Molecular Structure and Physiological Functions of GABA$_B$ Receptors

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I. Introduction

GABA is the major inhibitory neurotransmitter in the central nervous system (CNS) and as such plays a key role in modulating neuronal activity. GABA mediates its action via distinct receptor systems, the ionotropic GABA$_A$ and metabotropic GABA$_B$ receptors. Unlike GABA$_A$ receptors that form ion channels, GABA$_B$ receptors address second messenger systems through the binding and activation of guanine nucleotide-binding proteins (G proteins; for other

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VI. Summary and Outlook
Dysfunction of GABA-mediated synaptic transmission in the CNS is believed to underlie various nervous system disorders. For example, hyperactivity of the GABA system was linked to epilepsy, spasticity, anxiety, stress, sleep disorders, depression, addiction, and pain. On the contrary, hyperactivity of the GABAAergic system was associated with schizophrenia (20). GABA research, because of its medical relevance, has always attracted a great deal of attention in academia and industry. Over the years pharmaceutical companies successfully exploited the GABA system and introduced a number of drugs to the market. However, despite considerable drug-discovery efforts, baclofen (β-chlorophenyl-GABA, Lioresal) currently remains the only available GABA<sub>B</sub> medication. Baclofen, a lipophilic derivative of GABA, was synthesized in 1962 in an attempt to enhance the blood-brain barrier penetrability of the endogenous neurotransmitter. Baclofen was introduced to the market in 1972 and is used to treat spasticity and skeletal muscle rigidity in patients with spinal cord injury, multiple sclerosis, amyotrophic lateral sclerosis, and cerebral palsy (44). GABA<sub>B</sub> agonists showed promising therapeutic effects in a whole range of other indications, but their side effects, including sedation, tolerance, and muscle relaxation, prevented further development (see sect. iv). Many researchers in the field assumed that a dissociation of the therapeutic effects from the side effects was achievable with more selective GABA<sub>B</sub> drugs. This assumption was based on a large body of literature suggesting the existence of pharmacologically distinct GABA<sub>B</sub> receptor subtypes in the brain (see sect. iiE). A more selective interference with the GABA<sub>B</sub> system appeared feasible but crucially depended on the cloning of the predicted receptor subtypes. Therefore, it was mostly commercial interests that were driving efforts to isolate a GABA<sub>B</sub> receptor cDNA. GABA<sub>B</sub> receptors were not cloned until 1997 and thus remained the last of the major neurotransmitter receptors to be characterized at the molecular level (160). At first glance this is quite surprising, considering that GABA<sub>B</sub> receptors were identified as early as in 1980 (46). In retrospect, it is clear that many cloning attempts failed because of the unexpected properties of GABA<sub>B</sub> receptors. We therefore review some of the strategies that were applied when trying to isolate GABA<sub>B</sub> receptors and discuss why these approaches failed (see sect. iiA1). Identification of the first GABA<sub>B</sub> cDNAs renewed commercial interests in these receptors for a number of reasons. Most importantly, the cloning generated the necessary tools to isolate the expected additional members of the GABA<sub>B</sub> receptor family. Moreover, high-throughput compound screening, using molecularly defined receptors and functional assay systems, suddenly became practicable. GABA<sub>B</sub> receptors were now also amenable to gene-targeting technology. This was supposed to help validate the most promising drug targets and at the same time provide a means to determine the specificity of GABA<sub>B</sub> compounds in vivo. Throughout this review emphasis is given to developments in the field that followed the initial cloning of GABA<sub>B</sub> receptors. However, where molecular findings extend or challenge earlier work, special attention is given to older literature.

As a consequence of intense research efforts that followed isolation of the first GABA<sub>B</sub> cDNAs, several laboratories reported the cloning of a second GABA<sub>B</sub> receptor cDNA, termed GABA<sub>B<sub>(2)</sub></sub> (see sect. iiA2). Several groups published a most important discovery related to GABA<sub>B</sub><sub>(2)</sub> receptors. As shown for the first time for a G protein-coupled receptor (GPCR), the GABA<sub>B</sub> receptor was not a single protein but instead consisted of two distinct subunits, neither of which was functional on its own. This finding was of interest to a large scientific community, and very quick progress was made in dissecting the roles of the individual subunits in receptor activation, assembly, and signaling (see sect. iiA4). Much of this review is devoted to this topic, as it fundamentally changed our view on the structure and functioning of GPCRs. The search for GABA<sub>B</sub> receptor subtypes did not lead to the expected identification of additional GABA<sub>B</sub> cDNAs, although a number of GABA<sub>B</sub>-related cDNAs were identified in the process. The apparent lack of molecular heterogeneity was a surprise to many in the field who expected a variety of pharmacologically distinct GABA<sub>B</sub> subunits, as predicted from work on native receptors. Efforts therefore turned toward identifying GABA<sub>B</sub> isoforms (see sect. iiA4), receptor-associated proteins (see sect. iIA9), receptor modifications (see sect. iiA10), and endogenous factors (see sect. iiA6) that possibly were responsible for generating pharmacological differences. Once more, some unexpected findings were made. Several groups reported that transcription factors, such as CREB2/ATF-4, are able to directly interact with GABA<sub>B</sub> receptors. At the same time, with the puzzling lack of pharmacologically distinct receptor subtypes, it became important to understand to which known GABA<sub>B</sub> functions the cloned receptor subunits contribute in vivo. To address this question, many laboratories studied the expression of cloned GABA<sub>B</sub> subunits in vivo (see sect. iiB3) or disabled GABA<sub>B</sub> genes in mice (see sect. iiB1), which greatly clarified the role of individual subunits in GABA<sub>B</sub> receptor physiology. The overt phenotypes of GABA<sub>B</sub> knockout mice also pointed at the neuronal systems that crucially depend on GABA<sub>B</sub>-receptor activity. A long-standing question in the field concerns the relationship between GABA<sub>B</sub> receptors and the receptors for γ-hydroxybutyrate (GHB), a metabolite of GABA and emerging drug of abuse. It is a matter of much debate whether specific GHB receptors exist and whether they are related to GABA<sub>B</sub> receptors. This question was addressed using molecular tools (see sect. iiB2). Following
receptor cloning, several studies tried to establish a link between polymorphisms in \( GABA_B \) genes and congenital human diseases (see sect. ivE). Taking recent developments in the field into account, we also touch on the most promising indications for \( GABA_B \) drugs (see sect. iv). Last but not least, the cloned receptors were used to establish high-throughput compound screens based on functional assays, which yielded the first allosteric compounds acting at \( GABA_B \) receptors (see sect. vA).

II. NATIVE \( GABA_B \) RECEPTORS

A. Coupling to \( G \) Proteins

Bowery et al. (46) were the first to discover that \( GABA_B \) reduces norepinephrine release by activating a bicuculline- and isoguvacine-insensitive receptor, which they named the \( GABA_B \) receptor. Evidence for a coupling of \( GABA_B \) receptors to \( G \) proteins came from the sensitivity of agonist affinity to GTP analogs (10, 144). Studies using \( N \)-ethylmaleimide (NEM), islet activated protein (IAP), pertussis toxin, or antisense knock-down provided evidence that \( GABA_B \) receptors predominantly couple to \( G_\alpha \)- and \( G_\beta_\gamma \)-type \( G \) proteins (9, 59, 122, 223, 228). It is now well established that presynaptic \( GABA_B \) receptors repress \( Ca^{2+} \) influx by inhibiting \( Ca^{2+} \) channels in a membrane-delimited manner via the \( G_\beta_\gamma \) subunits (see sect. iiB). Postsynaptic \( GABA_B \) receptors trigger the opening of \( K^+ \) channels, again through the \( G_\beta_\gamma \) subunits (see sect. iiC). This results in a hyperpolarization of the postsynaptic neuron that underlies the late phase of inhibitory postsynaptic potentials (IPSPs) (201). Besides modulating ion channels through \( G_\beta_\gamma \), \( GABA_B \) receptors activate and inhibit adenylyl cyclase via the \( G_\alpha_i/G_\alpha_o \) and \( G_\beta_\gamma \) subunits (see sect. iiD). Recent work by Hirono et al. (145) shows that \( GABA_B \) activity enhances mGlu1 responses at excitatory synapses in the cerebellum. This facilitation is suggested to require \( Ca^{2+} \) release from internal stores through a mechanism that involves \( G_\alpha_i/G_\alpha_o \)-linked phospholipase C (PLC) activation and a cooperative upregulation of GPCR signaling by the \( G_\beta_\gamma \) subunits. This is reminiscent of synergistic interactions seen with \( GABA_A \) and \( G_\beta_\gamma \)-adrenergic receptors (42). Importantly, \( G \) protein-independent \( GABA_B \) effects on neurotransmitter release were also suggested (132).

B. Coupling to \( Ca^{2+} \) Channels

Presynaptic \( GABA_B \) receptors are subdivided into those that control \( GABA \) release (autoreceptors) and those that inhibit all neurotransmitter release (heteroreceptors). In most preparations, \( GABA_B \) receptors mediate their presynaptic effects through a voltage-dependent inhibition of high-voltage activated \( Ca^{2+} \) channels of the \( N \) type (\( Ca_{2.2} \)) or \( P/Q \) type (\( Ca_{2.1} \)) (4, 61, 223, 225, 236, 261, 265, 299, 320). Both types of \( Ca^{2+} \) channels are expressed in presynaptic terminals and were shown to trigger neurotransmitter release (349). A postsynaptic inhibition of \( Ca^{2+} \) channels by \( GABA_B \) receptors was also postulated (130). It was shown that \( GABA_B \) receptors couple to different types of \( Ca^{2+} \) channels depending on the input site (266). The inhibition of \( Ca^{2+} \) inward currents is voltage dependent and varies between 10 and 42% among studies (73, 78, 294). Since \( Ca^{2+} \) influx and transmitter release are correlated with a third to fourth power law (349), \( GABA_B \) agonists frequently inhibit more than 90% of neurotransmitter release with a less than 50% inhibition of \( Ca^{2+} \)-channel activity. This inhibition is modulated by the action potential frequency, where strong depolarization relieves \( Ca^{2+} \) channels from their \( G_\beta_\gamma \)-mediated inhibition (140, 152, 354). This particular property of presynaptic \( Ca^{2+} \) channels may differentially modulate action potential trains, depending on their frequency (53). \( GABA_B \) receptors are also described to either inhibit (4, 202, 208) or facilitate (309) L-type \( Ca^{2+} \) channels. The latter effect was shown to be indirect and to depend on protein kinase C (PKC) activity. Similarly, \( GABA_B \) receptors also inhibit or disinhibit T-type \( Ca^{2+} \) channels (83, 107, 219, 303, 304).

C. Coupling to \( K^+ \) Channels

\( GABA_B \) receptors induce a slow inhibitory postsynaptic current (late IPSC) through activation of inwardly rectifying \( K^+ \) channels (GIRK or Kir3) (201, 300). Accordingly, \( GABA_B \)-induced late IPSCs can be inhibited by the Kir3 channel blocker Ba\textsuperscript{2+} (159, 264, 325), and they usually exhibit a reversal potential similar to the \( K^+ \) equilibrium potential (188, 220). The physiological effect of Kir3 channel activation is normally a \( K^+ \) efflux, resulting in a hyperpolarization. The time course of the late IPSC, with a time to peak of 50–500 ms and decay times of 100 and 500 ms, clearly differs from that of the fast IPSC, which is \( GABA_A \) receptor mediated (188, 249). Baclofen-induced outward currents in hippocampal neurons are absent in Kir3.2 and \( GABA_B^{(A)} \) knockout mice, corroborating the prominent role of Kir3 channels in mediating the effects of \( GABA_B \) receptors (201, 300). The rectification properties of synthetically evoked late IPSCs differed between studies. On the one hand, the stimulus-evoked and spontaneous late IPSCs in dopaminergic neurons are inwardly rectifying and similar to those activated by baclofen (134). On the other hand, baclofen also induces linear or even outwardly rectifying conductances, suggesting that channels other than Kir3 can contribute to the late IPSC. These other channels may include fast inactivating, voltage-gated \( K^+ \) channels (289) and small-conductance \( Ca^{2+} \)-
activated K⁺ channels (SK channels) (116). Accordingly, the fast inactivating A-type K⁺-channel blocker 4-aminopyridine inhibits a baclofen-induced current in guinea pig hippocampal neurons (153, 243). Moreover, GABA activates Ca²⁺-sensitive K⁺ channels (36) and small-conductance K⁺ channels (36, 89) in rat hippocampal neurons. Possibly, GABA receptors enhance the activity of SK channels by inhibiting the production of cAMP after an action potential-induced Ca²⁺ influx (116). In addition to the well-documented coupling of GABA receptors to postsynaptic K⁺ channels, GABA receptors also appear to activate Ba²⁺-sensitive K⁺ channels at presynaptic sites (325). Likely these presynaptic K⁺ channels are of the Kir3 type, devoid of the Kir3.2 subunit and assembled from Kir3.1 and Kir3.4 subunits (201).

D. Coupling to Adenylyl Cyclase

All of the known nine adenylyl cyclase isoforms are expressed in neuronal tissue. Gα and Gαα proteins, the predominant transducers of GABA receptors, inhibit most of them (311). Many studies have reported that GABA receptors inhibit forskolin-stimulated cAMP formation, but others also observed a stimulation of cAMP production (45, 55). Gα and Gαα proteins inhibit adenylyl cyclase types I, III, V, and VI, while Gβγ stimulates adenylyl cyclase types II, IV, and VII. This stimulation depends on the presence of Gα, which results from the activation of GPCRs by, e.g., norepinephrine, isoprenaline, histamine, or vasoactive intestinal polypeptide (311, 321). Therefore, the stimulatory action of GABA receptors on cAMP levels is a consequence of G protein cross-talk and depends on the expression of adenylyl cyclase isoforms together with GABA receptors and Gαα-coupled GPCRs. Both the inhibition and enhancement of cAMP levels by GABA receptor activation were confirmed in vivo using microdialysis (133). Many ion channels are targets of the cAMP-dependent kinase (protein kinase A or PKA). Accordingly, a GABA receptor-mediated modulation of K⁺ channels via cAMP was reported (116). Significantly, the activity of GABA receptors on neuronal function on a longer time scale (see sect. IIIA).

GABA receptors were repeatedly implicated in synaptic plasticity (87, 229, 246, 247, 334). Until recently, it was unclear whether GABA receptors can influence plasticity processes through the cAMP pathway. Recent experiments now demonstrate that G protein-mediated signaling through GABA receptors retards the recruitment of synaptic vesicles during sustained activity and after short-term depression (290). This retardation occurs through a lowering of cAMP, which blocks the stimulatory effect of the increased Ca²⁺ concentration on vesicle recruitment. In this signaling pathway, cAMP and Ca²⁺/calmodulin cooperate to enhance vesicle priming.

E. Pharmacology of Native GABA receptors

Bowery et al. (45) recently published a comprehensive and detailed review on currently available GABA receptors (45). Here, we only focus on one particular aspect of GABA receptor pharmacology: the discrepancies in the potency of agonists and antagonists in different biochemical and physiological paradigms. Numerous biochemical studies indicate pharmacological differences between auto- and heteroreceptors and even within auto- and heteroreceptors (15, 39–41, 248, 323). However, the proposal of presynaptic receptor subtypes based on neurotransmitter release experiments has been open to dispute (336). Electrophysiological and release experiments suggest distinctions between pre- and postsynaptic GABA receptors as well (65, 76, 88, 96, 115, 262, 268, 325, 352). Accordingly, published half-maximal effective concentrations for baclofen in pharmacological studies vary considerably and range between 100 nM and 100 μM (Table 1).

The rank order of agonist and antagonist binding affinities at GABA receptors is identical (169). This, together with the reasons outlined in sections IIIA4 and IIIA5, makes it unlikely that molecularly distinct GABA receptor subtypes or isoforms underlie these pharmacological differences. Obviously, the ratio of receptors and effectors can determine the apparent potency of receptor agonists (174). Agonist potency may also depend on the concentration of divalent cations in the extracellular buffer (see sect. IIIA6), the association with lipid rafts (18), the phosphorylation state of subunits (see sect. IIIA10), or the type of G protein that is present in the cell. These factors may also explain why the agonist affinity at GABA receptors increases 10-fold during postnatal development (206). This said, all experiments with native GABA receptors reporting changes in the rank order of ligand efficacies remain unexplained (40, 85). These experiments would normally clearly argue in favor of pharmacologically distinct receptor subtypes.

III. CLONED GABA RECEPTORS

A. Molecular Structure

1. Expression cloning of GABA receptors

GABA receptors were cloned in 1997 (169), close to 20 years after their discovery by Bowery et al. (46). In retrospect, we understand the reasons that prevented an earlier isolation of GABA cDNAs. To begin with, a purification of GABA receptor proteins proved difficult. There were no radioligands that bound irreversibly or with high affinity to the receptor. Moreover, receptor function was inevitably lost in the presence of solubilizing...
concentrations of detergents, most likely because of the dissociation of GABA_B(1) and GABA_B(2) subunits. This made it impossible to trace the protein during the purification steps using functional assay systems, such as, e.g., GTPγS[35S] binding. Nevertheless, the purification of a putative 80-kDa GABA_B-receptor protein was reported (233). The molecular mass of the 80-kDa GABA_B protein does not match the molecular mass of cloned GABA_B subunits, and a relationship with the latter is unlikely. No amino acid sequence of the 80-kDa protein was disclosed, and hence, its molecular structure remains enigmatic. As a consequence of the problems associated with purifying a GABAB-receptor protein, no antibodies for cloned GABAB subunits were available prior to cloning.

In pregenome times, expression cloning was the most successful approach for isolation of a neurotransmitter receptor. In essence, expression cloning circumvents the requirement for protein purification and instead uses a specific biological activity as the basis of cDNA identification. An inherent advantage of such a screening approach is that the isolated cDNA clones are usually full-length and immediately available for functional studies. *Xenopus* oocytes are the expression system of choice when using electrophysiological screening techniques. This procedure was used to isolate a number of G protein-coupled neurotransmitter receptors, including mGlu receptors (218). Several laboratories, including ours, explored strategies using *Xenopus* oocytes when attempting to clone a GABA_B cDNA. Because GABAB receptors do not couple to PLC, it was impossible to apply expression-cloning strategies based on increases in intracellular [Ca2+] and subsequent activation of Ca2+-activated Cl− channels. Others and we therefore supplied *Xenopus* oocytes with effector Kir3 channels (201) or promiscuous G proteins that permitted the detection of weak functional GABAB responses (306). We eventually abandoned expression cloning in *Xenopus* oocytes because the electrophysiological GABA_B responses were unreliable and were lost in the process of splitting the brain cDNA pool into smaller pools. This loss of functional responses is readily explained by the fact that both GABA_B(1) and GABA_B(2) subunits are required to assemble a functional receptor. The concomitant serial isolation of the two distinct cDNA clones is virtually impossible because the functional response is missing whenever the two cDNAs segregate into different pools in the course of narrowing down the active cDNAs.

We reasoned that for the isolation of a GABA_B cDNA, a screening approach using a radioligand binding assay rather than an electrophysiological read-out would be more promising. Such a screening does not depend on a functional coupling of the receptor and solely relies on

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**TABLE 1. Potencies of GABA_B compounds**

<table>
<thead>
<tr>
<th>Region</th>
<th>Read-Out</th>
<th>Drug</th>
<th>IC50/EC50, μM</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>mIL cells</td>
<td>Ca2+ channel inhibition</td>
<td>Baclofen</td>
<td>3</td>
<td>74</td>
</tr>
<tr>
<td>PAG neurons</td>
<td>Ca2+ channel inhibition</td>
<td>Baclofen</td>
<td>1.3</td>
<td>78</td>
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<td>Midbrain cultures</td>
<td>IPSCs</td>
<td>Baclofen</td>
<td>7</td>
<td>285</td>
</tr>
<tr>
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<td>IPSCs</td>
<td>CGP55845</td>
<td>0.5</td>
<td>285</td>
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<tr>
<td>Spinal cord</td>
<td>Glu release</td>
<td>Baclofen</td>
<td>0.1</td>
<td>39</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>Glu release   3-APPA</td>
<td>CGP44532</td>
<td>0.05</td>
<td>39</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>Glu release</td>
<td>CGP5348</td>
<td>&gt;100</td>
<td>39</td>
</tr>
<tr>
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<td>10</td>
<td>39</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>GABA release</td>
<td>Baclofen</td>
<td>&gt;100</td>
<td>39</td>
</tr>
<tr>
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<td>0.09</td>
<td>39</td>
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<td>39</td>
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<td>242</td>
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<td>Contraction</td>
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<tr>
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<td>5 (K_d)</td>
<td>308</td>
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<td>Rat brain</td>
<td>Binding [H]GABA</td>
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<tr>
<td>Rat brain</td>
<td>Binding [H]GABA</td>
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<td>4.3</td>
<td>158</td>
</tr>
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</table>

PAG, periaqueductal gray; IPSCs, inhibitory postsynaptic currents; EPSCs, excitatory postsynaptic currents; K_d, dissociation constant.
the exposure of a high-affinity binding site. With the development of high-affinity GABA_B radioligand antagonists, such as ^125_I-CGP64213 ($K_d = 1.2 \pm 0.2 \text{ nM}$), expression cloning using a binding assay became feasible and allowed the isolation of GABA_B(1a) and GABA_B(1b) cDNAs (169). The two encoded proteins derive from the same gene and differ in their extracellular NH₂-terminal domains (see sect. m4A5). The molecular structure of the cloned GABA_B proteins revealed all the hallmarks of a GPCR. Specifically, the sequence of GABA_B(1) subunits exhibited seven transmembrane domains and similarity with Family 3 (also named Family C) GPCRs. It is now known that Family 3 GPCRs comprise metabotropic glutamate (mGlu), the Ca²⁺-sensing (CaS), vomeronasal, and taste receptors (Fig. 1).

2. GABA_B receptor heteromerization

While GABA_B(1) subunits showed many of the expected features of native GABA_B receptors in terms of structure and distribution, they surprisingly did not efficiently couple to their effector systems (214). Moss and colleagues (80) were the first to demonstrate that GABA_B(1) proteins are retained in the endoplasmatic reticulum (ER) when expressed in heterologous cells. This is taken to explain the 100- to 150-fold lower affinity for agonists that is observed with recombinant GABA_B(1) subunits compared with native GABA_B receptors (169). Presumably, the failure of GABA_B(1) to traffic to the cell surface in the absence of GABA_B(2) prevents the interaction with the G protein in the plasma membrane, which is necessary to stabilize the high-affinity conformation of the binding site (see sect. m4A8). The search for a missing factor, which traffics GABA_B(1) subunits to the cell surface and renders them functional, therefore became an important objective. The search ended with the remarkable publication of three consecutive papers in *Nature* by groups from GlaxoWellcome, Novartis, and Synaptic Pharmaceuticals, as well as three subsequent papers from BASF-LYNX Bioscience, Merck, and the Laboratory of Molecular Neurobiology at Boston University (163, 170, 185, 216, 239, 343). All six papers describe the identification of the GABA_B(2) subunit, which must be coexpressed with GABA_B(1a) or GABA_B(1b) subunits to form a functional receptor. This finding represented the first compelling evidence for heteromerization among the GPCRs. Recombinant heteromeric GABA_B(1,2) receptors couple to all prominent effector systems of native GABA_B receptors, that is, adenylyl cyclase, Kir3-type K⁺ channels, and P/Q- and N-type Ca²⁺ channels (98, 100, 214). When the GABA_B(2) subunit is coexpressed with GABA_B(1), agonist potency more closely approximates that of native receptors (214). Kaupmann et al. (170) still observed a 10-fold lower affinity of recombinant GABA_B(1,2) receptors as opposed to brain receptors, which may be explained by limiting amounts of the G protein in the heterologous cells (170).

The reason for the intracellular retention of GABA_B(1) subunits is the presence of an ER-retention signal, the four-amino acid motif RSRR, in its cytoplasmic tail (210, 250). ER-retention signals of the RXR type were...
also observed in other multisubunit proteins, such as, for example, the K$_{\text{ATP}}$ channels (355) or N-methyl-d-aspartate (NMDA) receptors (302). It was recently proposed that the sequence context of the RSRR motif in GABA$_B$(1) is crucial for ER retention, and the motif was extended to include the sequence QLQXRQQLRSRR (125). The ER-retention signal in GABA$_B$(1) is masked from its ER-anchoring mechanism through the interaction with the COOH terminus of GABA$_B$(2), thus allowing for delivery of the GABA$_B$(1,2) complex to the cell surface. This ER-retention mechanism is suggested to prevent incorrectly folded GABA$_B$ receptors from reaching the cell surface and to represent a quality control mechanism. Some laboratories identified GABA$_B$(2) in the yeast two-hybrid system when using GABA$_B$(1) COOH-terminal sequences as bait, directly demonstrating that the COOH-terminal domains of GABA$_B$(1) and GABA$_B$(2) interact with each other (185, 343). Subsequent deletion analysis mapped the interaction to $\alpha$-helical coiled-coil domains of 32–35 amino acids in length. Coiled-coil domains are dimerization motifs that are found in numerous structural, trafficking, and regulatory proteins, such as, e.g., in leucine-zipper transcription factors. Mixing equimolar amounts of recombinant GABA$_B$(1) and GABA$_B$(2) peptides corresponding to the predicted coiled-coil domains indeed produced parallel coiled-coil heteromers under physiological conditions (166). In contrast, individual GABA$_B$(1) or GABA$_B$(2) peptides folded into relatively unstable homodimers or remained largely unstructured, suggesting that homodimeric receptors are not efficiently formed through coiled-coil interaction. Mutational analysis confirmed that the COOH-terminal coiled-coil interaction of GABA$_B$ receptors is essential for surface trafficking of the heteromer (57, 210, 250). Surprisingly, however, this interaction is not an absolute requirement for assembly of the receptor complex. It was demonstrated that COOH-terminally truncated GABA$_B$(1) and GABA$_B$(2) subunits can form fully functional receptors when expressed in heterologous mammalian cells (57, 250). This indicates that the transmembrane domains and/or extracellular domains (ECDs) encode surfaces that are sufficient for heteromerization, which is in agreement with findings with the COOH-terminally truncated GABA$_B$(1e) splice variant (301). Furthermore, this agrees with data that suggest that other Family 3 GPCRs, e.g., the mGlu1, mGlu5, and CaS receptors, homodimerize in their ECDs (278, 286, 328).

Challenging the general assumption that GABA$_B$ receptors necessarily need to heteromerize for function are infrequent responses seen with receptor subunits expressed in isolation. For example, GABA$_B$(2) was found to couple to adenyl cyclase in the absence of GABA$_B$(1) (185, 216), which is at odds with the proposal that only GABA$_B$(1) subunits are able to bind ligands (179). Similarly, when expressed alone in heterologous cells, GABA$_B$(1) yields infrequent electrophysiological and small biochemical responses (169, 171). This suggests that GABA$_B$(1) could be functional either alone or in combination with an unknown protein. It is difficult to reconcile functional GABA$_B$(1) responses with the efficient ER retention observed with this subunit. Even when wild-type GABA$_B$(1) is artificially targeted to the cell surface by masking the ER-retention signal with the COOH-terminal domain of GABA$_B$(2), functional responses are not observed (250). It may be speculated that the weak and infrequent coupling of homomeric GABA$_B$(1) or GABA$_B$(2) to cAMP and Kir3 channels depends on presently unknown endogenous factors. On the other hand, it remains unclear whether occasional endogenous expression of the partner subunit in heterologous cells could be responsible for the rare responses that were seen when GABA$_B$(2) or GABA$_B$(1) was transfected alone. In this context it is interesting to compare GABA$_B$ receptors with NMDA receptors that are similarly assembled with subunits that contain the RXR-type ER-retention signal (302). For unknown reasons, and similar to GABA$_B$ subunits, the NMDA receptor subunit NR1 occasionally generates functional responses in HEK293 cells or Xenopus oocytes in the absence of the masking subunit (222).

Despite several observations suggesting that GPCRs could form dimers or higher-order oligomers (7), the conventional perception was that GPCRs exist at the cell surface as monomers that couple to G proteins. For the most part it is the characterization of GABA$_B$ receptors that changed our view. It is now generally agreed that GPCRs can form homo- and heterodimers. In the last couple of years, it became more and more evident that heteromerization between GPCRs is not that rare a phenomenon. There are now several examples where even distantly related GPCRs form heteromeric complexes (1, 117, 164, 284). This raises fascinating combinatorial possibilities and may generate a level of regulatory and pharmacological diversity that we did not anticipate. Because GPCRs are major pharmacological targets, this recognition will have important implications for the development and screening of new drugs.

3. Invertebrate GABA$_B$ receptors

Development and organization of the nervous system are substantially different between vertebrates and invertebrates. Neurotransmitters evolved the ability to activate a range of ion channels and second messenger systems, but their relative importance as sensory, inter- or motor-neuron signaling molecules varies widely between phyla. Although the evidence is still sketchy, it currently appears that most mammalian neurotransmitter receptors have counterparts in invertebrates (337). Invertebrate GABA$_B$ receptors were described in echinoderms (90, 91), mollusks (11, 287), and arthropods (101, 226, 254, 276, 335). Invertebrate GABA$_B$ functions were mostly studied at the

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neuromuscular junction of echinoderms, where GABA elicits inhibitory and excitatory responses (90). GABA B responses were also observed in invertebrate CNS neurons (150, 287). Like their mammalian orthologs, invertebrate GABA B receptors activate G proteins and regulate K+ and Ca2+ currents (226, 287).

The only cloned invertebrate GABA B receptors are those of *Drosophila melanogaster* (224). Two of the three subunits that were described, d-GABAB(1) and d-GABAB(2), show clear sequence identity to the mammalian subunits. A third subunit, d-GABAB(3), seems to be specific for insects and is of unknown function. Surprisingly, *Drosophila* GABAB receptors do not contain the sushi domains that are found in the mammalian GABA B1a subunit (see sect. mA4A), although sushi domains exist in the *Drosophila* genome. For example, sushi repeats are found in hikaru genki, a *Drosophila* protein that is secreted from presynaptic terminals during the period of synapse development (148). Analysis of the expression pattern in the embryonic nervous system revealed that d-GABAB(1) and d-GABAB(2) are expressed in overlapping regions. Upon coexpression in *Xenopus* oocytes or mammalian cell lines, d-GABA B(1) and d-GABA B(2) form a functional heteromeric d-GABA B(1,2) receptor that couples to Gα/Gα- and Gβ-Gα-type G proteins. Therefore, heteromerization is not only a prerequisite for mammalian but also for invertebrate GABA B function. d-GABA B(1,2) receptors exhibit a unique pharmacology. GABA and 3-aminopropylphosphonous acid (3-APPA) are, as expected, d-GABA B(1,2) agonists. However, baclofen has no longer agonistic properties and instead acts as an antagonist. Furthermore, neither saclofen nor CGP35348 antagonizes d-GABA B(1,2) receptors, in contrast to their activity at mammalian receptors. d-GABA B(1,2) receptors closely reproduce the pharmacology that was described for insect GABA B receptors (12, 150, 295) and therefore do not suggest the existence of additional GABA B subunits.

Physiological or pharmacological approaches have not found any evidence for GABA B receptors in helminths (337). GABA-induced muscle relaxation in the nematode *Caenorhabditis* only depends on the unc-49 gene products, which form GABA B-like ionotropic receptors (14). However, the *Caenorhabditis* genome database reveals the presence of orthologs for both GABA B(1) and GABA B(2) (179). Most residues of the GABA binding-pocket in GABA B(1) are conserved from *Caenorhabditis* to human (179). This is in contrast to the complete lack of evolutionary constraint placed on the binding pocket of GABA B(2), which suggests that this subunit does not constitute a binding site for an endogenous ligand (see sect. mA5).

4. GABA B receptor isoforms

When it became apparent that probably all GABA B receptors in the vertebrate brain are the sole products of the GABA B(1) and GABA B(2) genes, much attention focused on subunit isoforms. Many in the field wondered whether isoforms encoded pharmacological differences and accounted for the heterogeneity observed with native GABA B receptors. Rapidly numerous GABA B isoforms were identified (recently reviewed in Ref. 29). A close inspection of GABA B gene structures indicates that not all of these splice variants are real and that some do not occur across different species. A summary of confirmed GABA B isoforms is shown in Figure 2. While in most laboratories mixing and matching of isoforms did not produce GABA B receptors with distinct functional and

![FIG. 2. Protein structure of vertebrate GABA B(1) isoforms. The sushi domains (SD), seven transmembrane domains (7TM), coiled-coil domains (CC), and the ER-retention signals (RSRR) are indicated. Unfortunately, unrelated variants identified in humans and rats both were named GABA B1c. Thus the variants described in the rat are better referred to as GABA B(1e-a) or GABA B(1e-b), depending on whether their amino-terminal domain is related to GABA B1a or GABA B1b, respectively. The 31-amino acid insertion between the second extracellular loop (ECL2) and the fifth transmembrane domain in GABA B(1f) corresponds to a 93-bp exon located between exons 19 and 20, which is not conserved in human and mouse. Dark gray segments represent the unique carboxy-terminal tails of GABA B(1d), GABA B(1e), and GABA B(1f). In GABA B(1f), skipping of exon 5 results in an in-frame deletion of 7 amino acids (Δ7aa). References for all published variants are shown in parentheses.](http://physrev.physiology.org/ by 10.220.33.6 on June 21, 2017)
pharmacological properties, others reported differences that are, however, highly controversial (see sect. II A4/A).

GABA<sub>B</sub> isomers may afford the means for a differential subcellular targeting and/or coupling to distinct intracellular signaling pathways. To some extent a coupling to different effector systems could mimic a differential pharmacology and explain some of the differences that were observed with native GABA<sub>B</sub> receptors.

A) PREDOMINANT SUBUNIT VARIANTS: GABA<sub>B(1a)</sub> AND GABA<sub>B(1b)</sub>. The most abundant GABA<sub>B</sub>-receptor isomers are GABA<sub>B(1a)</sub> and GABA<sub>B(1b)</sub>, which exhibit dissimilarity in the ECD (169). The GABA<sub>B(1a)</sub> and GABA<sub>B(1b)</sub> isomers are the only variants that are highly conserved among different species (Fig. 3). The first 147 amino acids of the mature GABA<sub>B(1a)</sub> isoform are replaced in GABA<sub>B(1b)</sub>, with a sequence of 18 amino acids. Contrary to the general assumption, GABA<sub>B(1a)</sub> and GABA<sub>B(1b)</sub> are not generated by NH<sub>2</sub>-terminal alternative splicing. The distinct ECD in GABA<sub>B(1b)</sub> results from the presence of an alternative transcription initiation site within the GABA<sub>B(1a)</sub> intron upstream of exon 6, thereby extending exon 6 at its 5'-end (Fig. 4A) (217, 260). Presumably, GABA<sub>B(1a)</sub> and GABA<sub>B(1b)</sub> use different promoters, with the GABA<sub>B(1b)</sub> promoter being buried within GABA<sub>B(1a)</sub> intron sequences. Alternative NH<sub>2</sub>-termini are rather exceptional for GPCRs and are not observed in any of the closely related Family 3 GPCRs. GABA<sub>B(1a)</sub> and GABA<sub>B(1b)</sub> primarily differ by the presence of a pair of sushi repeats in the GABA<sub>B(1a)</sub>-specific domain (28, 135). Sushi repeats, also known as short consensus repeats (SCRs), were originally identified in complement proteins as a module that is involved in protein-protein interactions. They are mostly found in proteins that are involved in cell-cell adhesion and were never before observed in a neurotransmitter receptor. Sushi repeats have yet to exhibit a function in the context of the GABA<sub>B</sub> receptor. It is tempting to speculate that GABA<sub>B(1a)</sub> is targeted to or retained at specific subcellular regions by means of interaction of its sushi repeats with proteins in the extracellular matrix or on the surface of neighboring cells. A recent report suggests that the GABA<sub>B(1a)</sub> sushi-repeats interact with the extracellular matrix protein fibulin (118). This proposal is of significant interest, also from a drug discovery point of view (see sect. VI).

Numerous studies indicate that GABA<sub>B(1a)</sub> and GABA<sub>B(1b)</sub> show differences in their spatial and temporal expression patterns (Fig. 4, B-D) (21, 32, 33, 104, 169-171, 194, 206, 267, 270). A pre- versus postsynaptic localization was suggested for both isoforms, but was never directly demonstrated (21, 32, 171). A striking example of a differential expression of GABA<sub>B(1a)</sub> and GABA<sub>B(1b)</sub> is found in the cerebellum (Fig. 4B) (33, 171). GABA<sub>B(1a)</sub> transcripts are confined to the granule cell layer that comprises the cell bodies of the parallel fibers, which are excitatory to the Purkinje cell dendrites in the molecular layer. By comparison GABA<sub>B(1b)</sub> transcripts are mostly expressed in Purkinje cells, the dendrites of which possess GABA<sub>B</sub> receptors that are postsynaptic to GABAergic basket and stellate cells or glutamatergic parallel fibers.

In dorsal root ganglia the density of GABA<sub>B(1a)</sub> transcripts is high as opposed to GABA<sub>B(1b)</sub> transcripts (32). GABA<sub>B(1b)</sub> protein is generally expressed at higher levels in the adult brain compared with fetal brain, whereas the opposite is seen during development (Fig. 4D) (56, 104, 206, 217). These spatial and temporal differences in the expression of the GABA<sub>B(1a)</sub> and GABA<sub>B(1b)</sub> subunits highlight the separate transcriptional regulation and suggest distinct functional roles.

A number of laboratories compared the pharmacology of GABA<sub>B(1a)</sub> and GABA<sub>B(1b)</sub> and did not detect significant differences (49, 121, 170, 206). However, there are isolated reports that claim that GABA<sub>B(1a)</sub> and GABA<sub>B(1b)</sub> can be separated by pharmacological or functional means (26, 192, 237). Especially, the proposal that the anticonvulsant gabapentin is an agonist at GABA<sub>B(1a)</sub>, but not at GABA<sub>B(1b)</sub>, attracted a great deal of attention (26, 27, 237). Not only was gabapentin suggested to be subunit specific, but it was also shown to selectively activate postsynaptic GABA<sub>B</sub> receptors. This remains highly controversial, as a number of other laboratories were unable to reproduce these findings, using similar and additional experimental approaches (161, 190, 255). In these latter studies, no clear effect of gabapentin on GABA<sub>B</sub> receptors was seen in recombinant systems, in brain slice preparations, or in vivo, even when using high concentrations of the drug.

B) MINOR SUBUNIT VARIANTS. Several additional GABA<sub>B</sub>-isoforms were reported. GABA<sub>B</sub> were identified in rat cDNA libraries (156, 260). GABA<sub>B</sub> is characterized by an in-frame insertion of 31 amino acids between the second extracellular loop and the fifth transmembrane domain. This extra sequence is not conserved in humans, casting doubt on its significance. Nonetheless,
the rat GABA\(_{B(1c)}\) protein forms a functional receptor when expressed with GABA\(_{B(2)}\) in heterologous cells (260). An isoform that differs at the NH\(_2\) terminus from the GABA\(_{B(1a)}\) isoform was identified in humans (56, 217). Unfortunately, this variant was also named GABA\(_{B(1c)}\). Human GABA\(_{B(1c)}\) is similar to GABA\(_{B(1a)}\) yet lacks one sushi repeat because the splice machinery skips exon 4. The human GABA\(_{B(1c)}\) mRNA expression pattern parallels that of GABA\(_{B(1a)}\). GABA\(_{B(1d)}\) results from the failure to splice out the last intron of the GABA\(_{B(1)}\) gene and has a divergent COOH terminus that deletes half the coiled-coiled domain, including the ER-retention motif. It is impossible to generate GABA\(_{B(1d)}\) in human and mouse due to poor sequence conservation, again rising doubts about the physiological relevance of this splice event. GABA\(_{B(1e)}\) encodes the extracellular ligand-binding domain of the GABA\(_{B(1a)}\) subunit (301). GABA\(_{B(1e)}\) is generated by skipping of exon 15 and is detected both in rats and humans. While the GABA\(_{B(1e)}\) transcript is a minor component in the CNS, it is very prominent in peripheral tissues. GABA\(_{B(1e)}\) is secreted into the culture medium when expressed in transfected mammalian cells. Additionally, it also forms stable heteromeric complexes with GABA\(_{B(2)}\) at the plasma membrane, providing additional evidence that the coiled-coil interaction is not the only dimerization interface between GABA\(_{B(1)}\) and GABA\(_{B(2)}\) (57, 125, 250). Should the GABA\(_{B(1e)}\) protein occur in vivo, then it could affect GABA\(_{B}\) receptor function in a dominant-negative manner. GABA\(_{B(1f)}\) was identified as a rat transcript. It contains the in-frame deletion of exon 5 resulting in a 21-bp deletion in the ECD, as well as a COOH-terminal insertion corresponding to intron 22 (156, 339). GABA\(_{B(1g)}\), an additional truncated GABA\(_{B(1a)}\) isoform, was identified in rat tissue (340). GABA\(_{B(1g)}\) is characterized by an insertion of 124 bp between exon 4 and 5, which generates a frameshift. GABA\(_{B(1g)}\) encodes a
COOH-terminally truncated polypeptide of 239 amino acid residues of unknown function. The distribution of GABA
B\(_{2(c-g)}\) isoform mRNA was exclusively studied using Northern blot, PCR, or in situ hybridization. For none of the GABA
B\(_{2(c-g)}\) splice variants the existence of a protein in vivo was demonstrated yet [in contrast to the GABAB\(_{1(a)}\) and GABAB\(_{1(b)}\) variants].

With regard to GABA\(_{2}\), all initial papers report a single transcript (163, 170, 185, 216, 239, 343). Subsequently, two additional transcripts, GABA\(_{2(b)}\) and GABA\(_{2(c)}\), were identified in human tissue (75). Analysis of human GABA\(_{2}\) genomic sequences reveals that the intron-exon boundaries required to generate these transcripts do not match known consensus sequences for splice junctions (217). GABA\(_{2(b)}\) and GABA\(_{2(c)}\) transcripts are likely to represent artifacts arising during cDNA synthesis and/or PCR amplification. Therefore, at the present time, there are no confirmed GABA\(_{2}\) splice variants.

5. The GABA binding site

All GABA\(_{B}\) agonists and competitive antagonists bind to the ECD of the GABA\(_{B(1)}\) subunit, as shown for other Family 3 GPCRs as well (207). The ECD of GABA\(_{B(1)}\) can be expressed as a soluble protein. The truncated protein mostly retains the binding properties of wild-type receptors, indicating that it folds independently from the transmembrane domains. Structural analysis of the GABA\(_{B(1)}\) ECD reveals a weak sequence homology with bacterial periplasmic binding proteins, such as the leucine-binding protein (LBP) (169). GABA\(_{B(1a)}\) and GABA\(_{B(1b)}\), which differ in their ECD (see sect. IIIA4A), share the entire bacterial homology domain (110). In all GABA\(_{B}\) subunits the LBP-like domain is linked to the first transmembrane domain via a short sequence that lacks the cysteine-rich region conserved between the other members of Family 3 GPCRs. In the mGlu receptors, this cysteine-rich region appears to be necessary for the LBP-like domain to bind glutamate (244).

The X-ray structure of periplasmic-binding proteins reveals a binding pocket that is made up by two globular lobes (lobes I and II) separated by a hinge region. The two lobes close upon ligand binding, similar to a Venus flytrap when touched by an insect (275). Homology models of the GABA\(_{B(1)}\) ligand-binding domain, based on the X-ray structures of the bacterial proteins, have guided mutational analysis of the GABA binding site (110, 111). Key for both agonist and antagonist binding are S246, S269, D471, and E465 in lobe I, as well as Y366 in lobe II (Fig. 5). GABA and baclofen are thought to bind via their carboxylic group to the hydroxyl groups of S246 and Y366. E465 is then believed to bind to the NH\(_2\)-terminal end of GABA. D471, which was originally proposed to undergo an ionic interaction with GABA, now appears more important for correct folding of lobe I. Mutation of S247 and Q312 increases the affinity of agonists while decreasing the affinity of antagonists. This supports a model where the LBP-like domain exists in two conformational states, an open and a closed state, where binding of ligands favors the closed state (110). According to the three-dimensional

![Fig. 5. Three-dimensional model of the Venus flytrap module (VFTM) of GABA\(_{B(1)}\). A model of GABA docked into the ligand-binding site of GABA\(_{B(1)}\). The putative interactions between GABA and the amino acid residues of GABA\(_{B(1)}\), are shown with dotted lines (see sect. IIIA5 for details). [Adapted from Kniazeff et al. (179); courtesy of Dr. J.-P. Pin.]

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model, a direct interaction with the second lobe is only possible in the closed form of the LBP-like domain, as shown for other receptors (8, 245). Some mutations differentially affect the binding of agonists. For example, the potency of GABA is decreased 30-fold by the S269A mutation, whereas the potency of baclofen remains unaltered (112). As is discussed in section II A6, this correlates with distinct effects of Ca²⁺ on GABA and baclofen binding (112). Interestingly, mutation of Y366 in lobe II not only decreases the affinity of GABA and baclofen, but also converts baclofen into an antagonist (111). The recently obtained crystal structure of the dimeric ECD of mGlu1 in the presence and absence of glutamate has essentially validated the homology models for GABA₂ subunits described above (180).

Most people will agree that GABA₂ does not bind agonists or antagonists and does not function when expressed alone (see sect. II A2 for conflicting reports). However, GABA₂ contains the large ECD that binds ligands in GABA₁, the mGlu and CaS receptors. It was speculated that an alternative, as yet unknown, ligand binds to the ECD of GABA₂. According to a recent phylogenetic analysis of GABA₂ subunits from various species, this appears rather unlikely (179). However, it is conceivable that the recently developed allosteric modulators bind specifically to the GABA₂ subunit (see sect. III A).

6. The Ca²⁺ binding site

GABA₂ receptors share sequence similarity with mGlu and CaS receptors that are sensitive to Ca²⁺. In the CaS receptor the primary determinant for Ca²⁺ recognition is in the ECD (129). The effect of Ca²⁺ on mGlu receptors is heavily disputed. Some reports show that Ca²⁺ can directly activate mGlu1, mGlu3, and mGlu5 receptors (183), whereas others claim that Ca²⁺ is an allosteric modulator rather than an agonist (234, 296). Because of the effects of Ca²⁺ on Family 3 GPCRs, two groups investigated the possible regulation of GABA₂ receptors by Ca²⁺ (112, 345). This led to the discovery of a Ca²⁺ binding site in the GABA₂ subunit as the first allosteric site of GABA₂ receptors. Accordingly, it was shown that Ca²⁺ potentiates GABA-stimulated GTPγ[S] binding in membranes expressing native or recombinant GABA₂ receptors. The effect of Ca²⁺ depends on the agonist, with baclofen being less sensitive than GABA or 3-APPA.

The residues that confer to GABA₂ receptors the ability to sense Ca²⁺ were identified (112). The S269A mutation (see sect. II A5) in the LBP-like domain of GABA₂ renders the otherwise functional GABA₂ receptor Ca²⁺ insensitive. S269 localizes to the GABA-binding pocket, next to S246 that interacts with agonists (111). S269 in GABA₂, which aligns with S170 in the CaS receptor, a residue that is involved in Ca²⁺ activation of the CaS receptor (48). Allosteric regulation of GABA₂ by Ca²⁺ is supposed to stabilize the activated closed conformational state (112). Possibly, Ca²⁺ compensates for the lack of the α-amino group in GABA, and the contact of Ca²⁺ with S269 optimizes positioning of the carboxylic group of GABA for contacting S246. Alternatively Ca²⁺ does not directly interact with S269 but affects the positioning of its hydroxyl group. This may allow the formation of an additional hydrogen bond with the carboxylic group of GABA. The EC₅₀ value for Ca²⁺ modulation of GABA₂ binding at GABA₂ receptors is with 37 μM rather low. Under normal physiological conditions, with [Ca²⁺] in the blood and cerebrospinal fluid in the millimolar range, the Ca²⁺ site of GABA₂ receptors is saturated. Allosteric regulation by Ca²⁺ may, however, become significant under pathological conditions, when extracellular [Ca²⁺] is low. This could be the case following ischemia (191) or epileptic seizures (138).

7. Molecular determinants of G protein coupling

A) MOLECULAR DETERMINANTS IN THE G PROTEIN. The cloning of GABA₂ receptors allowed characterizing the G protein interaction in heterologous systems. Franek et al. (103) used chimeric G proteins to identify the specific regions of the Gα/Gqα subunits that are important for their interaction with GABA₂ receptors. HEK293 cells expressing recombinant GABA₂ or GABA₂ alone or in combination do not activate PLC through Gqα-type G proteins. However, heteromeric GABA₂ receptors do activate PLC via chimeric Gqα and Gqα proteins, in which the five COOH-terminal residues of Gqα replace those of Gα. Therefore, like other GPCRs, GABA₂ receptors recognize the very COOH terminus of Gα subunits (35). The amino acid residue at position -4 in the COOH terminus of Gα proteins was shown to be most important for the coupling to GABA₂ receptors. B) MOLECULAR DETERMINANTS IN GABA₂ RECEPTOR SUBUNITS. Questions were raised as to what domains of the heteromeric GABA₂ complex are involved in the interaction with G proteins. Two reports showed that neither the COOH-terminal intracellular domain of GABA₂ nor that of GABA₂ is needed for coupling to chimeric G proteins in a heterologous system (57, 250). Both groups expressed receptor constructs together with chimeric Gqα or Gqα proteins in HEK293 cells and measured the increase of the intracellular [Ca²⁺] following PLC activation. These findings were confirmed by others who found that the COOH termini of GABA₂ and GABA₂ influence G protein coupling but that they are not an absolute requirement for function (125). In contrast, it was reported that the deletion of the GABA₂ COOH terminus impairs the ability of recombinant receptors to activate Kir3-type K⁺ channels in Xenopus oocytes (211). The reason for this discrepancy...
is unclear but may relate to the fact that PLC is activated by Ga, whereas Kir3 channels are activated by Gβγ. There is now good evidence that the heptahelical region of the GABA_{B(2)} subunit directs the coupling to G proteins. With the use of chimeric GABA_{B(1)} and GABA_{B(2)} subunits with swapped ECDs, it was shown that only the heptahelical region of GABA_{B(2)} is absolutely necessary for G protein signaling (109, 212). However, the heptahelical region of GABA_{B(1)} significantly improves coupling efficacy. These studies further showed that both the GABA_{B(1)} and GABA_{B(2)} ECDs are required for function. It was later found that all GABA_{B(2)} intracellular loops are important for receptor coupling to Kir3 channels, whereas those of GABA_{B(1)} could be replaced with those of GABA_{B(2)} without affecting function (97, 211, 280). Particular attention was set on addressing the role of the second intracellular (i2) loop since there is evidence that this region is critical for G protein coupling in Family 3 GPCRs (119). Exchanging the i2 loops between GABA_{B(1)} and GABA_{B(2)} did not result in the formation of functional receptors. Hence, the i2 loop of GABA_{B(2)} needs to be correctly positioned with respect to the other intracellular domains. Sequence comparison between the i2 and i3 loops of GABA_{B} and the related mGlu receptors highlighted clear differences between GABA_{B(1)} and GABA_{B(2)}. Mutational analysis confirmed the functional importance of conserved residues (K586, M587, and K590) in the i2 loop of GABA_{B(2)} and indicates that the GABA_{B(1)} i2 loop lacks the requirements for interaction with G proteins (97, 280). Mutation of K686, a basic residue in the i3 loop of GABA_{B(2)} that plays a critical role in G protein coupling of mGlur1 and CaS receptors (66, 102), suppresses functional coupling to G proteins in HEK293 and cerebellar granule cells, corroborating a similar role for this residue (97).

The question arises as to how many G proteins a dimeric GPCR can interact. It was shown that the cytoplasmic surface of monomeric rhodopsin is too small to anchor both the Ga and Gβγ subunits (195). Only a rhodopsin homodimer provides sufficient interface to anchor both Ga and Gβγ. Although not generally accepted yet, the data on rhodopsin imply that all GPCRs need to homodimerize for function. We therefore expect that the GABA_{B(1,2)} heterodimer binds one G protein only; one GABA_{B} subunit probably interacts with Ga, while the other subunit interacts with Gβγ.

8. Intra- and intermolecular events controlling receptor function

As described above, a large body of work has aimed at defining the structural requirements for ligand binding, subunit interaction, and G protein coupling (see sect. ii, A5–A7). The interdependence of heteromerization, surface trafficking, and effector coupling makes it difficult to assign a defined molecular function to structural elements. Nevertheless, a number of laboratories reached similar conclusions regarding the sequence of intra- and intermolecular events that take place when activating a GABA_{B} receptor (109, 125, 253, 280). A scheme that accommodates most of the available data predicts that the ECD of GABA_{B(1)} is the only determinant for GABA binding, while the ECD of GABA_{B(2)} is necessary for receptor activation and for increasing agonist affinity. The heptahelical region and the cytoplasmic tail of GABA_{B(2)} is the prime determinant of G protein coupling, but GABA_{B(1)} is clearly necessary to optimize the coupling efficiency. A model was proposed where a conformational change within the dimeric ECDs of GABA_{B(1)} and GABA_{B(2)} is responsible for the stabilization of an active dimeric form of the transmembrane domains (Fig. 6). Hence, there is an allosteric interaction between the ligand-binding domain and the effector transmembrane domains. It is assumed that the GABA_{B} heteromer differs from the homodimers formed by other Family 3 GPCRs with respect to the functional coupling between binding and effector domains. In the GABA_{B} receptor the conformations of the effector and binding domains are probably tightly coupled, that is, the two domains are either both in an active or inactive conformation (253). This model is reminiscent of a two-state model for receptor activation.

9. Interacting proteins

The discovery that receptor activity modifying proteins (RAMPs) can change the pharmacology of a GPCR triggered an intense search for GABA_{B} receptor-associated proteins (221). Many people in the field wondered whether interacting proteins could account for the pharmacological differences that were observed with native GABA_{B} receptors (see sect. ii). A number of candidate proteins were identified in yeast two-hybrid screens, using the COOH-terminal domains of GABA_{B(1)} or GABA_{B(2)} as baits (Fig. 7). There are no reports claiming pharmacological changes upon expression of these proteins together with GABA_{B(1)}, GABA_{B(2)}, or GABA_{B(1,2)}. This aside, interacting proteins constitute potential targets for ligands that modulate GABA_{B} function in a more specific way.

Three laboratories described that the COOH-terminal domain of GABA_{B(1)} interacts with members of the ATF/CREB family of transcription factors, that is, ATF4/CREB2 and ATF6 (235, 333, 342). Gadd153, also known as CHOP, is an additional leucine-zipper transcription factor that was described to bind to GABA_{B} receptors (297). These findings are both intriguing and provocative, as they suggest for the first time a direct interaction between a GPCR and transcription factors. The interaction between CREB2/ATF-4 and GABA_{B(1)} takes place between the leucine-zipper and the coiled-coil domain, respectively. Although it is formally not ruled out that this
interaction is artificial, increasing evidence points toward a physiological relevance. Colocalization between GABA₆ receptors and ATF4/CREB2 is observed in the soma and dendrites of cultured hippocampal neurons as well as in retinal amacrine cells in situ (235, 333). Translocation of ATF4/CREB2 either into or out of the nucleus was seen following GABA₆ receptor activation (333, 342). The reason for these opposing effects is unclear, but possibly relates to differences in the neuronal cultures that were used. Furthermore, stimulation of GABA₆ receptors re-
sulted in transcriptional activation of ATF4/CREB2-responsive reporter genes (235, 342). Paradoxically, in one case pertussis toxin blocked transcriptional activation, whereas in the other case pertussis toxin was ineffective. GABA<sub>B</sub> signaling through CREB proteins was already proposed before cloning (16, 157). In these earlier studies baclofen silenced transcription instead of activating it. These earlier findings are more in agreement with the well-known inhibitory effect of baclofen on cAMP production. Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) genes may well be the targets of GABA<sub>B</sub>-mediated transcriptional regulation. Both the production of NGF and BDNF are stimulated after treatment of rats with GABA<sub>B</sub> receptor antagonists (137). Taken together, the data suggest a novel and unique mechanism of signal transduction to the nucleus that results from activation of GABA<sub>B</sub> receptors. Although still conflicting, the data linking GABA<sub>B</sub> Receptors to transcription factors suggest that these receptors mediate long-term metabolic effects requiring new protein synthesis. Additionally, GABA<sub>B</sub> receptors may signal through G protein-independent effector pathways that modulate neurotransmitter release (132). A G protein-independent signaling was recently also proposed for the related mGlu receptors (142, 143) and for CASK/Lin2 (149).

The intracellular COOH terminus of GABA<sub>B</sub> receptors associates with two members of the 14–3–3 family of signaling proteins (81). The interacting region of GABA<sub>B</sub> overlaps with the coiled-coil domain. Consequently, 14–3–3 proteins could interfere with GABA<sub>B</sub> heteromerization. 14–3–3 proteins were implicated in GPCR signaling before (23, 269). For example, they directly influence G protein coupling efficiency and act as scaffolds that recruit proteins modulating G protein activity, such as protein kinases and regulators of G protein signaling (RGS). In addition, owing to their dimeric nature, 14–3–3 proteins might be directly implicated in the clustering of GABA<sub>B</sub> receptors at pre- or postsynaptic sites. The functional consequences of 14–3–3 binding to GABA<sub>B</sub> receptors are, however, unknown.

There are additional scaffolding proteins that interact with GABA<sub>B</sub> receptors. NEM-sensitive factor, or NSF, an ATPase critical for intracellular trafficking, interacts with the COOH terminus of GABA<sub>B</sub> (341). NSF also interacts with GABA<sub>A</sub> receptors through its binding of GABARAP (177) and possibly provides a structural link between the ionotropic and metabotropic GABA receptor systems. Tamalin is yet another scaffolding protein that interacts with GABA<sub>B</sub> as well as mGlu receptors (176). Tamalin is also proposed to interact with guanine nucleotide-exchange factor cytohesins and to promote intracellular trafficking and cell-surface expression of associated receptors. Tamalin comprises multiple protein-interacting domains, including a 95-kDa postsynaptic density protein (PSD-95)/discs-large/ZO-1 (PDZ) domain (131), a leucine-

zipper region and a COOH-terminal PDZ-binding motif. PDZ domain proteins are important for the scaffolding of receptors at synapses and in epithelia (182). MUPP1, a multivalent PDZ protein, interacts with a stretch of 10 amino acids proximal to the coiled-coil domain of GABA<sub>B</sub> (214). Interestingly, this sequence does not conform to the PDZ domain-binding consensus sequence. MUPP1 was initially shown to interact with another GPCR, the 5-HT<sub>2C</sub> receptor (17). MUPP1 may serve as an adaptor protein, linking GABA<sub>B</sub> to various signaling molecules. Intriguingly, the GABA<sub>B</sub> COOH terminus contains a putative PDZ domain-binding consensus sequence, LYK. However, no PDZ domain protein is reported to interact with GABA<sub>B</sub>. Finally, the actin-binding protein β-actinin is proposed to tether GABA<sub>B</sub> receptors via the GABA<sub>B</sub> subunit to the cytoskeleton (341).

Two reports suggest that GABA<sub>B</sub> receptor extracellular domains interact with other proteins. Fibulin, an extracellular matrix protein, apparently binds to the sushi repeats of the GABA<sub>B</sub> subunit (118). This finding is of special interest, as we still do not know whether mechanisms are in place to target GABA<sub>B</sub> to different subcellular regions, e.g., pre- and postsynaptic sites. The other report provides evidence that the HNK-1 carbohydrate carried by many neural extracellular matrix proteins, among them tenascin-R and tenascin-C, binds to GABA<sub>B</sub> receptors (288). HNK-1 was proposed to regulate GABA<sub>A</sub> receptor-mediated perisomatic inhibition by suppression of postsynaptic GABA<sub>B</sub> receptor activity. HNK-1 does not bind to the sushi repeats of GABA<sub>B</sub>. GABA<sub>B</sub> receptors were found to be associated with lipid rafts, specialized plasma membrane microdomains that function as platforms for signaling complexes (18). Lipid rafts are enriched in cholesterol/sphingolipids and contain specific populations of membrane-associated proteins, such as G proteins and other signaling molecules. For example, G<sub>α</sub>o- and G<sub>α</sub>x-type G proteins, the main transducers of GABA<sub>B</sub> receptors, are enriched in the lipid-raft fraction extracted from cerebellar membranes. It remains to be seen whether lipid rafts can segregate GABA<sub>B</sub> receptor populations, e.g., GABA<sub>B</sub> and GABA<sub>B</sub>, or in some ways account for the functional heterogeneity observed in vivo.

10. Phosphorylation and desensitization studies

Modulation of GPCR activity by intracellular kinases is well-known and usually the trigger for activity-dependent desensitization (327). In general, phosphorylation of the receptor protein is followed by interaction with cytoplasmic accessory proteins called β-arrestins, which interfere with the receptor to G protein coupling and promote rapid endocytosis. PKC is reported to suppress GABA<sub>B</sub>-mediated inhibition of neurotransmitter release (322). Similarly, PKA is described to desensitize GABA<sub>B</sub>
receptors expressed in *Xenopus* oocytes (353). Subsequently, it was observed that PKC- and PKA-dependent signaling pathways mediate the modulation of GABA<sub>B</sub> activity in response to estrogen (173, 189). More recent molecular studies contrast these earlier findings. Moss and colleagues (82) reported that PKA phosphorylation of S892 in the cytoplasmic tail of GABA<sub>B(2)</sub> reduces rather than increases receptor desensitization, probably as a result of stabilizing the receptor complex at the cell surface. This challenges the conventional view that phosphorylation is a negative modulator of GPCR signaling. S892 phosphorylation in GABA<sub>B(2)</sub> was also observed in another experimental paradigm (350). It was shown that withdrawal from repeated cocaine treatment produces an increase in the basal level of extracellular GABA in the rat accumbens (see sect. IV). The increase in extracellular GABA is paralleled by diminished Ser-892 phosphorylation, suggesting a functional desensitization of GABA<sub>B</sub> autoreceptors. At first glance contradictory, Bouvier and colleagues (258) identified the G protein-coupled receptor kinase 4 (GRK4) as the kinase that promotes desensitization of GABA<sub>B</sub> receptors. Surprisingly, however, this desensitization occurred in the absence of ligand-induced receptor phosphorylation and could be promoted by GRK4 mutants deleted of their kinase domain. Again, these results are at odds with the generally accepted model linking the kinase activity of GRKs to their role in receptor desensitization. GABA<sub>B</sub> receptors can also change the phosphorylation state of other proteins by influencing intracellular signaling pathways. For example, GABA<sub>B</sub> receptors increase the activity of P<sub>2</sub>X-type ATP receptors or the calcium-dependent K<sup>+</sup> current (i<sub>AMP</sub>) through a downregulation of cAMP levels, resulting in decreased PKA activity and subsequent dephosphorylation of the ion channel (116, 120). Possibly the interplay between the GABA and ATP neurotransmitter systems could be exploited for the treatment of neuropathic pain (see sect. IV D).

B. Molecular Studies on Native GABA<sub>B</sub> Receptors

1. GABA<sub>B</sub>-deficient mice

Given that cloning efforts did not substantiate the claim for receptor heterogeneity, it became important to understand to which GABA<sub>B</sub> functions the cloned receptors can contribute in vivo. To address this question, three laboratories disabled the GABA<sub>B(1)</sub> gene in mice (272, 274, 300). Strikingly, the GABA<sub>B(2)</sub> subunit is heavily downregulated in all three GABA<sub>B(1)</sub><sup>−/−</sup> mouse lines. This requirement of GABA<sub>B(1)</sub> for stable expression of GABA<sub>B(2)</sub> supports that in vivo most GABA<sub>B(2)</sub> protein is associated with GABA<sub>B(1)</sub>, in agreement with biochemical studies (21). Only the GABA<sub>B(1)</sub><sup>−/−</sup> mice generated on the Balb/c genetic background are viable (300). The GABA<sub>B(1)</sub><sup>−/−</sup> mice generated on other genetic backgrounds die within 3–4 wk after birth, thus precluding behavioral analysis of adult animals (272, 274). Strain differences in viability of knockout mice are not uncommon (256). The overt phenotype of all GABA<sub>B(1)</sub><sup>−/−</sup> mouse strains includes spontaneous epileptic seizures, and these seizures may be suppressed to some extent in the Balb/c genetic background. A reduced seizure activity, in turn, may rescue mice from lethality. Upon GABA<sub>B</sub> agonist application, adult Balb/c GABA<sub>B(1)</sub><sup>−/−</sup> mice show neither the typical muscle relaxation, hypothermia, nor delta electroencephalogram (EEG) waves. These behavioral findings are paralleled by a loss of all detectable biochemical and electrophysiological GABA<sub>B</sub> responses in GABA<sub>B(1)</sub><sup>−/−</sup> mice. This demonstrates that GABA<sub>B(1)</sub> is an essential component of pre- and postsynaptic GABA<sub>B</sub> receptors in the CNS. This finding rules out the presence of functional homomeric GABA<sub>B(2)</sub> receptors and is consistent with the proposal that the GABA<sub>B(1)</sub> subunit is solely responsible for binding GABA (179). Knockout studies further indicate that the GABA<sub>B(1)</sub> subunit is an essential requirement for GABA<sub>B</sub> receptor function in the peripheral nervous system (PNS) and the enteric nervous system (293). Therefore, gene-targeting experiments do not substantiate the existence of GABA<sub>B</sub> subtypes in the periphery, claimed by studies in which different rank orders of GABA<sub>B</sub> agonist affinities were reported (see sect. IV E). GABA<sub>B(2)</sub>-deficient mice should further clarify whether molecular subtypes of GABA<sub>B</sub> receptors exist in vivo, and whether GABA<sub>B(1)</sub> can participate in GABA<sub>B</sub> receptors in the absence of GABA<sub>B(2)</sub>.

Theoretically, it remains possible that additional GABA<sub>B</sub> subunits exist, which could explain the discrepancy between the historically diverse receptor pharmacology in vivo on the one hand, and the lack of pharmacologically distinct cloned receptors on the other hand. However, with most of the human genome sequence available, and despite extensive data-mining efforts, no GABA<sub>B</sub> genes other than GABA<sub>B(1)</sub> and GABA<sub>B(2)</sub> were identified. All GABA<sub>B</sub>-related proteins that were isolated do not form functional GABA<sub>B</sub> receptors when expressed together with GABA<sub>B(1)</sub> and/or GABA<sub>B(2)</sub> (47, 50, 54, 69, 72, 281). Altogether, this indicates that the likelihood of identifying additional GABA<sub>B</sub> receptor genes in the future is small.

2. GHB activity at GABA<sub>B</sub> receptors

GHB is a metabolite of GABA that is present at micromolar concentration in the brain (24). An abundance of high-affinity [H]<sub>3</sub>GHB binding sites is present in the cortex and hippocampus, but the physiological role of endogenous GHB is unclear. Behavioral effects are usually only observed when endogenous GHB levels increase above physiological levels, either due to a genetic disease
or to exogenous administration of GHB. Patients suffering from GHB aciduria, a congenital enzyme defect causing such a GHB accumulation, exhibit psychomotor retardation, delayed or absent speech, hypotonia, ataxia, hyporeflexia, seizures, and EEG abnormalities. When administered exogenously, acute increases in GHB induce a large spectrum of behavioral responses. Since 1990, GHB has been more and more abused for its euphoric, sedative, and anabolic (body-building) effects (241). In the past GHB was clinically used as an anesthetic, while in recent times it is shown to normalize sleep patterns in narcoleptic patients (241, 329). Preliminary preclinical and clinical data suggest that GHB is also useful in the therapy of alcoholism, nicotine, and opiate dependency (108), similar to baclofen (see sect. ivA).

Some actions of GHB clearly involve GABA B receptors (162, 109, 279). GHB binds to native and recombinant GABA B receptors, however with significantly lower affinity than to its cognate high-affinity [3H]GHB binding sites in the brain (198). Therefore, GHB is only expected to activate GABA B receptors under abuse conditions, where high concentrations of GHB are reached. If endogenous GHB is involved in active signaling, high-affinity GHB receptors, likely related to brain [3H]GHB binding sites, are expected to mediate these effects. The availability of GABA B-deficient mice provided the opportunity to study GHB effects in the absence of coincident GABA B effects (168, 274). After GHB application, GABA B(1)/- mice showed neither the typical hypolocomotion, hypothermia, increase in striatal dopamine synthesis, nor EEG delta-wave induction seen in wild-type mice. This indicates that these GHB effects are all mediated by GABA B receptors. Autoradiography reveals a similar spatial distribution of [3H]GHB binding sites in brains of GABA B(1)/- and wild-type mice, demonstrating that GABA B subunits are not part of high-affinity [3H]GHB binding sites. Millimolar concentrations of GHB induce small GTPγS responses in brain membrane preparations from wild-type, but not from GABA B(1)/- mice. The GTPγS responses in wild-type mice are blocked by the GABA B antagonist CGP54626, but not by the GHB antagonist NCS-382. This, together with additional data (63), suggests that the GHB-induced GTPγS responses are exclusively mediated by GABA B receptors and not by the high-affinity [3H]GHB binding sites, as proposed in earlier studies (277, 315).

Recently, the cloning of a putative GHB receptor from a rat hippocampal cDNA library was reported (6). However, several findings related to this putative GHB receptor require clarification. For example, the NH2-terminal part of the cloned GHB receptor identifies it as a member of the tetraspannin gene family, with four transmembrane regions and no significant similarity to GPCRs. Nevertheless, the cloned protein is reported to exhibit seven transmembrane regions and to activate G proteins. No additional transmembrane domains to achieve the typical GPCR topology are visible in the COOH-terminal part of the protein, which exhibits no significant homology to known proteins. All the more puzzling, the mRNA of the putative GHB receptor is particularly abundant in the cerebellum, where GHB binding sites are low or absent (24). Furthermore, the GHB receptor antagonist NCS-382 has no activity at the cloned GHB protein. In summary, these findings make it unlikely that the cloned protein corresponds to the high-affinity [3H]GHB binding sites in the brain.

3. Cellular and subcellular distribution

A) mRNA distribution. GABA B(1) and GABA B(2) transcripts are broadly expressed in the CNS of various vertebrate species (2, 22, 25, 32, 33, 75, 95, 163, 171, 194, 200, 231, 232, 307, 326, 356). In the rat brain, GABA B(1) mRNA is detectable in almost all neuronal cell populations, with highest levels of expression in hippocampus, thalamic nuclei, and cerebellum (Fig. 8). High levels of GABA B(2) mRNA are seen in the piriform cortex, hippocampus, and medial habenula. Moreover, GABA B(2) mRNA is abundant in all layers of the cortex, in the thalamus, and in cerebellar Purkinje cells (25, 95, 163, 170). In the in situ hybridization patterns of the GABA B(1) and GABA B(2) subunits are mostly overlapping (Fig. 8) and qualitatively parallel those of GABA A agonist and antagonist binding sites (33, 171, 326). From the subunit expression patterns it is obvious that heteromeric GABA B(2) receptors constitute the majority of native GABA B binding sites. This does not exclude that the subunits also form receptors independent of each other. Several studies tried to find evidence for this by looking for differences in the expression pattern of GABA B(1) and GABA B(2) transcripts. The most striking difference is seen in the striatum/caudate putamen region, where high GABA B(1) mRNA levels contrast low to nondetectable GABA B(2) mRNA levels (Fig. 8) (25, 75, 95, 170). However, immunohistochemical analysis shows that the GABA B(2) protein is significantly expressed in this structure (see sect. ivB3b). PCR approaches detect GABA B(1) mRNA, but not GABA B(2) mRNA, in most peripheral human tissues analyzed (56). The reason for this is unclear.

B) Receptor immunohistochemistry and autoradiography. Antibodies directed against COOH- and NH2-terminal sequences of GABA B(1) and GABA B(2) were produced by several laboratories and are available from a number of commercial sources. Additionally, GABA B(1) subunits can be traced using the photoaffinity cross-linker 125I-CGP71872 (169) or the radioligands [3H]CGP54626 (33) and [3H]CGP64239 (172, 326). The only radioligand that is currently commercially available is [3H]CGP54626.

In general, the immunohistochemical localization of GABA B(1) and GABA B(2) subunits (56, 71, 213, 313) correlates well with physiological and autoradiographic data.
on the distribution of native GABA_B receptors (170). The level of GABAB protein expression in the molecular layer of the cerebellum is not uniform (104, 213, 267). The pattern of intensely stained bands of Purkinje cells coincides with anatomical and functional compartments previously defined by zebrin, a brain-specific aldolase with no obvious link to GABAB receptors (104). It is thus conceivable that the regulatory elements governing the gene expression of zebrins may also entrain the expression of GABAB receptors. Where analyzed, the pattern of GABAB(1) protein expression matches well with that of GABAB(2). Significantly, electron microscopy confirmed a colocalization of GABA_B(1) and GABAB(2) protein at synaptic and extrasynaptic sites (104, 170, 184). In general, GABA_B(1) immunoreactivity appears to be abundant in the cytoplasm of neurons (313). It was therefore speculated that the GABA_B(2) protein is the limiting factor that determines the level of expression of the functional heteromer at the cell surface. Several studies suggested a mismatch of GABA_B(1) and GABA_B(2) mRNA expression in the basal ganglia (25, 75, 95, 170), but it appears that significant levels of GABA_B(2) protein are nevertheless present in this area (56, 71). A differential expression of GABA_B(1) and GABA_B(2) protein was observed in subpopulations of striatal neurons (240). However, a recent study comparing GABA_B(1) and GABA_B(2) protein expression in different brain regions found no evidence for a GABA_B(1) subunit expression in the absence of GABA_B(2) (71). In conclusion, it appears that most, possibly all, GABA_B receptors are of the heteromeric GABA_B(1,2) type. This is in good agreement with biochemical data (21) and GABA_B(1) knockout studies (206). In the neostriatum, GABA_B(2) and GAD67 do not often colocalize (240). Instead, GABA_B(1) protein was mostly detected on glutamatergic and dopaminergic terminals. GABA_B(2) is also expressed on glutamatergic and dopaminergic terminals in monkey brain (68). This localization implies that GABA_B receptors are activated by spillover GABA from adjacent synapses. Immunohistochemical data therefore question whether GABA_B(1) can fulfill autoreceptor functions. However, GABA_B(1) knockout studies clearly demonstrate that this subunit is necessary for regulating GABA release (272, 300). Most likely, GABA_B protein expression levels at GABAergic terminals are below the detection limit of immunohistochemical methods. In this context it is interesting to note that GABA_B receptors are mostly located extra- or perisynaptically (170, 184), a finding that is consistent with the observation that inhibitors of GABA uptake, which promote diffusion of GABA out of the synaptic cleft, greatly enhance IPSPs (155).

GABA_B(1a) and GABA_B(1b) proteins are differentially regulated during postnatal maturation (Fig. 4D) (104, 206). GABA_B(1a) expression is highest within the first postnatal days (P0–P5) and then decreases to adult levels. In contrast, immunoreactivity for GABA_B(1b) increases from P5 onwards and reaches a maximum at P10, followed by a gradual decline to adult levels. A similar expression profile was observed using photoaffinity labeling of GABA_B(1a) and GABA_B(1b), followed by gel electrophoresis and quantitative autoradiography (206). In the

FIG. 8. Comparison of the distribution of GABA_B(1) and GABA_B(2) subunit mRNA in the rat brain. Transcripts of GABA_B(1) and GABA_B(2), are coexpressed at similar levels throughout the brain, with a few exceptions. For example, GABA_B(2) transcripts are less abundant than GABA_B(1) transcripts in the caudate putamen (CP) and the olfactory bulb (OB).
adult rat brain GABA_{B(1b)} protein levels exceed GABA_{B(1a)} levels in most structures. Exceptions are the olfactory bulb and the striatum where higher levels of GABA_{B(1a)} than GABA_{B(1b)} protein are detected.

Two laboratories succeeded in obtaining antibodies that discriminate between the GABA_{B(1a)} and GABA_{B(1b)} isoforms (104, 184). Although these antibodies work well on immunoblots, the GABA_{B(1a)} antibodies are not suitable for use in fixed tissue. Nevertheless, a distinct and highly selective immunohistochemical distribution for each of the two isoforms could be inferred by subtractive analysis using GABA_{B(1b)} and pan GABA_{B(1)} antibodies (104). GABA_{B(1b)} is predominantly expressed in Purkinje cells and GABA_{B(1a)} mostly confined to granule cells (30, 104, 151, 267). This was taken to suggest a pre- versus postsynaptic localization of GABA_{B(1a)} versus GABA_{B(1b)}, respectively (32, 33). Conversely, GABA_{B(1a)} appears to be located postsynthetically on the cell bodies in thalamocortical circuits (271). In dorsal root ganglia the density of GABA_{B(1a)} immunoreactivity is much higher than that of GABA_{B(1b)} (267). This supports the association of GABA_{B(1a)} with presynaptic receptors in the primary afferent terminals. In biochemical experiments, however, GABA_{B(1a)} protein accumulated in the postsynaptic density fraction (21). While some additional studies suggested a differential pre- and postsynaptic function of the GABA_{B(1a)} and GABA_{B(1b)} isoforms (104, 194, 267), it emerges that they probably both contribute to pre- and postsynaptic functions (71). GABA_{B(1a)} receptors are also expressed on nonneuronal cells of the brain (70). Specifically, GABA_{B(1a)} subunits were found on astrocytic processes surrounding both symmetric and asymmetric synapses in the CA1 hippocampal region and on activated cultured microglia. In this context it is interesting to note that an activity-dependent potentiation of inhibitory synaptic transmission between hippocampal interneurons and CA1 pyramidal neurons was described (167). It was suggested that interneuronal firing elicits a GABA_{B(1a)} receptor-mediated elevation of Ca^{2+} in surrounding astrocytes, which in turn potentiates inhibitory transmission. Activation of GABA_{B(1a)} receptors on astrocytes may therefore be necessary for activity-dependent modulation of inhibitory synapses in the hippocampus.

C) GABA_{B(1)} RECEPTORS IN PERIPHERAL TISSUES: GABA_{B(1)} receptors are also expressed in many peripheral tissues. Using reverse transcriptase-PCR analysis, GABA_{B(1)} mRNA expression was detectable in all peripheral organs examined, including heart, spleen, lung, liver, small intestine, large intestine, kidney, stomach, adrenal, testis, ovary, and urinary bladder (64, 156, 301, 339). This supports a physiological role for GABA_{B(1)} receptors in the control of a wide variety of peripheral organs. GABA_{B(1)} protein expression in peripheral tissues was confirmed using 125I-CGP71872 photoaffinity labeling (19). These experiments showed that GABA_{B(1a)} and GABA_{B(1b)} are coexpressed in stomach and testis. In the same study GABA_{B(1b)} was selectively detected in kidney and liver, while GABA_{B(1a)} was expressed in the adrenal gland, pituitary, spleen, and prostate. Immunoblot analysis demonstrated the presence of the GABA_{B(1)} protein, but not the GABA_{B(2)} protein, in uterus and spleen (56). Surprisingly, an earlier Northern blot analysis revealed no evidence for significant GABA_{B(1)} mRNA expression in the spleen (169). Studies with GABA_{B(1)}−/− mice confirmed that, as in the CNS, the GABA_{B(1)} subunit is an essential requirement for GABA_{B(1)} function in the enteric nervous system and the PNS (293). Specifically, it was shown that the effects of baclofen in both the ileum and urinary bladder were absent in GABA_{B(1)}−/− mice.

Peripheral expression of GABA_{B(1)} subunits is not restricted to cells of neuronal origin. As in neurons, both GHB (see sect. III.B2) and baclofen activate an inwardly rectifying K+ channel in rat cardiomyocytes (199). Expression of GABA_{B(1a)} and GABA_{B(1b)} in cardiomyocytes was suggested by immunoblotting and in comcomparative studies. In rat ventricular myocytes, the two subunits are expressed in the sarcolemma and along the transverse tubular system. GABA_{B(1a)} and GABA_{B(1b)} mRNA were detected in rat testis and sperm (136). GABA_{B(1a)} protein localizes to the head of sperm cells, as shown in immunofluorescence experiments. It was speculated that GABA_{B(1)} receptors play a role in fertilization, e.g., in the induction of the acrosome reaction by GABA. However, male Balb/c GABA_{B(1)}−/− mice are fertile (B. Bettler and M. Gassmann, unpublished observation), casting doubt on this proposal.

IV. IMPLICATION IN DISEASE

Baclofen, the only GABA_{B(1)} drug on the market, is prescribed as an antispastic agent and muscle relaxant for the treatment of patients suffering from multiple sclerosis as well as hemi- or tetraplegia. For severe cases of spasticity, baclofen is administered intrathecally. This is also the route of administration for stiff-man syndrome patients that suffer from rigidity of skeletal muscles. Baclofen is further used in the treatment of spasticity due to traumatic/hypoxic brain injury, dystonia, and in cerebral palsy movement disorders. Therapy with baclofen efficiently alleviates the rigidity associated with Lewy-body dementia and the spasms following tetanus infections. It is generally assumed that baclofen exerts its therapeutic effects by inhibiting the release of excitatory neurotransmitters and neuropeptides in the spinal cord.

Over the years, a number of clinical observations suggested that GABA_{B(1)} compounds might offer the means to ease the symptoms of drug withdrawal (see sect. IV.A) and to treat neuropathic pain (see sect. IV.D). Furthermore, GABA_{B(1)} compounds are of therapeutic interest for...
the treatment of intestinal (see sect. ivC) and pulmonary disorders (67, 94), bladder dysfunctions (257), epilepsy (see sect. ivB), and memory loss (227). Indeed, mice with a disruption in the GABA_B(1) gene support that GABA_B drugs could be used to manage pain, strengthen memory, and treat epilepsy (272, 300). Moreover, GABA_B(1)−/− mice pointed at indications that were not necessarily linked to the GABA_B system. For example, GABA_B(1)−/− mice exhibit a hyperactive phenotype reminiscent of dopamine-transporter DAT knockout mice that are similarly aroused by novelty and respond with hyperlocomotion to a new environment (300, 316). It is commonly assumed that hyperactive behaviors are related to a hyperdopaminergic state. It is therefore conceivable that the loss of GABA_B control over dopamine release triggers the behavioral abnormalities in GABA_B(1)−/− mice. A GABA_B brake on dopamine release could possibly be exploited to attenuate motor problems of Parkinson’s disease or attention-deficit hyperactivity-disorder (ADHD) patients. Furthermore, GABA_B(1)−/− mice show enhanced prepulse inhibition compared with littermates, suggesting that GABA_B(1) knockout mice exhibit sensorimotor gating abnormalities (272). Compounds interfering with GABA_B receptors may therefore be beneficial in schizophrenia. Below we review some of the most promising indications for GABA_B drugs.

A. Addiction

There is now good evidence, for the most part both in humans and animals, that GABA_B agonists can reduce the craving for drugs such as cocaine, heroin, alcohol, and nicotine (79). Preliminary clinical studies with cocaine-abusing patients reported a reduced craving for cocaine after baclofen administration (51, 197). GABA_B agonists are also effective in clinical studies of alcohol abuse (3). Regardless of these highly promising findings, the pharmaceutical industry is reluctant to enter the drug-abuse market. One reason is that there are no GABA_B agonists with lasting patent protection available. Another reason is that clinical trials are considered highly problematic and risky because of multidrug abusers, dropouts, and possible lawsuits.

Therapeutic effects of baclofen in humans are supported by experiments with animals. A number of studies showed that baclofen suppresses intravenous cocaine self-administration in rodents at low doses of injected cocaine, but not at high doses (>1.5 mg/kg) (52, 58, 282, 310). Baclofen is well-known to cause motor side-effects and to reduce locomotion (252). It is therefore important to rule out that the therapeutic actions of baclofen on drug self-administration are mediated by these side effects. To address this question the effects of GABA_B agonists on cocaine- and food-reinforced responses were compared. Baclofen and CGP44532, a high-affinity GABA_B agonist, are much more effective on cocaine-reinforced than on food-reinforced responding, thus validating the therapeutic concept (52, 282, 310). In rats, withdrawal from repeated cocaine treatment produces an increase in the basal levels of extracellular GABA in the accumbens (350). The increase may be mediated by GABA_B autoreceptor desensitization, likely the result of diminished S892 phosphorylation of the GABA_B(2) subunit (see sect. iiA10). Baclofen is reported to reduce self-administration of heroin (351). Rats that received a low dose of baclofen (0.5 mg/kg) resumed self-administration of heroin when a 5-day treatment with baclofen was followed by 2 baclofen-free days. This is in contrast to rats that received a high dose of baclofen (1 mg/kg), which did not resume drug administration. This suggests that high and low doses of baclofen activate distinct cellular mechanisms. Low doses of baclofen (3 mg/kg) reduce alcohol intake in alcohol-prefering Long-Evans rats (86). A reduction of alcohol intake after baclofen treatment was also seen in Wistar rats (77). However, Smith et al. (314), using Long-Evans rats and 5 days of baclofen treatment before the start of the dark cycle, reported that baclofen reduced alcohol intake only during the first hour and increased intake over the next 23 h (314). Possible motor impairing and sedative effects of baclofen on alcohol and heroin self-administration were addressed. As shown in the experiments with cocaine, baclofen did not impair food intake, and it reduced alcohol intake if an ethanol versus water choice was given to the animals (77, 86, 314).

Other GABAergic compounds reproduce the therapeutic effects of baclofen on drugs of abuse. γ-Vinyl-GABA inhibits the GABA transaminase and thereby elevates GABA levels. Like baclofen, γ-vinyl-GABA reduces the reinforcing properties of cocaine, heroin, and ethanol in animals, at doses that do not decrease locomotion (178, 338, 351). γ-Vinyl-GABA also reduces the acquisition and expression of condition place preference by cocaine and nicotine (92, 93). It further alleviates the effects of cocaine in an electrical brain stimulation paradigm (187). GHB, an endogenous metabolite of GABA (24), shows promising effects in situations involving opiates withdrawal, alcohol, and nicotine dependence (108). GHB is also shown to decrease cocaine self-administration in rats (215). The effects of high doses of GHB are almost certainly mediated by GABA_B receptors (see sect. iiB2). More recently, GHB has become a drug of abuse itself (241). Studies are therefore needed to address whether GABA_B agonists reduce craving and withdrawal symptoms, or whether they just mimic the effects of the abused drug.

There is evidence that GABA_B agonists attenuate the reinforcing effects of abused drugs by influencing the mesolimbic dopamine system (93, 282). Drugs of abuse increase extracellular dopamine levels in the accumbens, a brain region that is believed to be involved in the reward
and reinforcement circuitry (346, 347). Activation of GABA<sub>B</sub> receptors in the accumbens reduces firing of dopaminergic cells and inhibits the release of dopamine (99). Similarly, γ-vinyl-GABA reduces the release of dopamine in the neostriatum and accumbens after cocaine and nicotine administration (92, 93). The GABA<sub>B</sub> antagonist SCH5091 blocks this effect, suggesting that γ-vinyl-GABA acts through GABA<sub>B</sub> receptors. Altogether, it appears that GABA<sub>B</sub> activity blocks the increase in dopamine release that is otherwise induced by drugs of abuse (351). Accordingly, rats reduce self-administration of cocaine after GABA<sub>B</sub> antagonists (351).

B. Epilepsy

GABA<sub>B</sub> receptors have repeatedly been implicated into the etiology of epilepsies. However, the first genetic link between GABA<sub>B</sub> receptors and human epilepsy was only provided very recently (113). It appears that a GABA<sub>B(1)</sub> polymorphism not only confers a highly increased susceptibility to temporal lobe epilepsy but also influences the severity of the disease.

GABA<sub>B</sub> antagonists suppress the absence seizures seen in the lh/lh lethargic mice (147) and the genetic absence-epilepsy rats from Strasbourg (GAERS) (209), while agonists exacerbate the seizures. Apparently, blocking of thalamic GABA<sub>B</sub> receptors reverses an excess of inhibition that is the cause of absence seizures (83, 305). It was proposed that GABA<sub>B</sub>-mediated IPSPs have a “priming” function towards the generation of low-threshold Ca<sup>2+</sup> potentials, thereby facilitating burst-firing of the type observed in absence epilepsy (83). The primary gene defect in lh/lh mice is a mutation in the Ca<sup>2+</sup> channel β-subunit, which affects the interaction with the Gβγ subunits of the activated G protein. The phenotype of lh/lh mice involves an upregulation of GABA<sub>B</sub> binding sites, which is expected to facilitate absence seizure development. Paradoxically, the overt phenotype of GABA<sub>B</sub> receptor-deficient mice includes spontaneous seizures, including sporadic absence-type seizures (272, 300). However, the absence-type seizures seen in the GABA<sub>B(1)</sub>-/- mice are not directly comparable to the “typical” absence seizures observed in the GAERS. The seizures in the GAERS are characterized by frequent and short EEG bursts, while the ones seen in the GABA<sub>B(1)</sub>-/- mice are rare, of much longer duration, and indicative of “atypical” absence seizures. A lack of GABA<sub>B</sub> signaling may also underlie the clonic-type seizures in the weaver mouse (126). The weaver mouse exhibits a mutation in the Kir3.2 K<sup>+</sup> channel gene, which leads to a postsynaptic loss of GABA<sub>B</sub> inhibition and to seizure development (201). Kainic acid-induced seizures in rats are characterized by a downregulation of GABA<sub>B</sub> receptor subunits (106, 127). In contrast, after induction of generalized seizures in rats with electroshock, an upregulation of the GABA<sub>B(1b)</sub> mRNA, but not of the GABA<sub>B(1a)</sub> mRNA, was observed (31). This suggests a selective involvement of GABA<sub>B</sub> splice variants in the regulation of electroshock-induced seizure activity.

To date, the data indicate that GABA<sub>B</sub> compounds could be most useful in the pharmacotherapy of absence seizures in children. The finding, however, that high doses of certain GABA<sub>B</sub> antagonists induce convulsions in rats renders clinical trials problematic (332). Clearly a wide therapeutic window will be a critical requirement when entering clinical trials with GABA<sub>B</sub> antagonists.

C. Gastrointestinal Disease

From a medicinal chemistry point of view it is often easier to develop peripheral drugs than central drugs. Peripheral GABA<sub>B</sub> drugs are expected to be largely devoid of the known side effects that are mostly of central origin and may well be superior to current therapies. The cloned GABA<sub>B</sub> subunits are expressed in the periphery (see sect. mB3c) and involved in the regulation of organs such as, e.g., bladder and ileum (203). GABA<sub>B</sub> receptors were also described on vagal-afferent terminals in the medullary brain stem region, which is important in the integration of sensory information from the stomach and the pharynx. Baclofen reduces the rate of transient lower esophageal sphincter relaxations and the number of reflex episodes, in humans and animals, which attracted a great deal of attention (34, 60, 146, 181, 193, 196, 318). GABA<sub>B</sub> compounds may therefore open new possibilities in the treatment of disorders such as gastroesophageal reflux disease (60, 196).

D. Nociception

Baclofen exerted antinociceptive effects in clinical trials involving trigeminal, glossopharyngeal, vagoglossopharyngeal and ophtalmic-postherpetic neuralgias, diabetic neuropathy, and migraine (44, 105, 139, 312). Although baclofen is used clinically to treat neuropathic pain and, when administered intrathecally, to attenuate pain associated with spinal injury (141) or stroke (319), its use as a general analgesic is limited because of its sedative properties and the rapid development of tolerance to its pain-relieving activity. Baclofen also exhibited antinociceptive properties in rodent models of acute pain, such as the tail-flick, acetic acid writhing, Formalin, and hot-plate tests (13, 273). Moreover, baclofen showed antinociceptive and antiallodynic actions in chronic pain models in rats (84, 344). Baclofen likely exerts its effects both in the brain (154, 160) and the spinal cord (204). One of the few cortical...
areas consistently activated by painful stimuli is the rostral agranular insular cortex (RAIC) where GABA robustly inhibits neuronal activity (160). Selective activation of GABA$_B$ receptor-bearing RAIC neurons produces hyperalgesia through projections to the amygdala, an area involved in pain and fear. In the dorsal horn of the spinal cord, baclofen inhibits the release of glutamate and substance P from both large and small fibers (255). This explains why intrathecal application of baclofen relieves central pain in patients with spinal lesions (141) or after cerebral strokes (319). Acute pain tests with GABA$_B$ receptor-deficient mice support that GABA$_B$ receptors participate in nociceptive pathways (300). GABA$_B^{−/−}$ mice exhibit pronounced hyperalgesia to noxious heat in the hot-plate and tail-flick test as well as reduced paw-withdrawal thresholds to mechanical pressure. Changes in the noxious thermal and mechanical threshold suggest that there is a loss of intrinsic GABA$_B$ tone in the nociceptive system of GABA$_B^{−/−}$ mice. The lack of GABA$_B$ receptors most probably results in an increased central hyperexcitability of the spinal nociceptive pathways.

E. Genetic Linkage Studies

The GABA$_{B1}$ and GABA$_{B2}$ genes map to human chromosomes 6p21.3 and 9q22, respectively (123, 238). Given the potential implication of GABA$_B$ receptors in the etiology of epilepsies (see sect. vB), a number of studies addressed a direct involvement of the GABA$_B^{R1}$ gene. The result of a recent study indicates that a GABA$_B^{R1}$ polymorphism, G1465A, indeed confers a highly increased susceptibility to temporal lobe epilepsy (113). This polymorphism also influences the severity of this common epileptic disease. This agrees with earlier studies that proposed a dysfunctional GABA$_B$ system as one of the causes of temporal lobe epilepsy (127, 298, 348). A possible involvement of GABA$_B^{R1}$ receptors in idiopathic generalized epilepsies was investigated in linkage and association studies (259, 292). Yet another study explored a link to childhood absence epilepsy (283). However, these latter studies provided no evidence for an involvement of GABA$_B^{R1}$ polymorphisms in these two other forms of epilepsy. Similarly, Anaya et al. (5) found no linkage between the GABA$_B^{R1}$ gene and epilepsy in Caucasian patients. Other linkage studies addressed a potential involvement of the GABA$_B^{R1}$ gene in conditions of alcohol dependence (292) and in panic disorders (291). However, GABA$_B^{R1}$ polymorphisms do not account for the genetic variance of alcohol dependence, nor is there an indication for increased vulnerability to panic disorders. The GABA$_B^{R2}$ gene maps in the vicinity to the locus for hereditary sensory neuropathy type-1 (HSN-1) (238). A possible involvement of GABA$_B^{R2}$ in the etiology of HSN-1 has not yet been investigated.

V. NOVEL GABA$_B$ COMPOUNDS

A. Allosteric Modulators

The side effects of baclofen, principally sedation, tolerance, and motor impairment, limit its utility for the treatment of many diseases. Novel GABA$_B$ drugs that largely lack these components are therefore much sought after. In the absence of pharmacological subtypes, alternative strategies for achieving selectivity must be considered. For instance, positive allosteric modulators may provide a means to dissociate the unwanted side effects seen with baclofen. Allosteric modulators discriminate between activated and nonactivated receptor states. They will enhance the endogenous activity of GABA, in contrast to agonists that will activate every GABA$_B$ receptor they reach, independently of synaptic activity. To be effective, allosteric GABA$_B$ drugs therefore rely on receptor activity stimulated by endogenous GABA. The demonstration of an intrinsic brake on memory impairment, locomotion, bladder activity, and nociception in GABA$_B^{R1}$ mice (293, 300) is therefore of great importance (see sect. mBJ). This suggests that under physiological conditions disease-relevant neuronal systems are under temporary or tonic control of GABA$_B$ receptors and that a treatment with allosteric modulators is possible.

Using the cloned GABA$_B$ receptors, researchers in several laboratories developed functional assay systems. For instance, the activation of G proteins by GABA$_B^{R1}$ can be measured using the GTP$^\gamma$S binding assay (330). Alternatively mobilization of intracellular Ca$^{2+}$ can be detected in cell lines expressing GABA$_B^{R1}$ in combination with chimeric G proteins (250). Functional assay systems permitted the identification of the first synthetic allosteric GABA$_B$ compounds (330, 331). CGP7930, CGP13501, GS39783, and related allosteric compounds are structurally distinct from GABA$_B$ agonists and markedly enhance agonist-stimulated responses at GABA$_B$ receptors (Fig. 9). Notably, these allosteric compounds have little or no intrinsic activity and do not directly activate GABA$_B$ receptors. Where analyzed, GABA concentration-response curves in the presence of fixed concentrations of the allosteric modulator indicate an increase of both the potency and the maximum efficacy of GABA at GABA$_B$ receptors. The published allosteric compounds do not discriminate between GABA$_B^{R1a,2}$ and GABA$_B^{R1b,2}$, the two predominant GABA$_B$ receptor populations in the nervous system (see sect. mA4A). The binding sites for allosteric modulators are unknown, but all compounds require the presence of GABA$_B^{R2}$ to exert their allosteric effect. Possibly, the compounds bind to the GABA$_B^{R2}$ subunit itself or to the GABA$_B^{R1a}$/GABA$_B^{R2}$ interface. All compounds are hydrophobic, and it is conceivable that they interact with the transmembrane do-
mains, similar to allosteric modulators at mGlu receptors (see below).

Recently, Kerr et al. (175) described the arylalkylamines as a different class of positive allosteric GABA\(_B\) modulators. Fendiline, prenylamine, and F551 potentiated hyperpolarizing responses to baclofen in rat cortical slices. Surprisingly, fendiline and F551 were inactive at GABAB receptors located on inhibitory terminals. Given that potentiation of ligand responses in acute slices can be mediated by a variety of mechanisms, including G protein cross-talk, second messenger modulation, or intercellular signaling, the interpretation of the experiments with arylalkylamines is difficult. Because there is currently no known molecular basis for a distinction of GABAB receptors on different terminals, these findings clearly need to be confirmed and extended to include studies with recombinant GABAB receptors. Phenylalkylamines, such as NPS467 and NPS568, are structurally related to arylalkylamines and allosterically modulate the Ca\(_\text{2+}\) receptors by binding to the hydrophobic transmembrane domains (128). It is therefore assumed that the arylalkylamines bind to the transmembrane domains of GABAB receptors as well. Positive allosteric compounds were recently also obtained for an additional member of the Family 3 GPCRs, the mGlu1 receptors (180). The residues that are critical for allosteric modulation of mGlu1 are again located in the transmembrane domains. It is likely that positive allosteric modulators act by stabilizing the active state of the transmembrane domains.

Positive allosteric modulation is not the sole possibility to fine-tune GABA\(_B\) receptors. High-throughput screening using functional read-outs allows identification of noncompetitive GABA\(_B\) antagonists as well, as, e.g., shown for the mGlu receptors (114). Noncompetitive mGlu antagonists, such as CPCCOEt, MPEP, and BAY96–7620, decrease the maximal effect of glutamate without changing its affinity, clearly indicating that they interact at a site other than the glutamate binding site. Similar to positive allosteric modulators, noncompetitive mGlu receptor antagonists interact with the transmembrane domains (62, 205, 251). It is to be hoped that in the near future noncompetitive GABA\(_B\) antagonists, based on novel chemical structures, will be identified as well.

B. Subtype-Selective Ligands

To date the only prominent molecular distinction in the GABA\(_B\) system is based on the two splice variants GABA\(_B\)(1a) and GABA\(_B\)(1b) (see sect. m(4A)). Essentially, these two variants represent the only means for directing the search for novel GABA\(_B\) drugs toward molecularly distinct receptor populations. The differential expression patterns of GABA\(_B\)(1a) and GABA\(_B\)(1b) point to a functional heterogeneity, but it is unknown which physiological effects relate to which isoform. Most importantly, no conclusion can be drawn regarding their specific contributions to muscle-relaxant effects and tonic brakes on locomotion, hyperalgesia, memory impairment, and bladder activity. Likewise, it remains unclear which splice variants are involved in pre- and postsynaptic functions (21, 32, 68, 71, 104, 267, 271).

Appreciating the unique roles played by GABA\(_B\)(1a) and GABA\(_B\)(1b) is essential to fully exploit GABA\(_B\) receptors for therapeutic uses. It was argued that the anticonvulsant, antihyperalgesic, and anxiolytic drug gabapentin is able to distinguish the two variants (26, 237). In particular, gabapentin was claimed to be active at GABA\(_B\)(1a,2) but not at GABA\(_B\)(1b,2) receptors. However, in most people’s hands gabapentin has no activity at GABA\(_B\) receptors (161, 190, 317). The reason for this discrepancy is unclear. Given the lack of truly selective ligands, it will probably be necessary to take a genetic approach to dissociate in vivo functions of GABA\(_B\)(1a,2) and GABA\(_B\)(1b,2) receptors. Mice with selective ablations of the GABA\(_B\)(1a) or GABA\(_B\)(1b) subunits should allow exposing the influence of splice variants on the manifestation of physiological and behavioral traits. Such mice will also help to evaluate whether compounds specifically targeted to GABA\(_B\)(1a,2) or GABA\(_B\)(1b,2) are likely to have distinct enough effects to warrant drug discovery efforts.
VI. SUMMARY AND OUTLOOK

Recent years have seen rapid advances in our understanding of GABA_B receptors. This has not only significantly advanced the GABA_B field but also fundamentally changed our view of the structure and signaling of GPCRs in general. Research on GABA_B receptors very much contributed to the now widely accepted idea that GPCRs form homo- or heterodimers. Although no consensus exists on the role of GPCR dimerization, structural studies on rhodopsin suggest that GPCRs necessarily need to dimerize to bind the G protein (195). In addition to GABA_B receptors, there are now several examples of GPCRs where heteromerization changes pharmacological and signaling properties (7, 43). Because GPCRs represent major drug targets and are the single largest family of orphan GPCRs, efforts to identify the expected variety of GABA_B subunits that function in combination with a distinct subunit. Searches in the human genome databases failed to identify the expected variety of GABA_B subunits that would readily explain the heterogeneity described for native receptors. While the existence of as yet unidentified splice variants and alternative quaternary assemblies is never totally ruled out as a source of receptor heterogeneity, it is now generally established that the pharmacological diversity proposed before receptor cloning can no longer be maintained. To date, no GABA_B compound unequivocally differentiates molecular variants of GABA_B receptors. Reported differences in the potency of agonists probably relate to differences in receptor reserve or in differences in the various downstream effectors that were analyzed. Additionally, the lipid environment or receptor modifications may wrongly suggest subtypes. As a consequence of the lack of heterogeneity, the possibilities for selective interference with the GABA_B system are now limited. Essentially there are only two GABA_B receptor populations, GABA_B(1a,2) and GABA_B(1b,2), which are abundant in vivo. However, it is still unknown whether these two receptors exhibit functional differences, for example, by localizing to pre- versus postsynaptic sites, or to inhibitory versus excitatory terminals. The search for GABA_B(1a) selective compounds will almost certainly necessitate the identification of protein(s) that interact selectively with one of the isoforms.

Despite the obvious lack of pharmacological subtypes, the availability of functional assays based on recombinant receptors has led to the discovery of novel GABA_B compounds. Notably the repertoire of GABA_B compounds was expanded to include positive allosteric modulators (175, 263, 330, 331). Chances are that positive allosteric compounds will be therapeutically effective, since a basal GABA_B activity is present in many disease-relevant neuronal pathways (272, 300). Functional high-throughput screens may allow the identification of non-competitive antagonists as well. Here the hope is that new antagonists with distinct chemical structures will have altered pharmacokinetic/distribution profiles that help to separate clinical efficacy from side effects. A big help for drug discovery efforts is the availability of GABA_B knockout mice, which allow optimizing receptor selectivity of novel GABA_B compounds.

Partial agonists that activate sensitized receptors at lower doses than inactive receptors mediating the side effects would represent another possibility for more selective interference with the GABA_B system. However, no laboratory reported the identification of new partial or full agonists (or competitive antagonists) in recombinant high-throughput screens. This is probably a consequence of the fact that only GABA_B(1) contains a GABA binding site. Compounds that are selective for the GABA_B site of GABA_B(1) were identified a long time ago, using radioligand antagonist displacement in rat brain membranes. A functional screening assay offers no particular advantages as opposed to previous binding assays for identifying competitive ligands. For these reasons we do not expect that many novel structures for competitive ligands will be reported as a consequence of functional high-throughput screens.

We thank Dr. J.-P. Pin for the contribution of figures and the anonymous reviewers for their suggestions.

B. Bettler is supported by the Swiss Science Foundation Grant 3100–067100.01. Address for reprint requests and other correspondence: B. Bettler, Pharmazentrum, Dept. of Clinical-Biological Sciences, Institute of Physiology, Univ. of Basel, Klingelbergstr. 50, CH-4056 Basel, Switzerland (E-mail: bernhard.bettler@unibas.ch).

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