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$GABA_B$ RECEPTOR PHARMACOLOGY

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INTRODUCTION

It is now 25 years since the establishment of γ-aminobutyric acid (GABA) as an inhibitory neurotransmitter within the mammalian brain (see refs. 1, 2). Although the presence of this neutral amino acid within the brain had been described more than 10 years earlier (3, 4), its role as a neurotransmitter was disputed at that time (e.g. 5, 6). While it produced profound depressant actions on central neurones, the nature of this effect was not considered to be consistent with synaptic inhibition (7). Only after Krnjević & Schwartz (8) equated synaptically mediated inhibition with the inhibitory action of GABA on the same cerebral cortex neurones was the transmitter role considered to be a reality. During the subsequent years much evidence was provided to support a synaptic role but perhaps the discovery in 1970 of the competitive receptor antagonist, bicuculline, by Curtis & colleagues (9) was the most crucial. This alkaloid was observed to be a selective antagonist of both the fast actions of GABA and synaptic inhibition in higher centers. Within the spinal cord much synaptic inhibition is mediated by the amino acid glycine through receptors that are insensitive to bicuculline. This insensitivity perhaps helped to explain why the determination of GABA as a CNS-inhibitory transmitter was such a problem, since many studies attempting to discover the natural transmitter were performed on neurones in the spinal cord where glycine is of major importance.

Bicuculline is now used routinely to define GABA-mediated processes where receptors are coupled to chloride conductance mechanisms in neuronal

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membranes. All the effects of GABA and GABA-related analogues on these receptors can be prevented by this antagonist.

In 1979, my colleague and I were performing experiments to establish the presence of GABA receptors on peripheral nerve terminals (10). In the course of our experiments we used bicuculline but it failed to prevent the action of GABA. This observation led us to the introduction of a novel receptor for GABA, the $GABA_B$ receptor. This review considers the significance of $GABA_B$ receptors in mammals.

The receptors are not confined to the periphery but are clearly functional within the central nervous system from where most data have emerged. Some comparisons with $GABA_A$ receptor mechanisms are made although much more is known about the $GABA_A$ receptor, including the receptor sequence from molecular biology studies (see ref. 11). No such information has been obtained thus far for $GABA_B$ sites.

HISTORY

As outlined above, it was while trying to establish the presence of receptors for GABA on peripheral nerve terminals that $GABA_B$ sites emerged. The purpose of the original experiments was to establish a peripheral model for receptors mediating synaptic inhibition. The existence of presynaptic inhibition had been known for many years and there was very good evidence that the inhibition was mediated by GABA. GABA neurones, present in the dorsal horn of the spinal cord, appear to form axo-axonic contacts with terminals of primary afferent fibers (12, 13). Activation of the receptors on these terminals decreases transmitter release to modulate the output of the sensory process. GABA released from the spinal interneurones is believed to depolarize (rather than hyperpolarize) the nerve terminal and the release evoked by nerve volleys would then be diminished (see ref. 14). We and others (15–17) had shown that GABA also depolarized peripheral sympathetic neurones and considered that if this effect was manifest on the terminals of ganglionic neurones as well as on the soma then the sympathetic system might form an effective model for presynaptic events in the spinal cord. Since it was not possible to record the electrical event in the nerve terminals, it was decided to measure the output of neurotransmitter instead. The rat isolated atrium was chosen for the study since much information was available on the release and uptake of the sympathetic neurotransmitter noradrenaline, using tritium-labeled material as a tracer (18). GABA decreased the transmurally stimulated release of ^{3}H-noradrenaline in a dose-dependent manner, as predicted (10, 19). However, the action was insensitive to bicuculline and other recognized GABA antagonists such as picrotoxin, and established GABA receptor agonists, such as isoguvacine and THIP, were inactive. This inhibition of ^{3}H-noradrenaline

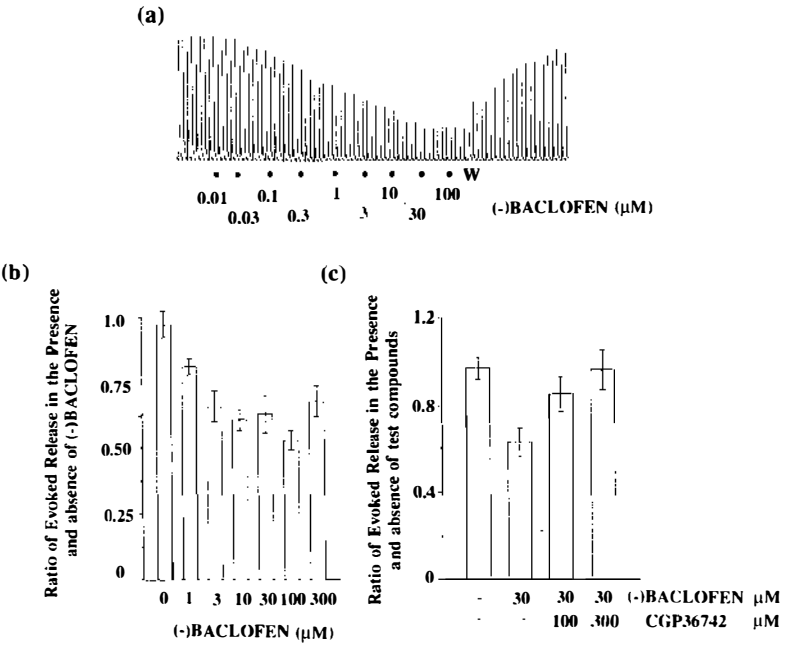

Figure 1 Effect of (−)baclofen on the electrically evoked twitch (*a*) and release of ^{3}H-noradrenaline from, (*b*) & (*c*), the rat isolated anococcygeus muscle. Anococcygeus muscles were set up in an organ bath, with or without prior incubation, in ^{3}H-L-noradrenaline (25OnM 38Ci/mmol for 40 min.). For measuring contractile responses (*a*) the tissue was stimulated for 1 sec, 0.5ms at 1OHz every 20s at supramaximal voltage. Increasing concentrations of (−)baclofen (to 10^{-8}–10^{-4}M) were added cumulatively to the bathing medium at the points indicated below the record.

In (*b*) and (*c*) the tissue was superfused at 0.5ml/min and transmurally stimulated for 10s at 0.5ms, 10Hz, and supramaximal voltage. Fractions were collected sequentially for 5 min periods. (−)Baclofen was present in the superfusing solution 90 s prior to and during stimulation. Each histogram bar represents the ratio of tritium release during and just prior to stimulation. In (*b*) all mean values were significantly lower ($p < 0.05$) than control. In (*c*) only the value obtained in the presence of 30μM (−)baclofen was significantly lower than control n = 3 or more in all cases. Yohimbine (2.5μM), pargyline (500μM) and ascorbate (500μM) were present throughout. [Data provided by J. J. Maguire.]

release could be observed equally in other peripheral tissues such as the rat anococcygeus. An illustration of this is shown in Figure 1 where the GABA analogue, baclofen (see later), depressed the evoked release of transmitter and the twitch response in a dose-dependent manner. The response seemed not to be mediated through an increase in Cl^- conductance and therefore depolarization seemed an unlikely explanation.

Thus, although we had set out to establish a model for terminal depolar-

ization and presynaptic inhibition, we had illustrated an inhibition of transmitter release by GABA that was not associated with membrane depolarization and was not via the familiar receptor.

After obtaining these data and characterizing the pharmacological profile of this, apparently novel, receptor (19), we used brain tissue to establish whether the receptor is present in the brain and not just an artefact within the periphery. We demonstrated that K^+ evoked release of radiolabeled noradrenaline from brain slices could be inhibited by GABA and this inhibition was not blocked by bicuculline (20). Moreover, the GABA analogues that were inactive in the peripheral model were also inactive in brain-slice release experiments. Ultimately, we developed a radiolabeled receptor binding assay in brain synaptic membranes that enabled us to distinguish two recognition sites for GABA with entirely separate profiles (21). At this point we introduced the terms "$GABA_A$ and "$GABA_B$" as there seemed little doubt that separate receptors exist.

AGONISTS

Our early studies soon demonstrated that many accepted GABA mimetics at bicuculline-sensitive receptors ($GABA_A$ sites) were inactive at $GABA_B$ sites, but at that stage only one compound had the reverse profile, i.e. active at $GABA_B$ sites but inactive at $GABA_A$. This compound, β-p-chlorophenyl-GABA or baclofen, had been synthesized and marketed as an antispastic agent some 10 years earlier (22, 23). It has been designed as a brain penetrating form of GABA that, it was hoped, would mimic the action of GABA probably after enzymic degradation. In fact, no evidence could be obtained to support either the GABA mimetic action or its degradation. It is known that baclofen is excreted largely unchanged by the kidney (24). Nevertheless, it is therapeutically active and remains a major treatment for spasticity by virtue of action within the brain to which it gains access by a selective transport process (25). Baclofen is virtually inactive at $GABA_A$ sites but is stereospecifically active at $GABA_B$ sites, (−)baclofen being the active form. A number of close-related analogues of baclofen and GABA have been shown to be agonists of $GABA_B$ sites (Figure 2). The most potent so far described are the phosphinic analogues 3-aminopropyl phosphinic acid and its methyl analogue, but although they exhibit 10–100 fold higher affinity for the $GABA_B$, binding site than (−)baclofen this affinity is not maintained in all functional assays (26–30).

The apparent inconsistency between the activities of the ligands in the binding and functional assays has invited consideration of heterogeneity within the $GABA_B$ receptor population. Surprisingly, however, it was not this discrepancy but rather the electrophysiological data that first led to the idea

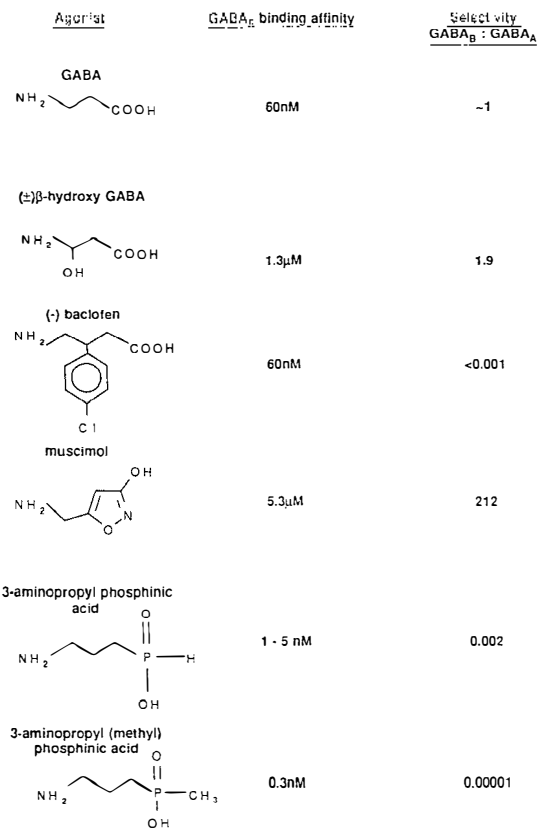

Figure 2 Structures of selected GABA$_B$ agonists and their binding affinities at GABA$_B$ sites in rat brain membranes. The ratios of their affinities for GABA$_B$ and GABA$_A$ sites are indicated in the right hand column.

of receptor heterogeneity. Although the evidence is very slender, it nevertheless warrants discussion and has therefore been included towards the end of the chapter.

Only a limited number of active GABA$_B$ agonists have so far been described, a reflection possibly of the narrow limits within which the structure can apparently deviate from GABA. Numerous predictions have been made based on the structure and the possible conformational state of baclofen but these have produced either inactive or only weakly active ligands. For example, Mann & colleagues (31) predicted that 1-(aminomethyl)-5-chloro-2,3-dihydro-1H-indene-l-acetic acid would be an optimal mimic of the

solid-state conformation of baclofen. Surprisingly, this compound was completely inactive.

Any substitution so far reported at the (α or γ positions in GABA or the phosphinic acid analogues markedly reduces agonist activity and affinity for the $GABA_B$ site (32) whereas substitution at the β position, of course, may not. Thus, the p-chlorophenyl (baclofen), hydroxy, or chloro analogues all retain appreciable activity (19). Substitution on the amino group always produces a significant loss of affinity (32). However, this does not appear to be the same for antagonists (see below).

ANTAGONISTS

Selective antagonists for the $GABA_A$ receptor complex have been known for many years and include compounds such as bicuculline, picrotoxin, and the bicyclophosphates (9, 33, 34). These compounds all act to decrease Cl^--mediated inhibition evoked by activation of the $GABA_A$ receptor even though their mechanisms of action may differ. For example, bicuculline is a competitive antagonist, whereas picrotoxin acts noncompetitively to prevent $GABA_A$ channel activation. The resulting decrease in neuronal Cl^- conductance produces convulsant activity with all of the antagonists.

None of these compounds has any effect on $GABA_B$ receptor mechanisms, a finding that, in part, provided the initial suggestion for a separate receptor. It was only some years later that the first selective $GABA_B$ antagonists emerged. The first substances to be reported, δ-aminovaleric acid and 3-aminopropane sulphonic acid (3APS) (35, 36), were not selective ligands. Although they exhibit weak $GABA_B$ receptor antagonism they are also effective $GABA_A$ *agonists* (3APS is at least as active as GABA at $GABA_A$ sites) and therefore cannot be used as tools to dissect out $GABA_B$-mediated events. However, the activity of 3APS provided a clue that Kerr and colleagues eventually pursued to produce the selective antagonist saclofen and its 2-hydroxy derivative (37). Prior to this, these same authors, inspired by the success of Watkins (38) who showed that ω-phosphono analogues of glutamate are antagonists of NMDA receptors, produced the phosphonic derivative of baclofen, phaclofen (39). Phaclofen was the first, albeit weak, selective $GABA_B$ antagonist.

The affinity of phaclofen for $GABA_B$ binding sites in rat brain synaptic membranes is in excess of 100μM (cf GABA and (−)baclofen ~50–80nM). When extrapolated to more functional assays this affinity means that concentrations in the region of 1mM are necessary to achieve sufficient receptor occupation to antagonize responses to the agonist. This was accomplished to good effect in hippocampal slices where data obtained with phaclofen provided the first evidence for a physiological role for $GABA_B$ receptors in mammalian

brain. Dutar & Nicoll (40) showed that phaclofen could prevent the production of late inhibitory postsynaptic potentials (ipsps) in hippocampal pyramidal cells in response to orthodromic stimulation of the stratum radiatum. Similar effects have now been demonstrated in other brain regions (e.g. 41–43) using phaclofen and the more potent derivatives saclofen and 2-hydroxy saclofen (44; Figure 3). These compounds are approximately tenfold more potent than phaclofen but, like the phosphonic derivative, do not readily penetrate the blood brain barrier. Since such high concentrations are required in the vicinity of the GABA$_B$ receptor to obtain antagonism coupled with the poor brain penetration, systemic administration of these antagonists will not produce any changes in brain GABA$_B$ receptor activation. Thus, although these compounds have provided much information in isolated preparations the introduction of a brain-penetrating antagonist has long been awaited.

CGP 35348 (Figure 3), the first compound in this class to be reported, acts competitively but has only low potency (45, 46). In binding studies its affinity for GABA$_B$ sites in brain synaptic membranes is in the region of 35μM (cf (−)baclofen ~50nM and 3APPA–10nM). Thus, to achieve any receptor antagonism in vivo large amounts must be injected systemically. Using 30–100mg/kg i.p. or 10-30mg/kg i.v., Olpe and colleagues (46) showed clear antagonism of responses to baclofen applied iontophoretically onto rat cerebrocortical neurones. The effect was selective and produced no change in the responses to certain other neuroactive agonists. Perhaps most importantly the antagonist produced no behavioral changes itself, which would suggest that GABA$_B$ receptor mechanisms are not subjected to a major endogenous activation under resting (physiological?) conditions. This lack of effect augurs well for possible future therapeutic use since adverse side effects may be limited. By comparison, GABA$_A$ antagonists produced profound effects following acute systemic administration, particularly convulsive activity. Clearly, GABA$_A$ receptors are constantly being activated and any interference in this activation places an imbalance in the system to produce excessive neuronal excitation. CGP 35348 has been used in numerous studies where GABA$_B$ mechanisms have been indicated and these applications are discussed below.

The most recent brain-penetrating antagonist to be introduced is CGP 36742 (Figures 1, 3), which appears to have greater potency in vivo than CGP 35348 and is brain available even after oral administration. The affinity of this compound for GABA$_B$ binding sites is 35μM (H. Bittiger, personal communication) and like CGP 35348 can block the effects of baclofen while producing no obvious effects in the absence of exogenous agonist. Future studies with this compound should prove exciting, particularly in relation to any therapeutic potential.

A limited number of antagonists that are benzofuran analogues of baclofen

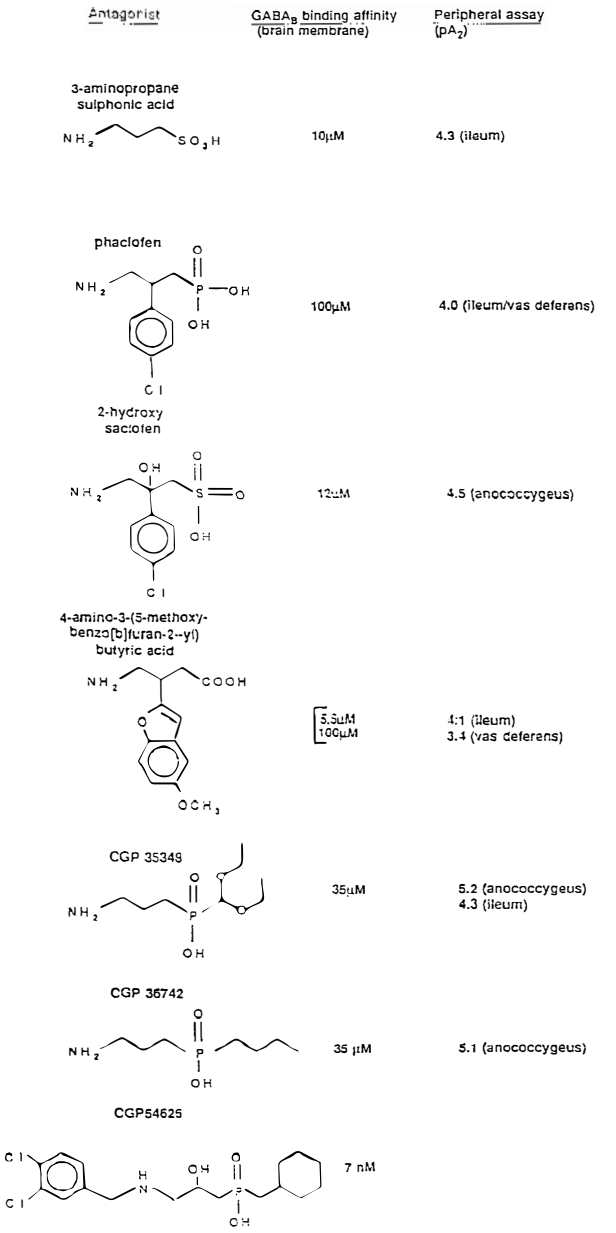

Figure 3 Structures and binding affinities of selected $GABA_B$ receptor antagonists. The pA_2 values derive from published and unpublished data (J. J. Maguire & N. G. Bowery) obtained in peripheral isolated tissue assays (guinea-pig ileum or rat anococcygeus). The binding affinities quoted are average values from the literature, except where the two values quoted differ by twentyfold in separate publications.

have been described by Debaert and colleagues (47, 48; Figure 3) but while they may or may not gain access to the brain, only relatively low potency has been noted. A series of thioether analogues of saclofen and phaclofen have also been examined, but in all cases they were less active than the parent compounds on isolated preparations (49). Perhaps the most exciting antagonists reported so far have been by Froestl et al (32). These authors have described results obtained with novel GABA$_B$ antagonists with nanomolar affinity for the receptor. By substitution with a monochloro- or dichlorobenzyl moiety on the nitrogen of the antagonist CGP 35348, affinity for the GABA$_B$ binding increased from 35μM to 1μM and 55nM respectively. Further substitution on the β carbon with methyl or hydroxyl groups increased the affinity still further (32). These observations are in striking contrast to those obtained with agonists where substitution on the nitrogen in all cases diminishes activity at the GABA$_B$ receptor.

LOCATION OF GABA$_B$ SITES

In mammals GABA$_B$ sites occur outside as well as inside the brain and, not surprisingly, are present in lower species too. For example, they have been described in the lobster at the neuromuscular synapse on both inhibitory and excitatory axon terminals where they appear to be coupled to K^+ channels via G-protein (50). Giant interneurones of the cockroach CNS also possess GABA receptors with characteristics reminiscent of the GABA$_B$ subtype, although the pharmacological characteristics are not exactly the same (51). In *Achatina fulica,* neuronal responses to GABA and baclofen have been described that were attributed to GABA$_B$ receptor activation, although the data were inconclusive (52).

The locations of GABA$_B$ receptor sites in mammals have been ascertained from functional as well as receptor autoradiography studies. In general, they are not confined to neurones but are also present on glial cells (53). Outside the brain, GABA$_B$ receptors have been described on axon terminals and ganglion cell bodies of the autonomic nervous system (e.g. 19, 54), on fallopian tube and uterine and intestinal smooth muscle cells (55–58), in the kidney cortex (59), urinary bladder muscle (60), and on testicular interstitial cells (61; Table 1). Within the brain, an uneven distribution of receptors occurs with regional variations in receptor density; receptor autoradiography has provided much of the evidence for this regional heterogeneity (62–64). In many brain regions GABA$_B$ and GABA$_A$ sites exist together, but in certain areas GABA$_B$ sites have been demonstrated with very few GABA$_A$ receptors being present. For example, in the interpeduncular nucleus the density of GABA$_B$ sites is high whereas that of GABA$_A$ sites is extremely low (62). By contrast, GABA$_B$ sites appear to be absent from the cerebellar granule cell layer whereas high affinity GABA$_A$ sites are in abundance (65). High densities

Table 1 Location of $GABA_B$ sites in mammalian periphery

Organ	Species	Response to receptor activation
Oviduct	Rabbit	Contractility ↑
Uterus	Rabbit	Contraction
Atria	Rat	Transmitter release ↓
Intestine	Rat/Guinea-pig	Relaxation
Anterior pituitary	Rat	Hormone release ↓
Superior cervical ganglion	Rat	Neuronal hyperpolarization/ Acetylcholine release ↓
Gall bladder	Guinea-pig	Contraction
Dorsal root ganglion	Rat	Ca^{++} conductance ↓
Pancreas	Rat	?
Urinary bladder	Rat/Guinea-pig	Relaxation
Adrenal medulla	Rat	Catecholamine release ↓
Vas deferens	Rat	Twitch response/ Transmitter release ↓
Anococcygeus	Rat	Twitch response/ Transmitter release ↓
Bronchioles	Guinea-pig	Relaxation

of $GABA_B$ sites also occur in the cerebral cortex and certain thalamic nuclei and these latter sites may be of therapeutic significance in the treatment of absence seizures (see below). Moderate densities of $GABA_B$ sites are present throughout the hippocampal formation although are low in the pyramidal cell layer. The presence of these sites in this brain region may be significant in cognitive functioning since it has been proposed that the induction of long-term potentiation is influenced by $GABA_B$ receptor activation/antagonism (66–68, but see also 69). The globus pallidus, habenula nucleus, superior colliculus, and amygdala also contain moderate to high densities of $GABA_B$ sites, but their possible significance in these regions has yet to be demonstrated. In many regions the primary location of $GABA_B$ sites appears to be presynaptic in origin. In the rat caudate putamen, for example, the density of $GABA_B$ sites is reduced following chronic decortication (70), which suggests a location on corticostriatal terminals. Also following electrolytic ablation of the habenula, $GABA_B$ site binding virtually disappears from the interpeduncular nucleus, which again indicates a presynaptic location on terminals of the retroflexus of Meynert that terminate in the nucleus (71). A further indication of a presynaptic location for $GABA_B$ sites occurs in the cerebellum where it has been suggested that they are located on parallel fibre terminals in the molecular layer (72). This does not exclude the possibility of a postsynaptic location on Purkinje cell dendrites as well (73). A dual location may also occur elsewhere, for example, in the rat spinal cord, where $GABA_B$ sites

predominate in the dorsal horn, denervation of primary afferents either by surgery or capsaicin injection in the neonate produced a 50% reduction in the density of GABA$_B$ sites (74). While the remaining sites may also be presynaptic in origin on terminals of interneurones or descending fibers, they may also be present at postsynaptic sites within the spinal cord. The significance of GABA$_B$ sites in the spinal cord in relation to spasticity and analgesia is discussed below.

Within the hippocampus GABA$_B$ sites also occur at pre- and postsynaptic sites. Electrophysiological data indicate their presence at both sites. Dutar & Nicoll (75) reported that the hyperpolarizing response to baclofen in CA1 pyramidal cells as well as the synaptically evoked late ipsp was antagonized by phaclofen and by G-protein ADP ribosylation with pertussis toxin. By contrast, the attenuation of evoked excitatory postsynaptic potentials (epsps) by baclofen in the same region was unaffected by either agent. Harrison (76) has produced similar data that suggest the possibility that not only are GABA$_B$ sites at these separate locations but that they may also be distinct forms of the receptor differentially linked to G-proteins at each site. Similar conclusions have derived from studies in the dorsal raphe nucleus (77), corpus striatum (78), and cerebral cortex (79).

The presence of presynaptic rather than postsynaptic receptors within the pyramidal cell layer of the hippocampus could explain data we have recently obtained using tetanus toxin. This endotoxin produced by *Clostridium tetani* selectively suppresses the release of inhibitory transmitter, e.g. GABA from nerve terminals (80, 81). Perhaps as a consequence, injection of the toxin into the CA1 region produced neurodegeneration with a loss of neurones in close proximity to the injection site some 7–10 days later (82, 83). A concomitant reduction in GABA$_A$ receptor binding also occurred in the same region and probably reflects the loss of neurones, which are presumed to be pyramidal cells possessing the binding sites on their surface. In complete contrast, GABA$_B$ binding in the pyramidal cell layer was completely unaffected by the toxin or subsequent loss of neurones. This result may indicate that the binding is restricted to nerve terminals within this hippocampal layer. The most obvious site would be the GABAergic nerve terminals of GABAergic basket interneurones, which innervate the pyramidal cells. These terminals might be unaffected by the toxin and thus GABA$_B$ binding occurs to the same extent as normal at autoreceptor sites. This hypothesis is supported by earlier evidence from Newberry & Nicoll (84) that electrophysiological responses to GABA$_B$ receptor activation can be readily detected on the dendrites of pyramidal cells, but not on the cell soma. By contrast, the sensitivity to GABA$_B$ agonists appeared to be constant over the soma and dendrites. Data from receptor autoradiography studies from normal hippocampus (62) have shown that GABA$_B$ sites are unevenly distributed across

Table 2 Comparative levels of $GABA_B$ binding in regions of mammalian brain

Region	Relative Density	Rat	Human	Monkey
Spinal cord	High	II	II	II
	Moderate	I & III	I & III	
	Low	Ventral laminae	Ventral laminae	
Cerebellum	High	Molecular layer	Molecular layer	
	Low	Granule cell layer	Granule cell layer	
Hippocampus	Moderate	Striatum radiatum Dentate gyrus molecular layer	Striatum radiatum Dentate gyrus molecular layer Striatum pyramidale CA1	
	Low	Subiculum	Subiculum	
Cerebral cortex	High	I–III		
	Moderate	IV		I–III
	Low			IV
Thalamus	High	Medial geniculate Dorsal lateral geniculate		
	Low	Ventral lateral geniculate		
Superior colliculus	High	Superficial grey layer		
	Low	Intermediate grey layer		

References 62, 62a, 62b, 63, 63a, 63b

hippocampus, with low densities in the pyramidal cells and with $GABA_A$ sites evenly distributed across the dendrites and soma.

Information on the distribution of $GABA_B$ sites in mammalian brain derives primarily from studies using rat tissue. However, these data compare favorably with those obtained so far in post mortem primate brain (Table 2). It is particularly worth noting that the distribution within the human spinal cord appears to be very similar to that in the rat despite the lack of well-documented evidence that baclofen acts as an analgesic in man (85, 86).

ELECTROPHYSIOLOGICAL ASPECTS

Ca^{++} Channels

Initial observations of $GABA_B$ receptor stimulation in neurones of peripheral origin, i.e. dorsal root ganglion cells and myenteric neurones, indicated that the primary effect of receptor activation was a diminution in membrane Ca^{++} conductance. This effect was manifest as a decrease in the duration of action

potentials in C and Aδ neurones of dorsal root ganglia (87, 88) and a reduction in the amplitude of Ca^{++} spikes elicited in myenteric neurones in vitro (89). That a decrease in Ca^{++} conductance occurs in ganglion cells was supported by subsequent voltage clamp studies in cat, mouse, and rat neurones (90–92). Such an effect on voltage-gated Ca^{++} channels would readily explain the original neurochemical evidence that baclofen and GABA can diminish the evoked release of neurotransmitters (19, 20). A diminution in Ca^{++} flux would provide an obvious explanation.

However, evidence solely for a Ca^{++} mechanism has not been established within the brain, where changes in K^+ conductance may be more important. Gähwiler & Brown (93) found no evidence for a direct involvement of Ca^{++} channels in the response to GABA$_B$ receptor activation in hippocampal slices. Their conclusion was that any changes in Ca^{++} conductance result from an initial increase in K^+ conductance. But there seems to be scope for implicating a decrease in Ca^{++} conductance in the mechanisms of presynaptic inhibition even within the hippocampus. Scholtz & Miller (94) have recently indicated that the calcium current responsible for mediating presynaptic inhibition in cultured hippocampal neurones is modulated by GABA$_B$ receptors.

Studies on dorsal root ganglia (DRG) have provided important information on possible Ca^{++} channel involvement. Holz et al (95) demonstrated with chick DRG cultures that pertussis toxin reduces the effect of GABA on action potential duration; this finding indicates a G-protein connection in the process. GABA$_B$ receptor activation appears to mediate a decrease in Ca^{++} conductance through a direct link with G-proteins (96).

GABA receptors have been considered to affect a variety of voltage-gated Ca^{++} channels in central and dorsal root ganglion neurones. Much evidence supports an association with "N" channels, although an effect on "L" channels has also been suggested (97). However, in studies using Ca^{++} flux measurements in rat cerebral cortex synaptosomes with the fluorescence indicator Quin 2, GABA$_B$ receptor activation diminished the voltage-dependent Ca^{++} signal in an additive manner with the L-channel antagonist nifedipine (98). Similarly, baclofen and nifedipine were additive in inhibiting the influx of Ca^{++} into cerebellar granule cells (99). This additivity might suggest an action for GABA$_B$ agonists independent of L-channels. Surprisingly, Thalman & Al-Dahan (100) observed a marked decrease in GABA$_B$ binding to brain synaptic membranes in the presence of an L-channel antagonist, but it seems unlikely that this reflects an affinity for L-channels.

The third type of Ca^{++} current, "T", may also be implicated in the response to GABA$_B$ receptor activation. This low threshold transient current can be influenced by baclofen in a manner dependent on the agonist concentration (101).

The overall effect of GABA$_B$ receptor activation on Ca^{++} mechanisms,

irrespective of a direct or indirect action, is undoubtedly of major significance in the pathophysiology of this receptor system. Whether a direct mechanism can be implicated in the control of transmitter release has yet to be established. However, such a mechanism seems most likely at primary afferent fibers in the spinal cord where $GABA_B$ receptor activation decreases the evoked release of putative neurotransmitter peptides such as substance P. Even in higher centers where preliminary evidence indicates that $GABA_B$ sites on excitatory nerve terminals are linked to K^+ channels, the possibility of implicating Ca^{++} channels at inhibitory nerve terminals remains (B. H. Gähwiler & S. M. Thomson, personal communication).

$GABA_B$-mediated inhibition of voltage-gated Ca^{++} currents has been observed in a variety of neuronal types and is not confined to sensory ganglia. In goldfish retinal ganglion cells under whole-cell patch clamp, (−)baclofen and GABA inhibited calcium currents activated by prolonged depolarization. However, phaclofen and 2-hydroxy saclofen failed to prevent the inhibition (102). In bovine chromaffin cells, (−)baclofen inhibited Ca^{++} signals in a pertussis-sensitive manner (103) and in freshly dissociated spinal cord neurones from the rat, baclofen inhibited the voltage-sensitive Ca^{++} current in a substantial fraction of cells. This effect appeared to be mediated via G-proteins (104). Inhibition of voltage-gated Ca^{++} channels in cultured spinal neurones has also been described by Kamatchi & Ticku (105). However, in these experiments Ca^{++} activated ^{86}Rb efflux was used as the marker. Only the voltage-sensitive Ca^{++} activated ^{86}Rb efflux was inhibited by baclofen whereas that produced by the Ca^{++} ionophore, A23187, was not.

K^+ Channels

Within higher centers of the mammalian brain an increase in K^+ channel conductance appears to be the primary neuronal response to $GABA_B$ activation and produces membrane hyperpolarization. This response has been described in numerous brain regions including the hippocampus cerebral cortex, thalamus, septum, and medulla (e.g. 40, 42, 84, 93, 106–112). This hyperpolarization appears to reflect the late inhibitory postsynaptic potential demonstrated in these regions after afferent fiber stimulation. The late ipsp and baclofen-induced hyperpolarization can be antagonized by phaclofen and CGP35348 and both events can be prevented by prior treatment with pertussis toxin. Injection of pertussis toxin into the rat lateral ventricle, 3 days prior to preparing hippocampal slices, prevented the hyperpolarization produced by baclofen as well as the evoked late ipsp (113, 114). The fast ipsp mediated via $GABA_A$ receptors through an increase in Cl^- conductance was unaffected. This result would support the notion that $GABA_B$ but not $GABA_A$ receptors

are linked through G-protein(s) to K^+ channels. Whether the same G-protein(s) is/are responsible for coupling K^+ and Ca^{++} channels is unknown but certainly both are of the Gi/Go class since both mechanisms are prevented by ADP ribosylation.

The question of which K^+ channel(s) is (arc) responsible for mediating the response to baclofen has been recently reviewed by Gage (115).

Current indications are that an A current may be responsible. This current is voltage-sensitive and is virtually inactivated at normal resting membrane potential. GABA$_B$ receptors appear to potentiate this current (116) and since these are present on presynaptic terminals they may be responsible for modifying transmitter release rather than any direct effect on Ca^{++} channels. Since activation of the A current was not prevented by pertussis toxin and the reported presynaptic effects of GABA$_B$ receptor activation also appear to be pertussis toxin-insensitive in rat hippocampus, the receptor may be coupled directly to K^+ channels in these terminals. This would contrast with the postsynaptic K^+ channels where activation is mediated through G-protein and second messengers (117).

The postsynaptic K^+ channel(s) modulated by baclofen in hippocampal neurones has been reported to be affected by numerous substances; these include the local anaesthetic QX-314, which appears to have no effect on Cl^- channels associated with GABA$_A$ receptors (118, 119), and quisqualic acid and kainic acid (120). In isolated substantia nigra neurones, GABA$_B$ receptors have been reported to activate what appears to be an ATP-regulated K^+ channel since the sulphonylurea, tolbutamide, blocked the hyperpolarization and increase in whole-cell conductance produced by baclofen (121).

The inhibitory effect produced by increasing membrane K^+ conductance on postsynaptic dendrites may be of even greater significance than the generally accepted influence of Cl^- conductance mechanism. Qian & Seynowski (122) predict that for inhibitory synapses to be effective on cortical spines "they should be mediated by K^+ through GABA$_B$ receptors". The traditionally held belief is that the profound hyperpolarization produced by GABA$_A$ receptor activation is faster and of greater magnitude. Perhaps the full potential of GABA$_B$ mechanisms has yet to emerge.

PRESYNAPTIC RECEPTORS

Undoubtedly, GABA$_B$ receptors on presynaptic terminals can have a major pharmacological influence on synaptic processing. As pointed out above, within the hippocampus baclofen can depress epsps consistent with an action on terminals of Schaffer collaterals in the CA1 region (see ref. 123). This heteroreceptor effect can also be detected in mouse hippocampal neurone

cultures, where epsps in one neurone are produced by stimulation of another neurone (124), as well as in hippocampal slices (75, 125). Suppression of the evoked release of glutamate appears to be the mechanism responsible for these observations. However, little evidence exists to support a physiological role for this phenomenon and, in fact, Morrisett et al (126) have concluded that, while the $GABA_B$ system constitutes a powerful mechanism for control of a major excitatory system in hippocampal pyramidal cells, no evidence exists for $GABA_B$-mediated presynaptic inhibition of excitatory transmitter release.

The presynaptic effect on glutamate release is not confined to the hippocampus but has been demonstrated in other brain regions; these include neurones of nucleus accumbens (127) and terminals of corticostriatal fibers in rat neostriatum (78) where the $GABA_B$ receptor may differ in its characteristics from the postsynaptic receptor (e.g. 76, 78). However, much of the evidence for this separation stems from an insensitivity to the antagonist phaclofen, which is a very weak antagonist even at the postsynaptic receptor. Interestingly, phaclofen completely blocks the inhibition of glutamate-evoked acetylcholine release produced by opioids in rat neostriatal slices (128). This action might reflect the presence of a pre- or postsynaptic $GABA_B$ receptor.

$GABA_B$ receptor-mediated inhibition of neurotransmitter release is manifest in a variety of systems, including those releasing catecholamines and serotonin (20, 129–133), peptides (134–137), and acetylcholine (see ref. 138; Table 3). In all cases, with the exception of substance P release in the spinal cord (137), there is little reason to believe that the effects are of physiological importance. However, by contrast, $GABA_B$-mediated inhibition of endogenous GABA release may be functional. The presence of $GABA_B$ autoreceptors in mammalian brain tissue has been well established by the use of neurochemical techniques (139–141) and they appear to be more abundant than presynaptic $GABA_A$ receptors. It has been suggested that these $GABA_B$ autoreceptors may differ from presynaptic $GABA_B$ sites on glutamate and somatostatin-containing nerve terminals (136) such that receptor subtypes may exist.

Waldmeier and colleagues (140, 142) cast doubt on the importance of $GABA_B$ autoreceptors. Firstly, they demonstrated that inhibition of GABA release occurs at low rather than high stimulation frequencies, whereas the firing rate of GABA neurones is normally expected to be high. Secondly, $GABA_B$ receptor antagonism by CGP 35348 failed to modify the K^+-evoked output of GABA within the rat striatum when measured in vivo by using a microdialysis technique (142). Although the experimental system may be inappropriate, the evidence suggests that $GABA_B$ autoreceptors may play little part in the physiological control of GABA release, at least in the corpus striatum.

Table 3 GABA$_B$-mediated inhibition of evoked transmitter/mediator release in isolated tissues

Transmitter/ Mediator	TISSUE		
	Higher centers	Spinal cord	Peripheral
Acetylcholine			√ (intestine, lung, sympathetic ganglia)
Catecholamines	√		√ (adrenals, heart, anococcygeus, vas deferens)
5-HT	√		
GABA	√		
Glutamate	√	√	
Substance P		√	√ (trachea, lung)
Somatostatin	√	√	
CGRP		√	√ (urinary bladder)
Prostanoids			√ (lung)

Despite this lack of neurochemical support for an endogenous control mechanism, other evidence points to presynaptic autoreceptor control. Mott et al (143) have demonstrated in the rat dentate gyrus that the reduction in recurrent inhibition produced by tetanic stimulation of mossy fibers can be reversed by phaclofen. Their data are consistent with the idea that GABA is released during tetanic stimulation and acts on GABA$_B$ sites located on inhibitory interneurones to reduce recurrent inhibition. The presence of GABA$_B$ receptors on such terminals is also supported by paired-pulse studies performed in hippocampal slices recorded from CA1 neurones (for example, see ref. 66).

Stimulation of afferent fibers in close proximity to the CA1 neurones in rapid succession normally produces a reduction in the second evoked response, depending on the interval between stimuli. This reduction can be decreased by GABA$_B$ antagonists, a result that has prompted Davies et al (144) and Mott & Lewis (68) to propose that GABA$_B$ mechanisms are influential in the genesis of long-term potentiation (LTP). This phenomenon, which is considered to be functional in learning mechanisms, is produced after periods of high frequency stimulus trains to depolarize the postsynaptic cells. Excitatory

amino acid receptor activation, notably of the NMDA receptor, has been implicated in this process and $GABA_B$ receptor activation may act as a modulation system. However, the nature of this participation appears to depend on the stimulus employed. At a frequency in the region of 5Hz, $GABA_B$ antagonists suppress the formation of LTP, which, it is suggested, stems from a loss of the disinhibition produced by activation of $GABA_B$ receptors on inhibitory terminals (68, 144). However, at higher frequencies of stimulation (100Hz) the reverse occurs (69) and $GABA_B$ antagonists facilitate the production of LTP. The significance of this distinction has yet to be determined.

RECEPTOR HETEROGENEITY

Electrophysiological studies on the presynaptic and postsynaptic $GABA_B$ mechanisms suggest that the receptors at these locations may differ. In the hippocampus presynaptic $GABA_B$ responses appear to be insensitive to phaclofen and pertussis toxin (75, 76, 78), whereas postsynaptic responses are prevented by both agents. In neocortical slices the hyperpolarizing action is not mimicked by 3-aminopropyl phosphinic acid (145), and Deisz et al (79) have suggested that $GABA_B$ receptors located on GABA-releasing terminals are pharmacologically distinct from postsynaptic $GABA_B$ sites and moreover, unlike the postsynaptic site, are not coupled to a K^+ conductance mechanism. By contrast, Seabrook et al (28) have indicated that the presynaptic $GABA_B$ site in caudate putamen on glutamate terminals and the postsynaptic receptor on neurones of the substantia nigra pars compacta are pharmacologically indistinguishable. The similarities in agonist potencies and antagonist affinities did not permit any separation. In addition, Thomson & Gähwiler (146) recently claimed that the pre- and postsynaptic receptors studied in area CA3 of hippocampal slice cultures are also the same. This finding appears to be in direct contrast to the data of Calabresi et al (78) obtained in slices of rat neostriatum. Could this disparity stem from differences between cultured neurones and slice preparations? Perhaps future experiments will clarify the issue.

Neurochemical release studies have not, so far, helped to establish a distinction between pre- and postsynaptic receptors. Release studies by Raiteri and colleagues (136) have suggested a possible distinction between receptors on different presynaptic terminals, but whether all or any of these differ from the postsynaptic recognition site has not been considered. In studies using the K^+-evoked release of endogenous glutamate from slices and synaptosomes prepared from rat brain cortex, we have observed that 3-aminophosphinic acid (3APA) does not mimic the inhibitory action of GABA and baclofen even though it is a potent $GABA_B$ agonist (147). The same conclusion was also

noted by Dolphin et al (96) in cerebellar granule cells. However, Seabrook et al (28) have demonstrated that 3APA is a partial agonist at glutamate terminals in the corpus striatum and Ong et al (148) indicate that 3APA mimics the presynaptic action of baclofen in hippocampal neurones in culture.

RECEPTOR HETEROGENEITY: SECOND MESSENGER STUDIES

An alternative basis for receptor heterogeneity may derive from studies on second messenger responses to GABA$_B$ receptor activation. It is well established that GABA$_B$ receptors are coupled via G-proteins to adenylyl-cyclase (72, 149, 150–156), although the association of G-proteins may not be absolute (157) and may fluctuate during development (158). Essentially, when GABA$_B$ receptors are activated in cerebrocortical slices an inhibition of basal or forskolin-induced cAMP formation or potentiation of β-adrenoceptor (or vasoactive intestinal peptide)-stimulated cAMP accumulation occurs. It has been suggested that these two systems may be pharmacologically distinct since certain GABA$_B$ agonists and antagonists fail to mimic or antagonize (−)baclofen in both assays (159, 160). In particular, CGP 35348 is only a very weak antagonist of the inhibitory effect of baclofen even though it does antagonize the potentiation of noradrenaline-induced cAMP formation at lower concentrations. However, the data are inconclusive and the distinctions insufficient to allow a separation to be made thus far.

Much evidence has been produced to indicate a coupling of GABA$_B$ sites with adenylyl cyclase through GTP-protein regulation sites (114, 152, 154, 161, 162) but other biochemical events have also been attributed to GABA$_B$ receptor activation. For example, weak stimulation of phosphotidyl inositol (PI) turnover in dorsal root ganglia (96) and enhancement of the turnover induced by NMDA in neonatal rat cerebellar slices (163) or noradrenaline in cerebral cortex slices (164, 165) have been reported. However, inhibition of the stimulation in PI turnover induced by 5HT and histamine has also been demonstrated (166, 167) although the effects are small and may be species-dependent.

It has also been reported that transmitter-associated enzymes can be affected by GABA$_B$ receptor activation. Systemically administered baclofen facilitates 5HT synthesis in vivo in the rat corpus striatum (168) and also stimulates dopamine synthesis in the same organ (169). Surprisingly, GABA$_B$ receptor activation has also been reported to inhibit tyrosine hydroxylase activity in rat corpus striatum (170), which would be expected to reduce rather than increase the catecholamine level.

Whether any of these biochemical changes plays a major role in GABA$_B$ receptor-mediated events is unclear. Do they participate in any of the

membranal responses or whole animal behavioral effects produced by $GABA_B$ receptor activation?

SPINAL CORD

In an attempt to answer this question, we (171) have recently studied the $GABA_B$-mediated changes in cAMP levels produced in rat spinal cord, in relation to the known antinociceptive effect of $GABA_B$ agonists. This analgesic effect is believed to be mediated through an action, at least in part, within the spinal cord, possibly through inhibition of the release of primary afferent transmitters such as substance P.

We have observed that (−)baclofen (0.1-100μM ED_{50} 4μM) can dose-dependently inhibit (up to 100%) the release of substance P from rat isolated spinal cord evoked by electrical stimulation of dorsal roots. By contrast, (+)baclofen produced less than 30% inhibition at 500μM (137; Figure 4).

The accumulation of cAMP produced by forskolin in spinal cord slices was inhibited by $GABA_B$ receptor agonists (171). However, the β-adrenoceptor-stimulated accumulation of cAMP was not potentiated by $GABA_B$ receptor activation. The $GABA_B$ receptor antagonist, CGP 35348, at doses up to

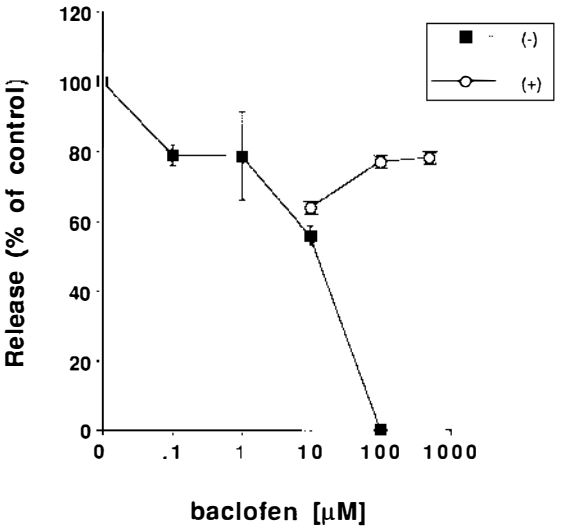

Figure 4 Effect of (−) and (+) baclofen on electrically evoked substance P release from rat isolated spinal cord. Each point represents release expressed as the mean percent (±s.e.m.) of basal outflow determined as an average of 3 samples collected prior to stimulation. Samples were collected for 8 min periods. Baclofen was present one minute prior to and during the stimulation period (8 min, 20V; 0.5 ms, 1Hz). Stimulation alone produced 70% increase in substance P release above basal. [Data from Ref. 137]

1.5mM failed to prevent the inhibition in forskolin-stimulated cAMP accumulation produced by (−)baclofen and GABA. Since CGP 35348 can prevent the antinociceptive action of baclofen in rodents (172), we concluded that the changes in cAMP accumulation produced by GABA$_B$ receptor activation are unrelated to the antinociceptive response. Presumably the decrease in release of primary afferent transmitter produced, perhaps, by a decrease in Ca^{++} flux (173) operates independently of changes in adenylyl cyclase activity.

Baclofen appears to have a primary action on terminals of afferent fibers within the spinal cord. While the highest density of receptors appears to be on small diameter fibers (74), there is also evidence for their presence on monosynaptic Ia afferents (174, 175) and perhaps to a lesser extent on descending fiber terminals (175). It seems plausible that the receptors on small diameter fibers mediate the antinociceptive response to baclofen and that those on large diameter fibers mediate the therapeutic muscle relaxant effect. Both effects are antagonized by GABA$_B$ antagonists (172, 176). However, it is also feasible that the effects are mediated additionally through other sites. For example, Stefanski et al (177) have suggested that muscle relaxation may also be produced via the nucleus accumbens septi since local injection of baclofen (1 and 2.4μg) into the brain region of the rat dose-dependently decreased muscle tone. Turski et al (178) have also indicated that supraspinal regions may be involved in muscle relaxation and have implicated the substantia pars reticulata as a possible site.

GENERAL EFFECTS ATTRIBUTED TO GABA$_B$ RECEPTOR ACTIVATION

Numerous side effects have been attributed to GABA$_B$ receptor activation in mammals as outlined in an earlier review (179) and summarized in Table 4. In the light of these actions, it is perhaps surprising that (−)baclofen does not normally produce multiple side effects when administered to patients. One reason may be that the majority of the reported effects in animals appear to derive from an action within the CNS and since there is normally only limited brain penetration of baclofen, the levels achieved are possibly insufficient to produce such effects. In fact, in certain patients the levels obtained in higher centers produce adverse effects at or below the therapeutic threshold dose in spasticity. This has prompted the introduction of intrathecal infusion systems to reduce the incidence of side effects while obtaining the required muscle relaxation (180–187). This site-directed application of the GABA$_B$ agonist is fast becoming a standard mode of administration, producing remarkable therapeutic results with few side effects.

Table 4 Consequences of $GABA_B$ receptor activation in mammalian tissue

Hormonal effects	Behavioral effects	Cellular effects
Corticotrophin-releasing hormone ↓	Epileptogenesis (in vivo)	Facilitation/Reduction of Long-term potentiation (depends on tetanic frequency)
	Panic attacks ↓	
Melanocyte-stimulating hormone ↓	Yawning	Synaptic late ipsps
	Catatonia ↓	
Gastric acid secretion ↓		Neuronal K^+ conductance ↑
Prolactin-releasing factor ↓	Memory retention and consolidation ↓	Neuronal Ca^{++} conductance ↓
Luteinizing hormone ↓	Antinociception	Inhibition/Potentiation of adenylyl cyclase activity
Androgen production ↑	Hypotension	Inhibition/Stimulation of phosphoinositide turnover
Lordosis ↓	Gastric motility ↑	
	Muscle relaxation	Decrease in cGmp
	Spike and wave discharges	
	Brown fat thermogenesis	
	Antitussive	
	Bronchiolar relaxation	
	Urinary bladder motility ↓	
	Food intake ↑	
	5HT-induced head twitch ↓	
	Ethanol and diazepam withdrawal symptoms ↓	
	Oviduct and uterus contraction	
	Intestinal peristalsis ↓	
	Induced gastric cancers ↓	
	Hypothermia/Hyperthermia	

THERAPEUTIC POTENTIAL OF $GABA_B$ LIGANDS

Agonists

The potential uses of baclofen-like compounds have yet to be explored in great detail but while higher centers of the brain may provide a focus of action for more potent $GABA_B$ agonists, the periphery may prove to be an equally important location. It has been proposed, for example, that a $GABA_B$ agonist

may be a useful antiasthma drug since GABA$_B$ receptor activation within the bronchioles decreases the release of acetylcholine and substance P (135, 188), which normally increase airways resistance, and also decreases bronchiole hyperreactivity (138), which is a feature of asthma. This contrasts with the action of GABA$_A$ agonists, which do not affect hyperreactivity. A potent and more aqueous soluble GABA$_B$ ligand may prove to be therapeutically effective.

GABA$_B$ agonists also decrease intestinal motility (189, 190) and this property may be an important indication for their use in overactive states without the side effects associated, for example, with muscarinic receptor antagonism.

One potentially significant CNS application may be the antinociceptive action referred to earlier. However, evidence for baclofen as an analgesic in man is lacking despite the unequivocal evidence for antinociceptive activity in rodents (85, 86, 172, 191–196). This effect in rodents appears to be mediated via G-protein coupled GABA$_B$ receptors since pertussis toxin as well as GABA$_B$ receptor antagonism can prevent the action of baclofen (172, 197, 198). Although the locus of this effect resides in the spinal cord (191), a supraspinal site of action may also contribute (86).

In humans, the trigeminal nucleus may be an important focus for the action of baclofen since trigeminal neuralgia has been successfully treated with (−)baclofen (199–201). The mechanism underlying this analgesic effect is unclear but GABA$_B$ receptors are certainly implicated.

Antagonists

Indications for the potential use of GABA$_B$ antagonists are far greater than for the agonists, assuming that the actions produced by baclofen are mimicking endogenous responses to GABA$_B$ receptor activation under physiological or pathological conditions. Even if only a limited number of the reported effects (Table 4) are relevant, the potential benefits of antagonists are high. It can be predicted that an antagonist will exhibit antiepileptic, antidepressant, nootropic, anxiolytic, and neuroprotective properties. However, the evidence for these effects with the exception of epilepsy is slender. Predicting a neuroprotective effect with a GABA$_B$ antagonist seems an unlikely proposition at first sight. If GABA$_B$ agonists suppress the release of glutamate (202, 203), which is considered to be a neurotoxin contributing to the production of neuronal damage in ischaemia (204), an agonist rather than an antagonist, might be expected to be neuroprotective. However, baclofen has been shown to be ineffective in the four-vessel occlusion model of cerebral ischemia in rats (205) and in global ischemia produced by carotid occlusion in mongolian gerbils (R. Gill & N. Bowery, unpublished observations). It was also ineffective against NMDA-induced neurotoxicity in the rat corpus striatum (206).

Focal injection of tetanus toxin, which suppresses the synaptic release of GABA (e.g. 80), induces neuronal degeneration in the vicinity of the site of injection and this can be prevented by NMDA receptor antagonists or ablation of excitatory afferents (207, 208). If the toxicity is due to an imbalance between GABA and glutamate then GABA agonists acting through $GABA_A$ receptors may be neuroprotective. If $GABA_B$ autoreceptor activation depresses GABA release (139) from GABAergic terminals then an autoreceptor antagonist should increase the overflow and the synaptic concentration. This antagonism might provide a novel basis for neuroprotective activity.

A similar argument could be made for the potential anxiolytic activity of a $GABA_B$ antagonist. If the synaptic release of GABA is increased to produce a greater response to $GABA_A$ receptors, GABAergic transmission could be enhanced in a manner synonymous with the action of benzodiazepines or the barbiturates.

The possibility of cognitive improvement stems from the observations of Olpe & Karlsson (69) that $GABA_B$ antagonists can facilitate long-term potentiation in hippocampal slices when provoked by high frequency stimulus trains. Schwartzwelder et al (209) also reported that baclofen can decrease memory acquisition and retention in rats. Presumably an antagonist might have the opposite effect, although no information is available at present. (See Note in Proof, page 147)

The basis for the potential antidepressant effect of a $GABA_B$ antagonist derives from evidence obtained in $GABA_B$ and β-adrenoceptor binding experiments after prolonged treatment with $GABA_B$ antagonists. Chronic administration (i.p.) of the brain penetrating $GABA_B$ antagonists, CGP 35348 and CGP 36742 for 21 days produced a significant down-regulation in β-adrenoceptor (^{3}H-iodopindolol) binding in rat frontal cortex (210; G. Pratt & N. G. Bowery, unpublished observations). The effect was comparable with that produced by administration of desipramine (p.o. or i.p.) over the same time period. $GABA_B$ binding was also upregulated in the same brain region, although the effect was only statistically significant following administration of CGP 36742.

Chronic administration of antidepressant drugs upregulates $GABA_B$ binding in the rat frontal cortex and hippocampus (211, 212) although this finding has been disputed by others (e.g. refs. 213, 214). Nevertheless, Lloyd et al (215) have reported that olfactory bulbectomy in rats, a model of depression, produces a decrease in $GABA_B$ binding in frontal cortex that can be reversed by antidepressant administration. Carbamazepine and lithium have also been reported to upregulate $GABA_B$ binding in rat hippocampus following chronic treatment (216).

In relation to these findings, it has been reported that $GABA_B$ receptor agonists may be beneficial in the treatment of clinical depression (217–220).

However, this conclusion has not been supported by other groups (e.g. 221, 222, 223, 224). In fact, an increase in depression was even apparent following (−)baclofen treatment in the study by Post et al (224).

Although no definitive evidence supports an antidepressant action with GABA$_B$ antagonists, the observed reduction in β-adrenoceptor binding and possible antagonism of the reduction in release of transmitter amines by GABA$_B$ agonists (20, 133) may provide a basis for further investigation.

The possible significance of GABA$_B$ receptors in convulsive epilepsy has been the focus of attention in many laboratories. In general, baclofen has been reported to exert both anticonvulsant and proconvulsant activity, depending upon the model employed and the dose administered (225–237).

In humans, GABA$_B$ receptor activation may induce seizure-like activity under certain circumstances, particularly after withdrawal (234, 238), but the incidence of such adverse reaction appears to be rare even in epileptic patients (239).

It has recently been suggested that GABA$_B$ mechanisms may be more important in nonconvulsive epilepsy or absence syndrome. Absence epilepsy appears to be of thalamocortical origin and manifests in humans as an abrupt decrease in motor activity without loss of consciousness. A variety of animal models for this syndrome exist, including the lethargic mouse (240), the Strasbourg genetic absence rat (241), and γ-hydroxybutyrate-induced syndrome in rats (242). In each of these models a spike and wave discharge consistent with that observed during absence syndrome in humans can be detected. GABA$_B$ receptor antagonism can suppress this discharge in all cases (240, 243, 244). Moreover, γ-hydroxybutyrate appears to act as a GABA$_B$ agonist at the high concentrations required to produce the syndrome in rats (245; R. Bernasconi, personal communication,). Even though other classes of drugs can reduce the spontaneous absence activity, Marescaux et al (243) suggest that GABA$_B$ receptor mechanisms are responsible for the genesis of the syndrome. However, the underlying mechanism(s) have yet to be discovered. The syndrome does not appear to result from an up-regulation of GABA$_B$ receptors since no evidence could be demonstrated in a receptor autoradiography study of brain sections obtained from these rats (246).

Crunelli & Leresche (247) have suggested that the electrophysiological events resulting from stimulation of afferents to the thalamus may provide clues to the underlying mechanism. These authors have demonstrated that the late ipsp recorded from thalamic neurones in response to optic tract stimulation can produce Ca^{++} spikes. These, they suggest, result from de-inactivation of "T" currents produced by the prolonged GABA$_B$ mediated hyperpolarization. Interestingly, Marescaux et al (243) indicate that suppression of the spontaneous spike and wave discharge in rats is only produced by GABA$_B$

antagonists injected systemically or directly at lower doses into specific thalamic regions. Discrete injections elsewhere in the brain produced no reduction in the pattern of discharges.

At present, only data from animal models are available and it remains to be seen whether $GABA_B$ antagonism proves to be effective in the human syndrome. However, the results obtained thus far make their potential application in humans an exciting prospect.

RECEPTOR STRUCTURE

Unlike the $GABA_A$ receptor no information is currently available about the structural sequence of $GABA_B$ receptors. However, it seems a reasonable assumption that the sequence will have greater homology with other G-protein linked receptors than with the 4 subunit pentameric structure of the $GABA_A$ receptor. Expression of the $GABA_B$ receptor(s) has been obtained in *Xenopus* oocytes using mRNA obtained from rat cerebral cortex (248) and cerebellum (249, 250). In the former experiments the expressed receptors mediated an increase in Cl^- conductance and a decrease in K^+ conductance, which contrasts with the native receptor mechanism(s). Presumably, the G-protein coupling associates the receptor with available channels that are not necessarily those expressed under normal conditions in mammals. Taniyama et al (249, 250) have used mRNA obtained from cerebellum to express $GABA_B$ sites in *Xenopus* oocytes. The channel coupling in their experiments was more consistent with the native mechanisms such that an increase in K^+ conductance was observed on receptor activation. However, only 5% of injected oocytes appeared to express the receptor-operated channel, suggesting that perhaps the G-protein coupling mechanism may limit functional expression in these cells. It would be interesting to know whether the expression rate for the uncoupled receptor binding site is greater.

Kuriyama and colleagues (251–253) have attempted to obtain a purified form of the $GABA_B$ receptor from bovine brain using baclofen as an affinity ligand. Baclofen was coupled to the aliphatic epoxy group of Sepharose 6B and receptor protein, solubilized from brain membranes, was applied to an affinity column containing the coupled ligand. A partially purified preparation of the $GABA_B$ receptor was obtained and a monoclonal antibody raised against it. Subsequent experiments with the antibody indicate a protein of about 80 kd. Incubation of the antibody with crude synaptic membrane overnight at 4°C reduced ^{3}H-GABA binding to the membrane pellet in a dose-dependent manner (253).

Monoclonal antibodies have also been raised against (−)baclofen conjugated with glutaraldehyde to hemocyanin (254). These have been used on brain tissue obtained from rats and monkeys previously injected with baclofen

to localize the ligand bound to its receptors. Overall the distribution of antibody binding was similar to that obtained by receptor autoradiography. It would be interesting to obtain an antibody to the baclofen antibody that might provide a selective receptor marker. However, the monoclonal antibody produced by Martinelli et al (254) appears to recognize the p-chlorophenyl moiety of baclofen. If this moiety does not form part of the molecule that binds to the recognition site then the anti-antibody may be of little value. Since GABA and 3-aminopropyl phosphinic acid are receptor ligands which do not contain a chlorophenyl moiety, the anti-antibody approach may be invalid.

Undoubtedly the sequence of the GABA$_B$ receptor will emerge in the near future to join the ranks of the receptor families already known. Whether or not this sequence will be homogeneous and homologous with other receptors is an important question since this may provide the basis for receptor heterogeneity, as suggested in functional receptor studies.

Functional homology with other receptor types is clear, not least with

Table 5 GABA$_B$ and adenosine receptors: Similarities

	GABA$_B$	A$_1$	A$_2$
Location	Pre & post	Pre & Post	Post
Actions	Hyperpolarization (postsynaptic)	Hyperpolarization (postsynaptic)	
pertussis toxin sensitive	K^+ conductance ↑	K^+ conductance ↑	
	Ca^{++} conductance ↓	Ca^{++} conductance ↓	
pertussis toxin insensitive	Decrease in epsp (reduced transmitter release)	Decrease in epsp (reduced transmitter release)	
Second messengers	cAMP formation (Gi protein) ↓	cAMP formation (Gi protein) ↓	Enhanced cAMP formation (G_s)
	Enhanced cAMP formation		
	Phosphotidyl inositol turnover ↓ ↑	Phosphotidyl inositol turnover ↑ ↓	
Distribution in CNS	GABA$_B$ and A$_1$ comparable		Rich in dopamine-containing areas

adenosine A1 receptors (Table 5). When the sequence is finally discovered, comparisons at the molecular level will be interesting to make.

SUMMARY

In conclusion, $GABA_B$ receptors appear to be of major importance in synaptic processing within the brain and are present at both post- and presynaptic sites. Their activation can hyperpolarize neurones and diminish neurotransmitter release from presynaptic terminals. We already know that drugs, i.e. baclofen, that mimic this activation are therapeutically useful, although the full significance of their use both inside and outside the brain has yet to be realized.

Drugs that interfere with $GABA_B$ receptor activation should also prove to be important therapeutic agents. A number of suggestions have been proposed but it will be many years before the potential effects can be consolidated or refuted in humans. Only now are brain-penetrating $GABA_B$ antagonists being discovered, due largely to the expertise of the research group at CIBA-Geigy, Basel. The emergence of such compounds makes future studies an exciting prospect. In particular, the discovery that $GABA_B$ antagonism can suppress absence seizures in rats has provided an important therapeutic target.

It is now just over ten years since we first designated the term $GABA_B$. Since then a wealth of information has been obtained, but perhaps the best is still to come.

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NOTE ADDED IN PROOF

Mondadori et al (1992) have recently demonstrated that GABA$_B$ receptor antagonism can improve cognition in mice (passive avoidance), rats (social learning), and rhesus monkeys (conditional spatial color test. *Pharmacol. Commun.* 2:93–97