$GABA_B$ receptors: targets for drug development

David I.B. Kerr and Jennifer Ong

GABA is a major inhibitory neurotransmitter in the CNS where it regulates neuronal excitability. These actions of GABA are mediated through GABA receptor subtypes, one of which is the $GABA_B$ receptor (GBR). The development of specific agonists and antagonists for GBRs has led to a better understanding of their functional roles, which mainly focus on the long-term modulation of neural activity. In this review, the authors emphasize the current knowledge of the structure–action profile of compounds active at GBRs, characterizing their physiology and pharmacology and highlighting their potential as targets for drug development.

Although GABA (4-aminobutanoic acid, γ-aminobutyric acid) was first synthesized in the late nineteenth century, and soon after found to be a product of bacterial metabolism[1], it was some time before GABA was found in the mammalian brain[2], and even longer before it became recognized as an inhibitory transmitter in the mammalian CNS. Indeed, it was not until the discovery by Curtis and Johnston of bicuculline as an antagonist of GABA that unequivocal acceptance of an inhibitory role for GABA became established[3]. In retrospect, the few dissenting voices were probably being prompted by bicuculline-insensitive actions of GABA in the CNS, mediated by, what were to become known as, GBRs.

That GABA might act through receptors other than the 'classical' GABA receptors was first pointed out by Dunlap and Fischbach[4], who found a bicuculline-insensitive GABA receptor that depresses calcium currents in chick sensory neurons. However, it was Bowery and his colleagues who found bicuculline-insensitive depressant actions of GABA on transmitter release from autonomic terminals in a variety of preparations[5]. They further showed that the GABA derivative baclofen [4-amino-3-(4-chlorophenyl)-butanoic acid (**7**); Figure 1] is a specific agonist for this new receptor, which they also demonstrated to be present in the brain and named the GBR (Ref. 6). This receptor was soon confirmed to be separate from the $GABA_A$ receptor[7,8], but any definitive role for GBRs in the CNS could not be established until our introduction of the first selective antagonists saclofen, 2-hydroxysaclofen and phaclofen (**18–20**; Figure 2)[9]. In particular, phaclofen (**20**) was used in a variety of central preparations[10–12] to demonstrate that the late, delayed inhibitory postsynaptic potential (IPSP) was mediated by GBRs, demonstrating postsynaptic GBRs for the first time.

Between 1980 and 1985, there was an emerging awareness of GBRs, based on information derived from neurophysiological, neurochemical and biochemical studies. Since then, we have seen the application of newly available GBR agonists and antagonists in a variety of biological systems, with further elucidation of the diversity and mechanism of action of these receptors. The future offers the prospect of building on this new information, and providing real insights into the physiology of GBRs, which may eventually become targets in the treatment of disease.

Characterization of GBRs

GBRs can be defined as a distinct class of bicuculline-insensitive GABA receptors for which baclofen (**7**) is a selective agonist and 2-hydroxysaclofen (**19**) is a selective antagonist.

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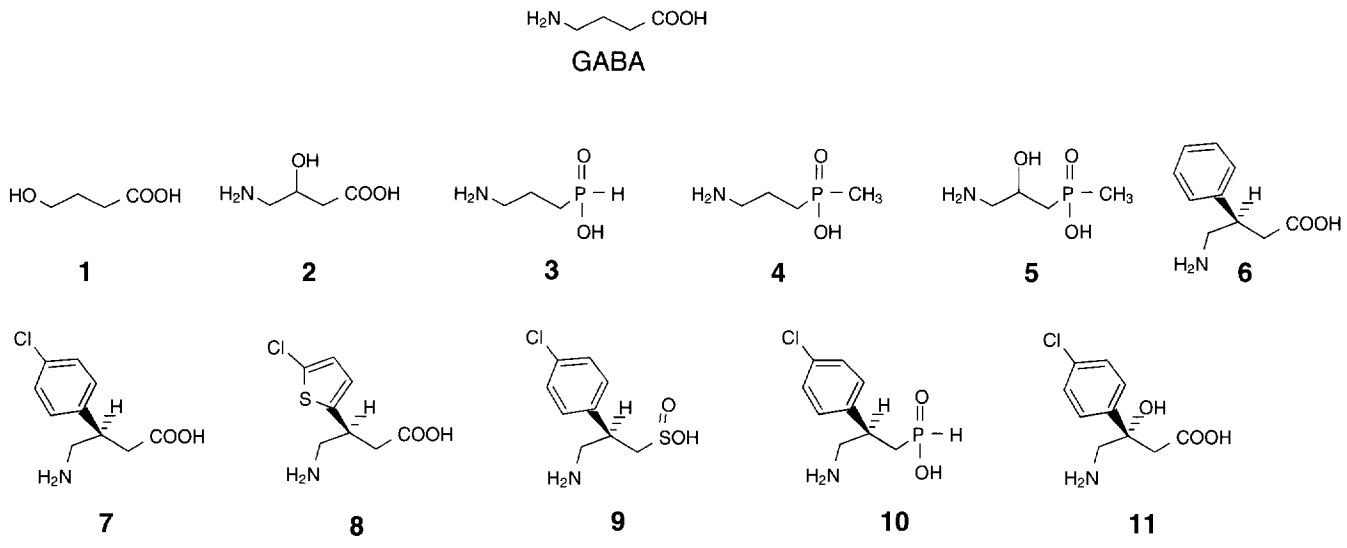

Figure 1. *$GABA_B$ receptor agonists.*

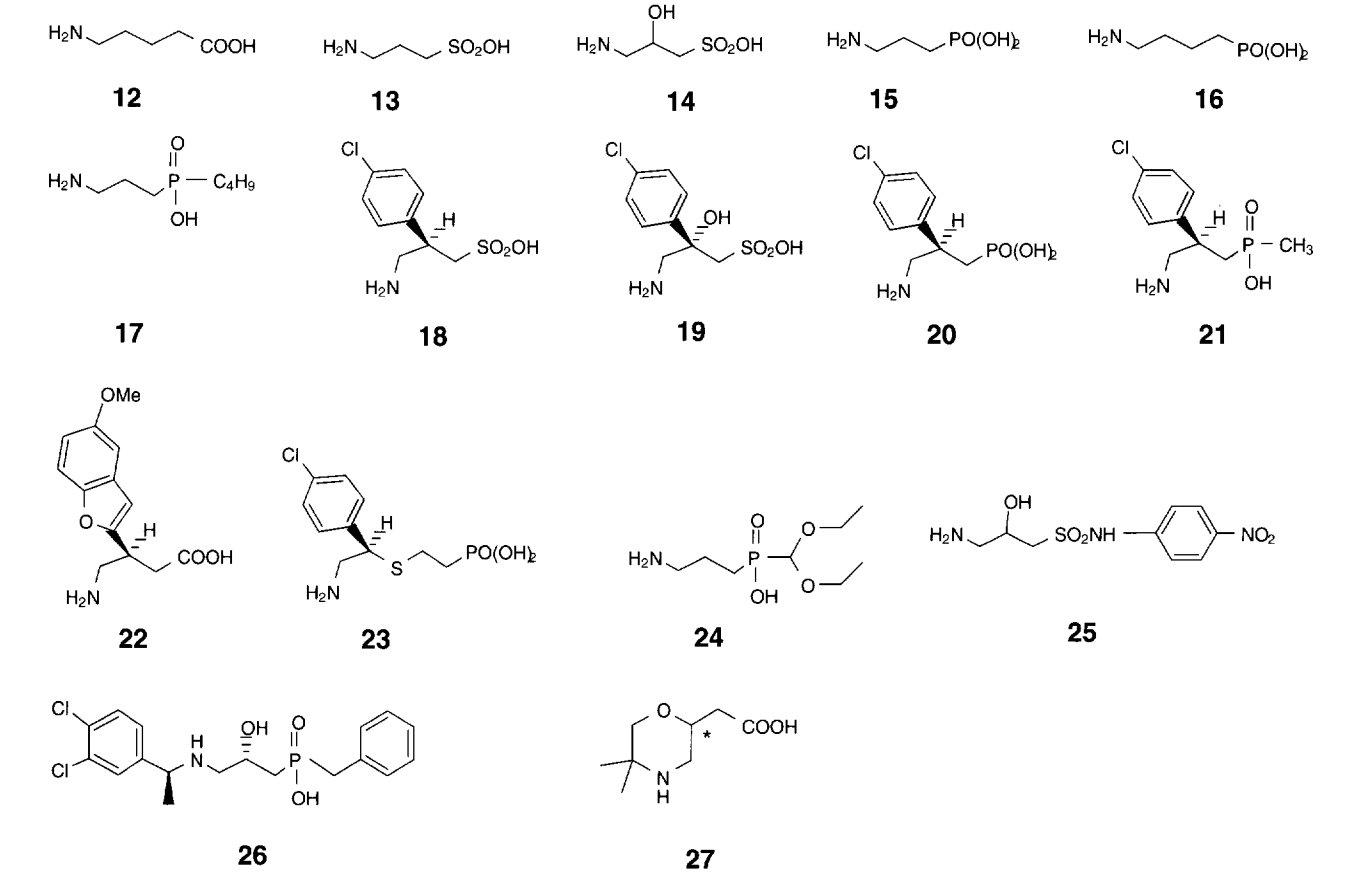

Figure 2. *$GABA_B$ receptor antagonists.*

GBRs have a totally different pharmacology from the better known and exhaustively studied $GABA_A$ receptors, as well as from the newly recognized $GABA_C$ receptors[13], which are a bicuculline-insensitive subset of $GABA_A$ receptors. The $GABA_A$ receptors form an integral part of a chloride channel, and are subject to modulation by a variety of tranquillizers and anaesthetic agents. On the other hand, GBRs show no such modulation but are 'metabotropic' receptors that belong to the superfamily of receptors linked to G proteins (guanine nucleotide binding proteins). These G proteins, in turn, provide intracellular effectors and second messengers that ultimately modulate calcium and potassium channels, controlling synaptic transmission and neuronal excitability[14].

Functionally, GBRs can be divided into several subtypes, distinguished by their anatomical locations and the responses that they mediate downstream from activation of their associated GTP-binding proteins[14]. Firstly, presynaptic GBRs modulate the release of a wide range of transmitters, and include autoreceptors that control the release of GABA itself. Paradoxically, when the autoreceptors are activated, they bring about excitatory disinhibition, because GABA release at inhibitory synapses is depressed. Such inhibition of transmission is due to a presynaptic GBR-mediated decrease in the calcium influx, coupled to transmitter release at the synapse. Secondly, postsynaptic GBRs activate an outward potassium current that mediates prolonged, delayed inhibitory postsynaptic potentials (late IPSPs). GBRs also produce metabotropic actions; for instance, GBRs potentiate adenylate cyclase activation by a range of other transmitters, such as noradrenaline at β-adrenoceptors, thus increasing cyclic AMP (cAMP) formation[14]. A further type of GBR is negatively coupled to adenylate cyclase activity, depressing cAMP formation during forskolin stimulation of cyclase activity. GBRs also activate phospholipase A_2, which leads to the release of arachidonic acid and its eicosanoid metabolites. The latter either act as second messengers within the cell, altering ionic channels, or diffuse to influence adjacent cells. In addition, GBRs activate phospholipase C, which hydrolyses phosphoinositide to inositol trisphosphate (IP_3) and diacylglycerol (DAG). These products are important, because IP_3 releases intracellular calcium which in turn activates protein kinase C, which is also activated synergistically by DAG, again modulating excitability. Examples of each of these metabotropic responses to GBR stimulation are known, but unfortunately have not been studied to any great extent. Overall, it is clear that a variety of neural responses are mediated through GBRs, which raises the question of heterogeneity among these receptors.

Although GBRs are, in all probability, G protein-coupled receptors, their sequence structures remain unresolved. Indeed, it is not even known to which of the three major subfamilies of G protein-coupled receptors[15] they may belong. Isolation of GBRs has been achieved by means of baclofen affinity and immunoaffinity column chromatography, which yielded an 80-kDa protein[16–18], but this receptor protein has not yet been sequenced. GBRs have also been expressed in *Xenopus* oocytes, using mRNA extracted from rat cerebral and cerebellar cortices. These expressed GBRs are activated by baclofen, and sensitive to 2-hydroxysaclofen and phaclofen[19,20]. However, only 10–20% of the oocytes are activated by baclofen, so they do not provide a suitable screening system.

GBR distribution in the CNS

Demonstration of the distribution of binding sites is a strong prerequisite for the identification of potential targets of a particular class of ligand. In autoradiographic and immunohistochemical studies, GBRs are found to be abundant, with regional variations throughout the CNS. The regional abundance of GBR binding sites strongly correlates with the distribution of GABAergic fibres and terminals that have been described previously[21]. Among the most densely labelling areas for GBRs is the cerebellum, where a characteristic distribution pattern is found predominantly in the molecular layer[21], while recent immunohistochemical evidence suggests that the 80-kDa GBR may, in fact, be exclusively localized at cerebellar presynaptic sites[22]. The functional significance of these cerebellar GBRs remains unknown.

Among the next most densely labelling regions for GBRs is the basal ganglia, particularly the globus pallidus, where their function is unknown. Within the thalamus, GBRs are present at high densities in the ventrolateral nucleus and areas of the lateral and medial geniculate nuclei, as well as the nucleus reuniens and the reticular nuclei; this system is associated with the generation of thalamic slow-wave activity[23]. Abundant GBRs also label in layers I–III throughout the neocortex and in the hippocampus, where they are involved in regulating and generating long-term synaptic plasticity. Possibly of significance in pain regulation, marked concentrations of GBRs are found at afferent regions of the dorsal horn of the spinal cord, including the equivalent region in the trigeminal spinal tract, and the vagal afferent relays of the tractus solitarius. A particularly prominent presynaptic accumulation of GBRs occurs on the habenulointerpeduncular tract, at the level of the interpeduncular nucleus. This

particular system, linking the basal forebrain and pineal to the midbrain, is of considerable phylogenetic antiquity, although surprisingly little is known of its functional importance.

Structure–action studies

GABA activates GBRs as well as $GABA_A$ receptors, because it is a flexible molecule with a large number of possible conformers. Attempts to limit this flexibility lead to considerable loss of agonist activity at GBRs. Indeed, the structural requirements for GBR activation are rather stringent. Apart from GABA itself, two metabolites, 4-hydroxybutyrate (**1**) and 4-amino-3-hydroxybutyrate (**2**), particularly the *R*-enantiomer, are weak agonists. The 3-(aminopropyl)-phosphinic acid (**3**), with a phosphinic isosteric replacement of the carboxylic function in GABA, is the most potent GBR agonist known, whereas 3-(aminopropyl)-methylphosphinic acid (**4**) is less potent but more stable and active *in vivo*. As Froestl and coworkers pointed out[24], **3** was originally described some 20 years ago as a flame retardant. In total, around 70 phosphinic analogues of GABA have been examined for therapeutic potential as GBR agonists. Among these is 2-(hydroxypropyl)-methylphosphinic acid (**5**), which was obtained by incorporating the β-hydroxy substituent of **2** in **4**. This is a more potent agonist than baclofen, it is useful *in vivo* and *in vitro*, and may serve to replace baclofen in therapy[24].

Although baclofen (**7**) is the prototypical GBR agonist, active *in vivo*, the congener 4-amino-3-phenylbutyric acid (**6**) was actually the first compound of this type to be prepared[25,26]. This 3-phenyl analogue has seen clinical use in the USSR for mood alteration[27], but has never entered Western psychiatry. Our own studies show that the activity of **6** resides in the *R*-enantiomer, which has the same absolute configuration as (*R*)-baclofen (**7**)[28,29]. In the rat CNS, **6** is some ten times weaker as a GBR agonist than (*R,S*)-baclofen, which contains the 4-chlorophenyl moiety at the 3-position; however, **6** is only a partial agonist/antagonist at GBRs in the guinea-pig ileum, whereas (*R,S*)-baclofen is a full agonist[29]. This difference in activity profile between **6** and its 4-chloro analogue **7** at GBRs is a curious feature of the structural requirements for agonist activity in such compounds. Systematic studies of substitution on the phenyl ring have shown that this 4-chloro substituent imparts maximal agonist activity. The same requirement is seen in 3-thienyl analogues of baclofen, which also have considerable aromaticity. In these, the most active congener is 4-amino-3-(5-chlorothien-2-yl)-butanoic acid (**8**), where the 5-chloro substituent on thiophene occupies the same region in space as the 4-chloro on the phenyl of baclofen[30,31]. The corresponding, less aromatic, 3-furanyl analogue is inactive, which confirms the necessity for an aromatic substituent at C-3 of GABA to give selectivity at GBRs (Box 1).

Two other baclofen analogues with reasonable GBR agonist activity are known; these have, respectively, a sulphinic (siclofen, **9**)[32] and a phosphinic (**10**) bioisosteric replacement of the carboxylic group in baclofen (**7**)[24]. Introduction of a 3-hydroxy substituent to give 3-hydroxybaclofen (**11**), analogous to 3-hydroxy GABA (**2**), substantially lowers the potency of baclofen. Evidently, such hydroxy substituents are not favourable for agonist activity at GBRs. Most recently,

Box 1. Potencies of selected agonists and antagonists at $GABA_B$-receptors

	Compound	EC_{50} (μM)[a]
Agonists		
GABA		10[b]
(*R*)-GABOB	**2**	70[b]
CGP 27492	**3**	0.8
CGP 35024	**4**	1
(*R*)-Baclofen	**7**	5
(*R*)-CTG	**8**	10[c]
(*R*)-Siclofen	**9**	14[d]
Antagonists		**pA_2**
GABA analogues		
3-APPA	**15**	4[e]
4-ABPA	**16**	4
CGP 36742	**17**	4.8
CGP 35348	**18**	5
(*S*)-2-OH-SAC	**19**	5.3
(*R*)-PHAC	**20**	4.3
Second generation antagonists		
AHPNS	**25**	4
CGP 55845	**26**	8
SCH 50911	**27**	5.8[d]

[a]$GABA_B$ receptor-mediated depression of ileal twitch.
[b]Non-selective ($GABA_A$ agonists).
[c]Estimation from (*R,S*)-CTG.
[d]$GABA_B$ receptors in trachea.
[e]Partial agonist.

an agonist pharmacophore for GBRs has been suggested[33] on the basis of X-ray crystal structures, NMR studies and conformational calculations of **7** and **8**, as well as their 3-furyl congeners. The combined data suggest that a positive charge on the ring sulphur of **8** is able to repel the ammonium functionality, bringing the latter into a favourable position for GBR agonist activity. Conversely, the furyl oxygen, bearing some negativity, attracts the ammonium into an unfavourable region, and these furyl compounds are inactive.

Characterization of GBRs, particularly of any functional subtypes, has been hampered by the restricted range of antagonists available for these receptors. The earliest identified GBR antagonists were the GABA homologue 5-aminopentanoic acid (**12**)[34,35] and the sulphonic analogue of GABA, homotaurine (**13**)[36], but neither is specific because they are also $GABA_A$ receptor agonists. This agonist property of **13** is reduced in the 2-hydroxy derivative **14**, but neither **13** nor **14** has seen significant use for characterizing GBRs (Box 1).

Our original lead, which was developed to give the first selective, albeit weak, GBR antagonist, was the corresponding phosphonic analogue of GABA, 3-aminopropylphosphonic acid (**15**), which behaves as a partial agonist/antagonist[37,38]. The homologue 4-aminobutylphosphonic acid (**16**), analogous to the antagonist (**12**), is a GBR antagonist with no partial agonist properties. Both these antagonists (**15** and **16**) are higher homologues of 2-aminoethylphosphonic acid, which was the first such compound bearing a C–P bond to be isolated from any organism, and is found in the brain[39]. Among GABA analogues showing GBR antagonist properties, phosphinic derivatives with bioisosteric replacements of the carboxylic moiety with a larger *P*-alkyl substituent (ethyl and above) related to **4** are important. In particular, the n-butyl derivative CGP 36742 (**17**) is an orally active GBR antagonist[40] (Box 1).

Originally, we introduced saclofen (**18**), 2-hydroxysaclofen (**19**) and phaclofen (**20**), the sulphonic and phosphonic analogues of baclofen, as GBR antagonists[9]. Although phaclofen was the first such antagonist based on baclofen, the sulphonic derivative (**18**) is the most potent, whereas **19** has seen the most use. Each of these has been resolved or prepared by chiral synthesis[41–43], all with an active configuration identical to that of (*R*)-baclofen[44]; notably, owing to the higher priority of oxygen, **19** is properly designated (*S*)-2-hydroxysaclofen. By analogy with the high potency of the phosphinic agonists **3–5**, phosphinic analogues of baclofen might be expected to be more potent antagonists than **18–20**. However, when converted to the corresponding baclofen analogue **21**, the agonist *P*-methyl derivative **4** becomes an antagonist, while the *P*-n-butyl antagonist **17** loses virtually all its activity[40]. This suggests that binding of the phosphinic head at the GBR is, in some way, incompatible with binding of the 4-chlorophenyl moiety in these baclofen analogues.

Apart from the GBR antagonist baclofen analogues **18–21**, based on an altered carboxylate moiety, there are two other analogues showing moderate activity. These are 4-amino-3-(5-methoxybenzo[*b*]furan-2-yl)-butanoic acid (**22**)[45], which was designed to explore the aromatic requirements of the 3-substituent in baclofen, and **23**, the thioether analogue of phaclofen (**20**)[46], intended to examine the influence of an increased chain length of phaclofen on GBR antagonism. As it happens, thioether **23** is no more potent than phaclofen itself, and it does not seem profitable to pursue this avenue further. However, benzofuran derivative **22** is of considerable interest because it is a partial agonist/antagonist[47], and provides some insight into the conformation that the aromatic moiety of **7–9** and **18–21** must assume for effective interaction with the GBR binding site. In these, X-ray crystallography, circular dichroism and energy calculations all point to a preferred conformation with the 4-chlorophenyl ring lying perpendicular to the plane of the GABA backbone in the *R*-configuration. On the other hand, **22** can assume a low-energy conformation where the benzofuran moiety lies in the plane of the backbone, and the ammonium functionality is folded towards the oxygen of the benzofuran, providing Van der Waals bonding between them. This suggests a possible antagonist conformation at the GBR, with the ammonium folded towards the 2-hydroxy substituent in **19**, **25** and **26**, and more particularly in SCH 50911 (**27**)[48].

Early in their studies of ligands for GBRs, the Ciba-Geigy group found that CGP 35348 (**24**), the *P*-(1,1-diethoxy)-methyl derivative of **3**, is an antagonist[49]. Actually, **24** is a precursor in the synthesis of **3**, based on the use of the Gallagher hypophosphorus synthon[50]. Thus, **24** provided the fundamental lead for the development of a series of greatly improved GBR antagonists, further optimized by a benzyl substituent at the nitrogen[40,51], as in **26**. Such refinements provided affinities in the low nanomolar range, compared with micromolar for the parent **24** (Ref. 52). In this important series of antagonists, the benzyl or equivalent P-substituent suggests a hydrophobic pocket adjacent to some basic functionality in the GBR that provides a counter ion for the phosphinic head. It was the latter notion that prompted

our development of the sulphonamide GBR antagonist AHPNS (**25**), which also contains the 2-hydroxy substituent on the propyl chain[53]. This sulphonamide series is being extended because it is rather versatile and capable of bearing a range of N-substituents at the sulphonamide to alter pK_a and lipophilicity.

In addition to the 2-hydroxy substituent on the propane backbone, the more potent Ciba-Geigy antagonists also incorporate an α-(*S*)-methyl on the dichlorobenzyl substituent on the nitrogen. These features seem to have provided Blythin at Schering Plough with the basis for the ring closure to provide the morpholino GBR antagonist SCH 50911 [(+)-5,5-dimethyl-2-morpholineacetic acid, **27**][48]. This is the first GBR antagonist with a stabilized ring structure, incorporating the hydroxy and amino functionality of **25** and **26** into a ring. Although chiral, the absolute configuration of **27** has not been revealed. Its pharmacological profile shows a greater potency than **24**, and it is an active antagonist at both peripheral and central GBRs (Ref. 54), as well as at metabotropic GBRs (Ref. 55).

A series of antagonists, based on **24** and **26**, has not provided any evidence for pharmacologically different presynaptic GBR subtypes. That is, no heterogeneity appears present among autoreceptors inhibiting GABA release as is reported for heteroreceptors inhibiting glutamate release[51], despite such heterogeneity being proposed by Lanza and coworkers[56], who, to the contrary, found **34** to be 100-fold more potent as an antagonist at autoreceptors than against heteroreceptors. These disparate results may be due to the use of different methods for evoking transmitter release by the two groups. Nevertheless, it seems likely that heterogeneity of GBRs does exist, and there are some structure–action results that indicate this more strongly. In particular, a series of baclofen derivatives (**28–30**, Figure 3), analogous to **18** and **20**, but based on 3-amino-3-phenylpropionic acid (**28**), are GBR antagonists in the guinea-pig myenteric plexus[57]. Indeed, **28** itself is an antagonist of gastric acid secretion, and of its enhancement by baclofen[58]. Yet these 'short-chain' sulphonic and phosphonic baclofen analogues (**29**, **30**) are totally without effect against baclofen-induced depression of evoked CA_1 field excitatory responses in rat hippocampal slices, or against baclofen-induced reduction of spontaneous activity in rat neocortical wedges (detailed elsewhere). In the same way, the 3-aminomethylbenzoisofuranones (**31** and **32**) are weak GBR antagonists in the guinea-pig ileum, but are completely inactive in the rat hippocampal slice or neocortical slice. These results can be interpreted as being due to GBR heterogeneity. Unfortunately, **28–30** are not at all potent as GBR antagonists, and no attempt has yet been made to increase their activity. There is stronger evidence for the heterogeneity of metabotropic GBRs. Using either SCH 50911 (**27**) or CGP 54626 (**33**) as an antagonist, a 10-fold difference between baclofen-induced GBR inhibition of forskolin-stimulated cAMP production and baclofen stimulation of cAMP production induced by other transmitters was found. On the other hand, CGP 52432 (**34**), which combines features of **24** and **26**, shows a 30-fold distinction between these actions, consistent with GBR heterogeneity[55]. These results suggest that further effort should be made to develop specific GBR subtype ligands, because these may represent important targets for drug development.

Therapeutic implications

Numerous physiological and pharmacological effects have been attributed to GBR activation, both peripherally and centrally. Nevertheless, when GBR antagonists such as CGP 35348 (**24**) are administered, animals show no overt behavioural actions. However, they do show signs of increased grooming, hyperactivity and stereotypy upon receiving excessive doses[59], which raises the question of any physiological role of GBRs in the intact animal. Possibly, these receptors may simply modulate neuronal activity, and only become more important in pathological rather than normal physiological states[60].

Spasticity and motor control

Baclofen (Lioresal) was first introduced some 30 years ago and became the drug of choice in the treatment of spasticity, particularly that caused by multiple sclerosis, or, more importantly, traumatic lesions arising from various forms of spinal injury[61]. Baclofen is generally administered orally, but direct intrathecal infusion of baclofen provides much better control of muscle spasms in patients refractory to oral baclofen[62], and eliminates unwanted central side-effects such as drowsiness, dizziness, nausea or vomiting, ataxia, muscle weakness, mental confusion, loss of consciousness and respiratory depression. Obviously, in severe cases of baclofen overdose[63], an antagonist would be useful in counteracting these unwanted effects.

Analgesia

GBRs are implicated in the modulation of neural responses to noxious stimulation[64], involving both supraspinal and spinal components. In a variety of behavioural tests,

(*R*)-baclofen, particularly when administered intrathecally, stereospecifically produces analgesic effects, attenuated by GBR antagonists such as phaclofen or 2-hydroxysaclofen[65]. In clinical practice, baclofen has not achieved conventional use as an analgesic agent[66], because studies of its usefulness as an analgesic in humans have yielded variable results[67]. Nevertheless, when administered as a single agent, baclofen may be effective in the treatment of trigeminal neuralgia[68] and atypical facial pain[69], as well as pain associated with acute low-back syndrome[70]. Interestingly, in postoperative dental pain in humans, baclofen enhances the analgesic effect of the μ-opioid agonist morphine, suggesting that agonist drugs targeted at GBRs may be useful in reducing the level and frequency of dosing for opioid analgesics needed in the treatment of postoperative pain[71].

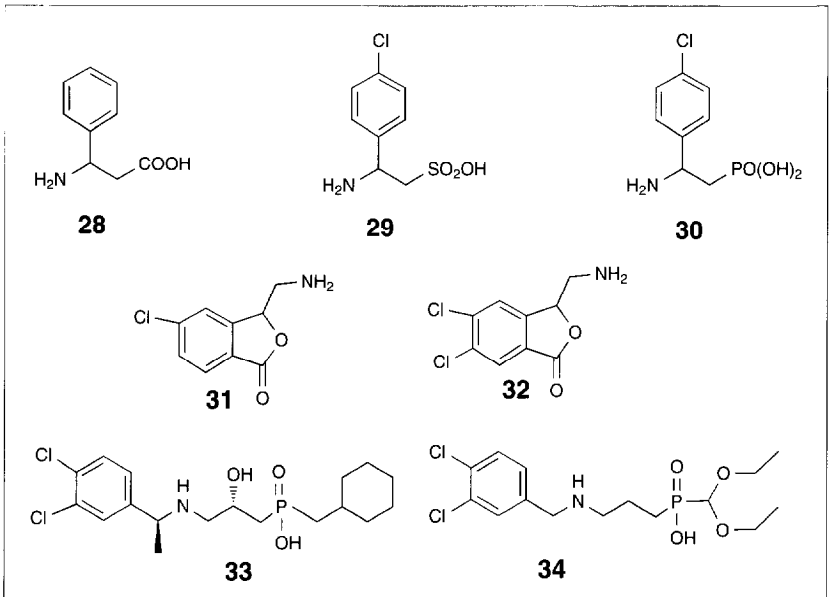

Figure 3. *$GABA_B$ receptor antagonists with an activity profile suggesting receptor heterogeneity.*

Epilepsy

Although alterations in synaptic inhibition are part of the pathology in epilepsy, baclofen has not proved to be an effective anticonvulsant in humans[72] because both pro- and anti-epileptic effects of baclofen have been reported[10]. Clinically therapeutic doses of baclofen can induce epilepsy[73], with toxic doses causing seizures even in non-epileptic patients[74,75]. In spite of the fact that clinical features of absence seizures (petit mal) have been recognized for decades, the underlying mechanisms are not well understood. In recent studies using genetically seizure-prone animals, the GBR antagonists CGP 35348 and 2-hydroxysaclofen blocked spontaneous absence seizures in lethargic mice[76,77], indicating that GBRs may represent effective therapeutic sites for pharmacological regulation of absence seizures in children. Recently, the new, systemically active GBR antagonist SCH 50911 (**27**)[48] has shown anti-absence activity in a variety of animal models[54], confirming the earlier work of Marescaux and coworkers in a rat model for absence epilepsy[78]. In the light of these results, compounds targeting the appropriate GBR sites may offer a new mode of treatment for patients who are refractory to treatment with traditional antiabsence drugs, or show adverse responses to them.

Systemic application of γ-hydroxybutyric acid (GHB; **1**), an endogenous product of GABA metabolism, has been shown to precipitate absence seizures[79], and to induce general anaesthesia at higher concentrations[80]. GHB also produces increased nocturnal slow-wave sleep[81,82]. This is potentially interesting for the development of more potent compounds in sleep disorders, particularly if a GBR subtype is involved. Low doses of GHB (25–40 mg/kg) are used to treat narcolepsy and alcoholism in humans[83,84]. These effects are likely to be mediated through activation of a tonic GBR-mediated hyperpolarization in central neurons[85].

Cognitive effects

In recent years, encouraging progress has been made in the biochemistry and electrophysiology of learning and memory. It has become clear that GBRs play a crucial role in memory acquisition and consolidation; for example, GBR activation by baclofen impairs learning and memory in a variety of tasks[86]. This is consistent with the observation that, following blockade of GBRs using the more potent antagonist CGP 36742 (**17**), there is an enhancement of cognitive performance in rats, mice and monkeys[87,88]. At the neurophysiological level, GBR antagonists can suppress or increase the induction of long-term potentiation depending on the stimulation conditions[89]. Baclofen is known to impair retention of passive avoidance tasks in rats[90], while improving memory retention in an active avoidance task[91], producing mixed cognitive effects. However, stimulation of GBRs in the basal forebrain selectively impairs memory of rats, an effect that is subsequently reversed by phaclofen[92]. In addition, baclofen

also produces dose-related working-memory impairments after intraseptal injection[93]. Thus, there is a possibility that the mnemonic effects of GBR antagonism might be useful in some clinical diseases associated with memory loss.

Pathological conditions

Interestingly, in tissues from patients with Alzheimer's disease, a significant decrease in GBR density has been detected, raising the possibility of using baclofen-like analogues as potential positron-emission tomographic ligands to scan patients with this disease[94]. GBRs are altered in other neurological disorders such as Huntington's disease[95]. In the cerebral cortex of rats with acute hepatic encephalopathy, there is a substantial loss of GBR binding sites, which may contribute to the disturbances in glutamatergic and GABAergic neurotransmission[96], suggesting that these receptors might have other potentially interesting therapeutic implications as drug targets.

Psychiatric disorders

Because GABA-containing synapses are abundant in the brain, GABA receptors are likely to play a pivotal role in various psychiatric disorders. GBRs are upregulated after long-term treatment with antidepressants[97], but downregulation of these receptors has also been shown[98]. The use of baclofen in panic disorder has been reported, decreasing the number of panic attacks and reducing the Hamilton Anxiety Score in nine individuals[99]. The baclofen congener **6** was actually the first such compound in clinical use for mood alteration[27]. However, GBRs may be involved in the pathophysiology of affective disorders, and their possible role in human depression, mood disorders and other behavioural states should be more adequately addressed in the future.

Alcohol dependence and withdrawal

Interactions of ethanol with GBRs have been implicated in the reversal of alcohol withdrawal symptoms by GBR activation; phaclofen (**20**), one of the original antagonists, blocks ethanol-induced hypothermia, motor incoordination, narcosis and locomotor stimulation[100]. Phaclofen also reverses the anticonvulsant action of alcohol against picrotoxin-induced seizures[101]. These are selective interactions through GBR sites. Baclofen itself reduces anxiogenic responses during alcohol withdrawal, suggesting a role of altered GBRs in physical ethanol dependence[102]. On the other hand, baclofen can also produce increased hyperexcitability in mice undergoing ethanol withdrawal[103]. Nevertheless, baclofen is effective in alcoholic patients with secondary affective disorders such as anxiety and depression[104].

Feeding behaviour

Other central effects of GBR activation include ingestive behaviour: baclofen elicits a dose-related increase in food intake[105]. In particular, the regulation of insulin secretion from the pancreas by GABA should be further explored, because GBRs have an inhibitory role on insulin secretion on β-islet cells[106].

Cardiovascular and respiratory functions

Although GBRs have been implicated in tonic inhibitory control of blood pressure[107], they have never been targeted for use in clinical practice involving cardiovascular abnormalities. While the GBR antagonist CGP 35348 blocks the pressor response induced by baclofen, it can also elicit a decrease in arterial blood pressure. This suggests that there is a tonic regulation of blood pressure by endogenous GABA acting on GBRs to attenuate the baroreceptor reflex[108]. Any possible therapeutic role of GBR agonists or antagonists in blood pressure control has not yet been explored, and further studies should be performed using the highly potent antagonists now available.

GBRs are also involved in respiratory function, with baclofen generally depressing inspiratory neurons[109], whereas the antagonists exert an excitatory effect by increasing spontaneous discharges of respiratory neurons, and at the same time block the depressant effects of baclofen on respiratory drive[110].

Peripheral functions

GBR function in peripheral nervous tissues has been extensively reviewed elsewhere[111–114]. In allergic inflammation of the airways, GABA inhibits allergen-induced contraction of guinea-pig trachea through activation of GBRs (Ref. 115). Recent studies have also found that GBR agonists such as baclofen and 3-aminopropylphosphinic acid (**3**) reduce microvascular leakage, inhibit a variety of neurally mediated responses in the airways and possess antitussive properties[115]. The latter is antagonized by SCH 50911 (**27**)[48]. GBRs may be significant in the pathogenesis of airway obstructive diseases such as asthma and other airway disorders. In view of the respiratory depressant effects of GBRs, caution is required when GBR ligands with central actions are given parenterally, in large doses. In consequence, CNS penetration could limit the therapeutic usefulness of a GBR agonist. By

contrast, GBR agonists that do not penetrate into the brain would not depress respiratory function, and may prove useful as peripheral antitussive agents. Indeed, GBR agonists that preferentially activate peripheral GBRs would be devoid of central side-effects, making them interesting targets for drug development.

Physiological roles for GBR in intestinal motility, and in the reproductive tract, gall bladder, pancreas, urinary tract and adrenal medulla, as well as in autonomic ganglia, have been well defined and reviewed elsewhere[114]. Centrally, baclofen increases and disrupts duodenal cyclic motility in the rat[116], while it stimulates rat gastric motility[117]. Indeed, GBRs are present at various levels of the gut where they modulate the release of acetylcholine and induce relaxation in the guinea-pig jejunum, duodenum and distal colon[113]. The targeting of GBRs that control peristaltic movements[8] may be of clinical significance in conditions of excessive or disturbed intestinal activity, as in irritable bowel syndrome, inflammatory bowel disease and Crohn's disease, particularly because anti-inflammatory actions of GBR activation are known. The emergence of newer and more potent GBR ligands makes future studies of GBRs in gastrointestinal motility an exciting prospect, with potential clinical applications.

In the rat, parenteral administration of baclofen stimulates acid secretion, which is associated with an increase in vagal efferent activity[118], presumably through central activation by disinhibition of the parasympathetic outflow to the stomach at the level of the nucleus tractus solitarius. Recently, 3-amino-3-phenylpropionic acid (**28**), a structural analogue of baclofen, has been shown to inhibit baclofen-induced gastric acid secretion in rats by a central action[58], implicating GBRs in the regulation of gastric acid secretion.

In general, GABAergic neurons are present in sympathetic ganglia of the autonomic nervous system. For example, in the adrenal medulla, activation of GBRs reduces catecholamine release that is evoked through activating the preganglionic, cholinergic, nicotinic innervation. Similarly, this same depressive effect is seen on the cholinergic innervation of ganglion cells in the superior cervical ganglion[114].

As for other peripheral tissues, GBRs appear to be located on smooth muscle in the rabbit oviduct, as well as on endocrine structures such as the pars intermedia of the pituitary gland, and pancreatic islet β-cells. From the presence of baclofen-binding sites in rat kidney, a role for GBRs in renal function has been suggested, perhaps in the modulation of calcium or potassium transport. Baclofen-sensitive GBRs are involved in regulating urinary bladder motility, by acting on autonomic nerves. In clinical practice, baclofen is used in the symptomatic treatment of motility disturbance of the urinary tract, and detrusor hyperreflexia secondary to spinal cord lesions[119]. Furthermore, these receptors may also modulate excitatory neurotransmission in rat pelvic ganglia, as well as micturition reflexes. In the rabbit detrusor muscle, baclofen inhibits contractile responses to electrical stimulation of the autonomic nerves, this action being reversed by the GBR antagonist 2-hydroxysaclofen[120], thus raising the possibility of using GBR ligands in the treatment of detrusor instability. All these have been extensively reviewed elsewhere[111–114].

Future directions

The contribution of classical pharmacology coupled with medicinal chemistry to define GBR subtypes has so far been hindered by the lack of variety and inadequate selectivity of available agonists and antagonists; this problem is acute where multiple receptor subtypes coexist on the same cell. Nonetheless, with current knowledge of the structure–action profiles, pharmacological characteristics and biochemical mechanisms linked to GBRs, it should shortly be possible to define which receptor subtype mediates a given functional effect of GBR stimulation, and how the effect is achieved. This should then pave the way for targeting particular receptor subtypes with selective therapeutic agents, to modify function in disease states. However, a wider range of ligands needs to be developed, targeting these receptor subtypes. Furthermore, the understanding of the functional diversity of GBR subtypes, and their structural components, can best be advanced by using molecular biological approaches. In particular, the GBRs need to be sequenced, and test systems developed based on cloned, expressed subtypes.

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