Advances in the molecular understanding of $GABA_B$ receptors

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The molecular nature of the metabotropic $GABA_B$ receptor was for some time a mystery, however it was recently discovered that two related G-protein-coupled receptors have to heterodimerize to form the functional $GABA_B$ receptor at the cell surface. This review discusses the most recent findings in the rapidly expanding field of $GABA_B$ receptor research, and includes a summary of all splice variants of both receptor subunits identified to date. It also evaluates emerging evidence that certain splice variants might play a role in determining pharmacologically distinguishable receptors, and reviews receptor localization at the sub-cellular level and involvement in neuronal development.

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Recent advances in GABA receptor biology have resulted in elucidation of the molecular structure of a $GABA_B$ protein with similarity to metabotropic glutamate receptors (mGluRs), $GABA_{B(1)}$ (Ref. 1), and the subsequent discovery by several laboratories that $GABA_{B(1)}$ heterodimerizes with $GABA_{B(2)}$ to form a functional G-protein-coupled receptor (GPCR) (Refs 2–7). For specific intervention in disorders such as epilepsy, pain and drug addiction, pharmacological subtypes would be therapeutically useful, and evidence from some pharmacological studies has indicated that subtypes do exist[8]. Since the initial cloning of $GABA_{B(1a)}$ and $GABA_{B(1b)}$, several other splice variants have emerged, in addition to splice variants of $GABA_{B(2)}$ (Refs 9–12). There is evidence to suggest $GABA_{B(1a)}$ and $GABA_{B(1b)}$ might represent pre- and postsynaptic subtypes, respectively, and evidence of functional heterogeneity has recently been published[13]. There are also indications that molecular subtypes might be pharmacologically distinguishable[14]. Investigation of structural determinants of ligand binding at $GABA_B$ receptors has indicated a venus fly-trap model, with specific serine and glutamine mutations differentially affecting agonist and antagonist binding, and serine 269 being crucial for the Ca^{2+} sensitivity of the receptor[15,16]. Interestingly, although both $mGluR_4$ and $GABA_{B(2)}$ can traffic $GABA_{B(1)}$ to the cell surface, only $GABA_{B(2)}$ and $GABA_{B(1)}$ can form a functional receptor[17]. It would be surprising if physiologically relevant $GABA_B$ receptor subtypes did not occur, and studies on the nature of ligand binding and splice variants will undoubtedly advance the quest for subtypes, which could confer different physiological and pharmacological properties.

$GABA_B$ receptor splice variants

Similar to the mGluR subtypes 1,4,5,7 and 8, $GABA_B$ receptor genes, $GABA_{B(1)}$ and $GABA_{B(2)}$ exist in different splice forms. The cloning of rat and human $GABA_{B(1)}$ showed that it could exist as two splice variants, $GABA_{B(1a)}$ and $GABA_{B(1b)}$ (Ref. 1). These differ at the N terminus, with the initial 147 amino acids of this region of $GABA_{B(1a)}$ being replaced with a different, shorter sequence of 18 amino acids in $GABA_{B(1b)}$. Human $GABA_{B(1a)}$ is encoded by 22 exons (20 exons in the rat[10]), the first four of which are missing from $GABA_{B(1b)}$, and the alternative N terminal region is produced from the fifth exon and fourth intron of $GABA_{B(1a)}$ (Ref. 18). There is a major difference between mGluR gene splicing and $GABA_B$ gene splicing – $GABA_{B(1)}$ can be spliced at the N terminus, whereas only C terminal splice variants of mGluRs have, as yet, been found[19]. More recently other $GABA_{B(1)}$ splice variants have emerged. Two variants based on rat $GABA_{B(1b)}$ were reported: a 31 amino acid insertion, which produces a variation between the fifth transmembrane domain (TMD) and the second extracellular loop [$GABA_{B(1c)}$], and a 566 base-pair insertion causing a divergent C terminus with a 25 amino acid insertion [$GABA_{B(1d)}$] (Ref. 9). The same $GABA_{B(1c)}$ variant was also found by other workers, and was shown to couple [in the presence of $GABA_{B(2)}$] to $K_{ir}3.1/3.2$ channels in *Xenopus* oocytes[10]. These workers showed that this insertion could occur in both $GABA_{B(1a)}$ and $GABA_{B(1b)}$. A different version of $GABA_{B(1c)}$ is reported in humans, which has a deletion in the N terminus of $GABA_{B(1a)}$. More recently, a $GABA_{B(1e)}$ variant has been reported, in which exon 11 is spliced out of $GABA_{B(1a)}$ in both rat and human tissue (Ref. 12). This induces a frame shift in exon 12, causing the introduction of two stop codons, and resulting in a truncated form of the $GABA_{B(1)}$ receptor (see Fig. 1 for splice variant summary).

There are currently three reported splice variants of the human $GABA_{B(2)}$ gene[11]. These differ in the extreme C terminal region; one has a deletion [$GABA_{B(2b)}$] from the original published sequence [$GABA_{B(2a)}$], and the other a divergent C terminus [$GABA_{B(2c)}$]. These variations do not affect the region of the coiled-coil, and are unlikely to disrupt the interaction of $GABA_{B(1)}$ and $GABA_{B(2)}$ and hence heterodimer formation, but interactions with proteins containing Gly-Leu-Gly-Phe motif PDZ domains could be affected. PDZ domains, which bind to short peptide sequences, are thought to be important for

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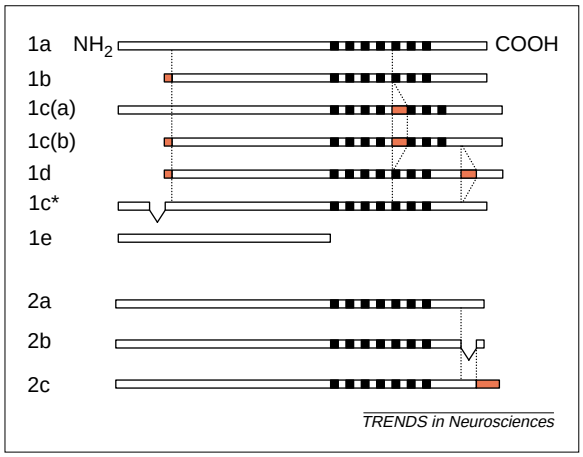

Fig. 1. Comparison of $GABA_{B(1)}$ and $GABA_{B(2)}$ splice variant protein structures. Black filled segments represent transmembrane domains, red filled segments represent spliced in regions, gaps linked by a 'V' represent spliced out regions. Dotted lines show points of identical sequence. 1c* has been found in human, but not rat. References: 1a/1b (Ref. 1), 1c(a)/1c(b) (Refs 9,10), 1d (Ref. 9), 2a/2b/2c (Ref. 11), 1e (Ref. 12).

the specific localization of proteins at synapses, including the insertion, endocytosis and recycling of target proteins[20].

Distribution and localization of $GABA_B$ receptor splice variants

The distribution of $GABA_B$ heterodimer component proteins, $GABA_{B(1)}$ and $GABA_{B(2)}$, is widespread throughout the rodent and human CNS (Refs 21,22), in agreement with receptor binding studies[23,24] (see Fig. 2). mRNAs encoding $GABA_{B(1)}$ and $GABA_{B(2)}$ colocalize in 95% of neurones in regions expressing both mRNA species[11]. mRNA encoding $GABA_{B(2)}$ is exclusively neuronal[11], but mRNA encoding $GABA_{B(1)}$ has been localized to both neurones and glia[1,11]. Brain regions in which $GABA_{B(1)}$ and $GABA_{B(2)}$ proteins do not coexist, might contain additional $GABA_B$ proteins that are capable of forming functional receptors. $GABA_{B(2)}$ mRNA is barely detectable in the rat caudate putamen (Fig. 2b), whereas $GABA_{B(1)}$ mRNA is relatively abundant in this region (Fig. 2a), leading to speculation as to the existence of further $GABA_B$ receptor proteins[11]. Both $GABA_{B(1)}$ and $GABA_{B(2)}$ immunoreactivity are present in the human caudate nucleus and putamen, and the diffuse non-cell body staining (neuropil) seen with a $GABA_{B(2)}$ antiserum, suggests primarily a nerve terminal localization[22]. Striatal output neurones and interneurones of different subclasses express $GABA_{B(1)}$ protein[25], however this has not been investigated in detail for $GABA_{B(2)}$. Low levels of $GABA_{B(2)}$ mRNA are also detected in hypothalamus, septum and brainstem (Fig. 2). These brain areas will be subjected to further close scrutiny, in case they contain potential protein partners for $GABA_{B(1)}$ or $GABA_{B(2)}$. Examination at the electron microscopic level using immunogold labelling techniques highlights receptor localization at the plasma membrane, but also there is some intracellular localization of $GABA_{B(1)}$ and $GABA_{B(2)}$ in the rat cerebellum[26] (Fig. 3). Much of the observed labelling is extrasynaptic, but both proteins are also detected in parallel fibre presynaptic terminals and at the edge of postsynaptic densities. $GABA_{B(1)}$ is also located both pre- and postsynaptically in the substantia nigra pars compacta (Fig. 3).

$GABA_{B(1a)}$ and $GABA_{B(1b)}$ mRNAs are widely distributed in the majority of brain regions, although at varying levels[28]. In the cerebellum, $GABA_{B(1a)}$ mRNA is predominantly located in the granule cells, and $GABA_{B(1b)}$ mRNA is mainly located in the Purkinje cells. Localization of the rat $GABA_{B(1c)}$ variant is similar to that of $GABA_{B(1a)}$ and $GABA_{B(1b)}$, which is not surprising, given that probes for $GABA_{B(1a/b)}$ variants would also detect the TMD5 insertion variants[10]. $GABA_{B(1b)}$ mRNA is found peripherally and centrally, whereas $GABA_{B(1d)}$ mRNA (divergent C terminal) only occurs in the eye, spleen and bladder outside the CNS. $GABA_{B(1a)}$ is the predominant mRNA species in the adult rat[29,30], whereas the predominant protein is $GABA_{B(1b)}$ (Ref. 31), which might be a result of the unknown stoichiometry between mRNA levels and protein synthesis. $GABA_{B(1e)}$ mRNA is distributed predominantly in peripheral organs such as lung, kidney and intestine, with much lower levels of transcript found in the CNS (Ref. 12).

Co-immunoprecipitation (co-ip) experiments using pan and splice variant-specific antibodies on solubilized native $GABA_B$ receptors, confirmed the association between $GABA_{B(2)}$ with either $GABA_{B(1a)}$

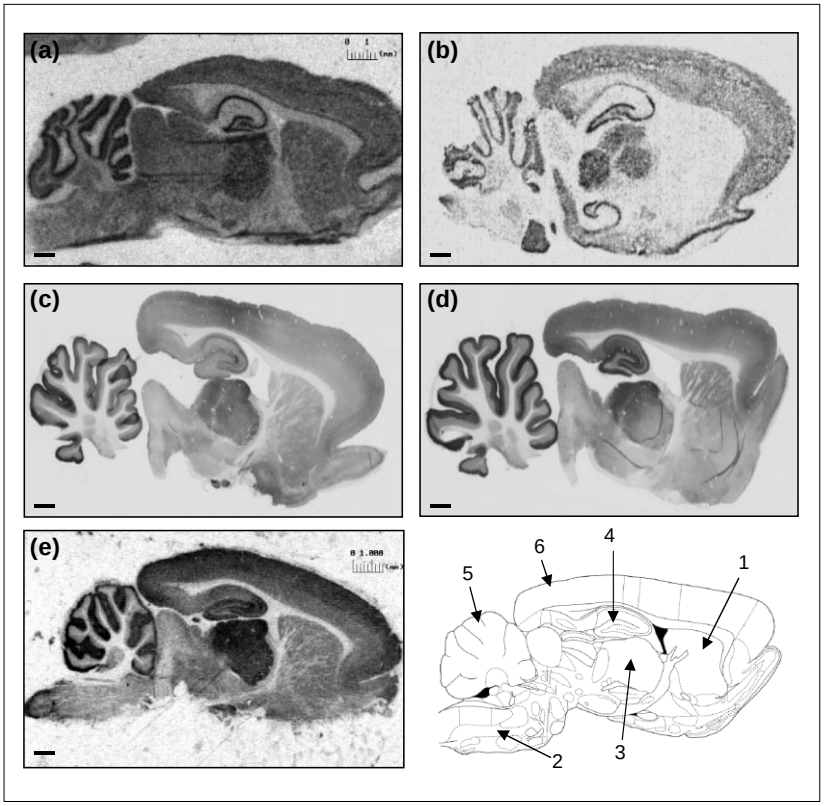

Fig. 2. Distribution of $GABA_{B(1)}$ and $GABA_{B(2)}$ mRNA by *in situ* hybridization using variant nonspecific oligonucleotides (a), (b), $GABA_{B(1)}$ and $GABA_{B(2)}$ proteins by immunohistochemistry using variant nonspecific antibodies (c),(d) and $GABA_B$ binding sites by receptor autoradiography (e). The mismatch of mRNA signals in the striatum is clear in (a) and (b). A comprehensive overlap of $GABA_{B(1)}$ and $GABA_{B(2)}$ protein localization is evident from immunohistochemistry with polyclonal antibodies (c) and (d), with cortex, hippocampus thalamus and cerebellum showing high levels of receptor subunit expression. Part (e) shows the binding of 0.5 nM [^{3}H]-CGP62349, a high affinity antagonist radioligand for $GABA_B$ receptors[42], which is in good agreement with immunohistochemical localization (c) and (d) [(e) image courtesy of Prof. N. Bowery, University of Birmingham, UK]. Labels: 1, striatum; 2, brainstem; 3, thalamus; 4, hippocampus; 5, cerebellum; 6, cortex. Scale bar, 1 mm.

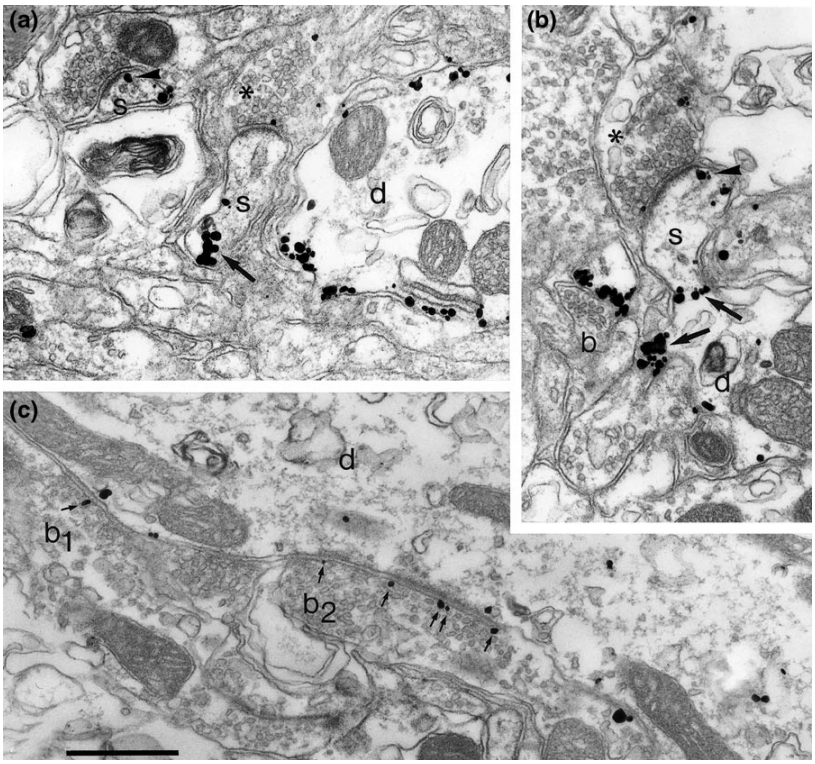

Fig. 3. Ultrastructural localization of immunolabelling for $GABA_B$ receptors in the molecular layer of the cerebellum (a) and (b) (Ref. 26) and in the substantia nigra pars compacta (c). (a) and (b) Immunolabelling for $GABA_{B(1)}$ (a) and $GABA_{B(2)}$ (b) in the molecular layer of the cerebellum is largely associated with Purkinje cell dendrites (labelled d) and spines (labelled s). Labelling is located at the plasma membrane but also at intracellular sites, including the spiny apparatus at the necks of spines (arrows). Immunolabelling also occurs presynaptically in terminals formed by parallel fibres (some indicated by asterisks). The immunolabelling is mostly extrasynaptic but sometimes occurs at the edge of postsynaptic densities (arrowheads). The small dendritic structure on the left in (b) is apposed by a bouton (labelled b), which is probably derived from a stellate cell or basket cell. Although not synaptic at this level, immunogold particles are associated with the membrane to which it is apposed. (c) Immunolabelling for $GABA_{B(1)}$ occurs at both pre- and postsynaptic sites in the substantia nigra pars compacta. The dendrite (labelled d) in longitudinal section has several immunoparticles associated with intracellular sites and the plasma membrane. The dendrite is apposed by several boutons but two of them (labelled b1 and b2) are also immunolabelled. Bouton b1 is in symmetric synaptic contact with the dendrite and contains a single immunoparticle at the active zone (small arrow). This bouton has the features of a terminal derived from the striatum or globus pallidus and is probably GABAergic[27]. Bouton b2 is in asymmetric synaptic contact with the dendrite and has several immunoparticles lined up against the presynaptic membrane (small arrows). This terminal has many of the features of a terminal derived from the subthalamic nucleus and is probably glutamatergic[27]. Scale bar, 0.5 μm.

or $GABA_{B(1b)}$ (Ref. 31). $GABA_{B(1a)}$ and $GABA_{B(1b)}$ proteins do not associate with each other, and repeated co-ip on resulting supernatants did not detect further $GABA_B$ protein, suggesting that virtually all $GABA_{B(1a)}$ and $GABA_{B(1b)}$ proteins were associated with $GABA_{B(2)}$ as heterodimers. Subcellular fractionation showed that $GABA_B$ receptors are localized to the synaptic membrane, and $GABA_{B(1a)}$ is enriched in the postsynaptic density (PSD), whereas $GABA_{B(1b)}$ was only weakly detected.

Are $GABA_B$ receptor subunits promiscuous?

Can $GABA_{B(1)}$ traffic to the membrane and form functional subtypes when expressed with other possible partners? Given that mGluR receptors can homodimerize[32], and the similarity between $GABA_B$ receptors and mGluRs, it is possible that an mGluR might dimerize with $GABA_{B(1)}$, conferring functional surface expression. Co-expression of $GABA_{B(1a)}$ and $mGluR_4$ in cell lines does indeed result in increased surface expression of $GABA_{B(1a)}$ at the cell membrane, although to a lesser extent than when expressed with $GABA_{B(2)}$ [four- to sevenfold increase with mGluR4 compared with an ~15-fold increase with $GABA_{B(2)}$] (Ref. 17). However, a heterodimer between $mGluR_4$ and $GABA_{B(2)}$ was not formed; nor was any function (inhibition of adenylate cyclase, K^+ channel activation) detected. These findings probably relate to the differences in $GABA_B$ and mGluR dimer formation: $GABA_B$ receptor dimerization is mediated via the C terminal coiled-coil, and mGluR homodimerization is mediated via N terminal cysteine disulphide bridges. The lack of functionality produced when $GABA_{B(1a)}$ is co-expressed with $mGluR_4$, might be the result of differential glycosylation. In addition, $GABA_{B(2)}$ might be required to alter the conformation of $GABA_{B(1)}$ to modulate ligand binding, or $GABA_{B(2)}$ might be required for correct G-protein coupling. Heterodimerization also occurs between δ- and κ-opioid receptors[33], and most recently hetero-oligomerization between different GPCR families [somatostatin receptor 5 (SSTR5) and dopamine D2 receptors] has become evident[34], and it is probable that other combinations of GPCRs will emerge. The possibility of $GABA_B$ receptor subunits interacting with other GPCRs remains an avenue for exploration.

The existence of the C terminal truncated $GABA_{B(1e)}$ splice variant is interesting, because in HEK293 cells, it can localize to the membrane in the presence or absence of $GABA_{B(2)}$ (Ref. 12). It can also heterodimerize with $GABA_{B(2)}$ via the extracellular domain and disrupt the normal $GABA_{B(1a)}$ – $GABA_{B(2)}$ interaction. However, $GABA_{B(1e)}$ does not bind the antagonist radioligand [^{3}H]-CGP54626, activate K^+ channels, and inhibit forskolin-stimulated cAMP formation[12]. It could in fact bind other ligands, and the absence of TMDs and C terminus could disrupt G-protein coupling, and alter the conformation of the binding domain. Interestingly, a truncated mutant similar to $GABA_{B(1e)}$ was detected by phototaffinity ligand binding using [^{125}I]-CGP71872 (see Ref. 1 for structure), suggesting that ligand binding is retained by C terminal truncated $GABA_B$ receptors[30]. The $GABA_{B(1e)}$ variant could play a role in regulating the availability of functional $GABA_{B(1)}$–$GABA_{B(2)}$ heterodimers, by competing with other $GABA_{B(1)}$ variants for $GABA_{B(2)}$. The ability of this truncated form of $GABA_{B(1)}$ to localize to the membrane in the absence of $GABA_{B(2)}$, occurs because in the absent part of the C terminus there is a retention sequence [RXR(R)], the absence of which allows membrane expression[35]. This retention sequence is masked by the formation of the coiled-coil between the C termini of $GABA_{B(1)}$ and $GABA_{B(2)}$ in heterodimerization, hence allowing surface expression of $GABA_B$ receptor heterodimers.

$GABA_{B(2)}$ probably plays a crucial role in the pharmacology and physiology of $GABA_B$ receptors. A single study has shown that $GABA_{B(2)}$ can inhibit

adenylate cyclase when expressed alone in CHO cells[7], but other studies found that neither conventional $GABA_B$ agonists nor antagonists bind to $GABA_{B(2)}$ (Refs 3,4), suggesting that $GABA_{B(2)}$ is simply a trafficking protein. This seems unlikely, given that a single transmembrane domain receptor activity modifying protein (RAMP) (Ref. 36) would equally perform the role without the need to produce a large, multiple TMD polypeptide. Furthermore, $GABA_{B(2)}$ [in addition $GABA_{B(1)}$], has a large extracellular domain incorporating a binding pocket, although the residues crucial for GABA and antagonist binding in $GABA_{B(1)}$ are not conserved in $GABA_{B(2)}$, perhaps suggesting that $GABA_{B(2)}$ is an orphan receptor, with an, as yet, unidentified endogenous ligand. The $GABA_{B(2)}$ C terminal splice variants might yet prove to mediate different intracellular signalling pathways.

Pre- and postsynaptic subtypes

The most widely studied splice variants are those that were first discovered: $GABA_{B(1a)}$ and $GABA_{B(1b)}$. The structural difference between the two proteins is the existence of complement-binding motifs (Sushi domains) in $GABA_{B(1a)}$ (Ref. 37), which are an integral part of the spliced-out N terminal region, and thus are absent from $GABA_{B(1b)}$. These domains might target $GABA_{B(1a)}$ to specific sub-cellular sites, or might modulate $GABA_{B(1a)}$ receptor pharmacology via interaction with extracellular proteins. Furthermore, mRNA localization studies suggest that, in some locations, $GABA_{B(1a)}$ and $GABA_{B(1b)}$ might represent pre- and postsynaptic $GABA_B$ receptors, respectively[38]. However, $GABA_{B(1a)}$ is found to be more abundant than $GABA_{B(1b)}$ in purified PSDs, suggesting that $GABA_{B(1a)}$ might be predominantly postsynaptic[31]. Functional studies have provided evidence that $GABA_{B(1b)}$ couples to K^+ channels in transfected cells, via which the classical postsynaptic $GABA_B$ receptor-mediated neurone hyperpolarization occurs[39], and $GABA_{B(1a,2)}$ couples to Ca^{2+} channels (resulting in presynaptic depression of neurotransmitter release) in sympathetic neurones[40]. However, both $GABA_{B(1a)}$ and $GABA_{B(1b)}$ will couple to K^+ or Ca^{2+} channels in transfected cells[12,40]. Other workers suggest that $GABA_{B(1b)}$ is located both pre- and postsynaptically using immunohistochemical studies[41]. The issue of $GABA_{B(1a)}$ and $GABA_{B(1b)}$ representing pre- and postsynaptic subtypes is not anatomically clear, and requires detailed immunohistochemical analysis at the electron microscope level with variant specific antibodies for clarification.

Pharmacological differentiation of recombinant $GABA_{B(1a)}$ and $GABA_{B(1b)}$ using conventional $GABA_B$ receptor ligands has proved difficult[42,43]. Most recently, the prescription anticonvulsant gabapentin [Neurontin; 1-(aminomethyl)cyclohexaneacetic acid], a structural analogue of GABA, was found to selectively activate $GABA_{B(1a,2)}$ heterodimers coupled to inwardly rectifying K^+ channels expressed in *Xenopus* oocytes, and not to activate $GABA_{B(1b,2)}$ or $GABA_{B(1c,2)}$ combinations[14]. This effect of gabapentin was not via presynaptic $GABA_B$ receptors, because monosynaptic $GABA_A$ IPSCs were not diminished in a rat hippocampal slice preparation. This data supports a postsynaptic location and function for $GABA_{B(1a,2)}$ receptors in the rat hippocampus, and is the first demonstration that molecular subtypes can be separated by pharmacological means. Recent evidence has also indicated differences in G-protein coupling between $GABA_{B(1a)}$ and $GABA_{B(1b)}$ (Ref. 13).

$GABA_B$ receptors are sensitive to Ca^{2+}

Both $GABA_{B(1)}$ and $GABA_{B(2)}$ share homology with the Ca^{2+}-sensing receptor and mGluRs, both of which are sensitive to extracellular Ca^{2+} concentration. The effect of including 1 mM Ca^{2+} in [^{35}S]-GTPγS binding assays was to decrease the EC_{50} of GABA ten-fold[44]. Ca^{2+} does not act as a ligand in its own right at $GABA_B$ receptors, and its effects are more prominent when added in combination with GABA, as opposed to in the presence of baclofen or $GABA_B$ receptor antagonists. The effect is exclusive to Ca^{2+} as opposed to other divalent cations. It has subsequently been shown that mutation of serine 269 to alanine (S269A) in lobe 1 of the binding pocket of $GABA_{B(1a)}$ abolishes the effects of Ca^{2+} (Ref. 16). This residue is conserved in *Caenorhabditis elegans* and *Drosophila melanogaster*, whereas other serine residues (S247, S268) are not conserved. The sensitivity of $GABA_B$ receptors to extracellular Ca^{2+} means that presynaptically, neurotransmitter release, and postsynaptically, neuronal excitability, can be regulated according to synaptic Ca^{2+} levels. For example, under conditions of low extracellular Ca^{2+}, such as immediately following ischaemia or seizure, $GABA_B$ receptor mechanisms might be impaired. However, 24 hrs post-ischaemia, $GABA_B$ receptor function, as indexed by the effect of baclofen on EPSPs, appears to be normal[45].

Structural determinants of ligand binding at $GABA_B$ receptors

mGluR receptors have a large extracellular N terminal domain, which is also a feature of $GABA_{B(1)}$ and $GABA_{B(2)}$. This large N terminal domain of $GABA_{B(1)}$ specifies agonist and antagonist binding to $GABA_B$ receptors as shown by the formation of truncated $GABA_{B(1)}$ mutants and $GABA_{B(1)}$–$mGluR_1$ receptor chimeras[30]. This domain has been modelled, and shares structural homology with the bacterial periplasmic leucine-binding protein (LBP), which forms two globular domains and a hinge region[15]. Point mutations of cysteine residues in this region caused a reduction in antagonist binding, and mutation of specific serine (S247) and glutamine (Q312) residues, lead to an increase in agonist binding affinity, and a decrease in antagonist binding affinity.

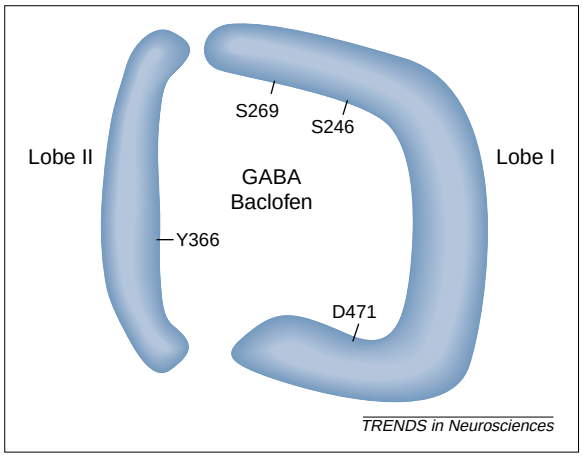

Fig. 4. View of the $GABA_B$ receptor showing the binding sites from above. Residues important for GABA and baclofen binding (serine S246, aspartic acid D471, tyrosine Y366), and Ca^{2+} sensitivity (serine S269) are shown.

The cysteine residues are probably involved in aiding stability of the binding pocket via disulphide bonds. The S247 and Q312 residues might play a role in the conformational state of the binding pocket, and could differentially affect agonist and antagonist binding by favouring the open or closed states of the binding pocket, respectively. Antagonist binding was abolished by mutation of S246, suggesting that this residue could interact directly with antagonist ligands. S246 also interacts via hydrogen bonding with the agonists GABA and baclofen, in addition to aspartate (D471), which when mutated affected the efficacy of GABA (Ref. 46). Both of these residues are present in lobe 1 of the binding pocket[46]. Tyrosine Y366 mutation in lobe 2, reduced the affinity of both GABA and baclofen, and converted baclofen into a competitive antagonist[46]. This evidence supports a 'venus fly-trap' model of binding, in which the two globular domains close upon agonist binding. Residues that are important for binding are shown in Fig. 4.

Developmental aspects

Brain $GABA_{B(1a)}$ and $GABA_{B(1b)}$ proteins differ in their abundance throughout the development of rats to adulthood. Initially, $GABA_{B(1a)}$ is more abundant until postnatal day 10 (P10) when $GABA_{B(1b)}$ becomes the predominant isoform[47]. Both proteins decline after P10 to adult levels, when $GABA_{B(1b)}$ is twice as abundant as $GABA_{B(1a)}$ (Refs 30,31). This decline is also evident from receptor binding studies[48,49]. The affinity of baclofen at both cloned $GABA_{B(1a)}$ and $GABA_{B(1b)}$ increases during development (P4 versus adult), with an approximately tenfold shift in IC_{50} values for both receptors; an effect which also occurs in native $GABA_B$ receptors[50]. Receptor levels are probably highest during the most intense period of brain development, and the switch from $GABA_{B(1a)}$ to $GABA_{B(1b)}$ might reflect differences in splice machinery regulation with development. Interestingly, $GABA_{B(1a)}$ and $GABA_{B(1b)}$ have different promoter regions, which allows these differential changes to occur[18]. $GABA_{B(1a)}$ [but not $GABA_{B(1b)}$] contain Sushi repeat motifs, which also occur in proteins associated with the extracellular matrix (for example neurocan[51]), and mediate protein–protein interaction or cell recognition and adhesion. Sushi repeats are also found in *hig*, a *Drosophila* protein which is secreted from presynaptic terminals during the period of synapse development[52,53]. Thus, Sushi repeats in $GABA_{B(1a)}$ might be involved in directing the protein to specific locations during synapse development.

Summary and conclusions

Undoubtedly further splice variants of both $GABA_{B(1)}$ and $GABA_{B(2)}$ will emerge. The challenge is to understand which neuronal systems they reside in, so that their functions can be elucidated. Such understanding will be aided via the development of specific and selective tools, such as antisera and knockout animals. Further work on pharmacological tools will be aided by mutagenesis studies of the binding pocket, and the effects of Sushi repeats on binding pocket conformation, which might provide the key to pharmacological differences. Also the truncated variant $GABA_{B(1e)}$ that lacks antagonist binding, might benefit from structural studies of the binding pocket. Whilst the work of Ng and colleagues[14] provides the first suggestion of pharmacological differences between splice variants, more specific tools are required to investigate the roles of heterodimers that contain subunits other than $GABA_{B(1a)}$. The finding that gabapentin only binds to $GABA_{B(1a)}$ is convincing, however the high-affinity gabapentin exhibits for the $\alpha_2\delta$ subunit of Ca^{2+} channels is not mentioned[54], and therefore its specificity as a pharmacological tool must be questioned. Unfortunately, the low-affinity nature of the interaction between $GABA_{B(1a)}$ and gabapentin, and the nanomolar affinity interaction between $\alpha_2\delta$ and gabapentin preclude the use of radioligand studies using [^{3}H]-gabapentin.

The issue of promiscuity amongst GPCRs might yet also have further bearing on $GABA_B$ receptor pharmacology. Interactions, such as that reported between SSTR5 and D2 receptors, must surely cloud the pharmacological effects of a given agonist. Co-expression of $GABA_{B(1)}$ and $mGluR_4$ resulted in membrane expression, without $GABA_B$ receptor function. The possibility of an interaction between $GABA_{B(1)}$ and other GPCRs remains a distinct possibility, and the possibility of splice variants having different interactions with GPCRs introduces another level of complexity to the situation.

The scene is set for $GABA_B$ receptor biology to advance in interesting and novel directions. The study of combinations of existing variants, and the possible discovery of additional proteins in this family, will provide more information about one of the more elusive targets in neuroscience, including its functional role and future therapeutic potential.

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