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Review

The metabotropic GABA receptor: molecular insights and their functional consequences

S. Blein[a], E. Hawrot[b]* and P. Barlow[a]

[a]Edinburgh Centre for Protein Technology, Joseph Black Chemistry Building, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ (United Kingdom)
[b]Department of Molecular Pharmacology, Physiology and Biotechnology, Brown University, Providence (Rhode Island 02912, USA), Fax +1 401 863 1595, e-mail: Edward_Hawrot@brown.edu

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Abstract. Recent years have seen rapid and significant advances in our understanding of the G-protein-coupled γ-amino butyric acid, B-type ($GABA_B$) receptor, which could be a therapeutic target in conditions as diverse as epilepsy and hypertension. This progress originated with the ground-breaking work of Bernhard Bettler's team at Novartis who cloned the DNA encoding a $GABA_B$ receptor in 1997. Currently, the receptor is thought to be an unusual, possibly unique, example of a heterodimer composed of homologous, seven-transmembrane-domain (7TMD) subunits (named $GABA_B$ R1 and $GABA_B$ R2), neither of which is fully functional when expressed alone. The large N-terminal domain of the $GABA_B$ R1 subunit projects extracellularly and contains a ligand binding site. The similarity of the amino acid sequence of this region to some bacterial periplasmic amino acid-binding proteins of known structure has enabled structural and functional modelling of the N-terminal domain, and the identification of residues whose substitution modulates agonist/antagonist binding affinities. The intracellular C-terminal domains of the R1 and R2 subunits appear to constitute an important means of contact between the two subunits. Alternative splice variants, a common and functionally important feature of 7TMD proteins, have been demonstrated for the R1 subunit. Notably $GABA_B$ R1a differs from $GABA_B$ R1b by the possession of an N-terminal extension containing two complement protein modules (also called SCRs, or sushi domains) of unknown function. The levels at which each of the respective variants is expressed are not equal to one another, with variations occurring over the course of development and throughout the central nervous system. It is not yet clear, however, whether one variant is predominantly presynaptically located and the other postsynaptically located. The existence of as yet unidentified splice variants, additional receptor subtypes and alternative quaternary composition has not been ruled out as a source of receptor heterogeneity.

Key words. $GABA_B$ receptor; G-protein-coupled receptor; seven-transmembrane-domain receptor; short consensus repeats; sushi domains; drug target.

Introduction

γ-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in mammalian brain. There are two distinct categories of target receptors for GABA, each of which mediates synaptic inhibition: the ionotropic GABA types A and C ($GABA_{A/C}$) receptors; and the metabotropic GABA type B ($GABA_B$) receptors.

Upon activation by two molecules of GABA, the pentameric $GABA_A$ receptor induces a rapid inhibition of

* Corresponding author.

neuronal electrical activity. This comes about because $GABA_A$ is an ion channel which opens upon appropriate ligation, allowing the inward flux of Cl^- and a subsequent hyperpolarisation of the postsynaptic membrane [1]. In contrast, activation of postsynaptic $GABA_B$ receptors produces a slower, more prolonged inhibition [2]. Presynaptic $GABA_B$ receptors also exist, and these may act as autoreceptors by suppressing GABA release from GABA-ergic nerve terminals, or they may act as heteroreceptors by blocking the release of other neurotransmitters, including L-glutamate (the main excitatory neurotransmitter), from the appropriate nerve terminals [3]. These effects are believed to be mediated by coupling of $GABA_B$ to the heterotrimeric guanine-nucleotide binding (G) proteins, G_i and G_o. $GABA_B$ receptors are thus able to modulate adenylyl cyclase activity, and to cause inwardly rectifying K^+ channels to open and voltage-dependent Ca^{2+} channels to close [4, 5].

$GABA_B$ autoreceptors in the hippocampus regulate the induction of the long-term 'potentiation' (LTP) of synaptic transmission [6]. The phenomenon of LTP represents an important model system for studies of associative enhancement of synaptic strength, as it is thought to underlie cognitive processes such as learning and memory. Other physiological actions have also been attributed to the stimulation of $GABA_B$ receptors, including analgesia, modulation of respiratory activity and muscle relaxation [2]. $GABA_B$ receptors are thought to be potential therapeutic targets in a range of conditions. Lioresal, a racemic mixture of baclofen, is a receptor agonist used clinically in the treatment of spasticity arising from multiple sclerosis or spinal injury [7]. Other possible clinical applications of $GABA_B$ agonists/antagonists include epilepsy [2, 8], drug withdrawal [9, 10], anxiety, depression, hypertension and cognitive dysfunction [4, 11].

The $GABA_B$ receptor was first classified and pharmacologically characterised by Hill and Bowery in 1981 [12]. Much additional pharmacological and biochemical information was gleaned over the subsequent decade and a half. Despite this, the cloning of a gene encoding a $GABA_B$ receptor proved very difficult. The much-awaited breakthrough came in 1997 with the first report of the successful cloning of complementary DNA (cDNA) encoding a $GABA_B$ receptor subunit ($GABA_B$ receptor type 1, $GABA_B$ R1). This work, carried out by a team led by Bernhard Bettler at Novartis in Basel [13], represents an important landmark. Significant progress in our understanding of the structure and function of the $GABA_B$ receptor has ensued rapidly, revealing several surprising and possibly unique aspects of the receptor. In the original report of the cloning work, two splice variants, named $GABA_B$ R1a and $GABA_B$ R1b, were identified. Subsequently, the gene encoding a second subunit of the receptor ($GABA_B$ R2) was cloned [14–20]. Both the R1a/b and R2 subunits are members of the family of G-protein-coupled receptors possessing seven transmembrane domains. Only when $GABA_B$ R1 (either R1a or R1b) and $GABA_B$ R2 are coexpressed is full activity obtained, and the $GABA_B$ receptor is probably a heterodimer composed of $GABA_B$ R1a/b and $GABA_B$ R2 subunits (fig. 1).

Despite these successes, the number of receptor genes cloned to date does not appear sufficient to explain the results of numerous studies obtained over the last 15 years which have suggested considerable heterogeneity of $GABA_B$ receptors [2, 21]. It is not unlikely that other splice variants, and perhaps even additional subunits, remain unidentified. The area is one of intense activity. We have used the opportunity afforded by this review to draw together relevant literature from 1997 through September 1999, and present a summary of these findings.

Cloning of the first subtype and its splice variants

The key to the first successful cloning of cDNA for a $GABA_B$ receptor was the development of a very high affinity ($K_d = 1$ nM), specific antagonist ([^{125}I]-CGP64213) [13]. Subsequent photoaffinity labelling revealed two candidate receptor glycoproteins with M_r's of 100 kDa and 130 kDa (90 kDa and 110 kDa, respectively after treatment with N-glycosidase) in the cortex, cerebellum and spinal cord of human, rat, mouse, chicken, frog and zebra fish. Expression cloning was then used to identify a 4.4-kb cDNA insert in a rat cortex and cerebellum library that had been transfected into COS-1 cells. The first gene to be identified encodes a 960-amino acid protein ($GABA_B$ R1a), including a 16-residue signal sequence and corresponds, after post-translational N-glycosylation, to the 130-kDa glycoprotein photoaffinity-labelled in native brain tissue. Subsequent low-stringency hybridization cloning using the $GABA_B$ R1a cDNA as a probe identified a 2.9-kb cDNA which encodes a protein corresponding to the 100-kDa glycoprotein ($GABA_B$ R1b), and which represents a splice variant of $GABA_B$ R1a. The sequence of the proteins is consistent with the presence of seven-transmembrane helices and indicates similarity with the metabotrobic glutamate receptors (mGlu R) [13] (fig. 2). Messenger RNA (mRNA) for both variants is abundant in brain, and transcripts for the receptor are found in all cerebral cortical areas, the pyramidal cell layers of the hippocampus, the granular cell layers of the dentate gyrus and basal ganglia. As confirmation that $GABA_B$ R1a/b indeed correspond to variants of a functional $GABA_B$ receptor, the cloned proteins, heterologously expressed in human embryonic kidney (HEK) 293 cells,

couple negatively to adenylyl cyclase. Further, the rank order of binding affinities for a series of agonists and antagonists is identical for the recombinant receptor and the rat cerebral $GABA_B$ receptor found in cerebral cortical membranes.

Classification of $GABA_B$ R1a and 1b, and sequence similarities

The primary sequence of $GABA_B$ R1, and analysis of its hydropathy plots, places it within the superfamily of G-protein-coupled receptors, all of which encompass seven putative transmembrane domains (7TMD). There are five classes of 7TMD receptors [22], each containing proteins that are related by sequence similarity, but there is no significant similarity between classes. Class A members are most numerous and related to rhodopsin, class B members are related to the vasoactive intestinal peptide and glucagon receptors, class C receptors are homologous to the metabotropic glutamate receptors (mGlu R) and include the Ca^{2+}-sensing receptor (CS R), class D contains the pheromone receptors and class E contains the adenosine 3′,5′-cyclic monophosphate (cAMP) receptors of *Dictyostelium* [22]. The sequence of $GABA_B$ R1a/b is 18–23% identical to the eight subtypes of mGlu R, and therefore it is grouped in class C [13]. The mGlu R subtypes are receptors for the major excitatory neurotransmitter [23], whereas CS R is involved in calcium homeostasis in humans and other mammals [24]. The similarity with mGlu R extends over

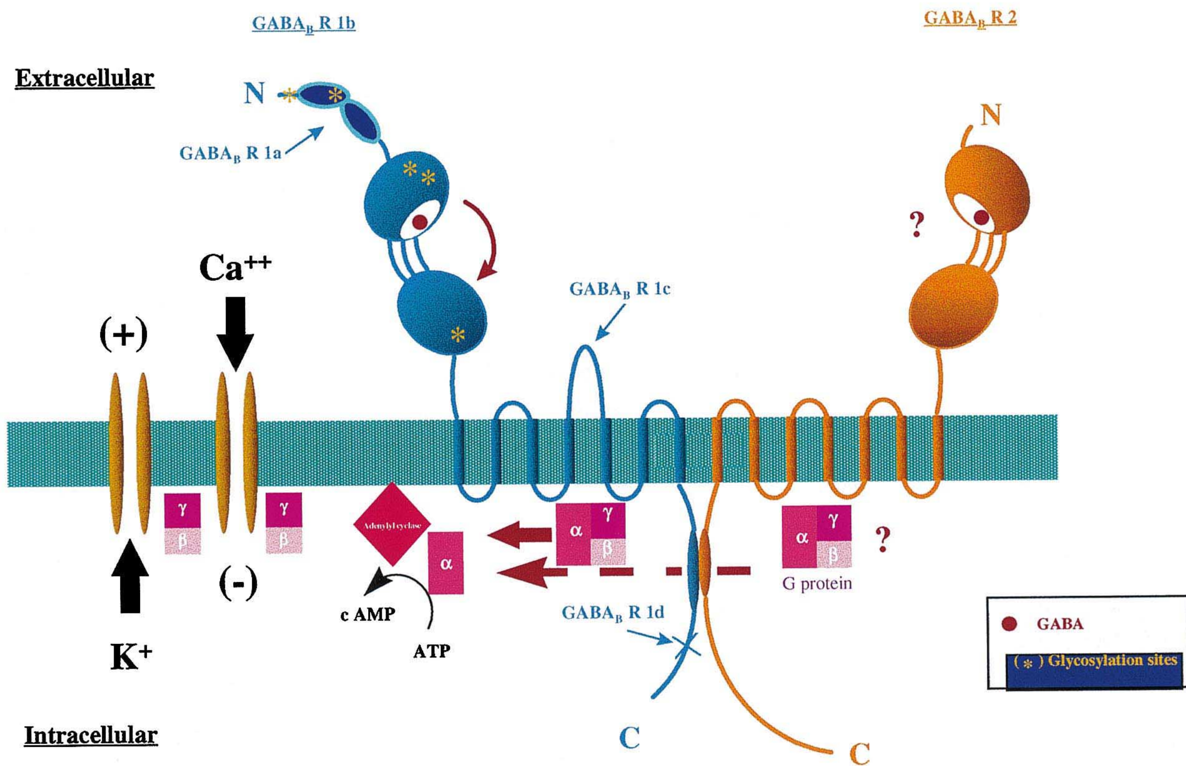

Figure 1. The $GABA_B$ heterodimer and splice variants. The $GABA_B$ R1 subunit is a protein of approximately 100 kDa. In the $GABA_B$ R1b subtype, the N-terminal extracellular domain is the ligand binding domain and consists of a region with sequence homology to a family of bacterial periplasmic binding proteins (LIVBP). The $GABA_B$ R1a splice variant differs at the N-terminus by the presence of a tandem pair of CP modules. The $GABA_B$ R1c splice variant differs in the fifth transmembrane region and the second extracellular loop by an additional 31 amino acids. $GABA_B$ R1d differs in the intracellular C-terminal domain where an insertion of 566 base pairs includes a stop codon which would generate a truncated receptor. The $GABA_B$ R2 subunit is 35% identical at the amino acid level to the $GABA_B$ R1 subunit but has a longer intracellular C-terminal tail (80 extra residues), and has no affinity for GABA or its analogues. Contact between the two subunits seems to be mediated by a “coiled-coil” region in each subunit. It has been suggested that upon ligand binding the amino-terminal domain undergoes a hinge-bending motion, as in LIVBP, which is described as a “venus fly trap” mechanism [27]. Receptor activation produces adenylyl cyclase inhibition through the involvement of the α subunit of $G_\alpha i/o$ type G proteins. Postsynaptic receptors cause inwardly rectifying K^+ channels to open allowing K^+ to move down its electrochemical gradient. This effect on K^+ channels is mediated by G protein β/γ subunits. It has been proposed that activation of presynaptic receptors results in the closing of voltage-dependent Ca^{2+} channels.

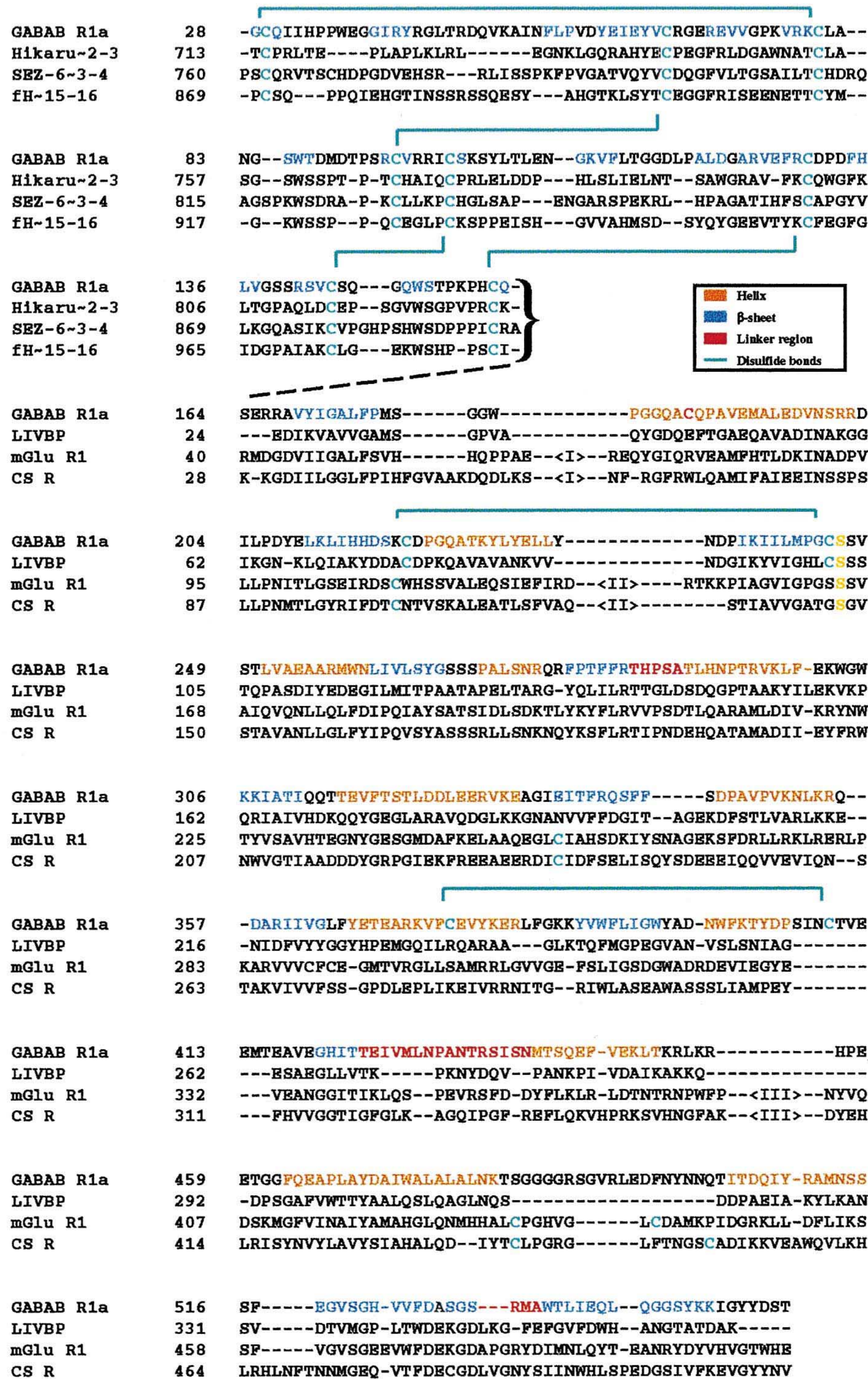

Figure 2. Top. One possible alignment of the $GABA_B$ R1a pair of CP modules with CP-like proteins found in the CNS; the second and third CP modules from the *Drosophila melanogaster* Hikaru genki protein, the third and the fourth CP modules from the rat SEZ-6 protein, and a well characterized pair of CP modules found in the complement system, the 15th and the 16th CP modules from the human factor H protein. Bottom. Alignment of the extracellular domains of rat $GABA_B$ R1a, LIVBP (*E. coli*), rat mGlu R1, and rat CS R according to Galvez et al. [27] and Brauner-Osborne et al. [32]. Note that Cys101 and Cys236 of the CS R align only with Cys109 and Cys254, respectively, of the mGlu R1—two residues involved in the covalent, disulfide-linked homodimerization of the CS R [50]. Amino acid numbering includes signal peptides. Three insertions found in the N-terminal domain of mGlu R1 have been removed and indicated by I-II-III [27].

the length of the sequence and is striking, since 27 out of 208 residues conserved within all mGlu R subtypes are present in $GABA_B$ R1. Seventeen of these are in the ~550-residue N-terminal, extracellular domain of $GABA_B$ R1. $GABA_B$ R1, however, lacks a domain present in the mGlu R's and in the CS R, which contains nine closely spaced cysteines [25]. This cysteine-rich domain lies just before the first transmembrane domain, and is essential for proper trafficking and function of the CS R. In fact, none of the consensus extracellular cysteines of the mGlu R group are retained in $GABA_B$ R1.

There is also a marked similarity between the N-terminal domains of class C members and a family of bacterial periplasmic amino acid-binding proteins which include the leucine-binding protein (LBP) and the leucine/isoleucine/valine-binding protein (LIVBP) [26] (figs 2 and 3). These binding proteins are found in Gram-negative bacteria and are essential to the uptake of low molecular weight compounds such as amino acids and sugars. The recognition and specific binding of these molecules is an essential first step for transmembrane transport. This theme is elaborated further below, since the similarity has been used as a basis for construction of a structural and functional model of the N-terminal domain of $GABA_B$ R1a [27]. There is a striking similarity between the N-terminal 143 residues of $GABA_B$ R1a and proteins of the mammalian complement system (figs 1 and 2) [28]. The complement protein (CP) category of protein modules (also called sushi domains) was first identified [and called the short consensus repeat (SCR)] [29] as a common feature in

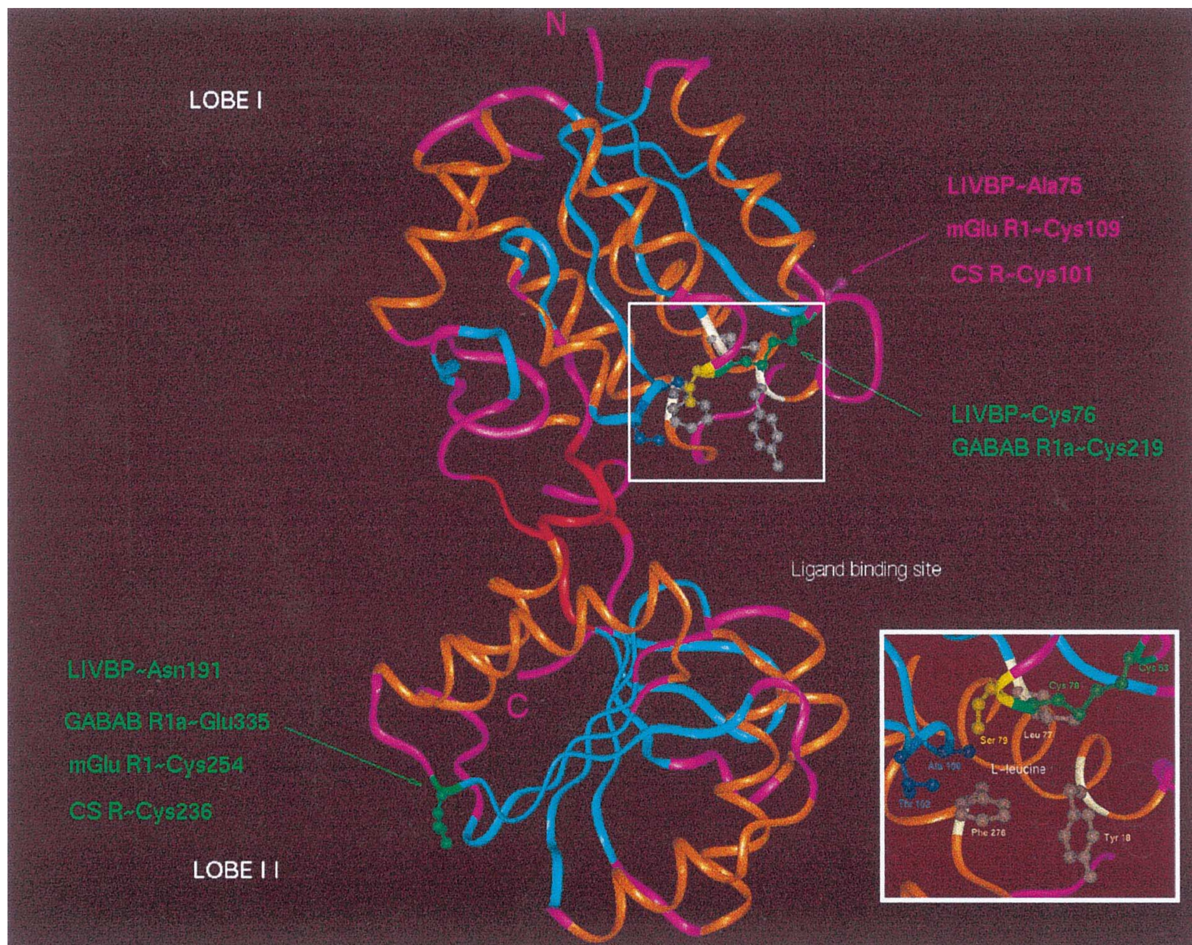

Figure 3. Crystal structure of the leucine/isoleucine/valine-binding protein from *E. coli* [77]. The secondary structure predictions for the $GABA_B$ R1 N-terminal domain were used, as indicated by the colored segments of the peptide backbone, to indicate the putative α-helical, β-sheet, and loop regions [27] (also, see figure 2). The extracellular domain is sufficient to specify agonist and antagonist binding [30].

the sequences of the family of proteins that regulate complement activation. The unexpected presence of two CP modules in $GABA_B$ R1a is discussed further below. In $GABA_B$ R1b, these 143 residues are replaced by an unrelated sequence of 18 amino acids.
Finally, the C-terminus of $GABA_B$ R1a/b contains several features of interest. There are consensus substrate sites for casein kinase and protein kinase C suggestive of modulation by phosphorylation [13]. There is also a putative 'coiled-coil' sequence of 35 residues (fig. 1) [16]. Such sites are often involved in protein-protein interactions, and this indeed seems to be the case for the subunits of the $GABA_B$ R.

The $GABA_B$ receptor subtype 1 as a G-protein-coupled receptor

All class C G-protein-coupled receptors share a similar molecular architecture consisting of a large extracellular amino-terminal domain encompassing a ligand binding site [30–32], followed by seven transmembrane domains and the intracellular C-terminal tail. Coupling to G proteins is mediated by the intracellular loops connecting the seven transmembrane domains and the C-terminal region. The short, third intracellular loop is highly conserved among class C 7TMD receptors and is essential for G protein activation [33]. Alternatively spliced insertions/deletions in this loop may affect differentially various receptor functions such as ligand binding, coupling to G protein and response to agonists.
As summarised by Kaupmann et al. [13] and Mott and Lewis [34], the G-protein-mediated effector systems that are believed to be regulated by $GABA_B$ receptors include adenylyl cyclase activity (inhibition), phospholipase A_2 (stimulation), potassium channels (activation), voltage-gated calcium channels (inactivation) and inositol triphosphate accumulation (inhibition). Amongst the specific G proteins that interact with the cytoplasmic region of $GABA_B$ receptors are $G_\alpha o$, $G_\alpha q$, $G_\alpha i$, and $G_{\beta\gamma}$. $GABA_B$ receptors play an important role in presynaptic nerve terminals as well as in the postsynaptic membranes of neurons [35], and distinct receptors may be involved [36]. Furthermore, different effector systems are thought to be coupled to the $GABA_B$ receptors localized to pre- and postsynaptic sites. The activation of presynaptic $GABA_B$ receptors (both autoreceptors and heteroreceptors) has been observed to suppress neurotransmitter release in a number of systems. The mechanism responsible appears to involve inactivation of voltage-gated calcium channels [37], although the involvement of the exocytotic machinery has also been proposed [38]. Studies of GIRK2 null mutant mice, and of *weaver* mice containing a mutation in the GIRK2 gene, have shown that these mice lack the outward potassium currents normally evoked by $GABA_B$ agonists [39, 40]. This has led to the conclusion that postsynaptic $GABA_B$ receptors produce characteristic late inhibitory postsynaptic potentials by G-protein-mediated activation of Kir3-type channels (these are also known as GIRK; G-protein-regulated, inwardly rectifying K^+ channels). Although the postsynaptic actions of GABA were abolished in these mice, the presynaptic inhibitory activity of GABA remained unaltered. These observations provide strong evidence for coupling of $GABA_B$ receptors to distinctly different effector systems in the pre- and postsynaptic membrane. Some recent studies have suggested that postsynaptic $GABA_B$ receptors may also couple to certain outward rectifying potassium channels, which may be localized to extrasynaptic sites [41].

Cloning of a second subunit: the metabotropic GABA receptor is a heterodimer

Despite the convincing demonstration by Kaupmann et al. [13] that $GABA_B$ R1a/1b fulfilled many of the criteria of a functional metabotropic GABA receptor, there remained some discrepancies between properties observed for the recombinant proteins, on the one hand, and the native brain receptor, on the other [13, 42]. Agonists showed significantly lower binding affinities for the recombinant receptors. This was attributed, at least in part, to inefficient coupling to G proteins within the heterologous expression system tested. Indeed, coupling to adenylyl cyclase was weaker than anticipated, and coupling to ion channels difficult to demonstrate. Subsequently, Couve et al. [43]. showed that heterologously expressed recombinant $GABA_B$ R1a/b were not transported efficiently to the cell surface and that these proteins were instead largely retained in the endoplasmic reticulum. There was thus considerable excitement when five groups simultaneously reported the existence of a second subtype of the $GABA_B$ receptor ($GABA_B$ R2) [14–18] (later reported by a sixth group as GPR51 [19, 20]). Not only did this discovery resolve many of the earlier difficulties raised by the work of Kaupmann et al. [13, 42] and Couve et al. [43], but it also opened a new chapter in the story of 7TMD receptors.
The discovery of a second subunit was made in 1998 using a combined bioinformatics and molecular biology approach. The human homologues of $GABA_B$ R1a/b had been identified and shown to be 98% identical to the rat sequences. The human cDNA sequence was then used to mine a database of expressed sequence tags, ultimately revealing a second receptor subunit ($GABA_B$ R2). The $GABA_B$ R2 subunit has 941 residues and is 35% identical and 54% similar to $GABA_B$ R1b. The N-terminal extension present in $GABA_B$ R1a is not found in the R2 subunit. Furthermore, the R2 subunit has an unusually

long, intracellular C-terminal tail containing 80 residues more than the equivalent region of $GABA_B$ R1a/b (fig. 1). However, it was reported that $GABA_B$ R2, when expressed alone, showed no affinity for GABA or its analogues.

Using a yeast two-hybrid system, White et al. [16] demonstrated that the C-terminal domain of $GABA_B$ R2 interacts specifically with the C-terminus of $GABA_B$ R1, most likely by virtue of coiled-coil sequences within the respective C-terminal regions. They and others went on to demonstrate by coimmunoprecipitation analysis that this interaction also occurs between recombinant $GABA_B$ R1 and $GABA_B$ R2 expressed in mammalian cells. In situ hybridization studies indicated that $GABA_B$ R2 colocalises with $GABA_B$ R1a/b in the human brain, consistent with a similar interaction occurring in vivo. These studies (along with those of others [15, 17, 20]) provide compelling evidence for heterodimerisation of the two receptor subunits. In further experiments, flow cytometry was employed to demonstrate that $GABA_B$ R1b only appears at the cell surface of HEK293 cells when cotransfected with $GABA_B$ R2 [14]. However, $GABA_B$ R1b is not required by $GABA_B$ R2 for transport to the cell surface. These findings were backed up by the observation that $GABA_B$ R2 appears to be fully glycosylated whether or not it is coexpressed with $GABA_B$ R1b—but in the absence of $GABA_B$ R2, $GABA_B$ R1b is only partially glycosylated [16]. Terminal glycosylation of a significant fraction of $GABA_B$ R1b (indicating passage through the Golgi body) occurs when $GABA_B$ R2 is coexpressed. When the two subunits were coexpressed in a 1:1 ratio in HEK293T cells [14], binding studies revealed agonist affinities comparable with the native brain receptors and 'robust' coupling to G proteins. Finally, and critically, when coexpressed in *Xenopus* oocytes, the two subunits produced a GABA-sensitive coupling with inwardly rectifying K^+ channels [14–17].

Thus it has been shown convincingly by several laboratories that $GABA_B$ R2 acts both as a translocator (or trafficking factor), and a binding partner, for $GABA_B$ R1a/b. It allows correctly processed $GABA_B$ R1a/b to reach the cell surface and subsequently to function at a level comparable (within an order of magnitude) with that of the native receptors. It has been suggested that $GABA_B$ R2 may also act independently under some circumstances, and coupling of $GABA_B$ R2 to adenylyl cyclase has been demonstrated [17, 18].

Other proteins that act as accessories for the membrane targeting of 7TMD receptors are known. For example, receptor-activity-modifying proteins (RAMP1 and RAMP2) are single-transmembrane-domain proteins that are essential for the correct processing, presentation at the cell surface and ligand selectivity of the calcitonin-receptor-like receptor, which is the receptor for calcitonin gene-related peptides [44]. The protein odorant-4 is an intracellular membrane protein required for the maturation and presentation of a group of olfactory receptors to the cilia in olfactory neurones of *Caenorhabditis elegans* [45]. In addition, other 7TMD receptors interact to form homodimers. Examples include the δ opioid receptor [46], β2-adrenergic receptor [33], mGlu R [47] and CS R [48–50]. The latter two class C receptors form homodimers via intersubunit disulphide bonds. One other example of a heterodimer involving two 7TMD receptors has recently come to light. This involves the association of two fully functional opioid receptors, κ and δ, resulting in a new receptor that exhibits ligand binding and functional properties distinct from either of the individual receptors [51]. The metabotropic $GABA_B$ receptor is the only example known, however, where two apparently nonfunctional 7TMD receptors dimerise. In general, the ability of receptors in the G-protein-coupled superfamily to form heterodimers with new functional properties would appear to be a previously unsuspected way of creating extra functional diversity from limited genetic material. This theme is expanded upon below in the discussion of splice variants.

Splice variants amongst the subunits of the metabotropic GABA receptor

As was mentioned above, two splice variants of $GABA_B$ R1 were recognised by Kaupmann et al. [13] upon sequencing of the first receptor clones. In $GABA_B$ R1b, the 143 residues following the signal peptide sequence of $GABA_B$ R1a are replaced by 18 residues of unrelated sequence. Otherwise the two variants are identical. Neither is a cloning artefact nor the result of a rare aberrant splicing event. In pharmacological profiles of $GABA_B$ R1a/b expressed in the absence of $GABA_B$ R2, the R1b variant had slightly lower affinities for all agonists and antagonists tested but otherwise exhibited identical pharmacological properties. Both variants form heterodimers with $GABA_B$ R2, and no differences in the properties of the resulting functional receptors have been reported [14–20]. This theme is expanded upon in the next section.

Two other splice variants, $GABA_B$ R1c and $GABA_B$ R1d, were subsequently identified [52]. $GABA_B$ R1c is identical to $GABA_B$ R1b but with an insertion of 93 bp that generates an additional 31 amino acids in the second extracellular loop and fifth transmembrane region, between Gly $\sim$ 654 and Ile $\sim$ 655 (fig. 1). Note that the fifth transmembrane domain is, in general, the most solvent-exposed domain according to modelling studies of 7TMD proteins [53] and is conceivably available for protein and drug interaction. A noncovalent dimerisation consensus motif (**L**MA**LG**F**LIG**YTC**L**, consensus residues in bold), originally found in the unrelated β-adrenegic receptors,

is present in the fifth transmembrane domain of the CS R and has been proposed to mediate homodimerisation via hydrophobic interactions [49]. The $GABA_B$ R1d variant has an additional insertion of 566 bp that generates a divergent C-terminal region (fig. 1). This insertion includes a stop codon that results in the replacement of 57 residues of R1b with 25 residues of unrelated sequence. The functional properties of these particular variants are currently unknown.

The importance of splice variants as a means of providing additional functional diversity amongst 7TMD receptors is now well established. In a review, Kilpatrick et al. [54] describe more than 30 examples amongst hormone and neurotransmitter 7TMD receptors. There may be as many as eight isoforms, they may be species-specific, and there may be cellular or temporal differentials in their distribution. In very few cases does splice variation affect ligand affinity. Where the splice variation is in the C-terminus (by far the most common location) or in transmembrane domains or intracellular loops, it may have profound effects upon signalling pathways and coupling efficiencies. To date the $GABA_B$ R1a/b pair of splice variants are unusual in that they differ in their possession of a clearly identifiable structural feature, and this raises interesting questions as to the role of this region.

The 143 residues distinguishing $GABA_B$ R1a from $GABA_B$ R1b form a tandemly arranged pair of CP modules [28]. This is the first example of CP modules occurring in a 7TMD receptor. There are two other 7TMD receptors known to possess extracellular modules. These belong to class B, the secretin receptor family, and have various numbers of epidermal growth factor (EGF)-like modules, rather than CP modules, in their large N-terminal domains [55]. In the case of one of these receptors, the leukocyte activation antigen, CD97, alternative splicing results in three, four or five EGF-like modules and a corresponding modulation in affinity for its ligand [56].

The CP modules are the predominant module type within several cell surface complement regulatory proteins with single transmembrane domains, such as CD46 (which is the measles virus receptor) and CD35. They are also found in a GPI-linked complement regulator (CD55) and in soluble proteins. In addition, CP modules have been found in many noncomplement proteins, including the IL2β receptor. There are several other examples of central nervous system (CNS) proteins that contain CP modules (fig. 2), including the *Drosophila* protein 'hikaru genki', which has four CP modules [57]; the human protein, neurocan, which has a single CP module [58]; and mouse SEZ-6, a single-transmembrane-domain protein possessing five CP modules [59, 60]. SEZ-6 was identified as a protein whose expression was enhanced by the perfusion of brain slices with convulsant drugs; the human equivalent is a hypothetical 87.6-kDa foetal brain protein which is 49% identical to the C-terminal 746 residues of SEZ-6. A CP-module-containing protein has also been found in the retina [61]. Although some examples of CP modules function as structural or spacer units in bigger proteins, wherever they occur towards the N-terminus of a well-studied cell surface protein, they have been shown to participate in specific protein-protein interactions. It is therefore possible that the N-terminal CP modules of $GABA_B$ R1a will prove to be of importance in recognising another, extracellular or membrane-bound protein. This putative interaction might be important for anchoring the receptor at a particular cellular location, or might have a modulatory influence on receptor activity and signal transduction. The only 7TMD receptor known to possess a cellular ligand is CD97, mentioned earlier. As it happens, its ligand has been identified as CD55, which is a GPI-anchored CP-module-containing protein [62]. The interaction is between an EGF-like module of CD97 and the N-terminal CP module of CD55 [63].

Differential roles and locations of $GABA_B$ R1a/1b and R2

Following the cloning of the $GABA_B$ R1 and R2 subunit genes, much effort has been directed towards determining whether the cloned sequences can account for all of the known functions of $GABA_B$ receptors in vivo [21]. A second related question has centred on whether the R1a and R1b isoforms might differ in their cellular localization; more specifically, do they correlate with the presynaptic and postsynaptic forms of the $GABA_B$ receptor?

As discussed above, $GABA_B$ R1a and R1b differ only in their N-terminal, extracellular region, a difference that should not affect directly the interaction with cytoplasmically localized G protein. R1a expressed stably in HEK293 cells produces a baclofen-evoked reduction in forskolin-stimulated cAMP production comparable to that observed in rat cortical slice preparations (30 vs 40% in slices). This effect is blocked by a $GABA_B$ antagonist and is abolished by pertussis toxin suggesting, as HEK cells are deficient in G_o, the involvement of G_i [13]. Another group failed, however, to detect a $GABA_B$ R1-mediated inhibition of adenylyl cyclase using a similar expression system [17]. Recombinant R2 receptors expressed in the absence of R1 can also couple to adenylyl cyclase [17, 18]. Coexpression of either R1a or R1b with Kir3.1/3.2 or Kir3.1/3.4 subunits in HEK293 cells leads to an inefficient but detectable coupling of these receptor isoforms with the physiologically relevant K^+ channels [13, 42]. More recently, several groups have demonstrated that the coexpression of R1 and R2 leads to a much more robust coupling to inwardly rectifying potassium channels [14–20]. In comparison, the expression of R2 alone is not sufficient to activate Kir3-type (GIRK) K^+ channels. $G_{\beta\gamma}$ would

be expected to play a critical role in the coupling of $GABA_B$ R1/R2 heterodimers to the inwardly rectifying K^+ channels as it is the binding of $G_{\beta\gamma}$ to recombinant GIRK1/GIRK4 channels that leads to their activation [64].

The coupling of recombinantly expressed $GABA_B$ R1 and R2 to voltage-gated calcium channels could serve as an important model system for investigation of the proposed presynaptic role of $GABA_B$ receptors. Such a coupling, however, has not been demonstrated to date. Studies using an antisense oligonucleotide targeted to a sequence common to R1a and R1b suggest that R1 may be involved in the inhibition of voltage-gated calcium channels in cultured melanotropes from the pituitary intermediate lobe [65]. On the other hand, overexpression of R1a in rat superior cervical ganglion cells does not enhance baclofen-evoked inhibition of voltage-gated calcium currents [43]. Based on the endogenous sensitivity of these cultured neurons to baclofen, they likely express some level of functional $GABA_B$ receptors. One possible explanation for the failure to obtain enhancement is that a limiting amount of R2 or some other required factor prevents these cells from using the introduced R1a receptor subunits to produce additional functional receptors on the cell surface.

In summary, it appears that the R1/R2 heterodimer is indeed the functional metabotropic $GABA_B$ receptor, presumably acting via $G_{\beta\gamma}$. Although coupling to voltage-gated calcium channels has not yet been demonstrated, there is no need to evoke the existence of further subunits (nor can this possibility be excluded).

The initial identification of the $GABA_B$ R1a and R1b splice variants led to the rather attractive hypothesis that the R1a and R1b variants might provide the basis for the distinction between presynaptic, and postsynaptic, $GABA_B$ receptors [5]. As these two splice variants have identical intracellular regions, a differential subsynaptic association would presumably be due to differential membrane targeting or coassembly with yet unidentified membrane proteins capable of distinguishing between the extracellular domains of R1a and R1b. A focal point for the investigation of this issue has been the rat cerebellum, where $GABA_B$ binding sites are at their highest density in the classically termed molecular layer of the cerebellum. Granule cell bodies located in the morphologically distinguishable granule cell layer send parallel axon fibres through this 'molecular layer', where they form excitatory presynaptic nerve terminals impinging upon the dendrites of Purkinje cells. The Purkinje cell bodies themselves form a highly characteristic morphological feature, the Purkinje cell layer. In situ hybridization studies were performed to determine whether there was a differential distribution of $GABA_B$ R1a and R1b mRNA in this region of the brain [66]. $GABA_B$ R1a mRNA was found to be expressed predominantly in the granule cell layer (approximately 16-fold higher grain density than in the Purkinje cell layer), whereas R1b mRNA was localized primarily to Purkinje cell bodies (approximately 8-fold higher grain density than in the granule cell layer). These results predict that R1a-containing receptor proteins are preferentially expressed in granule cells, the source of the presynaptic terminals, whereas on the other hand, $GABA_B$ R1b-containing receptor proteins are differentially produced in the postsynaptic Purkinje cells, whose dendrites form synapses, albeit excitatory ones, with the granule cell terminals. These observations appear to support the hypothesis of a differential association of $GABA_B$ R1a with presynaptic structures and R1b with postsynaptic membranes.

At that time, the only available immunohistochemical study had not shed much additional light on the presynaptic versus postsynaptic question [67]. A polyclonal antibody (serum 722) raised against a synthetic peptide corresponding to residues common to R1a and R1b, along with another serum (174.1) unable to distinguish between $GABA_B$ R1a and $GABA_B$ R1b (described in [68]), allowed localization of immunoreactivity to various synaptic structures in the rat retina. Immunostaining was observed on presynaptic, autoreceptor-containing structures in horizontal and amacrine cells. It was also observed in postsynaptic structures in amacrine and ganglion cells. Furthermore, staining was observed that supported an extrasynaptic localization of $GABA_B$ receptors. Using other antibodies (gb1a,b922–944), including one raised against a synthetic peptide corresponding specifically to the first 18 residues in mature R1b, Fritschy et al. [69] carried out an immunohistochemical study of rat brain including the cerebellum. In general, the distribution of immunoreactivity in brain sections was largely consistent with the previously published studies describing $GABA_B$ receptor binding sites in the brain and suggested that the two isoforms, R1a and R1b, together can account for most of the binding sites in the brain. In other words, all $GABA_B$ receptors capable of binding ligands are likely to contain either the R1a or R1b splice variant. These authors also report the unexpected observation that, although there clearly were instances of postsynaptic association, much of the $GABA_B$ R1b immunostaining in the cerebellum was localized to extrasynaptic sites in Purkinje dendrites and spines. The authors suggest that these extrasynaptic sites may play a role in GABA spillover from other synapses or that they may be involved in taurine action in the cerebellum. Despite the reported abundance of $GABA_B$ R1a mRNA in granule cells [66], only low quantities of R1a protein expression in this region were deduced from the relatively poor level of immunoreactivity observed with the pan-antibody (gb1a,b922–944). Hence current im-

munohistochemical studies do not reinforce the in situ hybridization studies with respect to presynaptic versus postsynaptic location, but neither do they contradict them. An antiserum (gb1a83–107) prepared against residues 83–107 in R1a, although effective in Western blotting, proved not to be useful for immunohistochemistry, and thus a direct examination with an R1a-isoform specific antibody could not be carried out.

In a biochemical approach to the question of differential presynaptic versus postsynaptic association of $GABA_B$ R1a and R1b splice variants [70], a subcellular fraction of postsynaptic densities (PSDs) from rat brain membranes was prepared and enriched by differential centrifugation. Immunoreactivity against both R1a and R1b was observed in fractions containing synaptic plasma membranes, suggesting a significant synaptic localization for both splice variants in vivo. A further purified PSD fraction, however, which is enriched in postsynaptic membranes and associated submembranous proteins, appears deficient in R1b immunoreactivity but gives a strong signal with the antibody specific for R1a. These results suggest, contrary to earlier expectations, that it is the R1a splice variant which may be preferentially associated with postsynaptic structures. In summary, the question of differential localization of R1b and R1a splice variants remains unresolved; a significant fraction of R1/R2 might be located extrasynaptically.

This study also presented evidence disfavouring a significant population of monomeric $GABA_B$ receptors in rat brain. Based on sucrose gradient centrifugation analysis of deoxycholate extracts of rat brain membranes, the size distribution observed was consistent with the major fraction of the native species being a heterodimer. A second discrete population of receptors has an apparent molecular weight in excess of 250 kDa. This larger material might consist of complexes between the heterodimer and an as yet unidentified associated synaptic protein. In addition, immunoprecipitation studies coupled with Western blot analysis were used to demonstrate that $GABA_B$ R1a and R1b are found in heterodimers with R2 in rat brain extracts and that R1a and R1b do not form heterodimers with each other. Furthermore, immunopurification studies failed to detect any significant amount of trimeric complexes containing R1a, R1b and R2. Finally, quantitative immunoprecipitation was used to rule out any significant population of monomeric or homooligomeric forms of R1a, R1b or R2 in rat brain extracts [70]. The results from this last experiment would also appear to argue against the presence of any significant amounts of an as yet unidentified $GABA_B$ receptor subunit that could substitute for either R1 or R2 in a heterodimer complex.

Structural insights into the metabotropic $GABA_B$ receptor

On the basis of sequence comparisons, the expression and characterisation of truncated fragments of $GABA_B$ R1a/b, and construction of chimeric receptors, a picture of the global architecture of $GABA_B$ R1 is emerging (see fig. 1).

The potential importance of the N-terminal 143 residues of $GABA_B$ R1a has already been discussed. A fragment of $GABA_B$ R1a corresponding to residues 24–159 (the numbering system used here corresponds to the sequence of $GABA_B$ R1a, as published, and includes the 16 residues of signal sequence) has been recombinantly expressed in *Pichia pastoris*, purified to homogeneity and shown to adopt a stable, soluble fold in isolation from the rest of the protein [28]. Biophysical analysis including measurements of circular dichroism and differential scanning calorimetry, when considered in conjunction with sequence comparisons (fig. 2), were consistent with the presence of two CP modules separated by a linker sequence of three amino acids—CP module 1 extends from residue 28–95, and CP module 2 from residue 99–158 [28]. In subsequent experiments [S. Blein and P. Barlow, unpublished], a similar result was obtained for a fragment also incorporating residues 17–23 (the first seven residues of the mature protein sequence, yielding the fragment 17–159). Furthermore, the presence or absence of N-glycosylation at site 83 did not have a noticeable influence on the structure of the fragment.

On the basis of homology with a tandem pair of CP modules for which a structure had been solved by NMR, a model was built for residues 28–158 [28]. This indicated that each module adopts a typical CP-module-like fold in which a compact hydrophobic core containing highly conserved residues is sandwiched between antiparallel β-sheets made up from short β strands. Four cysteines, disulphide-linked in the pattern 1–3 (i.e. Cys ~ 29-Cys ~ 80/Cys ~ 99-Cys ~ 144) and 2–4 (i.e. Cys ~ 66-Cys ~ 95/Cys ~ 130-Cys ~ 156), stabilise each module. Module 1 is a less typical example of a CP module than module 2 and has a large insertion of 12 residues (Arg ~ 43-Asn ~ 54) that appears as a looped-out segment in the model. On the basis of precedent, the two modules are probably joined flexibly in an end-to-end fashion, generating a highly extended structure with a large surface area available for binding with another domain within $GABA_B$ R1a, or with another protein. N-glycosylation sites are surface-exposed at positions Asn ~ 23 and Asn ~ 83. The recombinant fragments deliver NMR spectra of good quality, and structure determination in solution is under way [S. Blein et al., unpublished].

The sequence immediately beyond the distinct N-terminal sequences of $GABA_B$ R1a/b, and prior to the first putative transmembrane domain, is referred to as the N-terminal domain (NTER). The role of these residues (Ala ~ 168-Gly ~ 550) has been investigated extensively. As with the 28–158 fragment, it is possible to express this domain as a recombinant protein that is both soluble and folded [30]. This recombinant protein is able to bind agonists and antagonists in a comparable fashion to the native receptor and is a candidate for X-ray or NMR-derived structure determination. Chimeric receptors [30], in which this domain was fused to the transmembrane and C-terminal domains of mGlu R1b, were also able to bind ligands but were nonfunctional, even in the presence of $GABA_B$ R2. This might be a consequence of the requirement for a C-terminal cytoplasmic domain suitable for formation of heterodimers. It might also reflect differences in the conformational changes that $GABA_B$ R's NTER undergoes upon ligand binding compared with the equivalent region of mGlu R. The truncated fragments had a higher affinity for agonists than wild-type receptor, although antagonist binding is comparable. This result may reflect the influence of receptor sequences beyond NTER on GABA binding, and is consistent with the well-known sensitivity of many 7TMD receptors to the presence of guanine nucleotides, an effect mediated by the cytoplasmic domain. Finally, a chimeric receptor in which the NTER of the $GABA_B$ receptor had been truncated at residue 530 rather than 550 did not bind ligand, an observation which helps to define domain boundaries within the receptor.

As mentioned above, the sequence of the NTERs of class C 7TMD receptors is significantly similar to that of a family of bacterial periplasmic amino acid-binding proteins. The three-dimensional structures of several of these bacterial proteins have been solved by Quiocho's group in Houston [71, 72], and these structures provide a basis for modelling the three-dimensional structure of the ligand-binding NTER of $GABA_B$ R1a (fig. 2). A similar exercise had already proved useful in the case of mGlu R [26].

The LIVBP (fig. 3) is a prolate ellipsoid with dimensions 35 Å × 40 Å × 70 Å, composed from distinct globular domains (lobes I and II). The domains are linked by three short stretches of residues which are widely spaced within the primary structure such that each domain contains contributions from both the N- and C-terminal halves of the sequence. The two lobes are similar in structure and composed of a seven-stranded β-sheet flanked by α helices. The linking sequences form the bottom of a cleft between the lobes which has a depth of about 15 Å and a base of 14 Å × 16 Å. The leucine binding site is in a crevice to the side of the cleft, within the lobe that contains the N-terminal residue (lobe I). L-leucine is held in place by hydrogen bonding of its $-NH_3^+$ and $-COO^-$ group (with the α-CO of Ala ~ 100/-OH of Thr ~ 102, and the -OH of Ser ~ 79, respectively), whereas its side chain lies in a depression lined with the nonpolar residues (Phe ~ 276, Tyr ~ 18 and Leu ~ 77).

A plausible model of the NTER of $GABA_B$ R1a/b was built using the program MODELER [28]. The model resulted in several interesting observations. There are five cysteines within the NTER of $GABA_B$ R1b (compared with 17 in the NTER of mGlu R), and in the model these are seen to pair up such that Cys ~ 219 and Cys ~ 245 could form a disulphide, as could Cys ~ 375 and Cys ~ 409; the remaining Cys, 187, is buried in lobe 1 and not in the proximity of the other Cys residues. The model also supplied predictions of the residues of $GABA_B$ R1a/b likely to be involved in binding of GABA, assuming that these are equivalent to the residues of LIVPB involved in binding leucine. For example, Ser ~ 246 and Ser ~ 269 of $GABA_B$ R1a/b are equivalent to Ser ~ 79 and Thr ~ 102 of LIVBP. These and other residues of $GABA_B$ R1a/b were mutated, and the functional consequences observed [28].

Mutation of Cys ~ 187 to Ala or Ser did not influence ligand binding; however, mutation of the four Cys residues proposed to form disulphide linkages resulted in abolition of ligand binding. This effect could not be explained fully by lack of protein expression. Surprisingly, in the light of these results, exposure of the wild-type receptor to conditions in which the disulphides should be reduced did not ablate ligand binding. This was reasonably interpreted to indicate that the disulphides were required in a critical folding or processing event preceding the appearance of the mature receptor on the cell surface. Neither of these disulphides could occur in mGlu R due to the lack of conservation of the Cys residues involved. Mutagenesis of residues at the putative binding site proved very instructive. S246A, and another mutant, Y470A, suppressed ligand binding altogether. Ser ~ 246 (Ser ~ 79 in LIVBP) is almost certainly involved in a direct interaction with ligand, and it is pertinent that the equivalent residue in mGlu R (Ser ~ 165) is also critical for ligand binding. Tyr ~ 470, on the other hand, probably has a structural role within lobe I. The mutation S269A exerted a small selective influence on ligand binding. The equivalent residue, Thr ~ 102 of LIVBP, is critical for H-bonding to the α-NH_3^+ of leucine, a moiety which is lacking in GABA, so the lack of a major effect for this mutation may be rationalised. The equivalent residue in mGlu R (Thr ~ 188) is functionally critical, as expected.

Hence mutagenesis studies supported the reliability of the model. This encouraged speculation that amongst these proteins there might be parallels between the molecular mechanisms underlying function. In the case

of the bacterial periplasmic amino acid-binding proteins, crystallographic evidence has accumulated for a ligand-induced hinge-bending motion. This so-called Venus flytrap mechanism in which binding of the ligand is followed by a conformational change which buries the ligand between the two domains, might exist also in $GABA_B$ R1 (and other class C receptors). Indeed, two mutants, S247A in lobe I and Q312A in lobe II, were proposed to manifest their functional effects—a small loss of antagonist affinity accompanied by a gain in agonist affinity—by influencing directly the hinge-bending motion. Ser ~ 247 is equivalent to Ser ~ 166 in mGlu R1, a residue critical for Ca^{2+}-activation [73].

Developmental regulation

The distinct sizes of the $GABA_B$ R1a and R1b splice variants, together with specific antibodies capable of distinguishing R1a, R1b and R2 on Western blots, allows investigation of the developmental regulation of these $GABA_B$ receptor subunits in the rat CNS. Using an antibody (Ab174.1) raised against a C-terminal stretch of 59 amino acids common to both R1a and R1b, Malitschek et al. [68] examined $GABA_B$ R1 expression in rats from postnatal days 2–28. The summed levels of R1a and R1b decrease in the cortex, the cerebellum and most dramatically in the spinal cord between postnatal day 7 and adulthood. In these regions, $GABA_B$ R1a levels appear significantly higher than R1b between days 2 and 14, although no quantitation of the Western blot immunoreactivity was included in this report. As $GABA_B$ R1a levels decrease over this time period, there appears to be a slight increase in R1b levels. After day 28, some reduction in expression of both R1a and R1b is observed in the midbrain but not in the striatum, brainstem or hippocampus.

Using the photoaffinity label [^{125}I]CGP64213, the developmental changes in the affinity of rat cortical membrane $GABA_B$ receptors for L-baclofen were examined in competition-binding assays. The apparent affinity for L-baclofen increases about 10-fold between postnatal days 1 and 60. Furthermore, SDS-gel analysis of the cross-linked membranes demonstrates that the R1a and R1b isoforms do not differ in their affinity for L-baclofen between day 4 and adulthood.

The issue of $GABA_B$ R1a and R1b developmental regulation was further examined with another antibody (gb1a,b922–944) prepared against a synthetic peptide corresponding to a sequence (922–944) common to both R1a and R1b [69]. It is expected that this antibody (like Ab 174.1, vide supra) would have identical affinity for both R1a and R1b on Western blots, and thus, following separation of the two isoforms on SDS gels, it could be used to quantitate expression levels. On the basis of the immunoreactivity observed in Western blots, it was shown that R1a levels are highest in rat brain at birth through postnatal day 5, during which time R1b levels are about fivefold lower. R1b levels then increase about threefold from day 5 to day 10 but decrease again between day 10 and adulthood to a level of about twice that seen at birth. R1a levels decline steadily after day 5, and in the adult rat brain there appears to be about twice as much R1b immunoreactivity as R1a.

The antibody (gb1a,b922–944) was used to examine the regional distribution of $GABA_B$ R1a and R1b in membranes prepared from various regions of the adult rat brain. R1b levels were highest in the cortex, thalamus and cerebellum, where R1a levels ranged from 50 to 60% of R1b levels. In the striatum and olfactory bulb regions, R1a levels exceeded R1b levels, but the immunoreactivity of R1a in these regions was only about 20% of that seen for R1b in the adult cerebellum.

A similar Western blot analysis with an antibody (AbC22) specific for R2 revealed that $GABA_B$ R2 is expressed at high levels in the cortex and cerebellum throughout postnatal development. In the cortex, $GABA_B$ R2 levels increase between days 2 and 28 with a slight apparent decrease in the adult (60 days) [15]. $GABA_B$ R2 levels in the cerebellum appear highest at 4 days, the earliest time point examined, and decline slightly after that. In contrast, R2 levels are detectable in spinal cord from postnatal days 7 (the earliest time examined) to 14 but decline sharply thereafter. It is of interest from the perspective of potential heterodimer configurations that while R2 levels are still increasing in the cortex between days 2 and 28 [15], the combined levels of R1a and R1b in the cortex decrease somewhat over this same period [68].

Some clinical implications

$GABA_B$ receptors may be involved in inherited neurological diseases. Physiological and pharmacological studies suggest that the $GABA_B$ receptor plays a major role in the epileptogenesis of absence seizures [2]. In animal models of absence epilepsy, alterations of $GABA_B$ receptor expression have been reported [8]. It is of interest that chromosomal localization of the gene(s) encoding $GABA_B$ R1, as revealed by fluorescence in situ hybridization, is on the human chromosome 6p21.3 [42, 74]. This location is close to the major histocompatibility complex (HLA-F), and in the vicinity of a susceptibility locus (EJM1) for subtypes of idiopathic generalised epilepsy (IGE), including juvenile myoclonic epilepsy, juvenile absence epilepsy and idiopathic generalised epilepsy with tonic clonic seizures on awakening. However, studies to test the hypothesis that the $GABA_B$

R1-encoding sequence represents a candidate for a gene predisposing a patient to EJM1 have not been conclusive so far. They failed to demonstrate that any of the variants or unidentified critical mutations within this locus were involved in the development of common IGE subtypes [75, 76].

The gene for $GABA_B$ R2 is located on a different chromosome. Two groups [18, 19] mapped it to chromosome 9, and one of these studies precisely localized it to region 9q22.1, underlining a putative evolutionary event in $GABA_B$ receptors. This particular region on chromosome 9 harbours a susceptibility locus for another inherited neurological disorder, hereditary sensory neuropathy type 1 (HSN-1). This disease is a common subtype of a spectrum of disorders of sensory neurons that lead to neuronal deficits by degeneration of dorsal root ganglion and motor neurons. Other disorders critical to neuronal development have been localized to this area. The HSN-1 susceptibility locus maps at 9q22.1–9q22.3 [77], but no further studies linking $GABA_B$ R2 and HSN-1 have been reported so far.

In humans, hypertension is associated with the activation of the sympathetic nervous system, but the mechanisms for this are unclear. $GABA_B$ receptors have been implicated at several levels. GABA-ergic axons are found throughout superior cervical (sympathetic) ganglia consistent with a neuromodulatory role for GABA in the peripheral nervous system [78]. Also, there is evidence that spinal $GABA_B$ receptors regulate sympathetic vasomotor tone [79]. Likewise, alterations in CNS $GABA_B$ receptors have been implicated in the regulation of cardiovascular function in hypertension.

Future directions

In order to evaluate the significance of the proposed extrasynaptic localisation of $GABA_B$ R1b receptors in the cerebellum [69] and also to address the outstanding issue of pre- versus postsynaptic association, it will be necessary to examine in detail the localization, at the electron microscope level, of $GABA_B$ R1a/b receptors. Unfortunately, although an antiserum specific for $GABA_B$ R1a has been described [69, 70], it does not recognise antigen in perfusion-fixed brain tissue, precluding an immunohistochemical analysis at the cellular or electron microscope level. The availability of an antibody that would permit such investigations would clearly be of great benefit.

Another issue that invites closer examination relates to the function and role of splice variants of $GABA_B$ receptors [52, 80]. An examination of the possible region-specific distribution of splice variants such as $GABA_B$R1c and $GABA_B$R1d [52] could help determine whether splice variant diversity underlies some of the reported heterogeneity in $GABA_B$ receptor subtypes which have been observed in pharmacological and electrophysiological investigations of the CNS.

A related priority is to determine the function of the pair of CP modules which distinguishes $GABA_B$ R1a from R1b. These are likely to be involved in a specific protein:protein interaction at the cell surface. Any such specific interaction could have a role in receptor assembly, receptor localization or the modulation of receptor activity. It is noteworthy that there is now evidence for the formation of a higher-order complex in situ involving the established $GABA_B$ receptor subunits and an additional, unknown protein (or proteins). However, it is not known whether this is an extracellular interaction [70].

Structural information is also a priority. A high-resolution three-dimensional structure of the intact $GABA_B$ R might not be feasible at present, but the N-terminal extracellular domains and the C-terminal intracellular domains of the two subunits will be more amenable to structural studies. These would confirm the structural similarity with other proteins mentioned above, and provide details of the interactions with ligand. This is likely to be an essential precursor to rational drug design. Structural information would also underpin an understanding of putative conformational changes on ligand binding and how these affect coupling to G proteins.

It is also of interest that although Northern blot analysis suggests that most tissues other than the brain and testis do not express significant levels of $GABA_B$R1a and $GABA_B$R1b mRNAs [13], an reverse transcriptase polymerase chain reaction (RT-PCR) analysis using primers that should detect both $GABA_B$R1a and $GABA_B$R1b mRNAs argues for a more widespread distribution in the periphery [52]. One peripheral tissue of particular interest is the sympathetic nervous system, as previous studies [78] have suggested a role for $GABA_B$ receptors in the rat superior cervical ganglion (SCG). In addition, in a preliminary account of studies in sensory nerves, $GABA_B$R1a transcripts, as measured by in situ hybridization, appear to show a high density over neuronal cell bodies in the dorsal root ganglia [5]. In contrast, the amount of $GABA_B$R1b transcript appears to be much lower in the same tissue. This information was used to suggest the association of R1a receptor subunits with presynaptic receptors in the primary afferent nerve terminals of sensory nerves.

Because of the important role of the sympathetic nervous system in cardiovascular regulation, it would be of considerable interest to examine the expression of $GABA_B$ receptor subunits in sympathetic tissue. In a study carried out with the assistance of Dr Diane Lipscombe in the Department of Neuroscience at Brown University, we have obtained preliminary indications

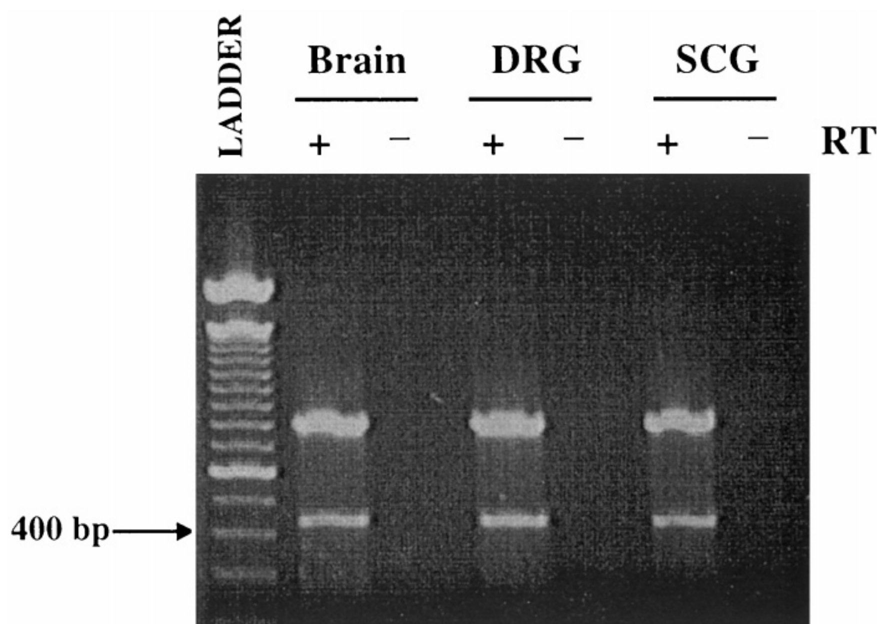

Figure 4. RT-PCR detection of rat mRNAs encoding $GABA_BR1a$. We obtained the PCR product at 439 bp by using primers selected to amplify the region corresponding to the CP-module pair in $GABA_BR1a$. The PCR product with decreased mobility (811 bp) was produced from primers designed to amplify a selected region in actin cDNA. The actin product serves as an indicator that comparable amounts of tissue mRNA were added to all of the reverse-transcriptase (RT) reactions. The ladder lane of marker DNA contains oligonucleotides differing by 100 bp increments. For each tissue pair of PCR reactions, we carried out a negative control where we omitted RT from the initial step of cDNA production. This control rules out genomic amplification and sample contamination.

suggesting an abundant presence of $GABA_BR1a$ transcripts in rat superior cervical ganglia. Using $GABA_BR1a$-specific PCR primers [28] in an RT-PCR-based analysis, we find that rat SCG tissue does indeed contain mRNA encoding $GABA_BR1a$ subunits. As shown in figure 4, these two primers gave rise to the expected 439-bp PCR product when rat brain cDNA (obtained from adult rats) is used as a template for the PCR. Rat dorsal root ganglion (DRG) tissue and rat SCG tissue (both from 5-day-old rats) also gave rise to a PCR product apparently of the same size as that found with brain tissue. Like the 'no-template' control, a 'no reverse-transcriptase control' was blank. Although not quantitative, these results suggest that $GABA_B$ R1a transcripts are likely to be at least as abundant in SCG as in the CNS. The physiological role of the $GABA_BR1a$ splice variant in the SCG and whether the other known $GABA_B$ R sequences or additional splice variants are expressed in the SCG remain to be determined.

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