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GABA_A Receptors: Subtypes Provide Diversity of Function and Pharmacology

Richard W. Olsen and

Department of Molecular & Medical Pharmacology Geffen School of Medicine at UCLA, Los Angeles, California, USA

Werner Sieghart

Division of Biochemistry and Molecular Biology Center for Brain Research, Medical University of Vienna Vienna, Austria

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GABA_A Receptors

Gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain, mediates rapid 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 & 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 & Pearce, 2004; Farrant & 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 & Lüddens, 1998; Olsen & Martin, 2000).

Heterogeneity of GABA_A receptors

GABA_A receptors are composed of five protein subunits that can belong to different subunit classes. There are 19 genes for GABA_A-R subunits (Simon *et al.*, 2004). These include 16 subunits (α 1–6, β 1–3, γ 1–3, 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 & Sieghart, 2008).

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Corresponding Author: Dr. Richard W. Olsen, Dept of Molecular & Medical Pharmacology, Geffen School of Medicine at UCLA, Room CHS 23-120, 650 Young Drive South, Los Angeles, CA 90095-1735 USA, Tel. 1-310-825-5093; FAX 1-310-267-2003; rolsenS@mednet.ucla.edu.

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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 & Sieghart, 2008). 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 heterooligomeric subtype as a native receptor, and our current opinion of which subtypes meet these criteria (Olsen & Sieghart, 2008).

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 $\alpha 1$, $\beta 1$, $\beta 2$, $\beta 3$, and $\gamma 2$ subunits are found throughout the brain, although differences in their distribution were observed. Subunits $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 6$, $\gamma 1$, and δ are more confined to certain brain areas and in some brain regions, a complementary distribution of $\alpha 2$, $\alpha 4$, $\beta 3$, and δ versus $\alpha 1$, $\beta 2$, and $\gamma 2$ subunits was detected (Sieghart and Sperk, 2002). The δ subunit is frequently co-distributed with the $\alpha 4$ 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 $\alpha 6$ 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 are composed of α , β , and γ subunits. Receptors composed of $\alpha 1$, $\beta 2$, and $\gamma 2$ 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 $\alpha 1\beta 2\gamma 2$ 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 $\alpha 3$ subunit immunoreactivity but are devoid of $\alpha 1$ subunit staining. In contrast, both the $\alpha 1$ and the $\alpha 3$ subunits are expressed in GABAergic neurons (Gao and Fritschy, 1993). In other studies it was demonstrated that 84–95% of the cholinergic neurons in the basal forebrain expressed the $\alpha 3$ subunit but not the $\alpha 1$ subunit. In contrast, parvalbumin-positive GABAergic neurons in these brain regions were frequently co-stained with the $\alpha 1$ -subunit and to a lesser extent with the $\alpha 3$ subunit antibody (Gao et al., 1995). The $\alpha 3$ 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 $\alpha 3$, θ , and ϵ GABA_A receptor subunits in the dorsal raphe and the locus coeruleus (Moragues et al., 2000; Moragues et al., 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.

Identification of native GABA_A receptor subtypes by their synaptic and extrasynaptic localization

Other studies indicated that the individual subunits exhibit a distinct subcellular distribution. For instance, in cerebellar granule cells $\alpha 1$, $\alpha 6$, $\beta 2/3$ and $\gamma 2$ 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 $\alpha 6$ and β subunits (Jechlinger et al., 1998; Pörtl 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 $\alpha 6\beta\delta$ 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 $\alpha 4\beta\delta$. 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 forebrain evidence has accumulated that such receptors might be composed of $\alpha 5\beta\gamma 2$ 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).

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 2 α , 2 β and 1 γ or 1 δ 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 2 α , 2 β and 1 γ subunit (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 $\alpha 1\beta 2\gamma 2$ subunits are the most abundant GABA_A-R in the brain. Similarly, $\alpha 2\beta\gamma 2$, $\alpha 3\beta\gamma 2$, $\alpha 4\beta\gamma 2$, $\alpha 5\beta\gamma 2$, $\alpha 6\beta\gamma 2$, $\alpha 4\beta\delta$ and $\alpha 6\beta\delta$ 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 an abundance comparable with that of major norepinephrine, dopamine, serotonin or peptide receptors.

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-HT₃ (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., 2008) show sequence homology on the order of 30 % identity, but even greater similarity at the level of secondary and tertiary structure. 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 ligands (Corringer *et al.*, 2000; Sine & Engel, 2006; Sigel & Buhr, 1997; Li et al., 2006; Bali & Akabas, 2004; Hosie et al., 2006). Each subunit consists of a long N-terminal extracellular hydrophilic region, followed by four trans-membrane (M) α -helices with a large intracellular loop between M3 and M4, and ends with a relatively short extracellular C-terminal domain, and M2 forms the lining of the ion channel. The structure of the Cys-loop pentameric LGIC superfamily has been resolved (currently 4 Å) for the nicotinic acetylcholine receptor of *Torpedo marmorata* obtained by cryo-electron microscopy and image reconstruction (Unwin, 2005). This has been combined with X-ray crystallography data on the homologous snail soluble acetylcholine binding protein (AChBP; Brejc *et al.*, 2001), recently followed by that of the water soluble portion of the muscle nicotinic acetylcholine receptor (Dellisanti *et al.*, 2007), and that of a related bacterial membrane LGIC receptor (Hilf & Dutzler, 2008).

The homologous sequence and structure allows homology modeling of the various functional domains of GABA_A-Rs using the structures of the AChBP and the nAChR as templates (Cromer *et al.*, 2002; Ernst *et al.*, 2003; Ernst *et al.*, 2005). The resulting models not only identified the absolute arrangement of the 1 γ , 2 α and 2 β subunits within the receptor, but also the structural features of the extracellular domain and their binding pockets. In the extracellular domain, there are 5 pockets at the 5 subunit interfaces. The two pockets at the β/α interfaces form the 2 GABA binding sites, and the pocket at the α/γ interface forms the benzodiazepine binding site. The GABA pocket is formed by the so-called “loops” A, B, and C of the β + (“principal”) side and the so-called “loops” D, E, and F of the α - (“complementary”) side (Ernst et al., 2003). Residues homologous to those in the agonist binding loops at the β/α interface, are homologous in all members of the superfamily (Smith & Olsen, 1995; Sigel & Buhr, 1997; Corringer *et al.*, 2000) and are also homologous to those of the respective “loops” A, B, C, of the α + and D, E, and F of the γ -side forming the benzodiazepine binding site (Ernst et al., 2003).

Homology modeling of the extracellular as well as the transmembrane domain of the GABA_A receptor was based on the 4 Å structure of the nAChR (Unwin, 2003), and indicated that GABA_A receptors contain additional cavities in the transmembrane domain in addition to those found extracellularly (Ernst et al., 2005). One set of putative pockets is actually an extension of the extracellular pockets into the lipid bilayer of the transmembrane domain. The extracellular and transmembrane intersubunit pockets, however, are not necessarily connected with each other and could also represent completely separate entities. Another type of cavity is found contained inside each of the subunits surrounded by the four

helices that make up the transmembrane domain. These multiple cavities are probably needed for conformational changes of the receptors, but could also serve as drug binding sites. Binding of drugs into any one of these sites could either stabilize or induce conformational changes of the receptor and thus, enhance or reduce GABA-induced chloride flux. These multiple binding sites at a single receptor subtype, thus, could explain the extremely complex pharmacology of GABA_A receptors (Ernst et al., 2005).

Identification of native GABA_A receptor subtypes by their pharmacology

Benzodiazepines

GABA_A-R are the site of action of a variety of pharmacologically and clinically important drugs. Their interaction with benzodiazepines has been most thoroughly investigated. The location of the benzodiazepine binding site at the α +/ γ - interface indicates that the benzodiazepine pharmacology of receptor subtypes is mainly determined by the α and γ isoform forming this site. The classical benzodiazepines, such as diazepam or flunitrazepam, predominantly interact with receptors composed of $\alpha 1\beta\gamma 2$, $\alpha 2\beta\gamma 2$, $\alpha 3\beta\gamma 2$, or $\alpha 5\beta\gamma 2$. They exhibit no activity on $\alpha 4\beta\gamma 2$ or $\alpha 6\beta\gamma 2$ receptors and a reduced activity on receptors containing $\gamma 1$ or $\gamma 3$ subunits (Sieghart, 1995; Hevers and Lüddens, 1998). However, the pharmacology of $\gamma 1$ and $\gamma 3$ containing receptors so far has not been extensively investigated. The classical benzodiazepines cannot distinguish between the benzodiazepine sites of different GABA_A-R subtypes, but over time, some compounds, such as the benzodiazepines quazepam and cinolazepam (Sieghart, 1989) or non-benzodiazepines such as zolpidem, CI218872, abecarnil, zaleplon and indiplon (for review see Möhler, 2006), have been developed that exhibit a preferential selectivity for $\alpha 1\beta\gamma 2$ receptors. Most of these compounds exhibit sedative and hypnotic properties. Other compounds, such as SL651498 (Griebel et al., 2003) or TPA023 (Atack et al., 2006) seem to exhibit a certain selectivity for $\alpha 2\beta\gamma 2$ and $\alpha 3\beta\gamma 2$ receptors, whereas L-838,417 (McKernan et al., 2000) exhibits a selectivity for $\alpha 2\beta\gamma 2$, $\alpha 3\beta\gamma 2$ and $\alpha 5\beta\gamma 2$ receptors. All these compounds exhibit anxiolytic properties. One of the few non-sedative, anxiolytic compounds that is not $\alpha 2$ subunit-selective is ocinaplon (Basile et al., 2004; Atack, 2005). The compound L-655 708 is a partial inverse agonist with preference for $\alpha 5\beta\gamma 2$ receptors and exhibits memory-enhancing properties (Sternfeld et al., 2004; Chambers et al., 2004), $\alpha 3$ IA is a weak inverse agonist at $\alpha 3\beta\gamma 2$ receptors that has anxiogenic actions (Atack et al., 2005). The overall selectivity of most of these drugs, however, is not yet sufficient for a selective activation of a single GABA_A receptor subtype, allowing the unequivocal identification of these receptor subtypes and their function in the brain.

Other benzodiazepine site ligands, such as the imidazobenzodiazepines flumazenil or Ro15-4513, are also able to interact with $\alpha 4\beta\gamma 2$ or $\alpha 6\beta\gamma 2$ receptors. These drugs have been used for identification of these receptor subtypes in the brain with ligand binding studies and autoradiography, using [³H]Ro15-4513 in the absence, or presence, of high concentrations of diazepam or flumazenil, to mask receptors containing the $\alpha 1$, $\alpha 2$, $\alpha 3$, or $\alpha 5$ subunits. (Korpi et al., 2002).

The function of at least some GABA_A-R subtypes in the brain recently has been identified using a combined molecular genetic and pharmacological approach (Rudolph & Möhler, 2004; Whiting, 2006). Thus, based on the evidence that most of the actions of diazepam are mediated via receptors composed of $\alpha 1\beta\gamma 2$, $\alpha 2\beta\gamma 2$, $\alpha 3\beta\gamma 2$ and $\alpha 5\beta\gamma 2$ subunits, a point mutation was introduced into the genes of the individual α subunits rendering the respective receptors insensitive to allosteric modulation by diazepam. A comparison of drug-induced behavioral responses in the mutated and wild-type mice then allowed the identification of diazepam effects that were missing or reduced in the mutant mice. Using this approach, it was demonstrated that $\alpha 1\beta\gamma 2$ receptors mediate the sedative, anterograde amnesic and in

part the anticonvulsant actions of diazepam (Rudolph *et al.*, 1999; McKernan *et al.*, 2000). The anxiolytic activity of diazepam is mediated primarily by GABA_A-Rs composed of $\alpha 2 \beta \gamma 2$ subunits (Low *et al.*, 2000), and, under conditions of high receptor occupancy, also by $\alpha 3$ GABA_A-Rs (Dias *et al.*, 2005; Yee *et al.*, 2005). The $\alpha 2 \beta \gamma 2$ receptors are also implicated in some of the muscle relaxant activities of diazepam (Low *et al.*, 2000). Receptors containing the $\alpha 3$ subunit seem to mediate the anti-absence effects of clonazepam, as indicated by the respective point mutated mouse (Sohal *et al.*, 2003) and the $\alpha 3$ global knockout mice displayed a hyperdopaminergic phenotype relevant for GABAergic control of psychotic-like symptoms (Yee *et al.*, 2005). The $\alpha 5 \beta \gamma 2$ receptors seem to influence learning and memory, shown by improved spatial memory in mice with knockout of $\alpha 5$ subunits (Collinson *et al.*, 2002), and improved trace fear conditioning in the point-mutated $\alpha 5$ knockin mouse (Crestani *et al.*, 2002). These and other studies for the first time indicated a possible function of specific GABA_A-R subtypes in the brain and provided additional evidence for their actual existence *in vivo*.

Other allosteric ligands

In addition to benzodiazepines, other allosteric modulators of GABA_A receptors such as general anesthetics of diverse chemical structure, including volatile agents and intravenous anesthetics, such as barbiturates, long-chain alcohols, neuroactive steroids, etomidate, propofol, as well as ethanol (Olsen & Sieghart, 2008) can be used for the identification of specific GABA_A receptor subtypes.

The differential sensitivity of GABA_A receptor subtypes to anesthetics led to the identification of residues in the trans-membrane region of α and β subunits that are critical for modulation of GABA_A-R function by volatile anesthetics like isoflurane, and high dose (100 mM) ethanol (Mihic *et al.*, 1997). These residues are located at TM2-15' and TM3-4' (helical numbering convention), and proposed to be part of a single intrasubunit water-filled binding pocket (Yamakura *et al.*, 2001), the existence of which has also been predicted by homology modeling studies (Ernst *et al.*, 2005). The TM2-15' had been found independently to affect sensitivity to loreclezole and the structurally related intravenous anesthetic etomidate, in β subunits (Belelli *et al.*, 1997).

The β M3-4' residue was further demonstrated to be part of the binding site for the intravenous anesthetic propofol (Bali & Akabas, 2004) and to be affinity labeled with an analogue of etomidate (Li *et al.*, 2006), which also identified a second residue in α M1 (11'). Residues α M1 (11') and β M3-4' were proposed to contribute to a single intersubunit binding pocket at the β/α subunit interface, 50 Å below the GABA binding pocket in the extracellular domain (Li *et al.*, 2006). The residues in α M1 and β M3 were shown to be facing each other by cross-linking of helical cysteine-substituted residues (Jansen & Akabas, 2006; Bali *et al.*, 2007) supporting the conclusion that residues α M1-11' and β M3-4' are likely to be positioned appropriately to participate in an intersubunit pocket. This conclusion is also consistent with homology modeling using the Unwin (2005) structure of the nAChR as a template. Further experiments will have to clarify whether β M3-4' contributes to an intrahelical or interhelical pocket.

The sensitivity of drugs towards the respective residues β M2-15' in $\beta 2$ - and $\beta 3$ -containing receptors led to the production of knock-in mice which exhibited a reduced sensitivity to the sedative ($\beta 2$ N265S: Reynolds *et al.*, 2003) and anesthetic actions of etomidate *in vivo* ($\beta 3$ N265M: Jurd *et al.*, 2003). These studies demonstrated not only that GABA_A-Rs mediate the action of the general anesthetic etomidate, but also that the behavioral effects depend differentially on $\beta 2$ - or $\beta 3$ -containing GABA_A-R, presumably due to their anatomical localization in brain structures contributing to the respective actions.

Another, related, mutagenesis study utilizing homology models of the TMD region of GABA_AR identified two potential binding pockets important for neurosteroid action: one intrasubunit site shared within α M1 and α M4 mediating enhancement of GABA, and a second, intersubunit site α M1/ β M3, involved in direct gating of channels, respectively (Hosie et al., 2006). The steroid and etomidate site models have the intersubunit pockets near each other but steroids do not inhibit etomidate binding (Li et al., 2006). It is possible that the residues needed for steroid action identified by mutagenesis are involved in conformational coupling and not binding. More information is needed to understand how these important residues participate in structure and function. Better models of GABA_AR structure will not only be useful for future rational drug design but also for aiding our understanding of their functional mechanism of action. The generation of knock-in mice carrying a mutation of the respective residues will be invaluable in a more detailed clarification of the action of these drugs and help to identify the receptor subtypes involved in their effects.

Which of the many possible GABA_A receptor subtypes have been unequivocally identified?

The frequent co-localization of α , β , and γ , or α 4/ α 6, β , and δ subunits in the brain and in synaptic or extrasynaptic sites of specific neurons, respectively, the evidence that these subunits could be co-precipitated from brain membrane extracts, that they form defined recombinant receptors with a specific pharmacology, their identification by receptor binding and electrophysiological studies and the identification of their function using molecular-genetic knock-in or knock-out and pharmacological experiments, suggest the actual existence of 8 different GABA_A-R subtypes (α 1-6 β γ 2, α 4 β δ / α 6 β δ).

The ρ subunits seem to be preferentially expressed in the retina (Enz et al., 1996; Koulen et al., 1998). mRNA encoding ρ subunits, however, is present also in the superior colliculus, dorsal lateral geniculate nucleus and cerebellar Purkinje cells (Boue-Grabot et al., 1998; Wegelius et al., 1998). In addition, pharmacological effects characteristic for ρ subunit containing receptors have been reported in various brain regions (for review see Sieghart and Sperk, 2002). The absence of antibodies or a pharmacology that could distinguish between receptors containing different ρ subunits, did not allow more thorough studies on their possible existence. Since the mere existence of a subunit and the fact that it can form receptors in recombinant systems does not necessarily imply that it actually forms receptors *in vivo* and since such subunits could also have other functions in the brain, at the time being it only seems safe to assume the existence of one ρ -containing native receptor in the brain.

Therefore, using a stringent classification, we only have 9 different GABA_A receptor subtypes (α 1-6 β γ 2, α 4 β δ , α 6 β δ , ρ) unequivocally identified in the brain (Olsen and Sieghart, 2008). To keep the possibility alive that there are also receptors composed of ρ 1, ρ 2, and ρ 3 subunits, we added these three receptor types to a list of “tentative receptors”. In this list we did not include receptors containing mixed ρ subunits, because only recombinant receptor studies so far provided evidence for their possible existence, and in such studies receptors also can be formed that might not be formed under native conditions (Olsen and Sieghart, 2008). Out of the same reason, we also did not include possible receptors formed from a combination of ρ and γ 2 or glycine receptor subunits (Pan et al., 2000; Milligan et al., 2004).

As mentioned above, not many studies have been performed investigating receptors containing any one of the minor GABA_A receptors subunits (γ 1, γ 3, θ , ϵ , π). These subunits have been demonstrated to have a distinct regional distribution, in some cases, co-

precipitation with other GABA_A receptor subunits has been demonstrated, and recombinant receptors have been generated exhibiting specific properties (for review see Sieghart and Sperk, 2002). But the lack of a selective pharmacology and function in the brain indicated that receptors possibly formed from these subunits at present are quite tentative, justifying their inclusion into the list of “tentative receptors”.

Finally, the question arose, whether receptors containing 2 distinct α and/or 2 distinct β subunits actually occur in the brain. The existence of such receptors is suggested by the overlapping regional and cellular distribution of the α and β subunits, and by co-precipitation studies (Sieghart and Sperk, 2002). However, neither an overlapping regional and cellular distribution nor a co-precipitation of subunits necessarily means that the subunits are present in the same receptor subtypes. In the latter case they also could be present in different receptors associated with each other either directly or via their associated proteins. In addition, receptors containing 2 distinct α and/or 2 distinct β subunit so far have not been demonstrated to exhibit a specific pharmacology and function in the brain. But since Benke et al. (2004), using knock-in mice containing point-mutated α subunits, provided additional evidence for the existence of receptors containing different α subunits and reported upon the abundance of these receptors in an in vivo system, we decided to include at least receptors containing 2 different α subunits on the list of “tentative receptors”.

At this time, however, we decided that the evidence for the existence of receptors containing 2 different β subunits, or containing five different subunits (Sieghart and Sperk, 2002) is not strong enough yet for justifying inclusion of such receptor types in any list of possible receptors. The same holds true for receptors containing two different γ , δ , or ϵ per pentamer, or those containing a γ and a δ subunit. So in summary, in the list of “tentative receptors” we only included 9 different receptors ($\rho 1$, $\rho 2$, $\rho 3$, $\alpha\beta\gamma 1$, $\alpha\beta\gamma 3$, $\alpha\beta\theta$, $\alpha\beta\epsilon$, $\alpha\beta\pi$, $\alpha x\alpha y\beta\gamma 2$).

However, Nusser et al., (1998) provided additional evidence for the existence of receptors composed of $\alpha 1\alpha 6\beta\gamma 2$ or $\alpha 1\alpha 6\beta\delta$ subunits by demonstrating separate as well as co-localization of $\alpha 1$ and $\alpha 6$ subunits in the cerebellum granule cells. This prompted us to place at least one receptor composed of $\alpha 1\alpha 6\beta\gamma$ / $\alpha 1\alpha 6\beta\delta$ into a third list of “receptors that exist with high probability” (Category B). This list, in between “receptors unequivocally identified” (Category A) and “tentative receptors” (Category C), defines receptors for which additional evidence for their existence has been accumulated, but for which evidence seemed not strong enough to include them into the list of “identified receptors”.

Additional evidence from co-localization, co-immunoprecipitation and neuronal electrophysiology studies also suggests that $\gamma 2$ or δ subunits partner with more than one β subunit (one at a time). In addition, point mutations in the M2 domain (M2-15') that can produce β subunit selectivity for loreclezole or etomidate (Cestari and Yang, 1996; Belelli et al., 1997; Rudolph and Antkowiak, 2004) provided evidence for the existence of receptor subtypes with specific β subunits. So far, these results did not provide evidence on whether the $\gamma 2$ or δ subunit-containing receptors, or both mediate the etomidate effects identified, although the δ -containing receptors are more sensitive to modulation. In addition, the respective α subunits present in these receptor also could not be identified.

The δ subunit-containing GABA_A receptors, partnered with the $\alpha 4$, or $\alpha 6$ subunits, and especially with $\beta 3$, are more sensitive than $\gamma 2$ -containing receptors to general anesthetics, neurosteroids, GABA analogs like THIP (Gaboxadol) (Wohlfarth et al., 2002; Brown et al., 2002; Chandra et al., 2006), as well as taurine (Jia et al., 2008) and ethanol (Wallner et al., 2003; Hancher et al., 2005). The $\alpha 4\beta 2\delta$ and $\alpha 4\beta 3\delta$ receptors differ in sensitivity to

modulators when recombinantly expressed in cells and both clearly occur naturally, because some brain areas express $\alpha 4$ and δ but not $\beta 2$, or $\beta 3$, subunits and display a pharmacology that distinguishes between these receptors. Thus, thalamic relay nuclei mainly express the $\beta 2$ subunit and the moderately ethanol-sensitive $\alpha 4\beta 2\delta$ receptor definitely mediates the tonic current (Chandra et al., 2006). Dentate granule cells express high levels of highly ethanol-sensitive, presumably $\alpha 4\beta 3\delta$ isoforms (Liang et al., 2006), but additionally express the etomidate-sensitive $\alpha 4\beta 2\delta$ isoforms (Herd et al., 2008). Partnering of either $\beta 2$, or $\beta 3$, with both the $\alpha 4\delta$ and $\alpha 6\delta$ subunits thus appears highly likely, even conclusive. Therefore, we added $\alpha 4\beta 2\delta$ and $\alpha 4\beta 3\delta$, $\alpha 6\beta 2\delta$ and $\alpha 6\beta 3\delta$ subtypes to the list of “receptors identified”, replacing the generic $\alpha 4\beta \delta$ and $\alpha 6\beta \delta$.

Recently, Glykys et al., 2007, demonstrated that the δ subunit can pair with the $\alpha 1\beta$ in hippocampal interneurons, based on co-localization with immuno-staining, electropharmacological properties and changes in knockout mice. While this single report is convincing, we decided that this subtype did not meet sufficient criteria to be included in the list of “identified” subtypes. We therefore included the $\alpha 1\beta \delta$ receptor on the category B list, “identified with high probability”.

Another candidate qualifying for this list is the $\alpha 5\beta 3\gamma 2$ receptor subtype. The $\alpha 5$ and $\beta 3$ subunits appear to be co-depleted in mice lacking either the $\beta 3$, or $\alpha 5$ subunits (Olsen and Homanics, 2000), and the properties of recombinant $\alpha 5\beta 3\gamma 2$ receptors appear to reflect those of a native subtype found in CA1 pyramidal neurons (that are enriched in both $\alpha 5$ and $\beta 3$ subunits) and are distinct from receptors in dentate gyrus granule cells (Burgard et al., 1996; Sur et al., 1998; McClellan and Twyman, 1999; Stell et al., 2003; Caraiscos et al., 2004). Such studies are not conclusive evidence for this combination, so we place this subtype in the second list. Another example in this category is the $\alpha 1\beta 3\gamma 2$ subtype. If we specify that $\beta 2$ is the most common partner of $\alpha 1\gamma 2$, and list it in category A, we need to ask whether $\alpha 1$ can partner with other β subunits. While strong evidence for $\beta 1$ is lacking, the properties of $\alpha 1\beta 3\gamma 2$ receptors in recombinant studies resemble those in certain neurons that express these subunits, some of which lack the $\beta 2$ subunit (Whiting et al., 2000; Sieghart and Sperk, 2002).

Although the $\beta 1$ subunit is less abundant than the other β subunits, it is likely that some $\beta 1$ -containing GABA_A receptors exist, based on regional distribution and coimmunoprecipitation data (Li and DeBlas, 1997; Jechlinger et al., 1998). A potential pharmacological fingerprint, salicylidene salicylhydrazide, has been reported to be a negative allosteric modulator selective for $\beta 1$ versus $\beta 2$ or $\beta 3$ subunit-containing receptors (Thompson et al., 2004). There is some evidence (Whiting et al., 2000) that cultured astrocytes express GABA_A-R with $\alpha 2\beta 1\gamma 1$ combination, and they are uniquely enhanced by the benzodiazepine site ligand DMCM, a β -carboline. However, more evidence is needed to establish if this subtype actually exists in the brain. We therefore placed just one subtype for $\alpha \beta 1\gamma$ / $\alpha \beta 1\delta$ in category B.

Finally, evidence accumulated for the existence of receptors composed of α and β subunits, only. First, Bencsits et al., (1999) demonstrated that a large part of the $\alpha 4$ receptors (about 50%) are not associated with γ , or δ , subunits and are possibly composed of α and β subunits only. This was in contrast to $\alpha 1$ receptors. Second, in several knockout mice it was demonstrated that receptors composed of only α and β subunits do exist, for example in $\gamma 2$ knockout mice (Günther et al., 1995), δ knockout mice (Tretter et al., 2001), or $\alpha 1$ knockout mice (Ogris et al., 2006). Third, in several brain regions α and β occur in the absence of γ and δ subunits. And fourth, Mortensen and Smart (2006) demonstrated by electrophysiological studies that there are extrasynaptic $\alpha \beta$ receptors on rat hippocampal

pyramidal neurons. In the absence of any evidence for a specific $\alpha\beta$ subunit combination, we placed one undefined $\alpha\beta$ subtype into category B.

Conclusions

The importance of GABA_A-Rs in different brain regions and behaviors has been reinforced in recent years, with recognition that GABA_A-Rs come in many different flavors differing in subunit combination, resulting in subtypes that vary in physiology, pharmacology, and most importantly, in location. Both the circuitry and the subcellular location on different postsynaptic membranes and extrasynaptic membranes is important for GABA_A-R control of neuronal excitability, circuit, and network activity.

As knowledge of structure and function of GABA_A-Rs increases, we find that allosteric modulation by clinically important CNS drugs can be explained by micro-domains within the receptor protein structure, which differ slightly but importantly among receptor subtypes. Modulation of GABA_A-R by drugs with anxiolytic, sedative/hypnotic, anesthetic, and antiepileptic profiles continues to provide a target for current and pharmaceutical pipeline candidates. These actions involve multiple categories of drug binding sites on GABA_A-R, the benzodiazepine sites and probably several other distinct sites for drugs like other anxiolytics, neurosteroids, general anesthetics, including barbiturates, etomidate, propofol, and volatile agents, and ethanol. The existence of receptor subtypes is now established, and exploitation for products with specific targets is in progress. It is expected that the NC-IUPHAR database on receptor classification and nomenclature will soon be expanded to the LGIC receptors, provided at no cost to the entire community.

The recent review on GABA_A-R (Olsen & Sieghart, 2008) provides a compendium of criteria for inclusion of candidate subtypes on a list of native receptors, and the resulting receptor list. Whether all these receptor subtypes as well as those still to be identified are biologically relevant currently cannot be answered. A specific subunit composition could allow, for example, specific targeting to a certain subcellular localization, specific regulation by protein kinases and phosphatases, or interaction with other associated proteins or other receptors. Thus we will need to continue to develop careful approaches for defining which receptor subtypes are native, especially the lower abundance subtypes. Three categories of subtypes are designated: Identified; Existence with High Probability; and Tentative. A 'working list' of receptor subtypes totaling 26 is provided in Olsen & Sieghart (2008), reproduced here (Table 1). The 19 subunit genes also are presented in Olsen & Sieghart (2008), and the subunit list for all LGIC genes is included in the introductory chapter for this volume (Collingridge et al., 2008).

Current evidence suggests that only 11 subtypes can be listed as conclusively identified, and these are reasonably abundant. Also listed are several subtypes for which the evidence is strong but not conclusive (6 subtypes). Finally, subtypes are included containing one of each of the minor subunits, evidence for whose native existence is tentative (another 8, plus one subtype with two kinds of α subunit, for a total of 9, and a grand total of 26). Note that one (ρ) overlaps with list A. An additional similar number of subtypes which are relatively rare, but are likely to exist, are not listed at this time, but the list will continue to grow as more information becomes available. Each subtype could play a significant role in the cells in which they occur. It should be noted that even these minor GABA_A-R subtypes are present in amounts comparable to, or greater than, receptor subtypes for other accepted brain neurotransmitters other than glutamate; that is the biogenic amines and acetylcholine. Clearly, the heteromeric ligand-gated ion channels offer much greater heterogeneity than other known receptor subtypes.

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Table 1GABA_A Receptor List 2008.

A. Identified			
$\alpha 1\beta 2\gamma 2$	$\alpha 4\beta\gamma 2$	$\alpha 5\beta\gamma 2$	$\alpha 6\beta 3\delta$
$\alpha 2\beta\gamma 2$	$\alpha 4\beta 2\delta$	$\alpha 6\beta\gamma 2$	ρ
$\alpha 3\beta\gamma 2$	$\alpha 4\beta 3\delta$	$\alpha 6\beta 2\delta$	
B. Existence with High Probability			
$\alpha 1\beta 3\gamma 2$	$\alpha 5\beta 3\gamma 2$	$\alpha\beta$	
$\alpha 1\beta\delta$	$\alpha\beta 1\gamma / \alpha\beta 1\delta$	$\alpha 1\alpha 6\beta\gamma / \alpha 1\alpha 6\beta\delta$	
C. Tentative			
$\rho 1$	$\alpha\beta\gamma 1$	$\alpha\beta\theta$	
$\rho 2$	$\alpha\beta\gamma 3$	$\alpha\beta\pi$	
$\rho 3$	$\alpha\beta e$	$\alpha x\alpha y\beta\gamma 2$	

The criteria for inclusion on the native receptors list and evidence for the subtypes were first described in Olsen & Sieghart, Pharmacol. Rev. 2008.