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Signal Transduction by $GABA_B$ Receptor Heterodimers

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$GABA_B$ receptors are G-protein-coupled receptors that mediate inhibition throughout the central and peripheral nervous systems. A single cloned receptor, $GABA_BR1$, which has at least three alternatively spliced forms, appears to account for the vast majority of binding sites in the brain for high-affinity antagonists. In heterologous expression systems $GABA_BR1$ is poorly expressed on the plasma membrane and largely fails to couple to ion channels. A second gene, $GABA_BR2$, which exhibits moderately low homology to $GABA_BR1$, permits surface expression of $GABA_BR1$ and the appearance of baclofen-sensitive K^+ and Ca^{++} currents. We review the data that supports a model of the native $GABA_B$ receptor as a heterodimer composed of $GABA_BR1$ and $GABA_BR2$ proteins. New data from mutagenesis experiments are presented that point to amino acid residues on $GABA_BR1$ critical for ligand activation of the heterodimer. The possible role of $GABA_BR2$ in signal transduction is also discussed. The interdependent nature of the two subunits for receptor function makes the $GABA_B$ receptor a useful model to explore the larger significance of GPCR dimerization for G-protein activation.
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Gamma-aminobutyric acid (GABA) is an abundant neurotransmitter that mediates inhibition throughout the nervous system via both ligand-gated channels ($GABA_A$ and $GABA_C$ receptors) and G-protein-coupled receptors ($GABA_B$ receptor). $GABA_B$ receptors have both pre- and postsynaptic actions (Bowery 1993; Price et al. 1984). Postsynaptic inhibition of neuronal firing is mediated primarily by the coupling of $GABA_B$ receptors to the activation of inwardly rectifying K^+ channels (GIRKs) (North 1989; Gahwiler and Brown 1985; Andrade et al. 1986). Presynaptic inhibition of neurotransmitter release by GABA is thought to occur by suppression of any of a number of identified high-threshold Ca^{++} channels (Harayama et al. 1998; Lambert and Wilson 1996; Mintz and Bean 1993; Dolphin and Scott 1987). The physiological importance of $GABA_B$ receptors has been widely appreciated in humans through the use of baclofen (Bowery et al. 1980), a selective agonist at $GABA_B$ receptors that is used clinically for the treatment of muscle spasticity and trigeminal neuralgia. The widespread distribution of $GABA_B$ receptors in both central and peripheral nervous systems reveals their importance in a variety of physiological processes.

There is now a substantial repertoire of ligands, having agonist or antagonist properties, that display high affinity and selectivity for $GABA_B$ receptors (Froestl and Mickel 1997). Although these chemical tools have been useful for studying the neurophysiology and molecular biology of $GABA_B$ receptors, they have failed for the most part to permit the identification of pharmacological subtypes. For example, using semi-intact tissue preparations it is possible to record robust pre- and postsynaptic responses to ba-

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clofen and high-affinity antagonists block these with roughly equal potency (Bon and Galvan 1996; Pozza et al. 1999; Seabrook et al. 1990). Although measurements of neurotransmitter release have permitted some groups to report selective antagonism (Fassio et al. 1994; Bonanno et al. 1996, 1997; Teoh et al. 1996; but see also Baumann et al. 1990), there is no general consensus about pharmacologically defined receptor subtypes (Bowery 1997). This is perhaps surprising for a receptor system that is both widespread and otherwise well characterized.

The identification of a gene encoding $GABA_B$ receptors proved to be at least as challenging as the pharmacological search for subtypes. Several groups attempted to expression clone a receptor using *Xenopus* oocytes (Uezono et al. 1998), or to biochemically characterize a protein from brain, affinity purified using a monoclonal antibody (Nakayasu et al. 1993). The cloning breakthrough came with the development of the photo affinity radioligand, CGP71872. Using this as a marker, Kaupmann and co-workers (Kaupmann et al. 1997) identified a clone, $GABA_BR1$, predicted to encode a heptahelical protein composed of 844 amino acids. This clone shares many features of other members of the metabotropic glutamate and Ca^{++}-sensing receptor family including a large extracellular, N-terminal domain having homology to bacterial amino acid binding proteins, such as LIVBP (O'Hara et al. 1993). When expressed heterologously in cell lines, the antagonist pharmacology of the cloned receptor was shown to be very similar to that of native receptors. In contrast, the apparent affinity of agonist ligands was about 100-fold less than expected from studies of brain membranes. When challenged with an agonist, $GABA_BR1$ also did not appear to be able to stimulate the expected cellular responses, such as K^+ channel activation or robust inhibition of the accumulation of cAMP, which suggested that a necessary component of signal transduction was missing.

The publication of $GABA_BR1$ set off a search by many groups for other genes having a related sequence. A query of public databases using the $GABA_BR1$ sequence led to the identification of a second, homologous protein, $GABA_BR2$ (Jones et al. 1998; Kaupmann et al. 1998a; Kuner et al. 1999; White et al. 1998; Martin et al. 1999; Ng et al. 1999). Like $GABA_BR1$, $GABAB_BR2$ was found to contain a region homologous to LIVBP within the large N-terminal extracellular domain.

Several groups have shown that when expressed in either oocytes or mammalian cells $GABA_BR2$ fails to produce the anticipated cellular responses to GABA (Jones et al. 1998; Kaupmann et al. 1998a; White et al. 1998; but see also Kuner et al. 1999; Martin et al. 1999). This deficit in coupling was especially surprising considering the lack of function activity of $GABA_BR1$. Hinting at a resolution of the functional expression problem was the spatial distribution of the mRNAs for both $GABA_BR1$ and $GABA_BR2$. The *in situ* localization patterns of both transcripts were highly convergent throughout the brain (Jones et al. 1998), suggesting that the two receptors were co-expressed in the same cellular regions. Double labeling of mRNAs and receptor proteins at cellular resolution provided proof that co-expression occurs in multiple cell types, including cerebellar Purkinje cells, hippocampal and cerebral cortical neurons, and the vast majority of neuronal somata within dorsal root ganglia (Jones et al. 1998; Kaupmann et al. 1998a; Durkin et al. 1999). We reasoned that fully functional $GABA_B$ receptors might somehow require the expression of both $GABA_BR1$ and $GABA_BR2$.

CO-EXPRESSION STUDIES

Co-expression of $GABA_BR1$ and $GABA_BR2$ in *Xenopus* oocytes and mammalian cells leads to the development of large amplitude GABA- and baclofen-sensitive GIRK currents (Jones et al. 1998; Kaupmann et al. 1998a; Kuner et al. 1999; White et al. 1998; Ng et al. 1999). These currents are rarely seen in cells expressing either gene alone (Kaupmann et al. 1998a, 1998b). Co-expression also permits robust inhibition of adenylyl cyclase (Kuner et al. 1999; Ng et al. 1999) as well as stimulation of GTPγS binding in cell membranes (White et al. 1998). The pharmacology of agonists at the $GABA_BR1/R2$ combination (Jones et al. 1998; Brauner-Osborne and Krogsgaard-Larsen 1999; Lingenhoehl et al. 1999) is comparable to that reported for native receptors (Bon and Galvan 1996; Seabrook et al. 1990). Antagonist affinity estimates for saclofen, CGP54626 and CGP55845 (Jones et al. 1998; Brauner-Osborne and Krogsgaard-Larsen 1999) are similar to values reported in previous electrophysiological studies using brain tissue (Bon and Galvan 1996; Seabrook et al. 1990), as well as to those obtained by measuring displacement of radioligand from cells expressing $GABA_BR1$ alone (Kaupmann et al. 1997).

Just as striking as the appearance of functional cellular responses is the shift in affinity for agonists. The co-expression of $GABA_BR2$ with $GABA_BR1$ now results in an apparent shift (10–30-fold) to a higher affinity state for the agonists GABA and baclofen (Kaupmann et al. 1998a; White et al. 1998). This observation led to the speculation that the two $GABA_B$ gene products might closely associate with one another (Kaupmann et al. 1998a). Additional evidence came from epitope tagging experiments that showed a high coincidence in the intracellular distribution of the two proteins when expressed heterologously (Jones et al. 1998).

PHYSICAL ASSOCIATION BETWEEN $GABA_BR1$ AND $GABA_BR2$

Using immunoprecipitation methods, several groups demonstrated that $GABA_BR1$ and $GABA_BR2$ specifically

associate in a protein complex, probably as heterodimers (Jones et al. 1998; Kaupmann et al. 1998a; Kuner et al. 1999; White et al. 1998). Furthermore, the heterodimers are concentrated on the plasma membrane, which suggests that the dimer is likely to be important for early events in signal transduction including ligand binding and G-protein activation (Jones et al. 1998). These studies provided the first compelling evidence that native G-protein-coupled receptors (GPCRs) can exist not only as homodimers, as is likely the case for other GPCRs (Gouldson and Reynolds 1997; Hebert and Bouvier 1998; Bai et al. 1998) but also as heterodimers composed of more than one receptor subunit.

Additional groups independently came to the conclusion that $GABA_B$ receptors are formed of heterodimers, and in the process provided a molecular mechanism for the subunit interaction. Experiments with the yeast two-hybrid system for identifying protein partners led to the discovery of domains on the extended C-termini of $GABA_BR1$ and $GABA_BR2$ that are responsible for their molecular association (White et al. 1998; Kuner et al. 1999). Both C-termini are predicted to contain coiled-coil motifs based on algorithms that reliably detect such features in the primary amino acid sequences of other more well known proteins that dimerize (Lupas 1997; Lupas et al. 1991). When either of the coiled-coil structures are deleted, the proteins no longer associate (Kuner et al. 1999). The yeast two-hybrid experiments also revealed that the associations are strictly heterophillic; there is no evidence that homodimers form.

The coiled-coil structure provides the first molecular substrate for GPCR dimerization; but among GPCRs this structure is so far unique to $GABA_B$ receptors (Table 1). In contrast, the coiled-coil is well conserved within $GABA_B$ receptors from different species (Figure 1). Additional types of protein–protein interactions must be responsible for dimerization of other GPCRs. In the case of the metabotropic glutamate/Ca^{++} sensor family of GPCRs, cysteine disulfide bridges are at least partly responsible for dimer formation. It is very likely that interactions between transmembrane alpha helices are also critical for dimer formation or stabilization (Gouldson and Reynolds 1997; Hebert et al. 1996; Maggio et al. 1996; George et al. 1998).

CONSEQUENCES OF HETERODIMERIZATION

The heterodimer model immediately raises a myriad of questions about the roles of the two receptor proteins in signal transduction. Does only $GABA_BR1$ bind ligand? Do both subunits bind G-protein? Does $GABA_BR2$ merely serve as a shuttle protein, or does it have an active role signaling? Does any part of the signal transduction cascade involve dissociation of the two subunits?

Answers to some of these questions are beginning to emerge. For example, heterodimerization appears to be important for receptor trafficking to the plasma membrane. Several groups have noted that $GABA_BR1$, when expressed by itself, does not reach the plasma membrane; instead, it accumulates within the cytoplasm, probably in association with the endoplasmic reticulum (Couve et al. 1998; White et al. 1998). Even when over-expressed in neurons, which might be expected to correctly process and transport neuronal proteins, there is a deficit of plasma membrane labeling (Couve et al. 1998). Using fluorescence activated cell sorting and antibodies that recognize an extracellular epitope of $GABA_BR1b$, White et al. (1998) observed that the inclusion of $GABA_BR2$ induces a significant increase in the surface expression of $GABA_BR1b$, and presumably, the heterodimer. In the absence of $GABA_BR2$, $GABA_BR1$ also exhibits an immature pattern of glycosylation (White et al. 1998). Thus, $GABA_BR2$ has an important role in regulating the expression of the mature, signaling heterodimer. In this regard, the assembly and transport of $GABA_B$ receptors may be similar to that of other proteins having multiple subunits, such as ion channels (Yu and Hall 1991). It is interesting to note that in a subset of neurons, such as hippocampal CA1 pyramidal cells and inhibitory interneurons,

Table 1. Analysis of GPCR Databases Using the COILS[a] Algorithm

GPCR Family	No. in Database[b]	Hits[c]	Description[d]
Rhodopsin	432	4	d1dr_fugru, m3dr_human, msr_xenla, opsd_octdo
Olfactory	491	0	
Secretin	29	0	
Chemosensor	8	0	
Frizzled	7	0	
mGluR/CaSR/$GABA_B$	32	7	$GABA_BR1$ (hum, rat, drosophila), $GABA_BR2$ (hum, rat, sponge, nematode)
Total	999	11	

[a] Lupas (1997).

[b] Search set includes GPCRs from all species in Genbank as of November 1999.

[c] Hits include those which show a >90% probability using a window size of 28 and weighting the first and fourth positions in the heptad repeat. Note that hits in the rhodopsin family do not extend to species homologues. Therefore, the significance for these receptors is less certain.

[d] Swiss Protein database nomenclature (except for $GABA_B$ receptors).

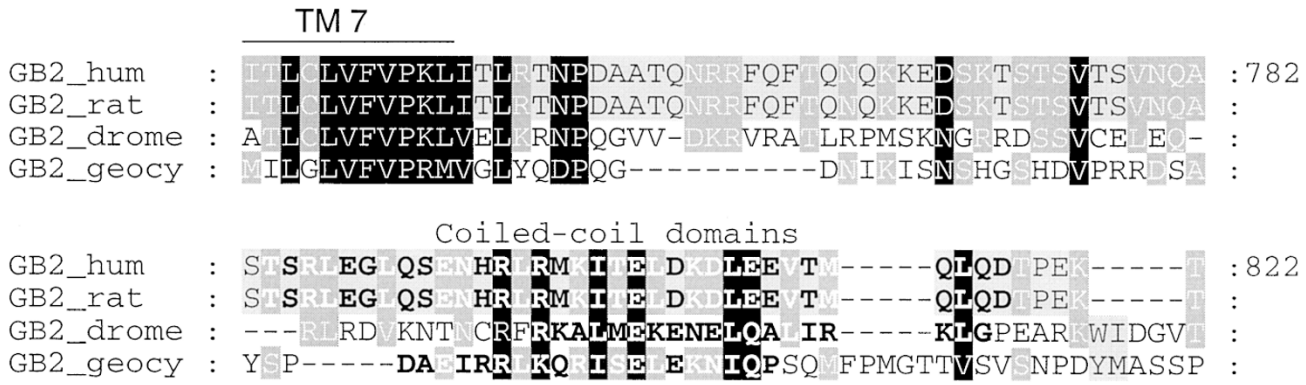

Figure 1. Alignment of portions of the C-terminal regions of $GABA_BR2$ from four species. Alignment was made using the default settings of Clustal W. Shading shows different levels of amino acid conservation. Residues in bold comprise the coiled-coil domain as predicted using the algorithm "COILS" (Lupas 1997). GB2_drome, $GABA_BR2$ from Drosophila melanogaster; GB2_geocy, Geodia cydonium (a marine sponge).

$GABA_BR1$-immunoreactive protein appears abundantly in the cytoplasm (Sloviter et al. 1999). Although the expression levels of $GABA_BR2$ protein have not been published for these cells, the CA1 cells do exhibit a much lower level of mRNA encoding this protein as compared to cells in nearby CA3 (Durkin et al. 1999; Kuner et al. 1999). These observations lead to the speculation that $GABA_BR2$ protein may be constitutively low in certain cell types resulting in an inefficient transport of $GABA_BR1$ to distal plasma membrane surfaces (Sloviter et al. 1999). The actual extent to which $GABA_BR2$ protein regulates expression of the functional heterodimer remains to be determined.

Immunochemical methods have demonstrated that native $GABA_B$ receptors in the nervous system exist largely as heterodimers. Throughout the CNS there is a striking degree of overlap of $GABA_BR1$ and $GABA_BR2$ immunoreactivity at a gross structural level (Benke et al. 1999). In the cerebellar molecular layer co-localization of $GABA_BR1$ and $GABA_BR2$ immunoreactivity has been observed in ultra-thin sections of Purkinje cell spines (Kaupmann et al. 1998a) providing support for co-assembly at particular postsynaptic structures. Since $GABA_B$ receptors are so widespread in the CNS, a useful approach to estimate the overall proportion of receptors that are composed of heterodimers versus homodimers is to perform quantitative immunoprecipitations on fractions containing solubilized receptors. Using this method, Benke and colleagues (Benke et al. 1999) observe that essentially all $GABA_BR1$ protein is immunoprecipitated with antibodies that recognize $GABA_BR2$. Thus, the proportion of receptors in the brain that exist either as $GABA_BR1$ or $GABA_BR2$ monomers is thought to be quite low.

FUNCTIONS OF THE HETERODIMER SUBUNITS

High-affinity agonists and antagonists bind the $GABA_BR1$ subunit in a region which exhibits structural homology to the ligand-binding domain of metabotropic glutamate receptors and bacterial periplasmic amino acid binding proteins (Galvez et al. 1999). And, since the antagonists are capable of completely blocking cellular responses to GABA or baclofen, it is clear that the ligand-binding domain on the N-terminus of $GABA_BR1$ is essential for initiating signal transduction by the heterodimer. $GABA_BR2$ does not bind radiolabeled $GABA_BR1$ antagonists, nor does it appear to bind [^{3}H]-GABA (Jones et al. 1998; Kaupmann et al. 1998; White et al. 1998). There have, however, been reports that $GABA_BR2$, when expressed alone, can be stimulated by GABA (Kaupmann et al. 1998a; Kuner et al. 1999; Martin et al. 1999). Does $GABA_BR2$ contribute a binding site for GABA or another ligand? The first possibility explored was sensitivity to Ca^{++} since $GABA_BR2$ exhibits some sequence similarity to the Ca^{++}-sensing receptor (Ruat et al. 1995). In fact, Ca^{++} can be seen to strongly modify the response of the heterodimer to GABA, but this effect can be attributed entirely to specific amino acid residues within the ligand-binding region of $GABA_BR1$ (Wise et al. 1999; Galvez et al. 2000).

To explore a ligand-binding role for $GABA_BR2$, we performed mutations within the region of $GABA_BR1b$ that encodes the ligand-binding domain, and then assessed the functional status of the heterodimer by expression with $GABA_BR2$. The mutations that were performed are described in Figure 2. Using [3H]-CGP54626 as a radiolabel, specific binding was reduced by more than 85% in membranes expressing any of the $GABA_BR1b$ mutants (data not shown). Others have shown that the S246A and Y266A mutations cause a >1000-fold decrease in affinity for antagonists using a different radioligand (Galvez et al. 1999). To determine if the mutations created a parallel decrease in functional activity, receptors were assayed for Ca^{++}-mobilization in a fluorescence plate reader using Fluo-3 dye and a chimeric G-protein (Conklin et al. 1993), $G\alpha_{q/i3(5)}$, that permits coupling of the heterodimer to activation of phospholipase C. Co-expression of non-

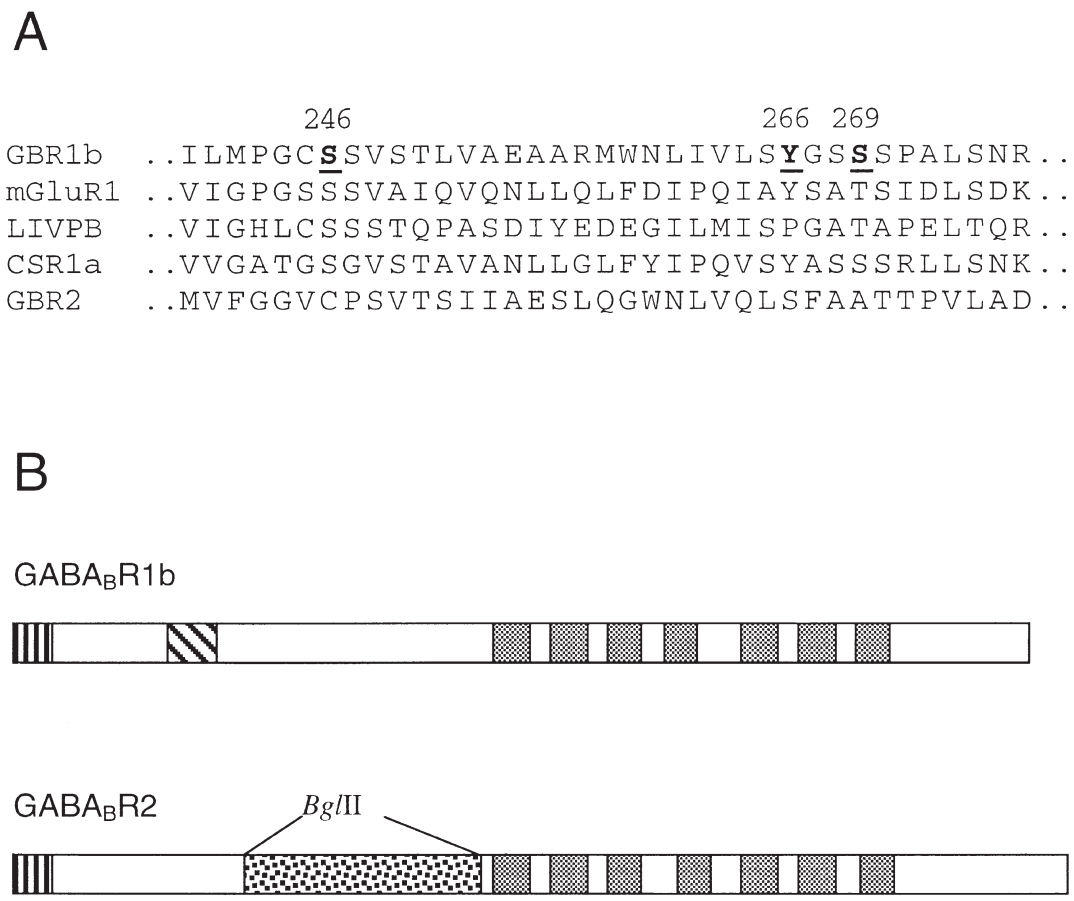

Figure 2. **(A)** Alignment of a portion of the ligand-binding domains of $GABA_BR1b$ (GBR1b), $GABA_BR2$ (GBR2), mGluR1, Ca^{++} sensor (CSR1a), and LIVBP. Bold letters in $GABA_BR1$ indicate residues that were mutated in this study. **(B)** Schematic of $GABA_BR1$ and $GABA_BR2$ showing the approximate locations of the region shown in detail in (A) (diagonals) and the *Bgl*II fragment (stippled region) deleted from $GABA_BR2$. *Bgl*II digestion of plasmid containing the $GABA_BR2$ cDNA removes 771 nucleotides encoding amino acids 226–482 in the protein. Signal peptide sequence (vertical stripes) and transmembrane regions (gray boxes) are indicated. All mutant and deletion constructs were verified by sequence analysis.

mutated $GABA_BR1b$ with $GABA_BR2$ resulted in concentration-dependent increases in liberated Ca^{++} with GABA and baclofen. EC_{50} values for these agonists were similar to those reported using other methods. In contrast, co-expression of either S246A or Y266A mutants with wild-type $GABA_BR2$ failed to stimulate Ca^{++} release using concentrations of GABA or baclofen up to 1000-fold above their EC_{50} values (Figure 3). These mutant receptors appeared to be expressed based on the appearance of cellular immunofluorescence using antibodies directed against the epitope-tagged C-terminus (data not shown). Thus, the most likely explanation for the lack of functional activity even at 1000-fold higher concentrations of agonist is that activation of the heterodimer is absolutely dependent on agonist binding to the $GABA_BR1b$ subunit. Others have shown that the S269A substitution induces a selective reduction in affinity to GABA while sparing baclofen binding (Galvez et al. 1999). When expressed with $GABA_BR2$, a similar shift in the EC_{50} of GABA, but not baclofen, was observed for the Ca^{++} mobilization response (Figure 3). The parallel changes in agonist potency measured either as binding to $GABA_BR1b$, or as activation of the heterodimer, provides additional support for the view that $GABA_BR2$ cannot directly contribute to receptor activation by either of these ligands.

The coiled-coil domain on the C-terminus of $GABA_BR2$ is necessary for binding $GABA_BR1$ and for formation of functional heterodimers (Kuner et al. 1999; White et al. 1998). Protein assemblies mediated by coiled-coil domains are generally thought to be thermodynamically quite stable. This and the observed predominance of $GABA_B$ receptor heterodimers over monomers (Benke et al. 1999) strongly suggests that the $GABA_B$ receptor is likely to remain as a heterodimer throughout the cycle of agonist binding, receptor activation and G-protein stimulation. What direct role, if any, does $GABA_BR2$ have in any of these events? The lack of conservation of the agonist-

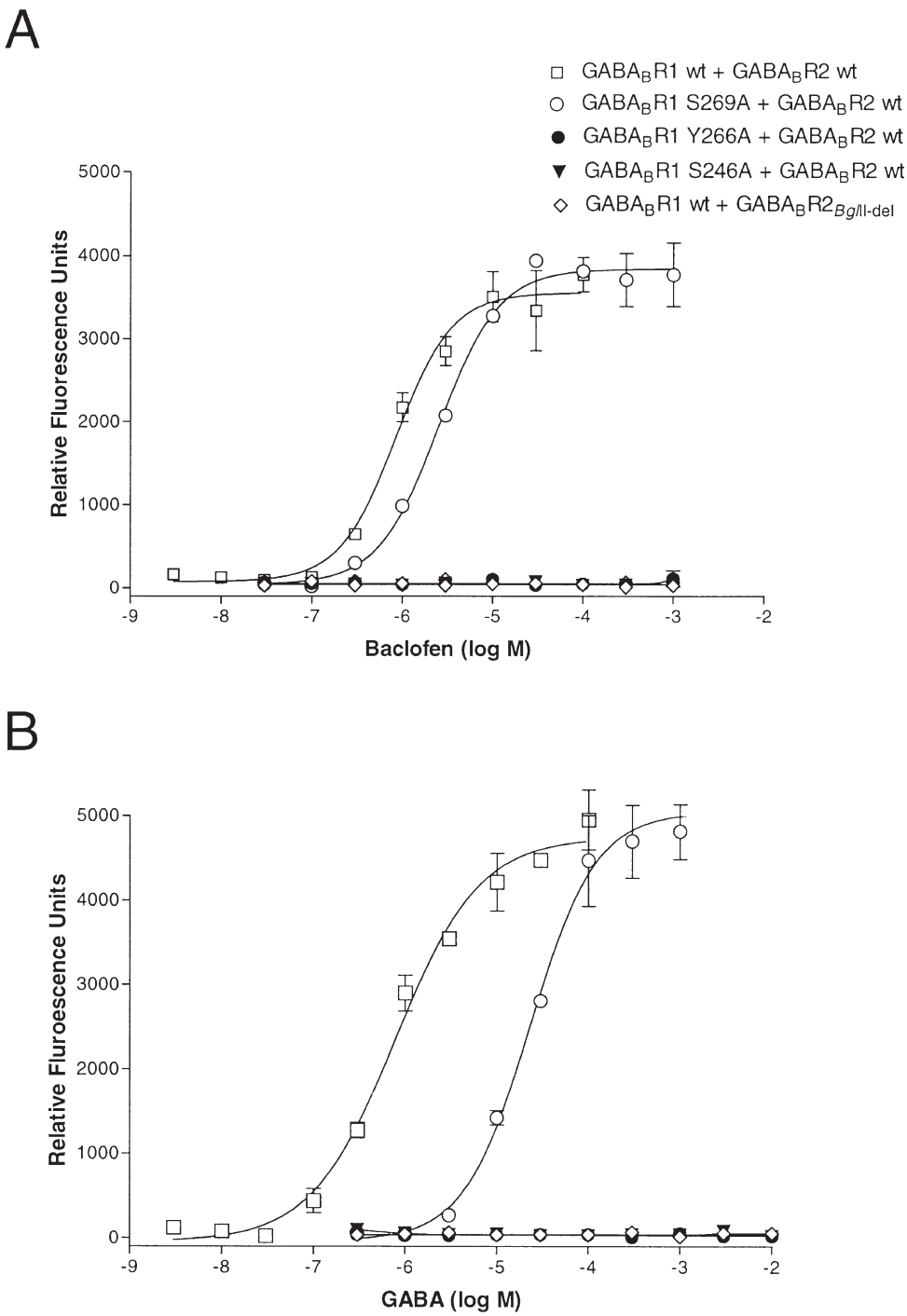

Figure 3. Activation of wild-type and mutant receptors by GABA and baclofen. Intracellular Ca^{++} release was measured by Fluo-3 dye fluorescence using a FLIPR (molecular devices). Cells were transiently transfected with plasmids containing wild-type or mutant GABA$_B$R1b and wild-type or truncated GABA$_B$R2 and chimeric $G\alpha_{q/i3(5)}$. Data are expressed as means ± S.E.M. of a quadruplicate determination from a single experiment. Similar results were obtained in three separate experiments.

binding domain of GABA$_B$R1 in GABA$_B$R2 would indicate that point mutations might not be an efficient way to map out domains important for signaling by GABA$_B$R2.

As a first step toward determining domains on GABA$_B$R2 important for signal transduction, we performed a deletion of 257 amino acids within the N-terminal domain of GABA$_B$R2 (Figure 2B). The expression pattern of this deletion mutant (GABA$_B$R2$_{Bgl}$II-del) was similar to that of wild-type GABA$_B$R2 as judged by the appearance of cellular immunofluorescence using antibodies directed against the epitope-tagged C-terminus (data not shown). In COS-7 cells co-expressing GABA$_B$R1b

and $GABA_BR2_{Bgl}$II-del no evidence was found for receptor activation by either GABA or baclofen using the Ca^{++}-mobilization assay (Figure 3). Loss of this region within the N-terminus of $GABA_BR2_{Bgl}$II-del may prevent heterodimerization, or it may produce a more subtle change in structure of the heterodimer that prevents stimulation of G-protein.

SUMMARY

$GABA_B$ receptors may be thought of as being composed of two subunits, a ligand-binding subunit, $GABA_BR1$, and a "structural" subunit, $GABA_BR2$. $GABA_BR1$ appears to determine the pharmacological properties of the receptor, while $GABA_BR2$ is important for proper expression of $GABA_BR1$ on the plasma membrane. Additional deletion and mutagenesis experiments will be required to determine to what extent $GABA_BR2$ actually participates in signal transduction events.

Suddenly it appears that the field of G-protein-coupled receptors is playing catch-up with its brethren plasma membrane proteins: ion channels, receptor tyrosine kinases and transporters, most of which occur as oligomeric assemblies. $GABA_B$ receptors are now joined by a growing list of GPCRs which are thought to exist as heterodimers, including receptors for endogenous opiates (Jordan and Devi 1999), dopamine and somatostatin (Rocheville et al. 2000) and serotonin (Xie et al. 1999). The recent finding that heterodimerization can change receptor affinities for agonists (Jordan and Devi 1999) indicates that the heteromeric nature of GPCRs is likely to have major implications for drug design in the near future. Given the fact that heterodimerization is now seen to occur in two divergent families of GPCRs, it seems likely that additional examples will become known with further study.

REFERENCES

Andrade R, Malenka RC, Nicoll RA (1986): A G protein couples serotonin and $GABA_B$ receptors to the same channels in hippocampus. Science 234:1261–1265

Bai M, Trivedi S, Brown EM (1998): Dimerization of the extracellular calcium-sensing receptor (CaR) on the cell surface of CaR-transfected HEK293 cells. J Biol Chem 273:23605–23610

Baumann PA, Wicki P, Stierlin C, Waldmeier PC (1990): Investigations on GABAB receptor-mediated autoinhibition of GABA release. Naunyn Schmiedebergs Arch Pharmacol 341:88–93

Benke D, Honer M, Michel C, Bettler B, Mohler H (1999): gamma-aminobutyric acid type B receptor splice variant proteins GBR1a and GBR1b are both associated with GBR2 in situ and display differential regional and subcellular distribution. J Biol Chem 274:27323–27330

Bon C, Galvan M (1996): Electrophysiological actions of GABAB agonists and antagonists in rat dorso-lateral septal neurones in vitro. Br J Pharmacol 118:961–967

Bonanno G, Fassio A, Schmid G, Severi P, Sala R, Raiteri M (1997): Pharmacologically distinct GABAB receptors that mediate inhibition of GABA and glutamate release in human neocortex. Br J Pharmacol 120:60–64

Bonanno G, Gemignani A, Schmid G, Severi P, Cavazzani P, Raiteri M (1996): Human brain somatostatin release from isolated cortical nerve endings and its modulation through GABAB receptors. Br J Pharmacol 118:1441–1446

Bowery NG (1993): GABAB receptor pharmacology. Ann Rev Pharmacol Toxicol 33:109–147

Bowery NG (1997): Pharmacology of $GABA_B$ receptors. In Enna SJ, Bowery NG (eds), The GABA Receptors. Totowa, NJ, USA, Humana Press, pp 209–236

Bowery NG, Hill DR, Hudson AL, Doble A, Middlemiss DN, Shaw J, Turnbull M (1980): (-)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. Nature 283:92–94

Brauner-Osborne H, Krogsgaard-Larsen P (1999): Functional pharmacology of cloned heterodimeric GABAB receptors expressed in mammalian cells. Br J Pharmacol 128:1370–1374

Conklin BR, Farfel Z, Lustig KD, Julius D, Bourne HR (1993): Substitution of three amino acids switches receptor specificity of Gq alpha to that of Gi alpha. Nature 363:274–276

Couve A, Filippov AK, Connolly CN, Bettler B, Brown DA, Moss SJ (1998): Intracellular retention of recombinant GABAB receptors. J Biol Chem 273:26361–26367

Dolphin AC, Scott RH (1987): Calcium channel currents and their inhibition by (-)-baclofen in rat sensory neurones: modulation by guanine nucleotides. J Physiol (Lond) 386:1–17

Durkin MM, Gunwaldsen CA, Borowsky B, Jones KA, Branchek TA (1999): An in situ hybridization study of the distribution of the GABA(B2) protein mRNA in the rat CNS. Brain Res Mol Brain Res 71:185–200

Fassio A, Bonanno G, Cavazzani P, Raiteri M (1994): Characterization of the GABA autoreceptor in human neocortex as a pharmacological subtype of the GABAB receptor. Eur J Pharmacol 263:311–314

Froestl W, Mickel SJ (1997): Chemistry of $GABA_B$ modulators. In Enna SJ, Bowery BJ (eds), The GABA Receptors. Totowa, NJ, USA, Humana Press, pp 271–296

Gahwiler BH, Brown DA (1985): GABAB-receptor-activated K+ current in voltage-clamped CA3 pyramidal cells in hippocampal cultures. Proc Natl Acad Sci USA 82:1558–1562

Galvez T, Parmentier ML, Joly C, Malitschek B, Kaupmann K, Kuhn R, Bittiger H, Froestl W, Bettler B, Pin JP (1999): Mutagenesis and modeling of the GABAB receptor extracellular domain support a venus flytrap mechanism for ligand binding. J Biol Chem 274:13362–13369

Galvez T, Urwyler S, Prezeau L, Mosbacher J, Joly C, Malitschek B, Heid J, Brabet I, Froestl W, Bettler B, Kaupmann K, Pin J-P (2000): Ca2+-requirement for high affinity GABA binding at GABAB receptors: involve-

ment of serine 269 of the GABABR1 subunit. Mol Pharmacol 57:419–426

George SR, Lee SP, Varghese G, Zeman PR, Seeman P, Ng GY, O'Dowd BF (1998): A transmembrane domain-derived peptide inhibits D1 dopamine receptor function without affecting receptor oligomerization. J Biol Chem 273:30244–30248

Gouldson PR, Reynolds CA (1997): Simulations on dimeric peptides: evidence for domain swapping in G-protein-coupled receptors? Biochem Soc Trans 25:1066–1071

Harayama N, Shibuya I, Tanaka K, Kabashima N, Ueta Y, Yamashita H (1998): Inhibition of N- and P/Q-type calcium channels by postsynaptic GABAB receptor activation in rat supraoptic neurones. J Physiol (Lond) 509:371–383

Hebert TE, Bouvier M (1998): Structural and functional aspects of G protein-coupled receptor oligomerization. Biochem Cell Biol 76:1–11

Hebert TE, Moffett S, Morello JP, Loisel TP, Bichet DG, Barret C, Bouvier M (1996): A peptide derived from a beta2-adrenergic receptor transmembrane domain inhibits both receptor dimerization and activation. J Biol Chem 271:16384–16392

Jones KA, Borowsky B, Tamm JA, Craig D, Durkin MM, Dai M, Yao W-J, Johnson M, Gunwaldsen C, Huang L Y, Tang C, Shen Q, Salon JA, Morse K, Laz TM, Smith KE, Nagarathnam N, Noble SA, Branchek T, Gerald C (1998): $GABA_B$ receptors function as a heteromeric assembly of the subunits $GABA_BR1$ and $GABA_BR2$. Nature 396:674–679

Jones KA, Borowsky B, Tamm JA, Craig DA, Durkin MM, Dai M, Yao WJ, Johnson M, Gunwaldsen C, Huang LY, Tang C, Shen Q, Salon JA, Morse K, Laz T, Smith KE, Nagarathnam D, Noble SA, Branchek TA, Gerald C (1998): GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2. Nature 396:674–679

Jordan BA, Devi LA (1999): G-protein-coupled receptor heterodimerization modulates receptor function. Nature 399:697–700

Kaupmann K, Huggel K, Heid J, Flor PJ, Bischoff S, Mickel SJ, McMaster G, Angst C, Bittiger H, Froestl W, Bettler B (1997): Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. Nature 386:239–246

Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, Mosbacher J, Bischoff S, Kulik A, Shigemoto R, Karschin A, Bettler B (1998a): GABA(B)-receptor subtypes assemble into functional heteromeric complexes. Nature 396:683–687

Kaupmann K, Schuler V, Mosbacher J, Bischoff S, Bittiger H, Heid J, Froestl W, Leonhard S, Pfaff T, Karschin A, Bettler B (1998b): Human gamma-aminobutyric acid type B receptors are differentially expressed and regulate inwardly rectifying K+ channels. Proc Natl Acad Sci USA 95:14991–14996

Kuner R, Kohr G, Grunewald S, Eisenhardt G, Bach A, Kornau HC (1999): Role of heteromer formation in GABAB receptor function. Science 283:74–77

Lambert NA, Wilson WA (1996): High-threshold Ca2+ currents in rat hippocampal interneurones and their selective inhibition by activation of GABA(B) receptors. J Physiol (Lond) 492:115–127

Lingenhoehl K, Brom R, Heid J, Beck P, Froestl W, Kaupmann K, Bettler B, Mosbacher J (1999): Gamma-hydroxybutyrate is a weak agonist at recombinant GABA(B) receptors. Neuropharmacology 38:1667–1673

Lupas A (1997): Predicting coiled-coil regions in proteins. Curr Opin Struct Biol 7:388–393

Lupas A, Van Dyke M, Stock J (1991): Predicting coiled coils from protein sequences. Science 252:1162–1164

Maggio R, Barbier P, Fornai F, Corsini GU (1996): Functional role of the third cytoplasmic loop in muscarinic receptor dimerization. J Biol Chem 271:31055–31060

Martin SC, Russek SJ, Farb DH (1999): Molecular identification of the human GABABR2: cell surface expression and coupling to adenylyl cyclase in the absence of GABABR1. Mol Cell Neurosci 13:180–191

Mintz IM, Bean BP (1993): GABAB receptor inhibition of P-type Ca2+ channels in central neurons. Neuron 10: 889–898

Nakayasu H, Nishikawa M, Mizutani H, Kimura H, Kuriyama K (1993): Immunoaffinity purification and characterization of gamma-aminobutyric acid (GABA)B receptor from bovine cerebral cortex. J Biol Chem 268: 8658–8664

Ng GY, Clark J, Coulombe N, Ethier N, Hebert TE, Sullivan R, Kargman S, Chateauneuf A, Tsukamoto N, McDonald T, Whiting P, Mezey E, Johnson MP, Liu Q, Kolakowski LFJ, Evans JF, Bonner TI, O'neill GP (1999): Identification of a GABAB receptor subunit, gb2, required for functional GABAB receptor activity. J Biol Chem 274: 7607–7610

North RA (1989): Drug receptors and the inhibition of nerve cells. Br J Pharmacol 98:13–23

O'Hara PJ, Sheppard PO, Thogersen H, Venezia D, Haldeman BA, McGrane V, Houamed KM, Thomsen C, Gilbert TL, Mulvihill ER (1993): The ligand-binding domain in metabotropic glutamate receptors is related to bacterial periplasmic binding proteins. Neuron 11:41–52

Pozza MF, Manuel NA, Steinmann M, Froestl W, Davies CH (1999): Comparison of antagonist potencies at pre- and post-synaptic GABA(B) receptors at inhibitory synapses in the CA1 region of the rat hippocampus. Br J Pharmacol 127:211–219

Price GW, Wilkin GP, Turnbull MJ, Bowery NG (1984): Are baclofen-sensitive GABAB receptors present on primary afferent terminals of the spinal cord? Nature 307:71–74

Rocheville M, Lange D, Kumar U, Patel SC, Patel RC, Patel YC (2000): Receptors for dopamine and somatostatin: formation of hetero-oligomers with enhanced functional activity. Science 288:154–157

Ruat M, Molliver ME, Snowman AM, Snyder SH (1995): Calcium sensing receptor: molecular cloning in rat and localization to nerve terminals. Proc Natl Acad Sci USA 92:3161–3165

Seabrook GR, Howson W, Lacey MG (1990): Electrophysiological characterization of potent agonists and antagonists at pre- and postsynaptic GABAB receptors on neurones in rat brain slices. Br J Pharmacol 101:949–957

Sloviter RS, Ali-Akbarian L, Elliott RC, Bowery BJ, Bowery NG (1999): Localization of GABA(B) (R1) receptors in the rat hippocampus by immunocytochemistry and high resolution autoradiography, with specific reference to its localization in identified hippocampal interneuron subpopulations. Neuropharmacology 38:1707–1721

Teoh H, Malcangio M, Bowery NG (1996): GABA, glutamate and substance P-like immunoreactivity release: effects of novel GABAB antagonists. Br J Pharmacol 118:1153–1160

Uezono Y, Akihara M, Kaibara M, Kawano C, Shibuya I, Ueda Y, Yanagihara N, Toyohira Y, Yamashita H, Taniyama K, Izumi F (1998): Activation of inwardly rectifying K+ channels by GABA-B receptors expressed in Xenopus oocytes. Neuroreport 9:583–587

White JH, Wise A, Main MJ, Green A, Fraser NJ, Disney GH, Barnes AA, Emson P, Foord SM, Marshall FH (1998): Heterodimerization is required for the formation of a functional GABA(B) receptor. Nature 396:679–682

White JH, Wise A, Main MJ, Green A, Fraser NJ, Disney GH, Barnes AA, Emson P, Foord SM, Marshall FH (1998): Heterodimerization is required for the formation of a functional GABA(B) receptor [see comments]. Nature 396:679–682

Wise A, Green A, Main MJ, Wilson R, Fraser N, Marshall FH (1999): Calcium sensing properties of the GABA(B) receptor. Neuropharmacology 38:1647–1656

Xie Z, Lee SP, O'Dowd BF, George SR (1999): Serotonin 5-HT1B and 5-HT1D receptors form homodimers when expressed alone and heterodimers when co-expressed. FEBS Lett 456:63–67

Yu XM, Hall ZW (1991): Extracellular domains mediating epsilon subunit interactions of muscle acetylcholine receptor. Nature 352:64–67