Abstract—In this review we attempt to summarize experimental evidence on the existence of defined native GABA_A receptor subtypes and to produce a list of receptors that actually seem to exist according to current knowledge. This will serve to update the most recent classification of GABA_A receptors (Pharmacol Rev 59:291–313, 1998) approved by the Nomenclature Committee of the International Union of Pharmacology. GABA_A receptors are chloride channels that mediate the major form of fast inhibitory neurotransmission in the central nervous system. They are members of the Cys-loop pentameric ligand-gated ion channel (LGIC) superfamily and share struc-
I. Introduction: Definition of GABA\(_A\) Receptors

\(\gamma\)-Aminobutyric acid (GABA\(^1\)), the major inhibitory neurotransmitter in the brain, exerts its action via ionotropic GABA\(_A\) and metabotropic GABA\(_B\) receptors. GABA\(_A\) receptors (GABA\(_A\)-Rs) are the major inhibitory receptors in the central nervous system (CNS). They 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\(_B\) receptors are activated by GABA and the selective agonist baclofen but are not sensitive to typical GABA\(_A\)-R ligands, such as bicuculline and muscimol. GABA\(_B\) receptors couple to several different effector systems, depending on the associated G protein (e.g., G\(_i/o\)), such as activation of inwardly rectifying K\(^+\) channels or inhibition of high-voltage-activated Ca\(^{2+}\) channels, involving regulation of cyclic AMP or inositol phosphate signaling (Bowery et al., 2002; Bettler et al., 2004).

The objective of this review is to define GABA\(_A\)-Rs and, in so doing, to summarize classifications and provide guidelines on nomenclature. This should serve to update the nomenclature suggested by Barnard et al. (1998), which remains useful and relevant, for most issues. GABA\(_A\)-Rs are chloride channels that are gated by GABA and mediate rapid phasic inhibitory synaptic transmission and also tonic inhibition by producing current in extrasynaptic and persynaptic locations (Mody and Pearce, 2004; Farrant and Nusser, 2005). They are abundant in the nervous system of all organisms with a nervous system, including invertebrates (Buckingham et al., 2005), which also express GABA\(_A\)-Rs on some muscle cells (Robinson and Olsen, 1988), where they also mediate Cl\(^-\) dependent inhibition. GABA\(_A\)-Rs are found, but in a limited capacity, in non-neural tissues [such as the pancreas (Borboni et al., 1994)], where their functional roles are still under study and their pharmacological relevance remains to be established. Consistent with the emphasis of the Nomenclature Committee of the International Union of Pharmacology (IUPHAR), the receptors considered here are limited to mammalian species, with emphasis on humans. Because of their widespread localization throughout the mammalian nervous system, GABA\(_A\)-Rs play a major role in virtually all brain physiological functions and serve as targets of numerous classes of drugs, used both clinically and important as research tools. The GABA\(_A\)-Rs are a family of probably many receptor subtypes, but so far only a few dozen subtypes have been identified with reasonable certainty. Because of recent advances in knowledge of their molecular makeup, identification of native subunit compositions, and relevance to pharmacological specificity, an update of the list of GABA\(_A\)-Rs is in order. This list is necessarily a continually changing work in progress.

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\(^1\) Abbreviations: GABA, \(\gamma\)-aminobutyric acid; GABA\(_A\)-R, GABA type A receptor; CNS, central nervous system; IUPHAR, International Union of Pharmacology; LGIC, ligand-gated ion channel; nAChR, nicotinic acetylcholine receptor; GlyR, glycine receptor; 5-HT, serotonin; 5HT\(_5\)-R, 5-HT\(_5\)- receptor; FRET, fluorescence resonance energy transfer; RT-PCR, reverse transcriptase-polymerase chain reaction; flumazenil, ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate; Ro15-4513, (3-ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4]-benzodiazepine-3-carboxylate; BZ, benzodiazepine; CL218-872, triazolopyridazine, 3-methyl-6-[3-(trifluoromethyl)phenyl]-1,2,4-triazol[4,3-\(b\)]pyridazine; L838,417, 7-tert-butyl-3-(2,5-difluorophenyl)-6-(2-methyl-2H)-[1,2,4]triazol-3-ylmethoxy)-1,2,4-triazolo[4,3-b]pyridazine; TP4003, 4,2’-difluoro-5’-[8-fluoro-7-(1-hydroxy-1-methyl)imidazo[1,2-alpyridine-3-yl]biphenyl-2-carbonitrile; TP4023, 7-(1,1-dimethyl)ethenyl)-6-(2-ethyl-2H-1,2,4-triazol-3-ylmethoxy)-3-(2-fluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine; a5IA, 3-(5-methylisoxazol-3-yl)-6-(1-methyl-2,3,4-triazol-4-yl)methoxy]-1,2,4-triazolo[3,4-a]pythalazine, SR95531, gabazine, 2-[(3’-carboxy-2’-propyl)-3-amino-6-p-methoxyphenylpyridazinium bromide); gaboxadol, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP).
increase in \([\text{Cl}^-]\) might shift the membrane potential toward the firing threshold, causing rebound excitation of neurons (Marty and Llano, 2005). \(\text{GABA}_A\)-R channels can conduct other anions with variable permeability ratios relative to \(\text{Cl}^-\). \(\text{HCO}_3^-\) flux could be physiologically relevant under certain conditions (Kaila et al., 1997). The importance of bicarbonate varies with tissue and is dependent on carbonic anhydrase activity, including the tissue isozyme expression, as well as anion pumps (Rivera et al., 2005).

**B. The Cys-Loop Pentameric Ligand-Gated Ion Channel Superfamily**

\(\text{GABA}_A\)-Rs are part of the Cys-loop pentameric ligand-gated ion channel (LGIC) superfamily, including nicotinic acetylcholine receptors (nAChRs) (Corringer et al., 2000; Lukas and Bencherif, 2006), glycine receptors (GlyRs) (Breitinger and Becker, 2002), ionotropic 5-HT receptors (5HT_3Rs) (Davies et al., 1999; Thompson and Lummis, 2006), and a Zn\(^{2+}\)-activated ion channel (Davies et al., 2003). They differ in structure from two additional LGIC families: the tetrameric glutamate receptors (P2X) (Chen and Wyllie, 2006) and the trimeric purine receptors (Khakh et al., 2001; Khakh and North, 2006). Whereas the nAChR, 5-HT_3R, and the Zn\(^{2+}\)-activated channels are cation-selective channels and thus excitatory, the \(\text{GABA}_A\)-R and GlyR families are anion-selective channels and, thus, with the exceptions noted above, mediate inhibition. All of the subunit members of the Cys-loop LGIC superfamily show sequence homology on the order of 30% identity but even greater similarity at the level of secondary and tertiary structure. Such receptors are all organized as pentameric membrane-spanning proteins surrounding a central pore that forms the ion channel through the membrane (Fig. 1). 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. Each subunit consists of a long N-terminal extracellular hydrophilic region, followed by four trans-membrane (M) \(\alpha\)-helices with a large intracellular loop between M3 and M4, and ends with a relatively short extracellular C-terminal domain. M2 forms the lining of the ion channel, with a possible contribution from M1 (Corringer et al., 2000; Sine and Engel, 2006). An \(\alpha\)-helical domain within the M3–M4 cytoplasmic loop has also been shown to influence ion conduction (Peters et al., 2005). The structure of the Cys-loop LGIC superfamily has been investigated by a large number of biochemical approaches, with current work emphasizing domains for ligand binding and coupling to ion channel gating, as well as subunit-subunit interactions and investigation of the role of intracellular domains. All of these data were confirmed and fell into place when combined with X-ray crystallography data on the snail soluble acetylcholine binding protein (Brejc et al., 2001), recently followed by that of the water-soluble portion of the nAChR \(\alpha\)1 subunit (Dellisanti et al., 2007). The complete channel structure has been progressively well resolved (currently 4 Å) for the nAChR of *Torpedo marinerata* obtained by cryoelectron microscopy and image reconstruction (Miyazawa et al., 2003, Unwin, 2005).

**C. The \(\text{GABA}_A\) Receptor Family of 19 Genes**

With the complete sequence of the genome for human and a few other vertebrate species, it is now clear that there are 19 genes for \(\text{GABA}_A\)-Rs (Fig. 2) (Simon et al., 2004). These include 16 subunits (\(\alpha\)1–6, \(\beta\)1–3, \(\gamma\)1–3, \(\delta\), \(\epsilon\), \(\theta\), and \(\pi\)) combined as \(\text{GABA}_A\) and 3 \(\rho\) subunits, which contribute to what have sometimes been called \(\text{GABA}_C\) receptors. Birds and probably some other species additionally express \(\beta\)4 and \(\gamma\)4, but lack \(\theta\) and \(\epsilon\) subunits, so also total 19. The sequence of \(\epsilon\) is closely related to \(\gamma\) subunits (Fig. 2) and \(\theta\) to \(\beta\), suggesting an evolutionary relationship, perhaps more evident in birds than mammals. The list is modified from Barnard et al. (1998) in that we include the \(\theta\), but not the \(\beta\)4, subunit.

**D. The \(\rho\) Subunits and the \(\text{GABA}_C\) Receptor Concept: Not Recommended**

“\(\text{GABA}_C\) receptors” were originally described precloning, based on a distinctive pharmacology, to encompass...
responses to GABA that did not fit either the “A” (blocked by bicuculline) or “B” (activated by baclofen) category. Such “nonA, nonB” GABA responses were found in numerous regions of the CNS. Attempts to find such a nonA, nonB GABA-activated current by expressing polyaRNA from brain in oocytes were unsuccessful but a cDNA expressing this sort of GABA-evoked current was isolated from retina (Woodward et al., 1992).

After cloning of the various GABA_A receptor subunit genes, it was demonstrated that the ρ subunits are closely related in sequence, structure, and function to the other GABA_A-R subunit families designated with other Greek letters and thus qualify for inclusion within that family. It is now clear that there are three subtype genes of ρ subunits that make homopentameric chloride channels. Such ρ-receptors show some of the pharmacological properties of the GABA_C receptors, such as sensitivity to the GABA analog, CACA (cis-aminocrotonic acid). They are relatively insensitive to bicuculline and also to GABA_A-R modulators such as benzodiazepines, barbiturates, and general anesthetics, at appropriate concentrations. GABA_C receptors are sensitive to picrotoxin, neurosteroids, and some other drugs, but the overall pharmacology differs from that of most traditional GABA_A-Rs (Bormann and Feigenspan, 1995; Johnston, 1996). The ρ subunits are not simply equivalent to GABA_C receptors, because some regions of the nervous system seem to lack ρ subunits and yet exhibit GABA_C (i.e., non-A, non-B) pharmacology. The ρ subunits are all expressed primarily in the retina, but unlike the ρ1, the ρ2 and ρ3 subunits are also found elsewhere (Johnston, 2002). At this time it is not clear whether ρ1–3 can combine with each other in heteromers. Enz and Cutting (1999) claimed that recombinant ρ1 and ρ2 subunits can form hetero-oligomers with distinct physical properties. In native receptors this is difficult to prove because there are no antibodies that distinguish between different ρ subunits. Nor is it established whether ρ1–3 can combine in nature with members of the other 16 GABA_A-R subunits. Some evidence suggests these possibilities but it is not decisive (Sieghart and Ernst, 2005). For example, coassembly of ρ and γ2 subunits was reported (Milligan et al., 2004; Pan and Qian 2005), and some cells in hippocampus show GABA_A-R-like properties intermediate between ρ and γ2-containing GABA_A-Rs (Hartmann et al., 2004). There is also some evidence for association of ρ with GlyRs (Pan et al., 2000).

The close structural similarities of ρ subunits to the other GABA_A-R subunits, the similarities in anion channel structure and function, the important fact that other subtypes of GABA_A-Rs differ in pharmacology, such as benzodiazepine sensitivity, from each other to a similar degree as do the ρ receptors, and the possibility of ρ subunits partnering with other GABA_A-R subunits, led to the decision of the Nomenclature Committee of IUPHAR to designate the GABA ρ receptors as part of the GABA_A-R family and to recommend against the use of the term GABA_C receptor. It is especially recommended that the name GABA_C receptor should not be used as the sole name for the ρ receptors in an article including, especially, the title and abstract. The ρ subunits are also discussed in section III.C.1.a.

II. Structural Basis of Receptor Classification

A. GABA_A Receptor Family and Superfamily (Homology of Structure and Function)

The sequence homology of all 19 GABA_A-R gene products to each other and to the Cys-loop pentameric LGIC superfamily lends itself to attempts to classify the receptors collectively. All members of the superfamily are homologous not only in the domains specifying membrane topology but also at the functional domain level, including ligand binding sites (Sigel and Buhr, 1997; Corringer et al., 2000; Olsen and Sawyer, 2004; Siné and Engel, 2006). Thus, homologous M2 residues are involved in channel structure and ion selectivity (Keramides et al., 2004), and the same multiple loops of extracellular sequence domains contribute to agonist/antagonist binding pockets (Galzi and Changeux, 1994; Sigel, 2002). This allows homology structural modeling.
of the various functional domains comparing GABA<sub>A</sub>-Rs and nAChRs (Cromer et al., 2002; Ernst et al., 2003; Ernst et al., 2005). Even the binding sites for some allosteric modulators also show considerable homology with agonist binding sites. For example, the major benzodiazepine binding site lies at the α/γ subunit interface and involves residues homologous to the agonist binding loops at the βα interface, which in turn are homologous in all members of the superfamily (Smith and Olsen, 1995; Sigel and Buhr, 1997; Corringer et al., 2000).

1. Subunit Gene List. The GABA<sub>A</sub>-R receptor subunit genes form a family of 19 (Table 1).

2. Subunit Splice Variants. Splice variants have been reported for only a few GABA<sub>A</sub>-R subunits, primarily γ<sub>2</sub> (Whiting et al., 1990; Kofuji et al., 1991). The γ<sub>2</sub> subunit splice variants differ in only an eight-amino acid stretch of the large intracellular loop that is present in the γ<sub>2</sub>L and missing in the γ<sub>2</sub>S subunit. The sequence includes a consensus protein kinase C phosphorylation substrate serine. No functional consequence of phosphorylation of the unique γ<sub>2</sub>L serine residue has been convincingly demonstrated. The γ<sub>2</sub> splice variants are both expressed and show differential abundance in different brain regions (Gutiérrez et al., 1994; Meier and Grantyn, 2004). In addition, they show differential aging-related changes in their level of expression (Gutiérrez et al., 1996). Brain membranes from genetically engineered mice expressing only the γ<sub>2</sub>S exhibit increased affinity for benzodiazepine agonists, an effect paralleled by increased sensitivity to "sleep" times (loss of righting reflex) in null allele mice (Quinlan et al., 2000). The subunit composition of the receptors responsible for such changes is unknown, and, indeed, receptors produced in compensation might include normally non-native oligomers.

Alternative splice products in the intracellular loop in β2 and γ3 subunits are found that, like γ<sub>2</sub>L, also include consensus phosphorylation substrate sequences. Alternative start sites create multiple mRNA species for β<sub>3</sub>, α<sub>3</sub>, and δ<sub>5</sub> subunits, and variants lacking one or more exons have been found for ρ<sub>1</sub>, α<sub>4</sub>, β<sub>2</sub>, and ε subunits (reviewed in Simon et al., 2004). To date, and this could change, none of these variant polypeptides have been demonstrated to be present within functional receptors, nor do they confer any unique function or pharmacology in recombinant expression systems. There is at least one report of RNA editing in the GABA<sub>A</sub>-R family (Ohlson et al., 2007), but no evidence so far for functional relevance.

### Table 1

<table>
<thead>
<tr>
<th>Subunit</th>
<th>Gene</th>
<th>Human</th>
<th>Mouse</th>
<th>Rat</th>
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<tbody>
<tr>
<td>α&lt;sub&gt;1&lt;/sub&gt;</td>
<td>GABRA1</td>
<td>5q34</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>α&lt;sub&gt;2&lt;/sub&gt;</td>
<td>GABRA2</td>
<td>4q12</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>α&lt;sub&gt;3&lt;/sub&gt;</td>
<td>GABRA3</td>
<td>15q13.2</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>α&lt;sub&gt;4&lt;/sub&gt;</td>
<td>GABRA4</td>
<td>5q34</td>
<td>14</td>
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<tr>
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<td>5q34</td>
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<td>10</td>
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<tr>
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<td>GABRB1</td>
<td>5q34</td>
<td>14</td>
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<tr>
<td>β&lt;sub&gt;2&lt;/sub&gt;</td>
<td>GABRB2</td>
<td>15q13.2</td>
<td>5</td>
<td>1</td>
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<tr>
<td>β&lt;sub&gt;3&lt;/sub&gt;</td>
<td>GABRB3</td>
<td>5q34</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>γ&lt;sub&gt;1&lt;/sub&gt;</td>
<td>GABRG1</td>
<td>5q34</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>γ&lt;sub&gt;2&lt;/sub&gt;</td>
<td>GABRG2</td>
<td>15q13.2</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>γ&lt;sub&gt;3&lt;/sub&gt;</td>
<td>GABRG3</td>
<td>5q34</td>
<td>11</td>
<td>10</td>
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<tr>
<td>δ</td>
<td>GABRD</td>
<td>4q12</td>
<td>14</td>
<td></td>
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<tr>
<td>ε</td>
<td>GABRE</td>
<td>Xq28</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>θ</td>
<td>GABRQ</td>
<td>Xq28</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>ρ&lt;sub&gt;1&lt;/sub&gt;</td>
<td>GABRP</td>
<td>5q34</td>
<td>11</td>
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<tr>
<td>ρ&lt;sub&gt;2&lt;/sub&gt;</td>
<td>GABRR1</td>
<td>5q34</td>
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<tr>
<td>ρ&lt;sub&gt;3&lt;/sub&gt;</td>
<td>GABRR2</td>
<td>15q13.2</td>
<td>11</td>
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### B. Heteropentameric Assembly Produces Complex Subtype Heterogeneity

The assembly of GABA<sub>A</sub>-R as heteropentamers produces complex subtype heterogeneity in structure, which is the major determinant of their pharmacological profile. These various subtypes differ in abundance in cells throughout the nervous system and thus in functions related to the circuits involved. A major factor in producing heterogeneity is the existence of the six different α subunit variants. Some of these physiological receptor subtypes containing specific α subunits can be distinguished by ligands that act at the benzodiazepine site (Barnard et al., 1998). In addition, there are now clear examples of β and γ or δ or other subunit selectivity for drug action on GABA<sub>A</sub>-R subtypes (see section III.B.2). Clearly it is the nature, stoichiometry, and arrangement of the subunits that determine details of pharmacological selectivity. Thus, pharmacology can often provide evidence for structural heterogeneity and informs us about the subunit composition of native receptor subtypes.

Evidence is greatly in favor of a pentameric receptor and most GABA<sub>A</sub>-R subtypes are formed from two copies of a single α, two copies of a single β, and one copy of another subunit, such as γ, δ, or ε (Sieghart and Sperk, 2002; Olsen and Sawyer, 2004). At this time the subunit composition, stoichiometry, and wheel alignment are not known for most pharmacological subtypes: tentative definitions of subtypes can be provided as a work in progress. Current knowledge allows elimination of most of the thousands of permutations theoretically possible for combinations of the known 19 subunits into five-part (pentameric) complexes. Barnard et al. (1998) suggested a maximum on the order of 800 combinations and probably far fewer in reality on the basis of current knowledge of identified subunit partnering and apparent rules of assembly in neurons. Very few combinations have been conclusively identified in situ. We suggest some criteria for inclusion in a list of native subtypes and begin to generate such a list. Current evidence suggests that only 11 subtypes can be listed as conclusively identified, and these are reasonably abundant. We have also listed several subtypes for which the evidence is strong but not conclusive (six subtypes). We finish by mentioning subtypes containing one of each of the minor sub-
units, evidence for whose native existence is tentative (another eight, plus one subtype with two kinds of α subunit), for a grand total of 26. 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\(_A\) subtypes are present in amounts comparable with or greater than receptor subtypes for accepted brain neurotransmitters other than glutamate, that is, the biogenic amines and acetylcholine. The heteromeric LGICs clearly offer much greater heterogeneity than other known receptor subtypes.

C. Criteria for Inclusion on a List of Native Receptor Subtypes

1. Subtypes Based on Structure, Pharmacology, and Function: Nomenclature Guidelines. Criteria are needed to define which receptor subtypes can be accepted as being native to neurons. We suggest basing the criteria on structure, pharmacology, and function. The list of subtypes will necessarily be a work in progress as information on all LGIC families is currently incomplete. We propose five major criteria: two for recombinant studies and three for native studies, each with subclassifications, for inclusion of a subunit combination on the native receptor subtype list (Table 2). None of these criteria by itself is sufficient. Because five criteria are rarely, if ever, met, it is the remit of a committee of experts (e.g., Nomenclature Committee of the IUPHAR subcommittee) to decide which candidates qualify. At this time we choose to be relatively strict regarding inclusion, including subtypes that either must, or are highly likely to, exist, rather than including all subtypes that might exist. We have decided to include more possibilities in the list for GABA\(_A\)-Rs (Table 3) by dividing it into three categories, depending on the number of criteria that are met: A) “identified,” B) “existence with high probability,” and C) “tentative.”

2. Discussion of the Criteria. A brief discussion of the available pertinent evidence and its value in determining the existence of native subtypes is in order. The expression of recombinant receptors, the determination of their subunit composition and arrangement, and their biophysical and pharmacological characterization is obviously an important part in the identification of native receptors. But expression of recombinant receptors is insufficient to prove their existence in vivo, because gross overexpression of subunit proteins can result in heterooligomeric combinations that are unlikely to occur in nature. For example, recombinant receptors containing both the γ2 and δ subunits have been generated and characterized (Saxena and Macdonald, 1994; Hevers et al., 2000), whereas the actual existence of such receptors in the brain is highly questionable (for discussion, see Sieghart and Sperk, 2002). In addition, coexpression of subunits does not always result in the exclusive formation of the expected receptors. Thus, several reports have shown that coexpression of α, β, and other (γ, δ, and ε) subunits in oocytes or cell lines can lead to considerable expression of αβ receptor channels, without γ, δ, or ε. One must either precipitate the receptors via the third component subunit measuring subunit composition by Western blots and pharmacological properties via binding studies or use a pharmacological test to demonstrate the expression of this subunit and its contribution to the channel currents. In any case, it has to be kept in mind that receptor heterogeneity might influence results of the subsequent characterization of recombinant receptors. To increase the formation of receptors containing α and β, as well as γ, δ, or ε subunits, higher amounts of mRNA or cDNA for the third component subunit often are used, as well as longer times for expression (Boileau et al., 2002, 2005; Olsen et al., 2007), despite the risk of non-natural or pathological pentamers with too many copies of a given subunit by the use of such extremes.

Thus, in recombinant expression studies, one must demonstrate that the subunits under study are indeed ex-

<table>
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<th>TABLE 2</th>
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<tr>
<td>Criteria for inclusion on a list of native receptor subtypes</td>
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<tr>
<td>I. Recombinant receptors</td>
</tr>
<tr>
<td>A. Evidence for their formation, subunit composition, and stoichiometry, using recombinant receptor expression in heterologous cell systems</td>
</tr>
<tr>
<td>1. The subunit polypeptides must be shown to be expressed</td>
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<tr>
<td>2. The subunit polypeptides must be shown to coassemble</td>
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<tr>
<td>a. Coimmunoprecipitation</td>
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<td>b. Physical demonstration of subunit interactions by FRET or similar technique</td>
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<tr>
<td>c. Formation of pentamers</td>
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<tr>
<td>d. Unique subunit arrangement, e.g., using concatemers</td>
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<td>3. The corresponding recombinant receptor subtype must be functional</td>
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<tr>
<td>B. Evidence for unique properties, including pharmacology</td>
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<tr>
<td>1. Unique biophysical characteristics</td>
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<tr>
<td>2. Unique pharmacology</td>
</tr>
<tr>
<td>a. Receptor subtype-selective agonists, antagonists, allosteric modulators</td>
</tr>
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<td>b. Receptor subtype-selective radioligands</td>
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<tr>
<td>c. Potency and efficacy for a series of ligands</td>
</tr>
<tr>
<td>d. Macrokinetic measures (e.g., apparent EC(_{50}) values and binding constants for a series of ligands)</td>
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<tr>
<td>II. Native receptors</td>
</tr>
<tr>
<td>A. Colocalization of subunits</td>
</tr>
<tr>
<td>1. Tissue colocalization</td>
</tr>
<tr>
<td>2. Cell colocalization (in situ, single-cell RT-PCR)</td>
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<tr>
<td>3. Subcellular colocalization (light and electron microscopy)</td>
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<tr>
<td>B. Physical demonstration of subunit interactions (e.g., by coimmunoprecipitation)</td>
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<tr>
<td>C. Functional demonstration:</td>
</tr>
<tr>
<td>1. Evidence that a given receptor is expressed in real neurons by showing properties (assessed with electrophysiology) corresponding with a recombinant receptor candidate; microscopy may complement</td>
</tr>
<tr>
<td>2. Evidence that a given subunit or subunit combination participates in a specific function in vitro or in vivo using genetically modified mice</td>
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pressed, combine into receptors, form pentamers with de-
finied subunit stoichiometry and arrangement, and have
function. If possible, the properties of receptors should be
compared with those of receptors with defined subunit
composition and arrangement using concatemers. One
might add that researchers also need to pay attention to
the source of their receptor subunit clones and specify the
source and sequences of those they use. A single point
mutation in the gene of a subunit isolated from the brain
could change the properties of the resulting recombinant
receptors (Sigel et al., 1992). Then one can examine the
receptor for unique biophysical properties using electrophysiology, assessing agonist and modulator mechanisms,
determined, for example, from single channel and macro-
scopic current kinetics, single channel conductance, and
possibly conductance substates. Finally, one can examine
the receptor for unique pharmacology, using subtype-selective
ligands, including radioligands, and measuring relative
affinities, potencies, and efficacies for a series of
ligands.

Expression in the brain at the mRNA (in situ hybrid-
ization, RT-PCR, single cell RT-PCR) or protein level
(immunostaining) is a good starting point to indicate
that two given subunits may be coexpressed in a given
cell and which types of cells express them. A combina-
tion of in situ hybridization and immunohistochemistry
is stronger than either technique alone. But colocaliza-
tion of two subunits, although necessary, is not suf-
cient to establish whether these subunits are partners
in a pentameric receptor subtype. Individual subunits
alone most often do not define a receptor and might even
have functions different from those of receptors. Micro-
scopic colocalization of subunit proteins is one of the
factors used in deciding native subtype composition, but
colocalization at the light microscope level is not conclu-
sive evidence for coexpression within a pentamer. Like-
wise, colocalization at the electron microscope level is
useful evidence but does not necessarily define receptor
subtypes, because even subunits located side-by-side
with immunogold labeling could belong to adjacent re-
ceptors. Anatomy compendia are being developed by ex-
erts, and these play a role in deciding on inclusion of
native subtypes. The questionable specificity of many
antibodies, however, suggests that great care must be
taken to analyze the published work on subunit local-
ization (Rhodes and Trimmer, 2006; Moser et al., 2007).
Because most antibodies used are polyclonal and be-
cause the composition of polyclonal antibodies is differ-
ent in each donating animal and even in each blood
sample, such verification of the specificity of the anti-
bodies has to go on until highly selective and well char-
acterized monoclonal or recombinant antibodies are
available. All antibodies used for these compendia
should thus be used in wild-type and knockout mice to
be sure that they unequivocally identify the correct pro-
tein only. But even if the data are correct, most data on
localization are not in themselves sufficient for defining
receptor subtype composition.

Coimmunoprecipitation of subunits comes close to defin-
ing a subtype because it indicates (but not necessarily proves) assembly. Here again, the antibodies used must be
demonstrated to have total subunit specificity as any cross-
reactivity contaminates the results. The antibodies must
be characterized by Western blotting on crude brain tissue
or cells and be shown to recognize the band of correct size,
and only that, in normal but not knockout mice, when such
mice are available. Specific antibody reagents are cur-
rently available for such an approach, and the provider of
such antibodies should be asked for data documenting the
absence of cross-reactivity. Nevertheless, incomple-
tely assembled intermediates could contribute, and
subunits or receptors could associate with each other
(natural interaction, artifactual aggregation, or associa-
tion via cytoskeleton proteins) without being in the same
receptors, weakening the strength of the evidence based
on the coimmunoprecipitation method. One needs to be
careful with the choice of detergent and solubilization
conditions to minimize nonspecific protein interactions
without destroying oligomers. If one could demonstrate
that the associated subunits were present in a deter-
gent-solubilized protein of the correct size for a pen-
tamer this would strengthen the argument, but this is
rarely, if ever, provided, and even such studies may be
ambiguous because of comigration of multiple hetero-
ooligomeric protein species, so the results in any case
must be regarded with caution. In the case of G protein-
coupled receptor heteromultimer possibilities, evidence
for true subunit-subunit association considers physical
techniques such as FRET (Pin et al., 2007), and this
approach may also find use for ligand-gated ion channel
receptors. However, the method is extremely difficult to
apply to native proteins, requiring the use of transgenic
mice. If it is performed with recombinant receptors in
a heterologous expression system, there is again the
possibility of identifying non-native combinations ow-
ing to subunit overexpression. But such studies again
would not provide a clear-cut answer because subunits
demonstrated to be close together by FRET techniques
could have assembled in the same receptor or be lo-
cated in two different receptors associated with each
other. The above considerations seem trivial, but they
are not, judged by proposals of acknowledged scien-
tists on how to unequivocally solve the problems of
establishing receptor subunit composition that do not
survive more thorough discussions.

Thus, the conclusion is that a combination of different
methods has to be used to identify a receptor subunit
composition of a native receptor, knowing the limits of
each technique. Even then, one cannot be absolutely
sure, but at least this is at the limit of the techniques
available. What is really needed is “in situ” electrophys-
iology with a channel characterization and a pharmaco-
logical fingerprint of the receptors to prove that the
subunits known to exist in that cell contribute to the receptor studied and its physiology. This is extremely difficult, and fingerprints for the different receptor subtypes are not always sufficiently selective, may be host cell-specific, or are lacking altogether. One must take care to be as certain as possible that the subunits meant to be expressed in a recombinant system for obtaining a fingerprint are actually present and contributing to the data and that the pentamer has correct stoichiometry (see discussion above). Recombinant expression suffers from uncertainty that perhaps other gene products present in neurons might not be present in the cells used for recombinant expression (e.g., associated proteins, proteins affecting trafficking, or post-translational modifications) or that they do not exactly match that in the native system because of host cell-specific factors (Birnir and Korpi, 2007). Even a biophysical characteristic as fundamental as single-channel conductance can be affected by the expression system (Lewis et al., 1997), and anecdotes abound regarding different results for the “same receptors” expressed in two kinds of cells. Concatenated subunits may be of use in determining specific subunit composition and wheel arrangement (Im et al., 1995; Baumann et al., 2002; Minier and Sigel, 2004; Boileau et al., 2005; Sigel et al., 2006), although this approach also has a number of problems (Ericksen and Boileau, 2007).

To summarize the strategy used: one first notes which subunits are expressed in a given cell type. Then one looks at evidence that a given pair or triplet of subunits on that list are associated, based on communoprecipitation, coregulation in cells, or knockout animals. The coexpression of α1β2γ2 suggests the possibility of coordinate regulation of expression of chromosomal clusters, but the exceptions are too abundant to consider this a factor in partnering. Then one determines by recombinant expression and electrophysiology the channel kinetics and pharmacology of those subunit combinations, keeping in mind the numerous caveats listed above. Because most of these caveats have not been taken into account previously, one clearly cannot believe everything published on the properties of recombinant receptors. Native receptors with the same unique properties are then sought in the neurons recorded from brain slices, dissociated single cells, or at least cells in culture.

Genetically engineered mice (knockout and knockin) provide some evidence indicating that certain oligomeric receptors really do exist because one can correlate the loss of certain receptor responses and behaviors with the receptor subtypes addressed (Jones et al., 1997; Rudolph et al., 1999; McKernan et al., 2000; Sur et al., 2001; Vicini et al., 2001). The function of deleted genes is sometimes obvious, whereas in other cases, subtle changes in behavior need to be evaluated properly. Evidence from global knockout mice is often complicated by possible compensation, causing the observer to miss important functions. For example, deletion of the α1 subunit of the GABA_A-R results in loss of nearly 50% of the total GABA_A-R population in mouse brain and ablates fast synaptic transmission mediated by the abundant α1 subunit-containing receptors, yet the null mutant mice display a phenotype that is grossly normal (Sur et al., 2001). Diverse transcriptional responses may act to abrogate the effect of the knockout and preserve neuronal excitability and network behavior (Ponomarev et al., 2006). Point mutated knockin mice with altered behavior/pharmacology are more convincing, and several important examples are included in the GABA_A-R field. For an overview and discussion, see Olsen and Homanics (2000), Rudolph and Möhler (2004), Sieghart and Ernst (2005), and Atack (2005).

### III. Working List of Native GABA_A Receptor Subtypes

#### A. Evidence for Subtypes from Localization, Abundance, Subunit Composition, and Stoichiometry Data

It was found early on in recombinant GABA_A-R studies that robust GABA-activated channel formation occurred with combinations of α and β subunits and also with α, β, and γ subunits. The latter turned out to be the prevalent native combination (Barnard et al., 1998). The vast majority of GABA_A-Rs in the CNS contain the γ2 subunit, and this is the most abundant subunit in rat brain and in most regions based on in situ hybridization of mRNA (Wisden et al., 1992; Laurie et al., 1992; Persohn et al., 1991, 1992) and immunostaining (Fritschy and Möhler, 1995; Pirker et al., 2000). The γ1 and γ3 subunits are rarer but have some role in discrete regions, probably with well prescribed subunit partners. Thus, approximately 75 to 80% of GABA_A-Rs contain the γ2 subunit (Whiting et al., 2000; Sieghart and Ernst, 2005).

The α1 is the most abundant of the α subunits and is often colocalized with its chromosome partners, the likewise highly expressed β2 and γ2 subunits (Sieghart and Sperk, 2002). As noted above, knockout of the α1 subunit causes total GABA_A-R content in mouse brain to decrease by 50% (Sur et al., 2001). The α2 and α3 subunits are moderately abundant and α5 is relatively rare except in the hippocampus, as indicated by regional distribution and immunoprecipitation studies (Pirker et al., 2000; Sieghart and Sperk, 2002). The α4 and α6 subunits are reasonably highly expressed in forebrain and cerebellum, respectively. Among the β subunits, β1 is least common, β2 is most abundant and most widespread (knockout results in a 50% reduction in GABA_A-Rs in mouse brain) (Sur et al., 2001), and β3 is reasonably highly expressed, but more discrete. Furthermore, it is more dense perinatally than in adult brain (Zhang et al., 1991; Laurie et al., 1992). The identity of the β subunit often cannot be determined, because on precipitation of GABA_A-Rs with α, γ, or δ subunit-specific antibodies in
most cases all three β subunits are coprecipitated. Only in some rare areas where there are cells with only one type of β subunit contributing to functional GABA<sub>γ</sub>-Rs (Persohn et al., 1991, 1992; Wisden et al., 1992; Laurie et al., 1992; Pirker et al., 2000) can the type of β subunit be predicted. In these areas, however, no subsequent immunoprecipitation or electrophysiological studies have been performed to investigate possible assembly partners, and, thus, actual assembly of defined receptors and their composition cannot be derived with certainty. For this reason, it has been concluded that all of the β subunits exist in functional receptors, usually with only one type per pentamer (Whitting et al., 2000).

Based on the frequent colocalization in the brain (Fritschy and Möhler, 1995; Pirker et al., 2000) and in neurons (Klausberger et al., 2002), on results from co-immunoprecipitation experiments (Jechlinger et al., 1998; Pöltl et al., 2003), and on the pharmacology described in section III.B, the α1β2-γ2 subunit combination is considered to exist in the brain, probably in large amounts (Benke et al., 1991; Somogyi et al., 1996; Whitting et al., 2000). Recombinant α1β2-γ2 receptors have been shown to have a 2α-2β-1γ stoichiometry [denoted (α1)2(β2)2(γ2)], and this stoichiometry is supported by coimmunoprecipitation results from rat or mouse brain (Sieghart and Sperk, 2002). It is generally assumed, without conclusive proof, that other α and β subunits also combine with γ2 in combinations of 2α-2β-1γ-1 (Sieghart and Ernst, 2005). Other α subunits are also colocalized with and have been shown to coprecipitate with all β and the γ2 subunits (Sieghart and Sperk, 2002). Receptors containing these subunits also have a specific pharmacology (see section III.B) and thus also seem to exist in the brain.

The other “minor” subunits, notably δ, are thought to be able to replace γ in the pentamer, as in the 2α-2β-δ combination (Barrera et al., 2008). However, α4 and α6 subunits are just as often combined with the δ subunit as with γ2 (Sieghart and Sperk, 2002). The δ subunit is obligatorily partnered with the α6 subunit in cerebellar granule cells and is primarily associated with the α4 subunit in forebrain areas including dentate gyrus, neostriatum, some layers of cortex, and a few other areas. The δ subunit seems to have a perisynaptic/extrasynaptic localization (Nusser et al., 1998; Peng et al., 2002; Wei et al., 2003). The ε subunit is rare but can substitute for γ or δ in some areas, such as hypothalamus, and the θ and π subunits are only sketchily characterized (Korpi et al., 2002; Sieghart and Sperk, 2002).

The γ2 subunit is required for synaptic localization of GABA<sub>γ</sub>-Rs, usually associated with the α1, α2, or α3 subunits. Nevertheless, substantial numbers, possibly a majority, of γ2 subunit-containing GABA<sub>γ</sub>-Rs are extrasynaptic, because of the much greater area of extrasynaptic membranes. This includes some of the α1/α2/α3 and the great majority of α4/α5/α6 combinations. In contrast, combinations in which other subunits, such as δ and ε, replace γ2 are considered to be exclusively nonsynaptic. The physiological and pharmacological importance of perisynaptically and extrasynaptically localized GABA<sub>γ</sub>-Rs has recently become increasingly appreciated (Mody and Pearce, 2004; Semyanov et al., 2004; Farrant and Nusser, 2005).

B. Pharmacological Evidence for Subtypes

1. Benzodiazepine Site Ligands Distinguish between Subtypes Based on α and γ Subunits. The typical GABA<sub>α</sub> receptor is positively modulated by diazepam-like benzodiazepines and binds radioligands for the GABA site (e.g., [3H]muscimol), the benzodiazepine site (e.g., [3H]flunitrazepam, [3H]flumazenil, or [3H]Ro15-4513, and the picrotoxin/convulsant/channel sites (e.g., [35S]t-butyl bicyclophosphorothionate) (reviewed by Macdonald and Olsen, 1994; Sieghart, 1995; Barnard et al., 1998; Korpi et al., 2002; Johnston, 2005). Barnard et al. (1998) listed approximately 20 chemical classes of ligand that bind to the benzodiazepine (BZ) site on GABA<sub>γ</sub>-Rs, including the structures of approximately 40 compounds. Additional chemical classes of ligands are listed in Gardner et al. (1993), Olsen and Gordey (2000), Sieghart and Ernst (2005), Atack (2005), and Whiting (2006), which should be consulted for chemical names and structures. In each of these chemical classes compounds are available that enhance (positive allosteric modulators or BZ site agonists) or reduce (negative allosteric modulators or inverse BZ site agonists) GABA-induced chloride ion flux via the same BZ binding site. In addition, in each of these chemical classes there are compounds that do not modulate GABA-induced chloride flux, although they interact with the BZ binding site (neutral BZ site ligands or BZ site antagonists). The latter compounds, however, are able to inhibit the action of BZ site agonists or inverse agonists. In between the full BZ site agonists or inverse agonists, there are compounds that elicit less drastic allosteric enhancement or reduction of GABA-induced chloride currents; these compounds are called partial agonists or partial inverse agonists at the BZ site, respectively.

The BZ binding site is located at the interface of an α and a γ subunit. Its pharmacology is thus influenced by both of these subunits, whereas β subunits, although needed to construct a channel, do not greatly affect the sensitivity of the GABA<sub>α</sub>-R to BZ site ligands (Hadingham et al., 1993). The traditional BZ site agonists (GABA-enhancing CNS depressants such as diazepam) are active on the GABA<sub>α</sub>-Rs containing a γ2 subunit (Pritchett et al., 1989), a β subunit, and one of the α subunits, α1, 2, 3, or 5. Receptors containing the γ2 subunit exhibit a higher BZ sensitivity than those containing the γ1 subunit (Sieghart, 1995; Khom et al., 2006); the γ3-containing GABA<sub>α</sub>-Rs are modulated by some BZ ligands but with altered selectivity from those incorporating the γ2 subunit (Sieghart, 1995; Hevers and Lüddens, 1998). The BZ-sensitive GABA<sub>α</sub>-Rs can
be further subdivided, in that receptors containing the α1 subunit have a higher sensitivity to a subpopulation of BZ site ligands, the benzodiazepines quazepam and cинolazepam (Sieghart, 1989) or nonbenzodiazepines such as zolpidem (an imidazopyridine) and a few others, including CL218-872 (triazolopyridazine), zaleplon, and indiplon, and abecarnil (β-carboline), (Olsen and Gordey, 2000; Korpi et al., 2002; Sieghart and Ernst, 2005). Furthermore, receptors containing the α2 or α3 subunit have an intermediate affinity for zolpidem, whereas those containing α5 have very low affinity for this drug. The differential zolpidem affinity demonstrated by recombinant GABA<sub>α</sub>-Rs containing different α subunits can also be found in the brain (Itier et al., 1996; Whiting et al., 2000; Sieghart and Sperk, 2002) and individual cells can be shown to exhibit more than one GABA<sub>α</sub>-R with varying affinity for zolpidem, depending on α subunit subtype expression (Criswell et al., 1997). Again, consult the cited reviews for chemical structures and analogs.

Receptors containing the α4 or α6 subunits, together with β and γ2, do not bind the traditional BZ agonists, including zolpidem, but demonstrate high affinity for some ligands, notably the imidazobenzodiazepines such as flumazenil and Ro15-4513, or bretazenil (Korpi et al., 2002). Both the potency and efficacy for BZ ligands depend on the nature of the α subunit.

The benzodiazepine site ligands so far available do not distinguish well between the α2 and α3 or between the α4 and α6 subunits. All four, however, can produce functional channels in vitro when coexpressed with other subunits, and their differential distributions in the brain suggest they will modulate different behavioral circuitry. Behavioral effects of drugs predicted from subtype selectivity in vitro has been successful in a few cases, such as the α2/3-selective triazolopyridazine anxiolytics based on CL218-872: L838,417, TPA003, or TPA023 (Atack, 2005; Morris et al., 2006; Atack et al., 2006).

Subtype selectivity for drugs in vivo has been further confirmed in subunit-specific genetically engineered mice (Rudolph and Möhler, 2004; Atack, 2005; Whiting, 2006). Thus, based on the evidence that most of the actions of diazepam are mediated via receptors composed of α1βγ2, α2βγ2, α3βγ2, and α5βγ2 subunits, a point mutation involving a histidine to arginine substitution 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. With this approach, it was demonstrated that α1βγ2 receptors mediate the sedative, anterograde amnestic and in part the anticonvulsant actions of diazepam (Rudolph et al., 1999; McKernan et al., 2000). These two independent studies showed exactly the same behavioral responses, provided the mice were tested under the same conditions (Crestani et al., 2000). The anxiolytic activity of diazepam is mediated by GABA<sub>α</sub>-Rs composed of α2βγ2 subunits (Low et al., 2000), and, under conditions of high receptor occupancy, also by α3 GABA<sub>α</sub>-Rs (Dias et al., 2005; Yee et al., 2005; discussed by Möhler, 2007). The α3-selective drug TP003 also implicates a role for α3βγ2 receptors in anxiolytic (Dias et al., 2005) and anticonvulsant action (Fradley et al., 2007). The α2βγ2 and α3βγ2 receptors are also implicated in some of the muscle relaxant activities of diazepam (Low et al., 2000) and the α3βγ2 in the antiabsence effects of clonazepam (Sohal et al., 2003). The α3 global knockout mice displayed a hyperdopaminergic phenotype relevant for GABAergic control of psychotic-like symptoms (Yee et al., 2005). The α5βγ2 receptors seem to influence learning and memory, shown by improved spatial memory in mice with knockout of α5 subunits (Collinson et al., 2000), and trace fear conditioning was facilitated in the point-mutated α5 knockin which unexplainedly showed major α5 subunit knockdown in the CA1 region (Crestani et al., 2002; Rudolph and Möhler, 2004). Furthermore, the α5-selective inverse agonists such as α5IA can improve cognitive function (Atack, 2005; Dawson et al., 2006). Mice lacking the α5 subunit were shown to exhibit reduced amnestic response to the intravenous general anesthetic etomidate but not to the immobilizing activity of the drug (Cheng et al., 2006). Knockout mice have also implicated several GABA<sub>α</sub>-R subtypes in the reinforcing effects of ethanol, including the α1 (Boehm et al., 2004), the α5 (Boehm et al., 2004; Stephens et al., 2006), the δ (Mihalek et al., 2001), the α4 (Liang et al., 2008), and the α6 subunit (Hanchar et al., 2005). Years of study on α subunit-dependent subtypes have led to drug candidates for modifying selective behaviors and clinical indications (Atack, 2005; Sieghart and Ernst, 2005; Whiting, 2006), and the genetically modified mice have supported this subtype selectivity. Possibly even more impressive than the drug candidates has been the clues generated regarding behavior involving specific receptor subtypes and thus specific brain circuitries (for review, see Rudolph and Möhler, 2004). Although this attribution of the varied behavioral actions of diazepam site ligands and ethanol to different receptor subtypes might only be a first approximation, and the evaluation may be somewhat tentative, it clearly indicates that receptors containing the respective subunits are present in the brain and exhibit distinct functions in different neuronal circuits, verified by consistent results with subtype-selective drugs.

In contrast to α1βγ2, α2βγ2, α3βγ2, and α5βγ2 receptors, α4βγ2 and α6βγ2 receptors are diazepam-insensitive but are still able to bind the imidazobenzodiazepines Ro15-4513, and flumazenil, which act as BZ site inverse agonist and antagonist, respectively. Thus, these receptors can be identified by 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 α1, α2, α3, or α5 subunits. Such studies have clearly identified diazepam-insensitive, but flumazenil-sensitive, binding sites in cerebellum and forebrain. These sites disappear in α6 or α4 null mutant mice, indicating that they represent α6β2, or α4β2 receptors, respectively (Korpi et al., 2002). In addition, there are GABA_A-R-mediated inhibitory currents recorded in hippocampus that are enhanced by Ro15–4513, an effect specific for α4β2 (Liang et al., 2006). So, even with limited pharmacology, some in vivo and in vitro information about selectivity, and the help of genetically modified mice, we can make receptor assignments; e.g., each α subunit defines at least one unique subtype, partnered with a β and a γ2 subunit. Thus, GABA_A-Rs exist with binding properties consistent with high, intermediate, and low affinity for certain BZ site ligands, such as zolpidem, and GABA_A-Rs are lost in the brain, based on tonic BZ-insensitive GABA_A-R currents, which are enhanced by Ro15–4513, an effect specific for α4β2 (Liang et al., 2006; Glykys et al., 2007). Such GABA_A-Rs have recently been shown to bind Ro15–4513, an effect specific for α4β2 receptors and with the α4β6 and α6β6 receptors showing even higher potency for GABA than the corresponding γ2 subunit-containing GABA_A-R (Wallner et al., 2003). The α5βγ2, α4β6, and α6β6 subtypes have recently been shown to be localized in extrasynaptic membranes, where they mediate a tonic inhibitory current, now recognized to have significant physiological relevance in controlling neuronal excitability, relevant to local circuitry and network activity (Bai et al., 2001; Caraiscos et al., 2004; Mody and Pearce, 2004; Farrant and Nusser, 2005). Many of these extrasynaptic GABA_A-Rs show a high affinity for GABA and are resistant to desensitization, appropriate for their role in tonic inhibition. In addition, GABA has a low efficacy for these receptors, making them susceptible to higher efficacy for modulation by allosteric drugs such as nonbenzodiazepines and especially general anesthetics (Brown et al., 2002; Wohlfarth et al., 2002; Stell et al., 2003; Wallner et al., 2003; Caraiscos et al., 2004; Belelli et al., 2005). Overall, these drugs produce a larger charge transfer via extrasynaptic than synaptic receptors, and, in contrast to earlier dogma, the actions of some important drugs seem to involve modulation of extrasynaptic rather than synaptic receptors. Extrasynaptic GABA_A-Rs show great heterogeneity with respect to subunit composition and include a significant fraction of the γ2-containing GABA_A-Rs, whereas those lacking γ2 are exclusively extrasynaptic.

The δ subunit, generally partnered with the α4 and α6 subunits, produces the seventh and eighth subtypes. This is supported by evidence of colocalization and coimmunoprecipitation (Jeclinger et al., 1998; Whiting et al., 2000; Pöltl et al., 2003; Sieghart and Ernst, 2005), accompanied by electrophysiological evidence for α6βδ GABA_A-Rs in cerebellar granule cells and α4βδ in forebrain, based on tonic BZ-insensitive GABA_A-R currents, which are lost in the δ knockout mice (Stell et al., 2003; Wei et al., 2003; Belelli et al., 2005; Hanchar et al., 2005; Liang et al., 2006; Glykys et al., 2007). Such GABA_A-Rs bind even fewer BZ site ligands. Nevertheless, these subtypes have recently been shown to bind Ro15–4513, flumazenil, and certain β-carbolines (Hanchar et al., 2006; Wallner et al., 2006). Thus, current evidence supports the existence of α4βδ and α6βδ receptors, and we conclude that these eight GABA_A-Rs should be placed in category A as conclusively identified.

2. Evidence from Other Ligands (GABA, Picrotoxin, General Anesthetics, and Ethanol) Confirms and Extends the List of Receptors. Heterogeneity of GABA_A-Rs was evident even before the cloning of the individual subunits as indicated by brain regional variation in radioligand binding, inhibition, and allosteric modulation involving the GABA, BZ, convulsant, and anesthetic sites as well as by photolabeling of GABA_A-R subunits using radiolabeled benzodiazepines (e.g., Sieghart, 1989; Olsen et al., 1990; Sieghart, 1995). Such evidence for subtypes was consistent with differential pharmacology assessed by electrophysiological recordings conducted using different cells (Macdonald and Olsen, 1994; Hevers and Lüddens, 1998). The potencies of GABA and antagonists such as gabazine (SR95531) vary slightly with the α subunit (Bai et al., 2001), being more potent at α4, 5, and 6, than at α1, 2, and 3 subunit-containing receptors and with the α4β6 and α6β6 receptors showing even higher potency for GABA than the corresponding γ2 subunit-containing GABA_A-R (Wallner et al., 2003). The α5βγ2, α4β6, and α6β6 subtypes have recently been shown to be localized in extrasynaptic membranes, where they mediate a tonic inhibitory current, now recognized to have significant physiological relevance in controlling neuronal excitability, relevant to local circuitry and network activity (Bai et al., 2001; Caraiscos et al., 2004; Mody and Pearce, 2004; Farrant and Nusser, 2005). Many of these extrasynaptic GABA_A-Rs show a high affinity for GABA and are resistant to desensitization, appropriate for their role in tonic inhibition. In addition, GABA has a low efficacy for these receptors, making them susceptible to higher efficacy for modulation by allosteric drugs such as nonbenzodiazepines and especially general anesthetics (Brown et al., 2002; Wohlfarth et al., 2002; Stell et al., 2003; Wallner et al., 2003; Caraiscos et al., 2004; Belelli et al., 2005). Overall, these drugs produce a larger charge transfer via extrasynaptic than synaptic receptors, and, in contrast to earlier dogma, the actions of some important drugs seem to involve modulation of extrasynaptic rather than synaptic receptors. Extrasynaptic GABA_A-Rs show great heterogeneity with respect to subunit composition and include a significant fraction of the γ2-containing GABA_A-Rs, whereas those lacking γ2 are exclusively extrasynaptic.

The synaptic γ2 subunit-containing GABA_A-Rs are all modulated by general anesthetics of diverse chemical structure including pentobarbital, etomidate, and propofol, as well as the neuroactive steroids, but the extrasynaptic δ subunit-containing GABA_A-Rs, and possibly the α5βγ2, are more sensitive to these drugs (Brown et al., 2002; Wohlfarth et al., 2002; Stell et al., 2003; Wallner et al., 2003; Hemmings et al., 2005; Herd et al., 2007). As mentioned, the extrasynaptic δ subunit-containing α4βδ and α6βδ GABA_A-Rs show a significant modulation by nonbenzodiazepine drugs. Recordings in native tissues suggest therefore that both γ2- and δ-containing GABA_A-Rs are clearly present and physiologically relevant. Glykys et al. (2007) demonstrated that the δ subunit can pair with α1β in hippocampal interneurons, based on colocalization with immunostaining, electropharmacological properties, and changes in knockout mice. Although this single report is convincing, we decided that this subtype did not meet sufficient criteria to be included in the list of identified subtypes. Rather than omit it, the α1βδ subtype is placed within a second category of native receptors.
(category B, existence with high probability), so that readers are informed of receptors being considered for inclusion and can evaluate for themselves if they believe one qualifies.

The γ2 and δ subunits may have selective β partners, but considerable evidence from colocalization, coimmunoprecipitation, and neuronal electrophysiology studies suggests that each partners with more than one β subunit (one at a time), so this provides a framework for additional subtypes. It was shown earlier that the nature of the α subunit might affect modulator dose-response curves, but no effect was found for varying the β subunit (e.g., Hadingham et al., 1993; Thompson et al., 1996; Smith et al., 2004). Nevertheless, mutation of residues in the channel M2 domain of any subunit, but definitely showing some subunit selectivity in the details, can change modulator affinity or efficacy (Tierney et al., 1996; Hill-Venning et al., 1997; Thompson et al., 1999). Thus, mutations of residues in the M2 domain of β subunits can produce β subunit selectivity for pharmacological agents (Cestari et al., 1996; Rudolph and Antkowiak, 2004).

In addition, recent evidence suggests that the β subunit can affect both the pharmacological and channel properties of the GABA_{A,R}, especially in the case of certain nonbenzodiazepine modulators and particularly for the extrasynaptic δ subunit-containing receptors. Thus, several modulatory ligands, such as the nonbenzodiazepine loreclezole and chemically related ligands, such as the general anesthetic etomidate, show some selectivity for β2 and β3 over β1 subunit-containing receptors (Wingrove et al., 1994; Hill-Venning et al., 1997): these authors identified a single amino acid residue 15′ in the M2 domain (counting from the intracellular N-terminal end of the channel-forming second transmembrane helix) responsible for this β subunit selectivity. Mutations of this residue were shown to affect sensitivity to the actions of etomidate in vitro (Belelli et al., 1997) and in vivo (Reynolds et al., 2003). A point mutation of the β2 subunit N265S that eliminates etomidate sensitivity in vitro loses the sedative-hypnotic effects of etomidate in the mouse knockin (Reynolds et al., 2003), whereas the β3 knockin N265M eliminates the immobilizing effect of a noxious stimulus of etomidate and suppresses loss of the righting reflex to this agent, typical of general anesthetics (Jurd et al., 2003).

The knockin mice showing either β3 or β2 insensitivity to etomidate in vivo allow detective work on which β subunit mediates the action of this drug on a given cell and current, and, along with other information about the physiology and subunit expression pattern in that cell, a good guess at the subunit composition of the functional receptors (e.g., which α subunits go with which β subunit) (Rudolph and Antkowiak, 2004; Belelli et al., 2005; Herd et al., 2008). So far, however, the results did not provide evidence on whether the γ2- or δ-containing receptors, or both, mediate the etomidate effects identified, although the δ-containing receptors are more sensitive to modulation.

This residue 15′ in the M2 “channel domain” of β subunits that affects etomidate sensitivity also affects sensitivity and to some degree β subunit selectivity of GABA_{A,R} to other anesthetic modulators such as propofol, barbiturates, and volatile agents (Cestari et al., 1996; Rudolph and Antkowiak, 2004), consistent with the earlier observation that this residue in (presumably all) α and β subunits affects sensitivity to volatile anesthetics and long-chain alcohols and may be part of a binding pocket for these drugs (Mihic et al., 1997; Ernst et al., 2005).

A few other ligands reported to show β selectivity include valerenic acid (Khom et al., 2007), some novel plant substances of a polyacetylene structure (Baur et al., 2005), tracazolate, and mefenamic acid (Korpi et al., 2002; Smith et al., 2004). This may be extended to ethanol (Wallner et al., 2003). The δ subunit-containing GABA_{A,R}s, partnered with the α4 or α6 subunits and especially with β3, are more sensitive than γ2-containing receptors to general anesthetics, neurosteroids, GABA analogs such as gaboxadol (Brown et al., 2002; Wohlfarth et al., 2002; Chandra et al., 2006), and taurine (Hadley and Amin, 2007; Jia et al., 2008) as well as ethanol (Wallner et al., 2003; Hanchar et al., 2005). The α4β2δ and α4β3δ receptors differ in sensitivity to modulators when recombinantly expressed in cells, and both clearly occur naturally, because some brain areas express α4 and δ but only β2 or β3 subunits and display a pharmacology that distinguishes between these receptors. Thus, thalamic relay nuclei mainly express the β2 subunit and the moderately ethanol-sensitive α4β2δ receptor definitely mediates the tonic current (Chandra et al., 2006). Dentate granule cells express high levels of highly ethanol-sensitive, presumably α4β3δ isoforms (Liang et al., 2006) but additionally express the etomidate-sensitive α4β2δ isoforms (Herd et al., 2008). This modulator selectivity for different β subunits in vitro and in vivo is supported by knockin mouse data. Partnering of either β2 or β3 with both the α4δ and α6δ subunits thus seems highly likely. To account for this additional evidence indicating the existence of α4δ and α6δ subunits combined with specific β subunits, we add α4β2δ and α4β3δ, α6β2δ and α6β3δ subtypes in category A, replacing the generic α4βδ and α6βδ and bringing our total to 10. Subtypes dividing the αγ2 subtypes depending on the β2 versus β3 partner, except for α1β2γ2, will not be listed until some compelling evidence is presented that the variants exist and differ in some property.

One strong candidate that nearly qualifies for category A is the α5β3γ2 subtype. The α5 and β3 subunits seem to be codepleted in mice lacking either the β3 or α5 subunit (Olsen and Homanics, 2000), and the properties of recombinant α5β3γ2 receptors seem to reflect those of a native subtype found in CA1 pyramidal neurons, cells enriched in both α5 and β3 subunits, and distinct from
receptors in dentate gyrus granule cells (Burgard et al., 1996; Sur et al., 1998; McClennan and Twyman, 1999; Stell et al., 2003; Caraiscos et al., 2004). However, the specific partnering of α5 with β3 has not been shown by coimmunoprecipitation or pharmacological changes in mutant mice, so we place this subtype in category B. Another example in this category is the α1β2γ2 subtype. If we specify that β2 is the most common partner of α1γ2 and list it in category A, we need to ask whether α1 can partner with other β subunits. Although evidence for β1 is lacking, the properties of α1β3-γ2 receptors in recombinant studies resemble those in certain neurons that express these subunits, some of which lack the αγ2 subunit (Pan et al., 2000; Hartmann et al., 2004; Miligan et al., 2004), does not qualify as subtypes for the list at this time.

The evidence for the existence of a native subtype containing any of the five minor subunits (γ1, γ3, ε, θ, and π) is not as compelling as that for the other, more abundant, subunits (α1–6, β1–3, γ2, and δ) already described. This is attributable to a scarcity of studies and the lack of pharmacological tools and in vivo investigations. However, these subunits belong to the GABA<sub>A</sub>-R family and can form recombinant GABA-activated chloride channels on coexpression with other subunits, (Sieghart, 1995; Hevers and Lüddens, 1998) and exhibit differential regional distribution in the brain (Laurie et al., 1992; Persohn et al., 1992; Wisden et al., 1992; Davies et al., 1997; Hedblom and Kirkness, 1997; Whiting et al., 1998; Bonnert et al., 1999; Pirker et al., 2001; Moragues et al., 2000, 2002, 2003; Sinkkonen et al., 2002). Thus, evidence is tending toward all of them forming receptors in nature. In few cases are the possible heteropentameric subunit combinations known for these uncommon subunits (e.g., from immunoprecipitation studies using antibodies directed against γ1 or γ3 subunits) (Mossier et al., 1994; Quirk et al., 1994; Tögel et al., 1994) or θ subunits (Bonnert et al., 1999) and thus the total number of subtypes may be large or small. Each subtype γ1, γ3, ε, θ, and π makes an unknown number of combinations with α and β subunits, but probably will be slightly more than the minimum. In fact, the mere existence of these subunits has not been extended to show that the cells where they are expressed have any unique pharmacological or physiological properties produced from these subunits, although few serious searches have been attempted. Benzodiazepine insensitivity in the rat nucleus tractus solitarii correlates with ε expression (Kasparov et al., 2001), and prolongation of inhibitory postsynaptic currents recorded from cultured hypothalamic neurons has been shown to be negatively correlated with the detection of the ε subunit by RT-PCR (Sergeeva et al., 2005). In vitro recombinant studies suggest it should be possible to identify these rare subtypes if they exist. Because this has not been done yet, we decided to put these subtypes, combined with α and β, in the third category, C, tentative. Unusual subunit combinations of these minor subunits remain possible. The ε subunit has been reported to partner with other combinations in recombinant expression (Davies et al., 1997, 2001; Moragues et al., 2000; Wagner et al., 2001; Jones and Henderson, 2007) but whether this occurs in nature has not been sufficiently investigated (Bollan et al., 2008). As mentioned above, “pathological” (non-native) subunit compositions can be forced to occur in recombinant expression systems. The partnership association of the θ subunit has not been extensively studied (Bonnert et al., 1999; Sinkkonen et al., 2000) and, π, as a peripheral tissue constituent, has not been well characterized, although it forms channels in vitro with other GABA<sub>A</sub>-R subunits (Hedblom and Kirk-
ness, 1997; Neelands and Macdonald, 1999; Korpi and Sinkkonen, 2006). Some of these subunits can be shown to express more than one type of channel in recombinant expression work. In these cases, it is particularly important to evaluate recombinant studies with caution unless the nature of the subunit composition of the expressed receptors is unambiguously determined.

b. $\alpha/\beta$ Pentamers. Evidence has accumulated for the existence of receptors composed of $\alpha$ and $\beta$ subunits, only. First, Bencsits et al. (1999) demonstrated that a large proportion of the $\alpha 4$ receptors (approximately 50%) are not associated with $\gamma$ or $\delta$ subunits and are possibly composed of $\alpha$ and $\beta$ subunits only. This is in contrast with $\alpha 1$ receptors. Second, in several knockout mice it was demonstrated that receptors composed of only $\alpha$ and $\beta$ subunits do exist, for example, in $\gamma 2$ knockout mice (Günther et al., 1995), $\delta$ knockout mice (Tretter et al., 2001), or $\alpha 1$ knockout mice (Ogris et al., 2006). This suggests that such receptors might develop under other physiological conditions also. It is possible that such receptors arise during epilepsy, hormone treatment, ethanol intake, or during development. Localization studies show that in several brain regions $\alpha$ and $\beta$ occur in the absence of $\gamma$ and $\delta$ subunits. Either these subunits have other functions or combine with other subunits or they form receptors of $\alpha$ and $\beta$ subunits only. Mortensen and Smart (2006) demonstrated by electrophysiological studies that there are extrasynaptic $\alpha\beta$ receptors on rat hippocampal pyramidal neurons. We could not find evidence for any specific $\alpha$ or any specific $\beta$ subunit in these $\alpha\beta$ combinations or for the existence of more than one such subtype, so have placed one $\alpha\beta$ subtype in category B (Table 3).

c. Multiple $\alpha$ or $\beta$ (other?) isoforms per pentamer. There is ample evidence from several different groups (Duggan et al., 1991; Lüddens et al., 1991; Khan et al., 1996; Sieghart and Sperk, 2002; Benke et al., 2004; reviewed in Whiting et al., 2000) for the coimmunoprecipitation of different $\alpha$ or $\beta$ subunits, whereas different $\gamma$ subunits in most studies were observed not to coimmunoprecipitate to a significant extent (for discussion, see Sieghart and Sperk, 2002). These findings suggest there is no unspecific aggregation of different receptor subtypes, and a variety of controls were performed to demonstrate specific coprecipitation (Jechlinger et al., 1998, Pöltl et al., 2003). Nusser et al. (1998) demonstrated separate locations but also colocalization of $\alpha 1$ and $\alpha 6$ in the cerebellum, suggesting the possibility that the two $\alpha$ subunits occur together in the same heteropentameric receptor. Benke et al. (2004) demonstrated the presence and abundance of GABA$_A$-Rs containing two different types of $\alpha$ subunits using point-mutated $\alpha$ subunits and reported on the abundance of these receptors in an in vivo system: The $\alpha 1$ predominantly forms $\alpha 1$-$\alpha 1$, whereas other subunits predominantly form hetero-$\alpha$. Finally, Minier and Sigel (2004) described the differential properties of receptors containing $\alpha 1$ and $\alpha 6$ subunits in a defined arrangement in a recombinant system. Each receptor configuration has its own pharmacological signature. We decided that the $\alpha 1\alpha 6\beta y/\alpha 1\alpha 6\beta \delta$ qualified as a subtype for the list in category B. Furthermore, we placed an additional species of unknown subunit composition $\alpha\alpha\alpha\beta\gamma 2$ in category C.

In addition, mixtures of $\beta$ subunits probably do exist (Li and De Blas, 1997; Jechlinger et al., 1998), and two articles (Khan et al., 1994; Quirk et al., 1994) suggested that two kinds of $\gamma$ subunit could be included. However, other researchers could not confirm the latter finding (Jechlinger et al., 1998; Pöltl et al., 2003).

d. Receptors containing 1, 2, 4, or 5 different subunits. The evidence for other combinations of subunits with other than three kinds of subunit (homo-oligimeric $\beta 3$ subunit channels and hetero-oligimeric channels containing 2, 4, or 5 different subunits), except $\rho$ and $\alpha\beta$ as discussed, is limited to recombinant expression work and to some reports indicating the existence of two different $\alpha$ or two different $\beta$ subunits in the same native GABA$_A$-R. None currently qualify for inclusion within the list (see discussion in Sieghart and Sperk, 2002).

2. Localization, Trafficking, Post-Translational Modifications, and Associated Proteins. Although these factors could alter receptor pharmacology (Kittler and Moss, 2003), little is known about such factors, no in vivo function has been identified unequivocally, and thus, none are accepted at this time.

### Table 3

<table>
<thead>
<tr>
<th>Category</th>
<th>GABA$_A$ receptor native oligomer list</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td>Identified</td>
</tr>
<tr>
<td>$\alpha 1\beta 2\gamma 2$</td>
<td></td>
</tr>
<tr>
<td>$\alpha 2\beta 2\gamma 2$</td>
<td></td>
</tr>
<tr>
<td>$\alpha 3\beta 2\gamma 2$</td>
<td></td>
</tr>
<tr>
<td>$\alpha 4\beta 2\gamma 2$</td>
<td></td>
</tr>
<tr>
<td>$\alpha 4\beta 2\delta$</td>
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</tr>
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<td>$\alpha 4\beta 3\delta$</td>
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<tr>
<td>$\alpha 5\beta 2$</td>
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</tr>
<tr>
<td>$\alpha 6\beta 2$</td>
<td></td>
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<tr>
<td>$\alpha 6\beta 3\delta$</td>
<td></td>
</tr>
<tr>
<td>$\alpha 6\delta 3\delta$</td>
<td></td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>Existence with high probability</td>
</tr>
<tr>
<td>$\alpha 1\beta 3\gamma 2$</td>
<td></td>
</tr>
<tr>
<td>$\alpha 1\beta \delta$</td>
<td></td>
</tr>
<tr>
<td>$\alpha 1\delta 3\gamma 2$</td>
<td></td>
</tr>
<tr>
<td>$\alpha 1\delta 1\gamma /\alpha 1\delta 1\delta$</td>
<td></td>
</tr>
<tr>
<td>$\alpha \beta$</td>
<td></td>
</tr>
<tr>
<td>$\alpha 1\alpha 6\beta 3\gamma /\alpha 1\alpha 6\delta \delta$</td>
<td></td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>Tentative</td>
</tr>
<tr>
<td>$\rho 1$</td>
<td></td>
</tr>
<tr>
<td>$\rho 2$</td>
<td></td>
</tr>
<tr>
<td>$\rho 3$</td>
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<tr>
<td>$\alpha 3\delta \gamma 1$</td>
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<tr>
<td>$\alpha 3\beta 3 \pi$</td>
<td></td>
</tr>
<tr>
<td>$\alpha 3\alpha \beta \gamma 2$</td>
<td></td>
</tr>
</tbody>
</table>
D. Tentative List of Naturally Occurring Receptor Subtypes

Three categories of entries are listed: identified, existence with high probability, and tentative.

IV. Concluding Remarks

This classification starts with the 19 human GABAA-R genes (Table 1) and some evidence for the likely subunit combination and stoichiometry of the abundant (α1)β2γ2 heteropentameric subtypes. Because of the emphasis of IUPHAR on the human clinic, the present discussion is limited to the human, rat, and mouse, acknowledging that GABAA-Rs exist in all organisms with a nervous system, including invertebrates. We further made the decision to concentrate on the usual location of GABAA-Rs, the central nervous system, realizing that there is expression of functional receptors in peripheral tissues.

In attempting to define native subtypes, we propose (Table 2) five criteria (two for recombinant studies and three for native studies) for inclusion of a given subtype combination on the list. We note that even for the 19 subunit genes, the evidence for some subunits participating in native subtypes varies considerably. Whereas there is some evidence that each of the 19 exists in at least one subtype, some of them meet few of the five criteria. We decided to use three different categories in making the list, consistent with the work in progress nature of such a list (Table 3). This is likewise the situation for the other members of the LGIC receptor superfamilies. The categories in the list are identified, existence with high probability, and tentative. We added the second category, existence with high probability, for those candidates that meet some but not a sufficient number of the criteria summarized here, allowing the reader to decide whether the evidence is convincing. Because the existence of native receptors containing the minor subunits did not meet even the criteria for this category but are likely to exist in at least one subtype each, we added the third category, tentative. One can see that each entry in the tentative category might actually exist as multiple discrete subtypes, and thus the number on the list will expand in the future, possibly by a substantial number, as suggested by Barnard et al. (1998). Our final list (Table 3) includes 11 subtypes in category A. We have 6 entries in category B. We placed 9 in category C, and if they all stay, that makes 26 in 2008, a number that is certain to increase over time.

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