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PERSPECTIVE

Positive Allosteric Modulators for γ-Aminobutyric Acid$_B$ Receptors Open New Routes for the Development of Drugs Targeting Family 3 G-Protein-Coupled Receptors

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The main inhibitory neurotransmitter in the central nervous system, γ-aminobutyric acid (GABA), activates two types of receptors, the GABA$_A$ and GABA$_B$ receptors. GABA$_A$ receptors are ligand-gated chloride channels responsible for the fast inhibitory action of GABA. These receptors are composed of five subunits and can be modulated by several allosteric modulators, such as the benzodiazepines, widely used for the treatment of several brain diseases including insomnia, epilepsy, and anxiety. The GABA$_B$ receptors are G-protein-coupled receptors (GPCRs) that are negatively coupled to adenylyl cyclase and regulate the activity of Ca^{2+} and K^+ channels. They modulate neuronal excitability and neurotransmitter release when located in presynaptic terminals. GABA$_B$ receptors are specifically activated by baclofen (Lioresal), used for the treatment of spasticity of patients suffering from multiple sclerosis (Bettler et al., 1998). GABA$_B$ agonists may also be useful for the treatment of epilepsy, depression, drug addiction, and pain (Couve et al., 2000). Recently, GABA$_B$ receptor knockout mice were shown to develop a generalized epilepsy that can result in premature death (Prosser et al., 2001; Schuler et al., 2001). However, the usefulness of GABA$_B$ receptor agonists may well be limited by the desensitization or down-regulation of the receptor resulting from its constant activation. In this issue of *Molecular Pharmacology*, Urwyler et al. (2001) describe a first series of positive allosteric modulators of the GABA$_B$ receptor, CGP7930 and CGP13501. These compounds do not activate the receptor on their own; rather, they potentiate the efficacy and affinity of agonists on the GABA$_B$ receptor, as observed with the benzodiazepines on the GABA$_A$ receptor. Such molecules open new possibilities for drug development, not only in the field of the GABA$_B$ receptor, but also in the field of the structurally related metabotropic glutamate (mGlu) receptors.

The molecular characterization of a functional GABA$_B$ receptor was achieved only recently (Kaupmann et al., 1997; Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998; Kuner et al., 1999; Marshall et al., 1999; Ng et al., 1999), and revealed a macromolecule much more complex than any other GPCR. Indeed, this receptor is the first GPCR that works exclusively as an heteromer constituted of at least two subunits, GB1 and GB2 (Fig. 1a). This heteromer is the main, if not the only, GABA$_B$ receptor in the mammalian brain (Kaupmann et al., 1998; Prosser et al., 2001; Schuler et al., 2001). Indeed, only splice variants of both subunits have been identified (Billinton et al., 2001) but no other subtype. Although no subtype selective ligands are expected, we will see below that the structural complexity of the GABA$_B$ receptor offers multiple possibilities for the development of GABA$_B$ modulators.

Except for the Sushi domains located at the N terminus of the GB1a variant (Kaupmann et al., 1997; Hawrot et al., 1998) (Fig. 1a), both GB subunits share sequence similarity with the mGlu and Ca^{2+}-sensing (CaS) receptors and have therefore been classified as members of the family 3 GPCRs (Bockaert and Pin, 1999). These receptors contain a 7TM region that is probably related structurally to rhodopsin (De Blasi et al., 2001), and a large extracellular domain (ECD) containing the ligand binding site. Modeling studies (O'Hara et al., 1993; Galvez et al., 1999; Bessis et al., 2000) and the recent determination of the structure of the mGlu1 ECD (Kunishima et al., 2000), revealed structural similarities between the ECD and bacterial periplasmic amino acid binding proteins (PBPs). Like PBPs, the ECD is composed of two globular lobes linked by a hinge region, allowing it to adopt an open or closed conformation. Binding of the agonist within the crevice that separates both lobes probably stabilizes the closed conformation of the ECD (Fig. 1a). Like the GABA$_B$

ABBREVIATIONS: GABA, γ-aminobutyric acid; GPCR, G-protein coupled receptor; mGlu, metabotropic glutamate; CaS, Ca^{2+}-sensing; ECD, extracellular domain; PBP, periplasmic binding protein; GB1, γ-aminobutyric acid$_B$1 subunit; 7TM, 7 transmembrane.

receptors, the other family 3 GPCRs have been shown to exist as dimers in vivo, although in those cases, only homodimers have been described (Romano et al., 1996, 2001; Bai et al., 1998, 1999; Ray et al., 1999; Ray and Hauschild, 2000; Tsuji et al., 2000).

Why are two subunits required to get a functional $GABA_B$ receptor? The interaction of GB1 with GB2 is necessary to mask an intracellular retention signal located in the C-terminal tail of GB1 (Margeta-Mitrovic et al., 2000; Calver et al., 2001; Pagano et al., 2001) (Fig. 1a). However, GB2 is needed not only for GB1 to reach the cell surface, but also for the formation of a functional receptor. Indeed, only the 7TM of GB2 was shown to activate G-proteins, whereas that of GB1 simply led to a higher coupling efficacy (Galvez et al., 2001). In addition, only the GB1 ECD contains a GABA binding site (Galvez et al., 1999, 2000a), but its association with the GB2 ECD increases agonist affinity (Galvez et al., 2001). Finally, the presence of both GB1 and GB2 ECDs is necessary to maintain the receptor in an inactive state and to allow GABA to activate the receptor (Galvez et al., 2001).

These observations fit nicely with a recent model for family 3 GPCR activation based on the structure of the mGlu1 ECD (Kunishima et al., 2000). Indeed, the structures with and without glutamate showed ECD homodimers in both cases, and a large change in conformation of the dimer upon glutamate binding. Glutamate would not only stabilize a closed state of at least one ECD in the dimer but also change the respective orientation of the two ECDs (Kunishima et al., 2000). Thus, the C-terminal ends of the ECDs (which are normally connected to the 7TM region) become closer by more than 25 Å. This large change in conformation would stabilize the active state of the dimeric 7TM regions (Fig. 1a).

Thus, the complex structure of the $GABA_B$ receptor offers multiple possibilities to develop drugs that modulate receptor activity, aside from drugs acting directly on the GB1 GABA binding site. Analyzing these different possibilities may give clues on the possible mechanism of action of the allosteric modulators described by Urwyler et al. (2001).

A first possibility would be to target the Sushi domains of the GB1a variant, leading to drugs selective for the GB1a-containing receptor. Although it is not yet possible to predict what would be the consequence of drugs acting at that level, the anticonvulsant GABApentin has recently been proposed to be a specific GB1a activator (Ng et al., 2001), suggesting that an action at the level of the Sushi domains may not

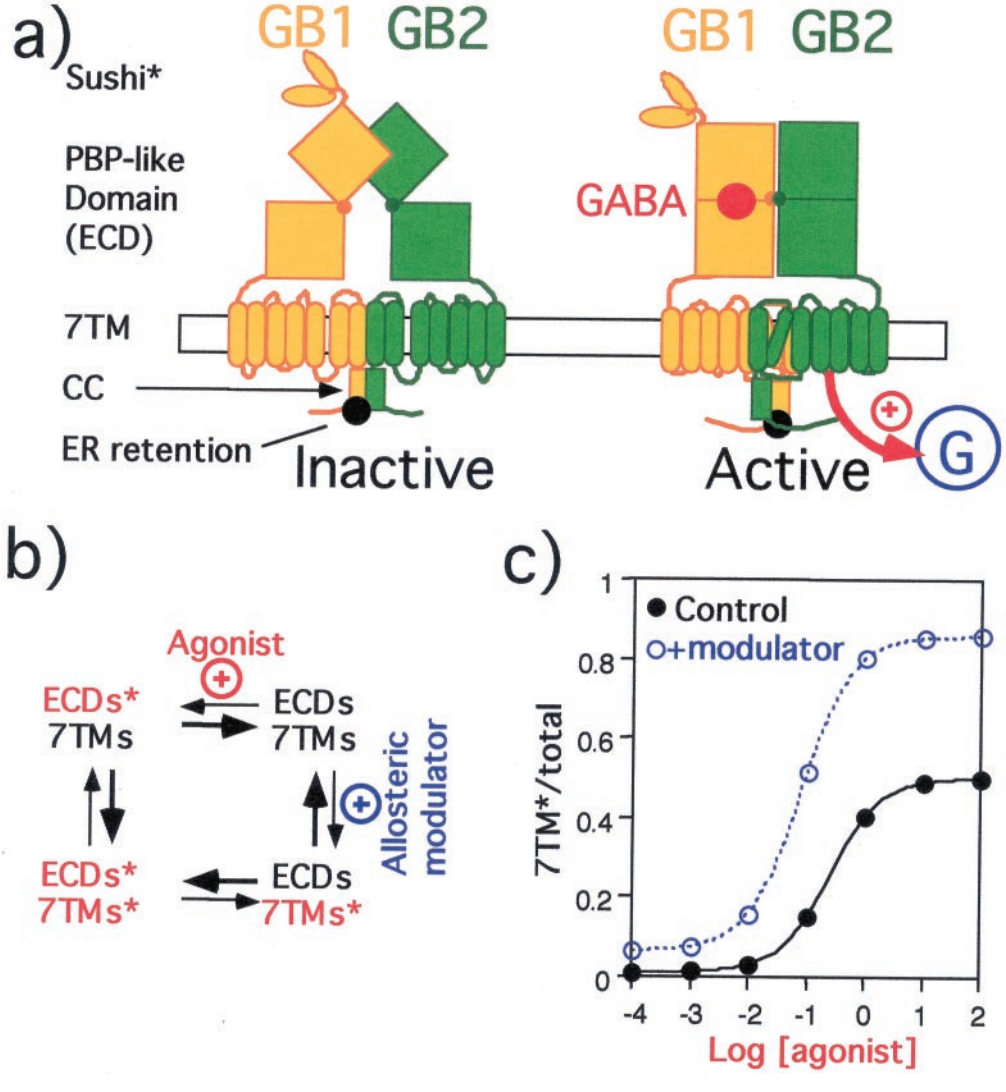

Fig. 1. General structure of the $GABA_B$ receptor and a possible mode of action of positive allosteric modulators. A, general structure of a dimeric form of the $GABA_B$ receptor showing the GB1 and GB2 subunits in orange and green, respectively. The Sushi domains specific for GB1a variant are indicated (*). The PBP-like ECD of both subunits are shown in an open conformation in the inactive state (left), and in a closed conformation in the active state (right). The helices involved in the coiled-coil interaction between the two subunits are indicated (CC), as well as the intracellular retention signal of the GB1 subunit (ER retention, black circle). On the right scheme, GABA is bound on the GB1 ECD exclusively, whereas the GB2 7TM activates the G-protein, as proposed recently. B, a simple model for $GABA_B$ receptor activation in which the dimeric ECDs exists either in an inactive (ECDs) or active (ECDs*) conformation. Similarly, the dimeric 7TM region exists in either an inactive (7TMs) or active (7TMs*) conformation. In this model, the active ECDs* stabilizes the active 7TMs (7TMs*). The action of an agonist will be to stabilize the active ECDs, whereas the positive allosteric modulator would stabilize the active 7TMs*. C, theoretical dose-response curves than can be obtained according to the model presented in B, without the positive modulator (black curve and symbols) or with a positive modulator (blue curve and symbols).

result in a positive allosteric action like that observed with the CGP compounds.

A second possibility is to stabilize a specific conformation of the dimeric GB1-GB2 ECD. Compounds further stabilizing the closed state of the agonist bound GB1 ECD would be expected to increase the agonist affinity. Indeed, Ca^{2+} was shown to increase GABA affinity, and a residue located in the GABA binding pocket (Ser269) is involved in this effect (Galvez et al., 2000b). It is possible that Ca^{2+} binds in the same cavity as that of GABA and further stabilizes the closed state of the GB1 ECD. However, in contrast to the CGP compounds, Ca^{2+} affects neither baclofen affinity nor coupling efficacy, indicating that the CGP compounds do not act like Ca^{2+}. Other compounds that may modify the functioning of the dimeric ECD are those that possibly interact in the GB2 ECD. These may affect the positive allosteric effect of GB2 on GB1. Such compounds may change the agonist affinity on GB1, as observed with the CGP compounds (Urwyler et al., 2001); however, it is difficult to predict their effect on the coupling efficacy.

The 7TM region of the $GABA_B$ subunits is probably structurally related to rhodopsin. A third possibility, then, will be to find compounds interacting within this region of either GB1 or GB2, such as most rhodopsin-like receptor ligands. Compounds preventing the formation of the active state of the 7TM region would be noncompetitive antagonists, but not positive allosteric modulators. Such molecules have been recently identified for group-I mGlu receptors (Pagano et al., 2000; Carroll et al., 2001) and shown to also display inverse agonist activity. They have been used recently to demonstrate that intracellular proteins (the Homer proteins) regulate the activity of these receptors in neurons (Ango et al., 2001), revealing the fascinating new possibility for a 7TM receptor to be activated by an intracellular signal.

Like agonists of rhodopsin-like receptors, compounds interacting in the 7TM regions of the $GABA_B$ subunits may also stabilize their active state (Fig. 1b). In the absence of agonist, such molecules are not expected to fully activate the receptor because, as mentioned earlier, the dimeric GB1–GB2 ECDs prevents the 7TM region from reaching the active state (Galvez et al., 2001). However, such compounds would facilitate the activation of the receptor by agonists, and further stabilize the active state of the full receptor complex. This may increase agonist affinity and efficacy (Fig. 1c). In agreement with this idea, positive allosteric modulators of the CaS receptor have been described and called calcimimetic (Hammerland et al., 1998; Nemeth et al., 1998). Such molecules have no significant agonist activity, but potentiate both the efficacy and potency of Ca^{2+} and interact in the 7TM region of the receptor (Hammerland et al., 1998). This hypothesis fits nicely with the data obtained with the CGP compounds (Fig. 1c), suggesting that these may act within the 7TM region of one of the $GABA_B$ subunits. Because the increase in agonist affinity is not observed when the GB1 subunit is expressed alone (Urwyler et al., 2001), it is likely that these modulators bind in the GB2 subunit. However, further work is necessary to clarify this fascinating issue.

The $GABA_B$ positive modulators represent the second example of artificial compounds potentiating the action of agonists at family 3 GPCRs. More work is required now to identify the mechanism of actions of such molecules; as discussed above, however, several possibilities exist, all offering new targets for the development of new $GABA_B$ modulators. Another important issue will be to examine whether endogenous molecules with similar properties exist. Finally, according to the structural similarities between $GABA_B$ and the other family 3 receptors, it is likely that similar molecules will be found for other receptors of this family, such as mGlu receptors. Agonists for group-II and group-III mGlu receptors are expected to have therapeutic application for the treatment of epilepsy, ischemia, anxiety, schizophrenia, Parkinson's disease (Conn and Pin, 1997; Pin and Bockaert, 2002). The therapeutic usefulness of agonists may well be limited by receptor desensitization. In contrast, positive allosteric modulators, by potentiating the action of endogenous agonists, may be much more efficient, as already exemplified by the benzodiazepines acting at the $GABA_A$ receptors.

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