mRNA is abundant and GABA_BR2 mRNA is undetectable, but in areas expressing GABA_BR2, GABA_BR1 is expressed in a majority of the same neurons, suggesting that native receptors are heterodimers in these cells^[92]. In the retinal ganglion cell layer, where GABA_BR2 mRNA is localized^[90], the latter may represent one of the two types of GABA_B receptors that have been earlier detected^[93]. The elevated levels of GABA_B receptor mRNA in the nucleus of the solitary tract of hypertensive rats point to an upregulation of GABA_B receptor function which could lead to alterations in cardiovascular function in chronic hypertension^[94]. In the Cacnb4^h mouse model which provides insights into molecular mechanisms underlying absence seizures, the GABA_B receptor mRNA expression is significantly increased in neuronal populations of 8 week-old mice, raising the possibility that these receptors may play a pivotal role in the generation of absence seizures in this strain^[95].

GABA_B RECEPTOR HETEROGENEITY

The isolation and functional characterization of GABA_BR2 may indicate, at least in part, multiple GABA_B receptors of distinct pharmacological sensitivities. The structural features, pattern of expression and plasma membrane targeting of GABA_BR2 distinguish it from GABA_BR1, again suggesting the possibility of GABA_B receptor subtypes, and consistent with a model in which either homomeric or heteromeric GABA_B receptors may couple to different cellular signal transduction systems through different cellular effectors. Defining GABA_B receptor subtypes is a major step towards a better understanding of the physiological role of GABA_B receptors in normal and pathological states. Multiple GABA_B receptor subtypes in various brain synapses have been suggested^[64,96-101], with a strong focus on differences between pre- and postsynaptic receptors^[99,100,102,103].

A number of pharmacologically distinct GABA_B receptors, each inhibiting a different transmitter has been demonstrated in synaptosomal preparations, as well as multiple receptors controlling adenylyl cyclase activity, based on differential sensitivities to agonists and antagonists^[97,104,105]. Some GABA_B receptor antagonists differentially affect GABA release at autoreceptors and re-

lease of other neurotransmitters at hetero-receptors^[51,64], whilst others display a different rank order of relative potencies on baclofen-induced inhibition of forskolin-stimulated cyclic AMP production in rat cortical slices, when compared to their reported binding affinities at GABA_B receptor sites^[97,106]. In the dorsal horn of the rat isolated spinal cord, a phosphinic acid antagonist CGP 56999A blocks baclofen-induced inhibition of GABA and substance P release, without affecting glutamate release^[107]. Extensive reviews on GABA_B receptor heterogeneity are available elsewhere^[33,34,38,39].

Using electrophysiological techniques in the neocortex, presynaptic GABA_B receptors modulating paired pulse depression are distinguished from postsynaptic receptors by their antagonist sensitivity^[102]. Also, three or more pharmacologically distinct GABA_B receptors are found within the dorsolateral septal nucleus, where there may be at least one presynaptic site on a glutamatergic terminal versus two postsynaptic receptors^[101]. These are distinguished by their pharmacological sensitivity to GABA_B receptor agonists and antagonists. Furthermore, the postsynaptic receptors within this nucleus are shown to be coupled to distinct K⁺ conductances, in agreement with a previous study where Pham *et al*^[108] reported that synaptic and extrasynaptic GABA_B receptors may be coupled to different K⁺ channels. The latter experiments indicate that synaptically released GABA only activates barium-sensitive K⁺ conductances, in contrast to exogenous GABA which can activate both barium-sensitive and insensitive K⁺ channels. This leads to the proposal that GABA_B receptors on subsynaptic sites are coupled to barium-sensitive K⁺ conductances, whilst GABA_B receptors on extrasynaptic sites are coupled to barium-insensitive K⁺ channels. Two different types of GABA_B receptors with high and low affinities for baclofen mediate Ca²⁺ current inhibition in thalamocortical neurons^[109], but whether this reflects true receptor heterogeneity is yet to be determined.

FUTURE PROSPECTS

Attempts have been made to develop highly potent compounds active at GABA_B receptor sites which will open up new avenues of physiological and therapeutic targets for this class of receptors. Although baclofen is used to treat spasticity, with sedation and tolerance as limiting side-effects, it has not achieved conventional use as an analgesic agent in clinical practice^[110]. Recently,

a potent GABA_B receptor agonist, CGP 44532, has been shown to be a substantially more effective analgesic agent than baclofen itself in a chronic pain model (formalin hindpaw injection), in blocking neurokinin-1 receptor gene expression which mediates nociceptive sensory information in the spinal cord^[111]. Thus, GABA_B receptor agonists with enhanced analgesic activity and minimal sedative and tolerance effects could be clinically useful in chronic pain management. Other promising pharmacotherapies and strategies may be extended to cocaine addiction^[101,112-114], cognition impairment^[115] and absence seizures^[116].

In the past, GABA_B receptor cloning has proved to be exceptionally difficult and slow due to a lack of suitable ligands, but since the sequence was recently revealed, there has been inescapable interest in GABA_B receptor heterodimers as mediators of signal transduction mechanisms. There is now reasonable evidence to support the view that multiple GABA_B receptors relevant to controlling the release of different neurotransmitter types exist, but whether these subtypes bear a relationship to GABA_BR1 or GABA_BR2 isoforms remains to be determined. Substantial insights might be gained from gene targeting in knockout experiments using GABA_B receptor-deficient animal models that allow clarification of some aspects of GABA_B receptor heterogeneity at the molecular level. It is still not clear what GABA_BR1 and GABA_BR2 represent in relation to their physiological versus pathological functions, or if different isoforms caused by alternative splicing or regulated by post-transcriptional processing could contribute to differences in their pharmacological and functional characteristics. This remains an entirely open field of investigation. Nonetheless, exciting and challenging advances have added new impetus to GABA_B receptor research, provoking much interest in the continuing development of selective agents as new targets for specific therapies such as drug addiction, nociception and absence seizures.

ACKNOWLEDGEMENTS To the Australian Research Council (ARC) for financial support. Jennifer ONG is an ARC Senior Research Fellow. We are grateful to Dr S Bexis for the illustrations.

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关键词 γ -氨基丁酸; GABA-B 受体; GABA_BR2 受体; 巴氯芬; GABA 受体激动剂; GABA 受体拮抗剂
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