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Review

$GABA_A$ receptors: Subtypes provide diversity of function and pharmacology

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ABSTRACT

This mini-review attempts to update experimental evidence on the existence of $GABA_A$ receptor pharmacological subtypes and to produce a list of those native receptors that exist. $GABA_A$ receptors are chloride channels that mediate inhibitory neurotransmission. They are members of the Cys-loop pentameric ligand-gated ion channel (LGIC) superfamily and share structural and functional homology with other members of that family. They are assembled from a family of 19 homologous subunit gene products and form numerous receptor subtypes with properties that depend upon subunit composition, mostly hetero-oligomeric. These vary in their regulation and developmental expression, and importantly, in brain regional, cellular, and subcellular localization, and thus their role in brain circuits and behaviors. We propose several criteria for including a receptor hetero-oligomeric subtype candidate on a list of native subtypes, and a working $GABA_A$ receptor list. These criteria can be applied to all the members of the LGIC superfamily. The list is divided into three categories of native receptor subtypes: "Identified", "Existence with High Probability", and "Tentative", and currently includes 26 members, but will undoubtedly grow, with future information. This list was first presented by Olsen & Sieghart (in press).

1. $GABA_A$ receptors

Gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain, mediates inhibition via $GABA_A$ receptors ($GABA_A$-R). These are ligand-gated chloride ion channels that were first identified pharmacologically as being activated by GABA and the selective agonist muscimol, blocked by bicuculline and picrotoxin, and modulated by benzodiazepines, barbiturates, and certain other CNS depressants (Macdonald and Olsen, 1994; Sieghart, 1995). $GABA_A$-R mediate rapid phasic inhibitory synaptic transmission, and also tonic inhibition by producing currents in extrasynaptic and perisynaptic locations (Mody and Pearce, 2004; Farrant and Nusser, 2005). Due to their widespread localization throughout the mammalian nervous system, $GABA_A$-R play a major role in virtually all brain physiological functions and serve as targets of numerous classes of drugs, both used clinically and important as research tools (Hevers and Luddens, 1998; Olsen and Homanics, 2000).

2. Heterogeneity of $GABA_A$ receptors

$GABA_A$ receptors are composed of five protein subunits that belong to different subunit classes. There are 19 genes for $GABA_A$-R subunits (Simon et al., 2004). These include 16 subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π) combined as $GABA_A$, and 3 rho (ρ) subunits, which contribute to what have sometimes been called $GABA_C$ receptors; the latter are considered by the Nomenclature Committee of IUPHAR to be subtypes of $GABA_A$-R containing the ρ subunits, and they recommend against using the term '$GABA_C$ receptors', especially in the title, abstract, or initial mention of these receptors in publications (Barnard et al., 1998; Olsen and Sieghart, in press).

The assembly of $GABA_A$-R as heteropentamers produces complex heterogeneity in their structure, which is the major determinant of their pharmacological profile (Barnard et al., 1998; Olsen and Sieghart, in press). The various receptor subtypes differ in abundance in cells throughout the nervous system and thus in functions related to the circuits involved. Available techniques do not allow us to unequivocally establish the subunit composition and arrangement of $GABA_A$ receptors in vivo, but this review summarizes our attempts to establish criteria for designating a proposed receptor hetero-oligomeric subtype as a native receptor, and our current opinion of which subtypes meet these criteria (Olsen and Sieghart, in press).

3. Identification of native $GABA_A$ receptor subtypes by their regional and cellular distribution

In situ hybridization (Wisden et al., 1992; Persohn et al., 1992) and immunohistochemical studies (Pirker et al., 2000; Fritschy et al., 1992) have indicated that α1, β1, β2, β3, and γ2 subunits are

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found throughout the brain, although differences in their distribution were observed. Subunits α2, α3, α4, α5, α6, γ1, and δ are more confined to certain brain areas and in some brain regions, a complementary distribution of α2, α4, β3, and δ versus α1, β2, and γ2 subunits was detected (Sieghart and Sperk, 2002). The δ subunit is frequently co-distributed with the α4 subunit, e.g., in the thalamus, striatum, outer layers of the cortex and in the dentate molecular layer. In the cerebellum, however, it is co-distributed with the α6 subunit (Pirker et al., 2000).

An important criterion for association of subunit isoforms into oligomeric native receptors is co-localization of the subunits. Immunocytochemical studies investigating the co-localization of subunits in $GABA_A$ receptor clusters on neuronal membranes (Fritschy et al., 1992; Bohlhalter et al., 1996), as well as electron microscopic studies (Nusser et al., 1995; Somogyi et al., 1996), indicate that the majority of $GABA_A$ receptors present in the brain is composed of α,β, and γ subunits. Receptors composed of α1, β2, and γ2 subunits are extensively co-localized among subsets of GABAergic interneurons in hippocampus and other brain regions, e.g., calretinin-, neuropeptideY-, somatostatin-positive cells, but not on calbindin-D28k-, cholecystokinin- and vasoactive intestinal peptide-containing cells (Gao and Fritschy, 1994), supporting the conclusion that α1β2γ2 receptors are the most abundant $GABA_A$ receptors in the brain (Pirker et al., 2000).

In the raphe nuclei the vast majority of serotonergic neurons express strong α3 subunit immunoreactivity but are devoid of α1 subunit staining. In contrast, both the α1 and the α3 subunits are expressed in GABAergic neurons (Gao et al., 1993). In other studies it was demonstrated that 84–95% of the cholinergic neurons in the basal forebrain expressed the α3 subunit but not the α1 subunit. In contrast, parvalbumin-positive GABAergic neurons in these brain regions were frequently co-stained with the α1-subunit and to a lesser extent with the α3 subunit antibody (Gao et al., 1995). The α3 subunit, however, not only is associated with serotonergic or cholinergic neurons, but also with noradrenergic and dopaminergic neurons in the brainstem (Gao et al., 1995). In addition, data indicating an overlapping distribution of α3, θ, and ε $GABA_A$ receptor subunits in the dorsal raphe and the locus coeruleus (Moragues et al., 2000, 2002), suggest that novel $GABA_A$ receptor subtypes that so far have not been studied in detail, may regulate neuroendocrine and modulatory systems in the brain.

4. Identification of native $GABA_A$ receptor subtypes by their synaptic and extrasynaptic localizations

Other studies indicated that the individual subunits exhibit a distinct subcellular distribution. For instance, in cerebellar granule cells α1, α6, β2/3 and γ2 subunits have been found by immunogold localizations to be concentrated in GABAergic Golgi synapses and also are present in the extrasynaptic membrane at lower concentration. In contrast, δ subunits could not be detected in synaptic junctions, although they were abundantly present in the extrasynaptic dendritic and somatic membranes (Nusser et al., 1998). Receptors containing the δ subunit also contain α6 and β subunits (Jechlinger et al., 1998; Poltl et al., 2003). Receptors containing δ subunits exhibit a smaller single channel conductance and a much longer open time, and do not desensitize on the prolonged presence of GABA (Saxena and Macdonald, 1994). Together with the exclusive extrasynaptic localization of these receptors, these properties indicate that tonic inhibition observed in these cells is mediated mainly by the persistent activation of α6βδ receptors by GABA that is present in the extracellular space of glomeruli (Nusser et al., 1998; Brickley et al., 1999).

Extrasynaptic receptors have been identified also in other brain regions. In the forebrain it is assumed that they are predominantly composed of α4βδ. Experiments indicating that tonic conductance sometimes can also be enhanced by benzodiazepines suggest that tonic inhibition can also be produced by gamma subunit-containing receptors. In the hippocampus evidence has accumulated that such receptors might be composed of α5βγ2 subunits. But it can be assumed that receptors with other subunit composition also can occur extrasynaptically (Semyanov et al., 2004). Due to the much larger cell surface area, the charge carried by the activation of tonically active $GABA_A$-R can be more than three times larger than that produced by phasic inhibition (Nusser and Mody, 2002; Rossi et al., 2003).

5. Identification of native $GABA_A$ receptor subtypes by their subunit composition

A variety of $GABA_A$ receptor subunit-specific antibodies have been generated and have been used for purifying $GABA_A$ receptor subtypes from brain membrane extracts by immunoprecipitation or immunoaffinity chromatography. These studies indicated an extreme promiscuity of the various subunits. Although the antibodies used were highly specific for the respective subunits, most if not all of the other subunits investigated could be co-purified with antibodies directed against an individual α or β subunit, suggesting that α and β subunits can combine with most of the other subunits to form a variety of different receptor subtypes. These studies also indicate that two different α and two different β subunits can be present in $GABA_A$ receptors. In contrast, most studies agreed that γ subunits could not be co-precipitated with other γ subunits. Similarly, δ subunits seem not to be present together with γ subunits in the same receptors (for review see Sieghart and Sperk, 2002). From these results a subunit stoichiometry of 2α, 2β and 1γ or 1δ subunit can be deduced for native receptors. This conclusion was confirmed in several recombinant receptor studies, some of which also were able to determine the subunit arrangement of receptors composed of 2α, 2β and 1γ subunits (Tretter et al., 1997; Baumann et al., 2002).

Studies investigating the abundance of $GABA_A$-R subtypes using subunit-specific antibodies confirmed that receptors composed of α1β2γ2 subunits are the most abundant $GABA_A$-R in the brain. Similarly, α2βγ2, α3βγ2, α4βγ2, α5βγ2, α6βγ2, α4βδ and α6βδ seem to be abundant, but to a lower extent (for review see Sieghart and Sperk, 2002). Given the promiscuity of subunits discussed above, and the receptor subtypes so far identified, it was estimated that more than 800 distinct $GABA_A$-R subtypes might exist in the brain (Barnard et al., 1998). Most of these receptors are not very abundant, but due to the widespread distribution and quantitative importance of the GABAergic system, even minor $GABA_A$-R subtypes probably exhibit abundance comparable with that of the major norepinephrine, dopamine, serotonin or peptide receptors.

6. Structural basis of $GABA_A$ receptor pharmacology

The $GABA_A$-R are members of the Cys-loop pentameric LGIC superfamily, including nicotinic acetylcholine receptors, inhibitory glycine receptors, and ionotropic 5-HT_3 (serotonin) receptors. They differ in structure from two additional LGIC families: the tetrameric glutamate receptors and the trimeric purine receptors (see mini-reviews on these families in this volume). All of the 44 subunit members of the Cys-loop pentameric LGIC superfamily (Collingridge et al., 2009) show sequence homology in the order of 30% identity, but even greater similarity at the level of secondary and tertiary structures.

All are organized as pentameric membrane-spanning proteins surrounding a central pore which forms the ion channel through the membrane. They all use similar sequences and functional domains to establish membrane topology, ion channel structure, agonist binding sites, and even binding sites for diverse allosteric