International Union of Pharmacology. XV. Subtypes of γ-Aminobutyric AcidA Receptors: Classification on the Basis of Subunit Structure and Receptor Function


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I. Introduction ............................................................................ 292
A. Earlier classifications of γ-aminobutyric acid receptors ................................................................. 292
  1. γ-Aminobutyric acidA and γ-aminobutyric acidB receptors .............................................................. 292
  2. γ-Aminobutyric acidC receptors ........................................................................................................ 292
  3. Benzodiazepine receptors .................................................................................................................... 293
  4. Excitatory γ-aminobutyric acidA receptors ........................................................................................ 296
B. Conclusion on γ-aminobutyric acid receptor types ........................................................................ 298

II. Approaches to the classification of the γ-aminobutyric acidA receptors ........................................... 298
A. Transductional criteria ......................................................................................................................... 298
B. Operational criteria ............................................................................................................................ 298
C. Structural criteria ............................................................................................................................... 298

III. The structures of the γ-aminobutyric acidA receptors ................................................................... 299
A. The repertoire of subunit types .......................................................................................................... 299
B. The subunit number per receptor molecule ....................................................................................... 300
C. The subunit isoforms in one receptor ................................................................................................. 301
D. Possibilities for subunit stoichiometry ............................................................................................. 302

IV. Principles of the classification ......................................................................................................... 303
A. Application of selectivities at the binding site for benzodiazepines and their functional analogs ........................................................................................................................................ 303
  1. The choice of a classification system .................................................................................................. 303
  2. Benzodiazepine-responsive γ-aminobutyric acid receptor subtypes .............................................. 305
  3. Notation ........................................................................................................................................... 306
B. Advantages and possible modification of the classification ............................................................ 307
C. Nomenclature for the site binding benzodiazepines and modulators with similar activity ............ 307
D. Benzodiazepine insensitivity .............................................................................................................. 308

V. Chromosomal localization of γ-aminobutyric acidA receptor genes ............................................... 309

VI. Other binding sites in relation to the receptor classification .......................................................... 309
A. Other modulatory sites ....................................................................................................................... 309
B. The γ-aminobutyric acid recognition site ........................................................................................ 309

VII. Conclusions ................................................................................................................................. 310

VIII. Acknowledgments ......................................................................................................................... 310
IX. References ...................................................................................................................................... 310

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I. Introduction

This article does not aim to review in detail the properties of γ-aminobutyric acidA (GABA\textsubscript{A}) receptors, because recent accounts of that topic are available. In this same journal, a review of the binding properties and pharmacology of these receptors has been published (Sieghart, 1995). Other reviews have dealt with their ion channel properties as well as their pharmacology (MacDonald and Olsen, 1994; Mohler et al., 1996a,b), whereas others have concentrated on their molecular biology and protein structure (Wisden and Seeburg, 1992; Smith and Olsen, 1995; Stephenson, 1995; McKernan and Whiting, 1996). Further, two recent books have provided many short review articles on the functional, behavioral, and psychopharmacological aspects of GABA receptors (Tanaka and Bowery, 1994; Enna and Bowery, 1997) and an account of these latter aspects will not be repeated here.

Building on that background, we will consider here how our knowledge of GABA\textsubscript{A} receptor structure and function could lead to a classification system. Such a system is not immediately obvious from those previous accounts, as it probably would have been with a one-subunit receptor of the G-protein-coupled class. It is surely no accident that all the present series of NC-IUPHAR reports in Pharmacological Reviews on the nomenclature of individual receptor types have so far concerned G protein-coupled receptors. Certainly the G protein-coupled receptor class covers by far the largest numbers of receptor types; it includes most of the cases in which known clinical drug applications can be related so directly to those types that they have given a great impetus to the receptor analyses. However, beyond those considerations, a major reason for the success in classification of those types must surely be the distinction which can be made therein between the subtypes of a receptor, based on the fact that each will be created by a single polypeptide with a pharmacology which is encoded solely by its own sequence. This is not to say that the classification of any of the receptors previously surveyed in this series has been entirely obvious or without complexities. Nonetheless, the problems involved are generally concerned with borderline cases in which the sequence data or the discriminatory pharmacological tools were historically less satisfactory. How one longs for such a one-to-one correspondence in the case of the GABA\textsubscript{A} receptors!

The discussion here, therefore, is the first in the classification series to tackle a receptor of their class, i.e., the multisubunit, heteromeric ion channels directly activated by the transmitter. The combinatorial principle of receptor construction (to be discussed below) for these ionotropic receptors, which also is used extensively in glutamate and nicotinic acetylcholine receptors, introduces a higher order of complexity. The functional unit is not the single polypeptide, and further, the functional properties contributed by a given subunit can vary with its interactions with the particular set of subunits in each receptor molecule. This complexity renders the recognition of the structures of receptor subtypes in their natural setting extremely difficult (in fact, at present, usually unattainable). Thus, it is not possible to construct a classification comparable with the comprehensive scheme for native receptor subtypes obtained in the previous articles in this series. Instead, a provisional version is presented which relies on the wealth of sequence and functional data available on the recombinant GABA\textsubscript{A} receptors.

A. Earlier Classifications of γ-Aminobutyric Acid Receptors

1. γ-Aminobutyric acidA and γ-aminobutyric acidB receptors. GABA has been accepted as a neurotransmitter (in mammals and down to crustacea) for several decades. It is now evident that GABA mediates most inhibitory transmission events in the vertebrate brain. It was long clear that the fast, bicuculline-blocked response to GABA observed was caused by direct activation of an intrinsic anion channel in an entity subsequently termed the GABA\textsubscript{A} receptor. GABA\textsubscript{A} receptors were recognized later as bicuculline-insensitive, baclofen-stimulated metabotropic GABA receptors (Hill and Bowery, 1981) linked to G proteins. Confirmation by the deoxyribonucleic acid (DNA) cloning of a GABA\textsubscript{B} receptor, as a 7-transmembrane domain protein, has been accomplished recently (Kaupmann et al., 1997).

The complete structural and functional distinction between GABA\textsubscript{A} and GABA\textsubscript{B} receptors has a clear parallel to that between nicotinic and muscarinic acetylcholine receptors, between 5-HT\textsubscript{3} and metabotropic serotonin receptors, ionotropic and metabotropic glutamate receptors, or ionotropic P\textsubscript{2X} and G protein-coupled P\textsubscript{2Y} receptors for nucleotides.

2. γ-Aminobutyric acidC receptors. A third type of GABA receptor, insensitive to both bicuculline and baclofen, was designated GABA\textsubscript{C} (Drew et al., 1984). The GABA\textsubscript{C} responses are also of the fast type associated with the opening of an anion channel; they are, however, unaffected by typical modulators of GABA\textsubscript{A} receptor
channels such as benzodiazepines and barbiturates (Sivilotti and Nistri, 1991; Bormann and Feigenspan, 1995; Johnston, 1996). Native responses of the GABA<sub>C</sub> type have occurred in retinal bipolar or horizontal cells across vertebrate species (Feigenspan et al., 1993; Quian and Dowling, 1993; Łukasiewicz, 1996) and can be expressed by rat retinal messenger ribonucleic acid (mRNA) injection in the oocyte system (Polenzani et al., 1991).

Although the term “GABA<sub>C</sub> receptors” still is used frequently for these bicuculline-insensitive ionotropic GABA receptors, we would argue that this terminology is no longer appropriate. The atypical GABA receptors at those retinal sites are mimicked when the recombinant ρ subunits are expressed, and ρ subunit mRNAs occur prominently in both human and rat retina (Cutting et al., 1991; Enz et al., 1995; Ogurusu et al., 1995, 1997; Zhang et al., 1995). ρ subunits are structurally part of the family of GABA<sub>A</sub> receptor subunits (Shimada et al., 1992; Kusama et al., 1993a,b), although their regulatory binding sites are obviously very distinctive. It would be unsatisfactory to separate these two branches of the ionotropic GABA receptor family as GABA<sub>A</sub> and GABA<sub>C</sub> receptors, with a metatopic family, GABA<sub>B</sub>, lying between them. Moreover, if the designation of GABA<sub>C</sub> were retained, then it would be difficult to refuse the extension to GABA<sub>D</sub>, etc., types for ionotropic receptors which do not match either of the previously recognized GABA<sub>A</sub> and GABA<sub>C</sub> specifications. This would further decrease the logic of the GABA<sub>A</sub>:GABA<sub>B</sub> classification scheme. Thus, Sato et al. (1996) have proposed such a “GABA<sub>B</sub>” type, for an embryonic brainstem ionotropic GABA receptor that is insensitive to both GABA<sub>A</sub> and GABA<sub>B</sub> antagonists and is activated by both GABA<sub>A</sub> and GABA<sub>B</sub> agonists. Again, Perkins and Wong (1996) have suggested, based on an anomalous current evoked by GABA in hippocampal pyramidal neurons, that a “GABA<sub>B</sub>” channel may occur there with a different ionic selectivity. We therefore recommend that the term GABA<sub>C</sub>, as well as sequential terms for any new classes for ionotropic GABA receptors, be avoided. The ρ-containing receptors are best classified as a specialized set of the GABA<sub>A</sub> receptors, as will be shown below.

3. Benzodiazepine receptors. The interaction with benzodiazepines (BZ) (fig. 1) has been a major influence in studies on GABA receptors because of the long history of therapeutic application of BZs as anxiolytics, anticonvulsants, sedative-hypnotics, and muscle relaxants. Although the BZs were introduced first into clinical practice in the early 1960s, it was not until 1975 that these drugs were recognized to act by potentiating the inhibitory action of GABA in the brain (Costa et al., 1975; Haefely et al., 1975). The presence of high-affinity, specific binding sites for BZs in the mammalian brain was then demonstrated (Braestrup and Squires, 1977; Mohler and Okada, 1977). Converging lines of evidence established that these sites are in the same macromolecule as the GABA sites and the chloride channel and that all three elements are coupled allosterically (Chang et al., 1981; Olsen, 1981; Paul et al., 1981; Sigel and Barnard, 1984). The term “GABA/BZ receptor” came into use for this complex (and is still encountered).

Progress in this field until recently was driven by the synthesis of a vast range of BZs and BZ-like drugs, all acting at brain GABA<sub>A</sub> receptors and possessing clinical anxiolytic or sedative potencies correlated to their binding affinities there (Haefely et al., 1985).

Based on the finding that all the BZs then tested displaced in a monophasic manner the binding of [³H]BZs in different brain regions, it originally was thought that there was a single class of BZ receptors. However, the subsequent availability of compounds (for structures see fig. 2) with non-BZ structure such as the triazolopyridazine CL 218872, imidazopyridines (e.g., zolpidem), and certain β-carbolines such as methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) or the propyl ester of β-carboline-3-carboxylate (β-CCP) (as well as 1-N-trifluoromethyl-benzodiazepines), which can displace [³H]BZ binding in a biphasic manner and possess a different affinity for BZ receptors in the cerebellum than those in the hippocampus or other brain regions, led to the concept of two BZ-receptor subtypes possessing a differential localization (Lippa et al., 1981; Braestrup et al., 1982; Leeb-Lundberg and Olsen, 1983; Sieghart and Schuster, 1984; Iorio et al., 1984; Arbilla and Langer, 1986; Corda et al., 1988). These were termed the BZ<sub>1</sub> and BZ<sub>2</sub> subtypes of the GABA/BZ receptor.

In addition to these two central BZ receptor types, diazepam binding sites with high affinity for many BZs but with pharmacological properties clearly distinct from those of the “central” BZ receptors were identified.
FIG. 2. Structures of ligands discussed in the text. A, acting at the BZ site; B, C, and D acting at other sites.
A) CHEMICAL STRUCTURES OF BZ/CC LIGANDS (continued)

3) Other structural types

CL 218872
CGS 8216
Ro 19-8022
CGS 20825

CGS 9895
NNC 14-0578
ICI 190622
Y-23684

U-93631
NNC 14-8198
NNC 14-0590
Zolpidem

Divapion
Ru 33-203

Zopiclone
Suriclone

RP 60503

FIG. 2. Continued
in several peripheral tissues (Braestrup and Squires, 1977). These were designated “peripheral type BZ binding sites” (BZp) (Basile and Skolnick, 1986; Verma and Snyder, 1989) and frequently became referred to as “peripheral BZ receptors.” These BZp receptors can be distinguished because they can be labeled selectively (at submicromolar levels) by a non-BZ ligand, the isoquinoline carboxamide PK 11195, and (in rodents but not in some other species; Basile et al., 1986) by an atypical BZ, 4'-chloro-diazepam (Ro5–4864) (Verma and Snyder, 1989), at sites that are insensitive to the antagonist BZ (fig. 1) flumazenil (Mohler and Richards, 1981).

The BZp receptors are unrelated to GABA receptors of any type, and the principal BZp type which has been identified by DNA cloning is a small protein that is associated largely with the mitochondrial membrane (Verma and Snyder, 1989). They are not relevant to the present classification scheme and we recommend that the term “BZ receptor” be dropped in relation to GABA receptors. The distinction made between central and peripheral BZ receptors will not be of value now, because BZp receptors subsequently have been found also in the brain.

Likewise, the term “GABA/BZ receptor,” although useful for two decades, now may be considered obsolete, because (a) a binding site of some form for BZs is not specific to GABAA receptors, as just noted; (b) the BZ site is only one of a set of regulatory sites now known on GABAA receptors, as defined below, so it does not uniquely define these receptors; and (c) as will be discussed below, some GABAA receptors are insensitive to BZs because of one of several distinct molecular causes. Thus “GABA/BZ receptors” is neither synonymous with “GABAA receptors” nor does it define precisely a single receptor subset. Similarly, the terms BZ1 and BZ2 for subtypes of the GABAA receptor no longer are recommended. As described below, evidence on recombinant subunits now indicates that there are many more than two subtypes of GABAA receptors. This is paralleled by biochemical evidence on brain GABAA receptor proteins, e.g., using photoaffinity labeling of their BZ sites by irreversible reaction (Mohler et al., 1980) with [3H]flunitrazepam, which showed that multiple subunits carry BZ sites (reviewed by Olsen et al., 1996). BZ2, as the term has been used in the literature, does not equate to any one molecular subtype alone.

4. Excitatory γ-aminobutyric acidA receptors. Yet another apparent distinction between sets of GABA receptors arises from observations that GABA can be an excitatory transmitter at certain loci in embryonic and early postnatal life in the mammal (reviewed by Cherubini et al., 1991; Ben-Ari et al., 1997). The excitatory response also may mediate the observed trophic role for GABA in nervous system development (Ben-Ari et al., 1997). Another form of excitatory GABA response is seen in tonically stimulated adult hippocampal pyramidal neurons (Staley et al., 1995; Perkins and Wong, 1996; Kaila et al., 1997). All the evidence on these excitatory
receptors indicates that they are GABA-activated anion channels, in general similar to the inhibitory GABA<sub>A</sub> receptors. GABA<sub>A</sub> receptors therefore should be classified as one general type, whether their transduction is a depolarization or a hyperpolarization of the cell membrane. The subunit composition of these excitatory receptors has not been determined yet. It is possible that a different subunit composition increases the permeability of bicarbonate relative to chloride through the receptor channel, or that the subtypes involved are not necessarily different from those well known in the adult but that the chloride gradient across the cell membrane is inverted at the sites in question. Either of these situations could explain the observed excitatory GABA<sub>A</sub> receptor activity. The relative bicarbonate permeability of the channel rarely has been measured for any identified GABA<sub>A</sub> receptor subtype, but the possibility that it is increased in a particular case has been supported by Staley <em>et al.</em> (1995) and Perkins and Wong (1996). It need not be assumed that this would involve a receptor outside the range of GABA<sub>A</sub> receptor subtypes. Indeed, Kaila <em>et al.</em> (1997) have shown that activity-induced changes in intracellular chloride and bicarbonate and extracellular potassium, along with normal GABA<sub>A</sub> receptor function, can account for the GABA-excitatory phase in the tonically stimulated adult hippocampus. Further, the intracellular chloride activity of developing neurons (of the rat nucleus basalis) has been measured using gramicidin-perforated patch recording and shows a large decrease from the immediately postnatal to the mature brain, sufficient to account for the excitatory and inhibitory responses, respectively (Akaike <em>et al.</em>, 1996). Likewise, the internal chloride concentration can be measured locally by confocal imaging based on a chloride-sensitive fluorescence, and this has shown that dendrites on some hippocampal or cortical neurons can exhibit a higher value than somatic locations (Inglefield and Schwartz-Bloom, 1996), confirming earlier suggestions of such a gradient. This also must be distinguished from subtype difference as a potential cause of the excitatory behavior of GABA<sub>A</sub> receptors on some dendrites. As further evidence for this, in the mature mammal the pituitary melanotropic cells are known to possess a very high internal chloride level, and activation of GABA<sub>A</sub>
receptors (of normal pharmacology) there also is depolarizing (Tomiko et al., 1983). For all these reasons, it is unnecessary to provide a specific designation for receptors that mediate excitatory neuronal responses to GABA.

B. Conclusion on γ-Aminobutyric Acid Receptor Types

All the available evidence suggests that GABA receptors can be classified simply as two types, i.e., ionotrophic (the GABA_A receptors) and metabotropic (the GABA_B receptors). The criteria for classification into subtypes will be very different for these two receptor families. The combinatorial basis of GABA_A receptor structure produces a remarkable diversity of receptor subtypes and requires a new form of classification scheme. The GABA_B receptors must be classified separately and will not be considered further here. Likewise, the “peripheral BZ receptors” are unrelated to any GABA receptors and will not be classified here.

II. Approaches to the Classification of the γ-Aminobutyric Acid\textsubscript{A} Receptors

It previously has been accepted in this series of receptor classifications (see Hoyer et al., 1994) that the most fruitful comprehensive system is one in which evidence from three approaches, operational, structural, and transductional, is applied. How can these be applied here?

A. Transductional Criteria

For an ionotropic receptor the transduction (intrinsic ion channel opening or closing) is by definition the same for all of its subtypes. The alternative intracellular pathways used in other receptor classes have no counterpart here. However, in principle subtle differences within this single transduction pathway still could occur. Thus, for two subtypes being activated by the same agonist it might be possible to measure different kinetic constants in the channel opening or closing steps, or different desensitization behaviors, or different distributions of the open and closed channel states. Such cases are known for subtypes of other transmitter-gated channels, e.g., glutamate receptors or P_{2X} nucleotide receptors (reviewed by North and Barnard, 1997). Differences in the kinetic properties among GABA_A receptor subtypes have been investigated rarely so far. In one case, Anglotti and Macdonald (1993) found that some difference in the single-channel properties could be discerned in two recombinant GABA_A receptors expressed in a nonneural cell line. Likewise, expressed recombinant receptors containing the α6 subunit exhibit, at least in some cases, distinctive channel properties (Ducic et al., 1995). However, it may be difficult in practice to find such discriminatory differences in channel properties for many of the subtypes, as well as cumbersome to apply those in classification. Further, in the native setting it will be difficult, if not impossible, to determine whether any such difference instead is not caused either by some intracellular secondary reaction (e.g., a phosphorylation) or by the availability of a native modulator. Therefore, we will not consider transductional criteria for classifying these receptors.

B. Operational Criteria

Selective antagonists have been the most powerful operational tools for discriminating subtypes in other receptor classes (Kenakin et al., 1992). However, for the GABA_A receptors, antagonists at the GABA site generally produce convulsions in vivo. Hence, therapeutic potential is limited and systematic exploration of antagonists has not been developed. A few compounds unrelated to GABA, such as certain arylaminopyridazines and cognate compounds (Heaulme et al., 1987; Melikian et al., 1992), have been developed as potent antagonists at GABA_A receptors generally. Olsen et al. (1990) have shown that the binding of such compounds can discriminate between some subtypes in the brain; their functional study to identify selective actions on recombinant subtypes could be rewarding.

GABA_A receptors are endowed with a variety of modulatory sites for which ligands have been found that can allosterically control the activation by GABA and/or the opening of the anion channel. With the possible exception of the N-methyl-D-aspartate subclass of glutamate receptors (another family of heteromeric ligand-gated ion channels ubiquitous in the brain), the number of different regulatory sites is greater than for any other receptor type. Modulatory sites offer the potential for discriminating among receptor subtypes, namely by the discovery or the design of agents that can act at these sites but can recognize differences in a given site as it occurs in different subunit combinations. Thus far, this possibility has been realized to some extent with the site at which BZ and molecules with BZ-like activity bind, as we shall see. Other established modulatory sites, which can exist on these receptors and which might be used thus include those for barbiturates, neuro-steroids, propofol, certain other anesthetics, furosemide, zinc, picROTOXIN, and some other channel blockers, loreclezole, substituted pyrazinoines, and dihydro-imidazoquinoxalines. Those compounds and the evidence of their interaction with GABA_A receptors are reviewed by Sieghart (1995), Im et al. (1993a,b), Wafford et al. (1994), and Korpi et al. (1995). Only occasional clues to subunit selectivity have been obtained for any of the latter sites.

C. Structural Criteria

The multisubunit compositions of the GABA_A receptors, which create the subtypes, are of primary importance in their classification. In practice, it is not a straightforward task to use the subunit sequences and the subunit assemblies as the primary basis of a classi-
III. The Structures of the \( \gamma \)-Aminobutyric Acid\(_A\) Receptors

A. The Repertoire of Subunit Types

Cloning from cDNA libraries or genomically so far has generated 19 related GABA\(_A\) receptor subunits in mammals, which are each encoded by different genes. These now comprise 6\( \alpha \), 4\( \beta \), 3\( \gamma \), 1\( \delta \), 1\( \epsilon \), 1\( \pi \), and 3\( \rho \) mammalian types (for references see Burt and Kamatchi, 1991; plus (\( \epsilon \)) Davies et al., 1997 and Whiting et al., 1997; (\( \pi \)) Heblom and Kirkness, 1997; (\( \rho_{1,3} \)), see Section I.A.2.; for database accession numbers see fig. 3). These polypeptides are all \( \sim 50,000 \) daltons in size, and each carries four putative transmembrane hydrophobic segments (TM1–4). Figure 3 illustrates the seven different sequence families into which these fall structurally and their relationships. A mammalian counterpart of the avian \( \gamma_2 \) subunit (Harvey et al., 1993) has not yet been isolated by cDNA cloning and so is not included here. However, the \( \beta_3 \) subunit gene, likewise discovered in the chicken (Bateson et al., 1991), has been shown more recently in humans (Levin et al., 1996).

This heterogeneity is increased by alternative exon splicing of the pre-mRNA, which generates two forms of the \( \gamma_2 \) subunit from one gene (Whiting et al., 1990; Koifu et al., 1991), which can be distributed differently in the brain (Glencorse et al., 1992). Two such forms are also known for the \( \beta_2 \) and \( \beta_3 \) subunits (Bateson et al., 1991; Harvey et al., 1994). In each case, the longer and shorter products were designated “L” and “S,” and differ by some form or other of a short peptide in the long intracellular loop between TM3 and TM4. Splicing also occurs to express two alternative forms of exon-1 of the \( \beta_3 \) subunit (Kirkness and Fraser, 1993). Three potential forms of the \( \alpha_5 \) subunit mRNA also exist (Kim et al., 1997) but with unchanged protein sequence. Another product of alternative splicing deletes a short sequence at the N-terminus of the \( \alpha_5 \) subunit (Korpi et al., 1994), although this abolishes the functional receptor activity in all the combinations tested so far. Therefore, in assessing possible combinations of subunit types (other than \( \rho \)) to form a GABA\(_A\) receptor, we must consider in a given mammalian species, including the splice variants, at least 7 \( \alpha \) forms, 7 \( \beta \) forms, 4 \( \gamma \) forms, 1 \( \delta \), 1 \( \pi \), and 1 \( \epsilon \) form. The recently discovered \( \epsilon \) and \( \pi \) subunits in each case can combine with \( \alpha \) and \( \beta \) subunits to form a functional, BZ-insensitive receptor (Davies et al., 1997; Heblom and Kirkness, 1997; Whiting et al., 1997). The \( \pi \) subunit has been detected clearly so far only in certain peripheral tissues (Heblom and Kirkness, 1997), and its range of combinations has not been defined yet. The GABA\(_A\) receptors in the central nervous system (CNS) are formed, on present knowledge, by combinations of both \( \alpha \) and \( \beta \) subunits with one or more of the \( \gamma \), \( \delta \) or \( \epsilon \) subunit types (or possibly, exceptionally, of \( \alpha \) and \( \beta \) types alone). In addition there are three known \( \rho \) subunits that occur in the retina: \( \rho_1 \) (Cutting et al., 1991); \( \rho_2 \)
(Cutting et al., 1992; Kusama et al., 1993b); \( \rho_3 \) (Ogurusu and Shingai, 1996; Shingai et al., 1996). In co-expressions, evidence was not obtained to show that a \( \rho \) subunit can participate in combinations with the aforementioned \( \alpha, \beta, \) or \( \gamma \) types (Shimada et al., 1992; Kusama et al., 1993a), although more recently a \( \rho_1 \gamma_2 \) heteromer forming in heterologous expression was suggested (Pan et al., 1997). In the rat retina, however, a recent study by immunofluorescence microscopy showed punctate localizations of non-\( \rho \) GABA\(_A\) receptors and of \( \rho \)-containing receptors, which occur at different synapses and do not overlap (Koulen et al., 1998). Hence, a pool of at least 20 subunit types may be used in forming combinatorially the CNS GABA\(_A\) receptors, plus at least 3 \( \rho \) subunit types which assemble in a restricted manner.

B. The Subunit Number per Receptor Molecule

To understand the construction of GABA\(_A\) receptor subtypes from this repertoire of subunits, it is necessary first to establish the total number of subunits in each receptor molecule, then to know whether this number is constant for all the native compositions, and finally to know the stoichiometry of the subunit types within that number. Regarding the number of subunits per receptor, the suggestion often has been made that this will be the same (five subunits) as for another transmitter-gated ion channel where the composition has been established unequivocally. Thus, the GABA\(_A\) receptor subunits share a low but definite (\( \sim 25\% \)) amino acid sequence homology with the subunits of the nicotinic acetylcholine receptors, both being in the same superfamily of the transmitter-gated ion channels (Schofield et al., 1987; Barnard, 1996b). The muscle type of that receptor occurs in Torpedo electric organ at such a high density in large postsynaptic membrane sheets that it is possible to prepare membranes containing a surface lattice of the receptors, from which a low-resolution three-dimensional structure of the molecule could be obtained by electron optical diffraction techniques (Toyoshima and Unwin, 1988; Unwin, 1993). Those studies clearly showed that the muscle type nicotinic receptor is pentameric, with the ion channel located in the center of a rosette formed by five homologous subunits (with the stoichiometry \( \alpha_2 \beta_2 \gamma_6 \)).

For the GABA\(_A\) receptors, the situation is necessarily more complex, because the unique situation in the Torpedo postsynaptic membranes does not recur in the mammalian CNS and because there are many types of subunits involved, in varying combinations, in the receptor population. It is preferable, therefore, to use the natural GABA\(_A\) receptor population from the brain rather than a selected recombinant composition expressed in a nonneural cell, which may or may not be representative of the native population; further, when direct analyses are made on the latter, these will not be limited by an assumption of the subunit classes to be taken as co-assembling. Using purified GABA\(_A\) recep-

![Fig. 4](Image 341x141 to 521x307)

**Fig. 4.** Evidence for the pentameric structure of native GABA\(_A\) receptors. The electron microscopic images of a population of pure GABA\(_A\) receptor molecules analyzed to yield dominant symmetry for each particle were used in plotting the histogram (for details see Nayeem et al., 1994). Particles with only one-fold apparent symmetry, which is trivial, were rejected. The form of the distribution seen around the peak at five-fold symmetry is consistent with 100% being pentameric, because the apparent spread to some lower symmetries can be caused by tilted particles. The distribution shown was confirmed on a large number of the particles (from Nayeem et al., 1994; E. A. Barnard, personal communication).
and (separately) it is presumed that the pentameric structure that has been observed, within experimental error, for GABA<sub>A</sub> receptors holds for at least the great majority of that receptor type.

It can be concluded that the repertoire of (at least) 24 mammalian subunit isoforms described above is drawn on to a total of 5 for each receptor molecule. Evidence discussed below will show that in most (but not all) GABA<sub>A</sub> receptors α, β, and γ subunits co-exist in one molecule, and that yet other combinations exist accommodating in special ways the other subunit types, δ, ε, π, and (separately) ρ. It is assumed that the p-containing receptors are also pentameric, but this question has not been studied as yet.

C. The Subunit Isoforms in One Receptor

The majority of GABA<sub>A</sub> receptors contain, as noted, α, β, and γ subunits, whereas the total number of subunits per receptor is five (fig. 4). Hence the receptors in this set can have at least one of three general compositions: 2α.2β.γ, 2α.β.2γ, α.2β.2γ. Here, a notation is introduced in which the numeral represents the number of molecules of a given subunit class (α, β, etc.) present in one receptor molecule and not the isoform identity within that class; separating points are then used, and are absent when the stoichiometry is not being indicated. Such additional cases as 3α.β.γ and α.β.3γ are theoretically possible, but measurements of an electrophysiological property determined quantitatively by the number of tagged recombinant subunits of each type forming the channel (Backus et al., 1993; Chang et al., 1996), in the cases of co-expression of the α<sub>3</sub>β<sub>2</sub>γ or α<sub>1</sub>β<sub>2</sub>γ subunits, have excluded (at least in those cases) the presence of three of any of those types in one receptor molecule. The next logical step in enumerating the potential combinations of subunits, therefore, is to ask whether two isoforms of α or of β or of γ can occur in one receptor molecule, e.g., to produce compositions of the type (α<sub>1</sub>α<sub>2</sub>).2β.γ.

In the α subunits, there is a variety of evidence for such a co-occurrence of isoforms in a minority of GABA<sub>A</sub> receptors. This evidence has come first from co-precipitation of a second α isoform when a brain-derived population of GABA<sub>A</sub> receptors is treated with an antibody specific for a first α isoform. Receptors containing at least the pairs α<sub>1</sub>α<sub>2</sub>, α<sub>1</sub>α<sub>3</sub>, α<sub>1</sub>α<sub>5</sub>, α<sub>2</sub>α<sub>3</sub>, and α<sub>3</sub>α<sub>5</sub> have been detected thus (each in a minority, with the majority of receptors in the population containing a single α isoform) (Duggan et al., 1991; Luddens et al., 1991; Zezula and Sieghart, 1991; Endo and Olsen, 1993; Mertens et al., 1993; Pollard et al., 1993; Khan et al., 1996; McKernan and Whiting, 1996). Further, for the α<sub>6</sub> subunit that occurs (in the mature brain) only in the cerebellar granule cells (Laurie et al., 1992; Thompson et al., 1992) and in the similar granule cells of the cochlear nucleus (Varecka et al., 1994), antibody reactivities show α<sub>1</sub> and α<sub>6</sub> co-occurring in one cerebellar receptor (Pollard et al., 1995; Khan et al., 1996), although not for all the α<sub>6</sub> subunits there. For the γ subunits, the similar use of isoform-specific antibodies has, on brain extracts or purified receptor preparations, shown evidence for the co-occurrence of γ<sub>2L</sub> with γ<sub>2L</sub> and also of γ<sub>2S</sub> with γ<sub>2S</sub> (Khan et al., 1994a,b, Quirk et al., 1994a).

A second method for investigation of possible co-occurrence of particular isoforms is the application of isoform-specific antibodies in situ, i.e., in light or electron microscopic studies (table 1). Thus, by confocal laser microscopy with double or triple immunofluorescent staining, Fritschy et al. (1992) and Mohler et al. (1996a) have found that certain α pairs were co-localized on the membranes of various neurons (table 2). Co-localization of α<sub>1</sub> and α<sub>6</sub> subunits in single synapses of rat cerebellar granule cells also has been demonstrated by double-antibody labeling in postembedding electron microscopy (Nusser et al., 1996), although when used in a freeze-fracture method on such cells in culture a co-localization of α<sub>1</sub> and α<sub>6</sub> was not seen (Caruncho and Costa, 1994). Third, some electrophysiological properties of recombinant αβγ assembly containing two isoforms of α can be distinct from those with either isoform separately, demonstrated with α<sub>1</sub>α<sub>6</sub> or α<sub>1</sub>α<sub>5</sub> pairings (Ebert et al., 1994; Verdoorn, 1994).

Further, the δ subunit often has been found to replace γ subunits: Quirk et al. (1995) found that δ and γ are completely separable by antibodies [although Mertens et al. (1993) found some co-existence]. δ subunits were present in only 11% of all the receptors in rat brain but

<table>
<thead>
<tr>
<th>Methods for recognition of γ-aminobutyric acid&lt;sub&gt;A&lt;/sub&gt; receptor subtypes in situ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method</strong></td>
</tr>
</tbody>
</table>
| 1. High-resolution labeling methods | a. Immunofluorescence  
b. Immunocytochemical reaction  
c. Freeze-fracture/antibody labeling  
d. Antibody labeling in postembedding electronmicroscopy<sup>a</sup> |
| 2. Single cell RT-PCR<sup>b</sup>, combined with patch-clamp recording | a. Only one subtype is present |
| 3. Use of an absolutely subtype-specific drug (e.g., furosemide, for α<sub>6</sub>β<sub>2S</sub>γ<sub>2L</sub>)<sup>c</sup> | a. Large cells are required  
b. All the receptor mRNAs present must give rise to the assembled receptor<sup>d</sup>  
c. Specific for one defined composition; cases will be rare |
| 4. Patch-clamping must be applicable, or the drug must be labeled, for in situ binding |

<sup>a</sup> For example, using sized gold particles (Nusser et al., 1996).
<sup>b</sup> RT-PCR, reverse transcriptase-polymerase chain reaction.
<sup>c</sup> Examples to the contrary are given by Williamson and Pritchett (1994).
<sup>d</sup> This specificity for this drug has been reported by Korpi et al. (1995), but application at the microscopic level of this or any other subtype-specific ligand has not been reported yet. Furosemide as a noncompetitive antagonist selects α<sub>6</sub>β<sub>3</sub>γ<sub>2</sub> receptors as well as α<sub>6</sub>β<sub>2</sub>γ<sub>2</sub> receptors, but is 14-fold less active at the former (Wafford et al., 1996).
in 27% of those in rat cerebellum, from which both α6β2δ and α6βδγ3 combinations can be isolated (Quirk et al., 1995) and where Caruncho and Costa (1994) found in situ that the receptors contain either a γ or a δ subunit, but not both, by a label-fracture method. The subunit ε (which has some similarities to δ) also may replace γ in some cells of the hypothalamus and hippocampus (Whitting et al., 1997).

Receptor gene knock-out can provide additional evidence. In favorable cases it can show the co-occurrence of certain pairs of subunits. Thus, the homozygous mice lacking the α6 gene also lack the δ subunit protein in the cerebellar granule cells and the results obtained support other evidence that α6 and δ are paired in receptors there and not α1 and δ without α6 (Jones et al., 1997). The specific pharmacology of α6-containing receptors was confirmed in vivo in this system (Mäkelä et al., 1997).

D. Possibilities for Subunit Stoichiometry

On the basis of the extensive evidence reviewed above, that two isoforms of the α subunit can sometimes occur in one receptor, the receptors are considered as having two α places in the pentamer. Pollard et al. (1995) supported this by quantitation in the α1α6-containing cerebellar receptor. Likewise, Khan et al. (1994a,b) and Quirk et al. (1994a,b) found that two different γ isoforms can co-occur, although Mossier et al. (1994) and Im et al. (1995) did not find this; if the former statement holds two γ places can also be in the pentamer. However, it is not known whether any conclusion of this type would apply to the entire native population of GABA_A receptors. Because δ has been observed at some sites (see above) to occur with α and β subunits only, a plausible model is that either γ or δ (and perhaps ε) subunits can occupy the γ places (in different receptors). We will give an illustration here, only of the basis on which the theoretical maximum number of receptor compositions may be assessed.

Some of the native GABA_A receptors may have the stoichiometry 2αβ2γ (with the γ subunits in some cases being replaceable by δ or by ε). Several lines of evidence support this. Thus, for the γ2-containing receptors, there is evidence from immunoprecipitation analyses (as noted above) that the γ2γ3 pairing within one receptor molecule can occur in some cases (Khan et al., 1994b; Quirk et al., 1994a) and also that a subset of receptors in the cerebellum has the composition α1α6-B.γ2S γ21 (Khan et al., 1994b, 1996). Further, Backus et al. (1993) have deduced, by incorporating mutant subunits with altered electrophysiological effects in the recombinant αβ2γ3 receptor [expressed in human embryonic kidney (HEK) 293 cells], that the 2αβ2γ composition best fitted the properties found.

On the other hand, Chang et al. (1996), using a similar principle (in oocytes and using α1, not α3 subunits), found that there the evidence apparently favors the 2α2β2γ composition. The same stoichiometry also was derived for α1β3γ2 receptors, when expressed in HEK 293 cells, from the staining ratios of those subunits when separated in Western blots (Tretter et al., 1997). Moreover, the co-occurrence of β1 with β3, and of β2 with β3 (but not β1 with β2), isoforms has been indicated in some of the receptors from rat cortex by immunopurification (Li and De Blas, 1997) and likewise in rat cerebellum (Jechlinger et al., 1998). Benke et al. (1994) compared the fractions from rat whole brain containing β1, β2, or β3 subunits by immunoprecipitation and also excluded the β1β2 combination; however, in contrast to the findings just noted, they found that the β1β3 or β2β3 pairings also were absent. Overall, it is desirable to allow for possible 2αβ2γ forms in the nomenclature. In view of this situation and of the evidence for 2αβ2γ combinations, Li and De Blas (1997) suggested that the ratios of the β and γ subunits in the molecule (within the total of 5) may vary with the isoforms selected.

In the expression of recombinant receptors in either cultured cells or oocytes, any ternary combination of the α1β1γ2 type tested so far can yield a functional receptor in the membrane (e.g., Kirsch et al., 1995). The limit to

<table>
<thead>
<tr>
<th>Neurons</th>
<th>Subunits</th>
<th>Possible subtypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olfactory bulb</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Mitral cells</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Granule cells</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Short-axon cells</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Periglomerular cells</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Pyramidal cells</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Dentate gyrus granule</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Most interneurons</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Thalamus</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Relay neurons</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Reticular nucleus neurons</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Supraoptic nucleus</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Ventromedial, arcuate nuclei</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Purkinje cells</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Granule cells</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Golgi type II cells</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Motoneurons</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
</tbody>
</table>

| TABLE 2

Some of the γ-aminobutyric acid (GABA) receptor subtypes in specified rat neurons*

<table>
<thead>
<tr>
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<th>Possible subtypes</th>
</tr>
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</tr>
<tr>
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<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
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<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
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<td>α2A3, A5a3</td>
</tr>
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<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Ventromedial, arcuate nuclei</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
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</tr>
<tr>
<td>Granule cells</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Golgi type II cells</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
<tr>
<td>Motoneurons</td>
<td>α1 α2 α3 β1 γ2</td>
<td>α2A3, A5a3</td>
</tr>
</tbody>
</table>

* Co-expressed subunits were visualized immunohistochemically at the cellular level. The subunits analyzed here are α1, α2, α3, α6, β2, β3, γ2, and δ (where the anti-β2 antibody used does not distinguish between β2 and β2_P2). In each combination the subunits of that set not detected are not indicated. Where multiple isoforms of α or β co-occur, they are not necessarily combined in one receptor molecule; for all the subunits, the co-occurrences shown are within a cell not necessarily within a molecule, and sometimes are within a cell type. (The localizations are from Mohler et al. (1996b).)
the number of ternary subtypes in vivo apparently is not set by barriers to the co-assembly in certain cases but by the program for gene expression of different isoforms in a given cell. However, in a case where this was tested (Angelotti and Macdonald, 1993), when such an $\alpha + \beta + \gamma$ set is expressed the ternary combination assemblies (as far as the subunits are available) and is maintained at the cell membrane to the exclusion of binary combinations. Within the ternary assemblies, there are no obligate combinations or exclusions of $\alpha \beta$ pairings known from the co-distribution data at the present resolution limits. However, some exclusions are known (see above) at the $\gamma \delta$ position. Moreover, the $\delta$ subunit has a more restricted expression in the brain than $\gamma$ subunits (Wisden and Seeburg, 1992) and has fewer co-occurrences with other subunits; the same is true for $\epsilon$ (Whiting et al., 1997), whereas $\pi$ is clearly detectable in certain peripheral tissues only (Heblom and Kirkness, 1997). Hence those subunits cannot be included on the same basis as the others in permutations of the possible compositions.

An enumeration is obtained on the basis that, for a given subunit set which will form one receptor, there will only be one arrangement and stoichiometry in the molecule. This is found to be so with all other heteromeric proteins containing tightly-bound subunits; for example, there is only one cyclic order of the subunits, $\alpha_1, \gamma, \epsilon, \alpha_1, \beta, \delta$, present in the population of *Torpedo* acetylcholine receptors (Karlin, 1991). Moreover, with those subunits one does not find that the same receptor type, in a variety of skeletal muscles, can contain another stoichiometry. That constancy and the circular order of subunits around the rosette are fixed by the interactions between the interfaces of different subunits. In the case of a GABA receptor (the recombinant $\alpha_1, \beta, \gamma_2$, supporting evidence for a single configuration in the population, from the homogeneity of the channel properties, has been reported (Angelotti and Macdonald, 1993).

Therefore we do not count all possible permutations ($n = 36$) of 2 $\alpha$ isoforms present (out of the 6), but only those for a fixed order ($n = 21$); likewise, for the others. Splice variants could increase these numbers on the same basis, except that two alternatives from one subunit cannot be assumed to be able necessarily to co-occur. From what is known so far of excluded compositions and the restricted co-occurrence of subunits in certain cases, Barnard (1996a) has suggested that a maximum of the order of 800 combinations, of the types observed so far, would then be calculated. The true number is likely to be far smaller than this, but still much larger than for other known receptor subtypes.

The $\rho$ subunits apparently assemble separately from the others (discussed below). They do not affect the enumeration above, but can add a few separate subtypes in the total noted above.

### IV. Principles of the Classification

#### A. Application of Selectivities at the Binding Site for Benzodiazepines and Their Functional Analogs

1. The choice of a classification system. As noted above, this modulatory site presently offers by far the richest pharmacology for distinguishing subtypes of the GABA$_A$ receptors. BZs have no intrinsic activity on mammalian GABA$_A$ receptors, unlike some of the other modulators such as anesthetics (although such a direct effect of some BZs has been found in invertebrate GABA$_A$ receptors; Zaman et al., 1992). Most BZs act to enhance the action of GABA by increasing the frequency of channel openings and their bursts (Rogers et al., 1994). This can be explained partly by the ability of BZs to increase the affinity of GABA at its binding site. However, in tonic activation of hippocampal neurons by low GABA concentrations they also can increase the channel conductance (Eghbali et al., 1997).

Some other drugs were found to act in the opposite direction at this site, i.e., to decrease the action of GABA at its receptor (Pole et al., 1982; Braestrup et al., 1982), for which the late Willy Haefely introduced the term “inverse agonist,” i.e., having negative efficacy at this site (fig. 1). One ligand class, exemplified by flumazenil, Ro14–7437, ZK 93426, or RP 60503, has such low efficacy (at most subtypes) that they effectively act as antagonists at this site (fig. 1). The wide range of BZs and other ligands active at the same site (table 3) that was examined has led to compounds which discriminate among some of the subtypes. When tested in recombinant subunits expressed (in either *Xenopus* oocytes or transfected mammalian cells) in various combinations, a variety of such effects can be found, as will be detailed.

#### TABLE 3

<table>
<thead>
<tr>
<th>Chemical class</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4-Benzodiazepines</td>
<td>Diazepam</td>
</tr>
<tr>
<td>1,5-Benzodiazepines</td>
<td>Clozabam</td>
</tr>
<tr>
<td>2,3-Benzodiazepines</td>
<td>GYKI-53222</td>
</tr>
<tr>
<td>Imidazobenzodiazepinones</td>
<td>Bretazenil; FG 8205; ZG-63a</td>
</tr>
<tr>
<td>Imidazobenzodiazepine carboxamides</td>
<td>Imidazenil</td>
</tr>
<tr>
<td>Heterocyclic, annelated 1,4-diazepines</td>
<td>Brotizolam; clotiazepam; Ro 19-4603</td>
</tr>
<tr>
<td>Triazolopyridazines</td>
<td>CL 218872</td>
</tr>
<tr>
<td>Pyrazoloquinolines</td>
<td>CGS 8216; CGS 9895</td>
</tr>
<tr>
<td>Quinolines</td>
<td>PK 9084</td>
</tr>
<tr>
<td>Imidazooquinolines</td>
<td>NNC 14-0578; U-93631</td>
</tr>
<tr>
<td>Imidazooquinazolines</td>
<td>NNC 14-8198b</td>
</tr>
<tr>
<td>Benzoquinolizones</td>
<td>Ro 19-8022</td>
</tr>
<tr>
<td>Pyrazolopyridines</td>
<td>CGS 20825; ICI 190622</td>
</tr>
<tr>
<td>Benzothiepinopyridazinones</td>
<td>Y-23684a</td>
</tr>
<tr>
<td>Thiencyclidines</td>
<td>NNC 14-0590</td>
</tr>
<tr>
<td>Imidazopyridazines</td>
<td>Zolpidem</td>
</tr>
<tr>
<td>Imidazopyrimidines</td>
<td>Divalpro; Ru 33-203</td>
</tr>
<tr>
<td>Cyclopentolone $\beta$-Carbolines</td>
<td>Zopiclone, surlcione</td>
</tr>
<tr>
<td>Cyclopentolone $\beta$-Carbolines</td>
<td>Zopiclone, surlcione</td>
</tr>
</tbody>
</table>

a The examples chosen are positive modulators on at least some of the GABA$_A$ receptor subtypes; some may also be negative modulators at other subtypes.

b ZG-63 (Wong et al., 1993); NNC 14-0578/NNC 14-8198 (Wong et al., 1995); Y-29684 (Yasumatsu et al., 1994).
### Classification of some of the γ-aminobutyric acidA receptors

<table>
<thead>
<tr>
<th>GABA&lt;sub&gt;A&lt;/sub&gt; receptor subtype</th>
<th>Composition&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Characteristic properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1a</td>
<td>α₁, β₁, γ₂</td>
<td>High affinities and efficacies for classical BZ agonists,&lt;sup&gt;b&lt;/sup&gt; CL 218872 (partial agonist), zolpidem, 2-oxoquazepam&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>A1b</td>
<td>α₁, β₁, γ₁</td>
<td>Same as A1a, but 400-fold less sensitive to zolpidem and affinities are lower for 2-oxoquazepam (in the same range as for A2, A3, and A5) and for classical BZ agonists&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>A1c</td>
<td>α₁, β₁, γ₁</td>
<td>Same as A1b, but flumazenil and Ro 15-4513 have low affinity and act, like β-carbolines (inverse agonists at A1a,b), as low-potency positive agonists&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>A2a</td>
<td>α₂, β₁, γ₂</td>
<td>Similar to A3 for the ligands noted there, but other properties not yet defined</td>
</tr>
<tr>
<td>A2c</td>
<td>α₂, β₁, γ₁</td>
<td>BZω agonists have 2- to 20-fold lower potency than on A2a, with FG2805 the most selective. The affinity of zolpidem is 5-fold greater on A2c but with very low efficacy. Insensitive to antagonists (e.g., flumazenil, CGS-8216, and Ro 15-4513). DMCM is an agonist&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>A3a</td>
<td>α₃, β₁, γ₂</td>
<td>High affinities and potencies for classical BZ agonists and β-carbolines, similar to those of A1, but intermediate for zolpidem, for CL 218872 and 2-oxoquazepam, 10-fold lower than on A1e&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>A4a</td>
<td>α₄, β₁, γ₂</td>
<td>Inensitive to classical BZ agonists, zolpidem and many other BZω agonists. Notable exceptions are brezatienz, CGS 20625, and some pyrazoloquinolines. Intermediate affinities for most β-carbonate inverse agonists (~10 times higher than at α₆ β₁ γ₂), but high affinity for DMCM. Flumazenil and Ro 15-4513 are agonists. The direct activation by propofol or pentobarbital is absent&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>A5a1</td>
<td>α₅, β₁&lt;sub&gt;c&lt;/sub&gt;, γ₂</td>
<td>A5: High affinity for classical benzodiazepine agonists but insensitive to imidazopyridines. Intermediate affinity for CL218872 and 2-oxoquazepam. Certain 8-acetylenic imidazobenzodiazepines (inverse agonists) and L-655,708 (BZω agonist) are highly selective</td>
</tr>
<tr>
<td>A5b3</td>
<td>α₅, β₁, γ₃</td>
<td>Affinities of A5b3 are as for A5a1, but triazolam and β-carbolines are ~10- to 30-fold weaker and CL 218872 is 10-fold stronger</td>
</tr>
<tr>
<td>A5a2</td>
<td>α₅, β₁, γ₂</td>
<td>A5a1 differs from A5a2 in its outward rectification and its slower desensitization at depolarized voltages&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>A6a1</td>
<td>α₆, β₁, γ₂</td>
<td>Insensitive to all BZω ligands except brezatienz and some other partial agonists; flumazenil and Ro 15-4513 become partial agonists and DMCM an antagonist (fig 5)</td>
</tr>
<tr>
<td>A6a2</td>
<td>α₆, β₁&lt;sub&gt;c&lt;/sub&gt;, γ₂</td>
<td>Same as for A6a1, but A6a2 is antagonized selectively by furosemide (see notes to table 1)</td>
</tr>
<tr>
<td>A16a2</td>
<td>α₁&lt;sub&gt;t&lt;/sub&gt;, β₁&lt;sub&gt;c&lt;/sub&gt;, γ₂</td>
<td>Combines the binding sites of A1a and A6a2&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>A0r</td>
<td></td>
<td>A0r: insensitive to all BZω ligands, but also to bicuculline and pentobarbital. Not activated by isoguvacine</td>
</tr>
<tr>
<td>A0r1</td>
<td>ρ₁</td>
<td>A0r12 (alone) has very low sensitivity to picrotoxin (in the rat) (for references: see Sections I.A. and IV.D.)</td>
</tr>
<tr>
<td>A0r2</td>
<td>ρ₂</td>
<td></td>
</tr>
<tr>
<td>A0r12</td>
<td>ρ₁ρ₂</td>
<td></td>
</tr>
<tr>
<td>A0r3</td>
<td>ρ₃</td>
<td></td>
</tr>
<tr>
<td>A01, A02</td>
<td></td>
<td>Insensitive to all BZω ligands, not because of ρ, but (e.g.) α + β + δ or α + β + ε. Sensitive to bicuculline</td>
</tr>
<tr>
<td>A01</td>
<td>α₁, β₁, δ</td>
<td>Same as above, and highly sensitive to zinc Generally similar to A01</td>
</tr>
<tr>
<td>A04</td>
<td>α₄, β₁, δ</td>
<td></td>
</tr>
<tr>
<td>A06</td>
<td>α₆, β₁, δ</td>
<td>Same as for A01; in cerebellar granule cells only&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>A10e</td>
<td>α₁e, β₁, ε</td>
<td>Generally similar to A01</td>
</tr>
</tbody>
</table>

---

<sup>a</sup> This means that, e.g., the GABA<sub>A</sub><sub>δ</sub> receptor has a pharmacology which mimics that of the co-expressed recombinants α₂β₁γ₂, where n = 1–3 (in tests so far), unless distinctions are known because of the β isoform present; β₁<sub>c</sub> means β₁ or β₁<sub>c</sub>. The stoichiometry within the assembly is not implied. Both binding affinities and effects on GABA-evoked currents are considered in the pharmacologies compared in column 3. For ease of comparison here, in the second column the isofrom numbers are not written as subscripts (which will be the correct general usage). Species differences in these receptors can occur within the mammals; where possible, data on the human receptors have been used here but, where not available, data on the rat are used. In general, the rule of preference, failing available human receptor pharmacology, will be rat, then other mammals, then birds.

<sup>b</sup> Classical BZ agonists are diazepam, flunitrazepam, clonazepam and other BZs of similar activity. Except where noted otherwise, flumazenil is an antagonist and Ro 15-4513 is a partial inverse agonist, both with high affinity.

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*Note: For "high affinity" for zolpidem would cover reported Kᵢ values (for the rat receptors), for α₁ β₁<sub>c</sub> γ₂ of 19 to 30 nM and "intermediate affinity" would cover 688 nM Kᵢ, or 650 nM EC<sub>50</sub> in potentiation of the GABA-evoked current (Faure-Halley et al., 1993; Luddens et al., 1994).

*<sup>c</sup> Yang et al. (1995); Hu et al. (1996); Knollfach et al. (1996). *<sup>d</sup> Schulze et al. (1990); Wafford et al. (1996).


*<sup>f</sup> A6: On A6a1/A6a2, partial agonists brezatienz and CGS-9895 and the antagonist flumazenil show 44 to 270 nM Kᵢ values (human, rat). A16a2 has high-affinity flumazenil and Ro 15-4513 binding sites, diazepam-sensitive. References: A6a1, A6a2, Luddens et al. (1990); Korpi et al. (1995); Yang et al. (1995); Hadingham et al. (1996); Hu et al. (1996); Nusser et al. (1996); Wafford et al. (1996). A16a2: Fullard et al. (1995); Khan et al. (1996).

below. For those cases where subtypes are established, the nomenclature for them ideally would be based only on molecular biology and would express the subunit composition, e.g., the \( \alpha_1\beta_2\gamma_2 \) subtype (which then could be abbreviated as \( \text{GABA}_{122} \), etc.). Even this scheme would be cumbersome to use, e.g., needing expansion to cover all the subunit forms, etc., as discussed in Section IV.B. below. It is acceptable for stating the composition of an experimentally expressed mixture of recombinant subunits (but this entails some simplifications, detailed in Section IV.B.). In contrast, it generally cannot be known in practice what this subunit composition is for the native receptors whose function is being measured by any current methodologies. In summary, a receptor composition (but not its stoichiometry) can be proposed in some cases of artificial co-expression in a heterologous system, but this does not classify native receptors.

Approaches are now being made to identifying some of the compositions of native GABA receptors in situ: the methods presently available for this are listed in table 1. It can be seen that these are very limited so far, and usually will not specify the stoichiometries of the subunits identified. In none of the cases where these methods have been used has an unambiguous correspondence to pharmacological activity in vivo been feasible. One potential exception would be method 2 of table 1, which was applied to single cells in the thin-slice recording system (Santi et al., 1994). In situ identifications of co-occurring subunits that have been obtained with method 1a are exemplified in table 2.

Thus, the methods listed in table 1 cannot deal as yet with the wide range of the subtypes nor overcome the resolution problems for most native situations of co-occurring multiple subtypes. Therefore, for an assignable and practical nomenclature, we are driven to using a pharmacologically based system. However, the hard information being obtained by recombinant receptor expression studies, e.g., that the \( \gamma_1 \) subunit always introduces atypical modulatory effects of BZs, acts as a constraint and must be kept in view when assigning the pharmacologoes. This is done in table 4, using the only site on the GABA\(_A\) receptors at which a sufficient pharmacology yet exists, the site binding BZs. When an in situ composition becomes firmly established for a given subtype, recombinant co-expression of that set of subunits to display the pharmacology of that subtype should establish or confirm its assignment in table 4.

Examples cited in this text show that there may not always be agreement on the evidence for co-assembly in vivo of a particular set of subunits. Independent confirmation thus is needed before a definite assignment is accepted.

2. Benzodiazepine-responsive \( \gamma \)-aminobutyric acid receptor subtypes. To obtain receptors expressing properties most closely resembling those of BZ-responsive GABA\(_A\) receptors on neurons, recombinant \( \alpha, \beta, \) and \( \gamma \) subunits are required to be co-expressed (as reviewed by Macdonald and Olsen, 1994 and McKernan and Whiting, 1996). Some \( \alpha \gamma \) or \( \beta \gamma \) combinations can reproduce many of those native properties but never in the full range that has been observed in vivo; the variety of functional patterns which has been observed with different \( \alpha \gamma \) or \( \beta \gamma \) combinations in vitro is detailed, with references, in Section III.B. of Sieghart (1995). It neither has been demonstrated nor excluded that some binary combinations exist in vivo, but if they do, this must be to a small extent in numbers or in locations. If such exist, the assumption is made that they are pentameric. If the \( \alpha_4 \) and \( \alpha_6 \) isoforms are used in \( \alpha \beta \gamma \) combinations, however, a different type of activity is produced; the effect varies with the modulator (table 4, fig. 5), but classical BZ full agonists such as diazepam and midazolam have greatly reduced affinities (Wisden et al., 1991; Kleingoor et al., 1991; Yang et al., 1995; Scholze et al., 1996). \( \beta \)-Carboline inverse agonists can be active on these subunit combinations but usually with much lower affinity, especially for the \( \alpha_6\beta_2\gamma \) combination (Kleingoor et al., 1991; Yang et al., 1995; Knoflach et al., 1996). From a wide range of recombinant studies of that kind, several

![Fig. 5. Modulation of EC\(_{20}\) responses to GABA by BZ and \( \beta \)-carboline ligands. a, Modulation of GABA EC\(_{20}\) responses by BZ ligands in oocytes injected with \( \alpha_6\beta_1\gamma_2 \), \( \alpha_6\beta_1\gamma_2 \), and \( \alpha_6\beta_1\gamma_2 \). b, Modulation of GABA EC\(_{20}\) responses by \( \beta \)-carboline ligands in oocytes injected with \( \alpha_6\beta_1\gamma_2 \), \( \alpha_6\beta_1\gamma_2 \), and \( \alpha_6\beta_1\gamma_2 \). All compounds were examined at a concentration of 1 \( \mu \)M, and the data shown are the mean \pm standard error for at least four oocytes. From Wafford et al. (1996). The three groups represent the A1a1, A6a1, and A4a1 subtypes of table 4.](image-url)
such ternary combinations have been distinguished, in fact; the α subunit isoform present exerts a major effect on the affinity and efficacy of ligands at the BZ site, as shown in table 4.

The BZs themselves are rather poor tools to make these distinctions; compounds of quite different structures but with BZ-like activities are more effective. These include (fig. 2; table 3) triazolopyridazines, pyrazolopyrimidines, heterocyclic annelated 1,4-diazepines, imidazopyridines, cyclopyrrolones, β-carbolines, etc. Table 4 shows that a variety of pharmacologies can be found using these modulators as probes, both those with positive and some with negative efficacy. These properties are produced experimentally by the recombinant subunit combinations shown; the main value of this approach for classification lies in the possibility of recognizing those individual pharmacologies in native receptors. We therefore recommend that the subtypes of the GABA_A receptor should be designated as a series, GABA_A1, GABA_A2, etc.

The isofoms of the γ subunit which is also present can modify the effect of the α isoform. The γ_2 subunit mRNA is much more abundant in the brain than that for γ_3 or γ_1, and the great predominance of the γ_2 subunit is confirmed by the results seen with γ_2-less transgenic mice, in which the BZ-binding sites and the BZ sensitivity of the GABA_A receptors are virtually totally extinct (Günther et al., 1995). Positive modulation by most BZ-type agonists is reduced when γ_2 is replaced by γ_1, and inverse agonists (β-carbolines) then become agonists (Puia et al., 1991; Giusti et al., 1993). Flumazenil is bound much more weakly when γ_1 is present (Ymer et al., 1990) and changes from an antagonist to a BZ agonist (Wafford et al., 1993). Hence the γ_1-containing receptors can be classified separately. A specific but lesser effect is known for the replacement of γ_2 by γ_3, as in the α_1- and α_3-containing receptors (table 4). If γ is replaced by δ (or ε) none of these pharmacologies apply and new (BZ-insensitive) subtypes are created (Wisden and Seeburg, 1992; Saxena and Macdonald, 1994, 1996). Native subtypes exist in the rat cerebellum which contain δ and have no high-affinity BZ binding (Quirk et al., 1995).

It will be only an initial simplification to classify the receptors on the basis of their α or γ or δ or ε subunit content, because of the other subunits present in the molecule. In future extensions, further subsubtypes would be listed based on a constant α subunit and variable β and γ, where these are shown to define specific subpharmacologies.

The subdivisions are made on the principle of the economy of classification, in that subcategories are not set up for every theoretical combination with γ or β isoforms (combinations which often may not exist in the nervous system), but only for those where functional discrimination is needed.

3. Notation. For identifying the subunits, the Greek letters already in use are acceptable for IUPHAR usage, for that purpose; their isoform numbers must be subscripts to them, as written in this text.

For the receptor subtypes notation, however, it should be remembered that we are constrained to keep to the IUPHAR general numbering scheme, intended to be uniform for all receptors and for ease of entering subtypes in information systems (Vanhoutte et al., 1996). Hence, Greek letters, further subscripts, internal separations by dots, and other typographical devices not used here are not permitted for the subtypes.

Conforming with these requirements, in the system used GABA is given as the receptor type and all the rest follows as a subscript to it. A is the designator of every GABA_A receptor. In the first position after this, for all of the “BZ-sensitive GABA_A receptors,” i.e., those responding in some manner to BZ/ω ligands, there is a numeral showing the α subunit isoform which would be needed for the behavior seen. In the successive positions (more accurately, fields) after this, an alternation is use of letters (lower case) and numerals for further pharmacological subsets. This allows more than one letter (or numeral) to be used in the same field where desired (particularly for any co-occurrence of two isoforms of one subunit) without causing confusion with the adjacent fields. Because a γ subunit is needed in all the known cases having a BZ site, in the second field there is a lower-case letter designating the γ subunit isoform also involved; these are named in order of abundance in the brain. Because γ_1 will rarely be involved, a denotes γ_2, b γ_3, and c γ_1.

In the third field after A, there is another numerical series, used only when the choice of β isoform has been shown to be significant. The number of the β isoform then is added here. Where, as often occurs, the β isoform present cannot be deduced from the pharmacology studied, this can be omitted, or n can be used. Where either of 2 alternative isoforms is known to give the described pharmacology (e.g., β_1/β_2), the lowest number is cited. If receptors are identified which are differentiated pharmacologically because of the presence of two isoforms of the β subunit, then both numerals are used, e.g., 13 denotes the co-occurrence of β_1 and β_3 subunits. Likewise, if pharmacologically differentiated receptors containing multiple isoforms of γ are identified, they can be designated by their two letters in the second field, e.g., ab for γ_2γ_3. According to present evidence (Khan et al., 1994b; Quirk et al., 1994a) that is the only pairing which occurs of the γ isoforms (apart possibly from pairs of γ_2 splice variants, encoded below). Present evidence generally indicates that a γ subunit does not co-occur with a δ or ε subunit in the BZ-sensitive receptors, but if such a case were to be established as a native receptor, then identifying English letters could be assigned to δ or ε when added in that field.

For splice variants, where a functional difference is known, (l) or (s) (for longer and shorter forms) can be added after the designating letter, because a single
length change specifies each case so far. This must be in lower case and in parentheses for the uniform system (Vanhoutte et al., 1996). This would need extension when more complex splicing is discovered.

The GABA<sub>A</sub> receptors insensitive to BZ/ω ligands are described as A0. Where this is because of δ or ε, for example, they then are numbered on the same principles as above. Where it is because of the ρ subunit, these are the A0ρ receptors, numbered as shown in table 4. If it is confirmed that a receptor can mix ρ and other subunit types, then additions based on the aforementioned principles can be made to the A0ρ numbers.

An example from the recent literature which can illustrate the functional classification is given in fig. 5 (Wafford et al., 1996). This shows the differences in the responses to various modulators at this site which define pharmacologies in the A1, A4, and A6 groups of table 4. Note how a given ligand can be an agonist, an inverse agonist, or an antagonist as the ρ subunit is varied.

B. Advantages and Possible Modification of the Classification

The advantages of the classification of table 4 are:

(i) At a glance this classification shows for which putative subunit compositions a pharmacological recognition of a subtype is possible with the present tools. The primary criterion for the classification is the defined functional behavior.

(ii) The many potential compositions for which no pharmacological distinction is known do not confuse the classification.

(iii) It indicates similarities or differences between structurally related receptors.

(iv) For a case to be listed in table 4, some evidence should exist, obtained (for example) by the methods of table 1 or by immunoprecipitations, that the receptor subtype noted occurs in vivo. For example, a general finding from such studies is that α<sub>1</sub>β<sub>2</sub>γ<sub>2L/S</sub> and α<sub>1</sub>β<sub>1</sub>γ<sub>2L/S</sub> are common compositions existing in situ, and hence A1a1 and A1a2 are confirmed subtypes.

Despite these considerations, some may prefer to refer to a given subtype by its putative composition from column 2 of table 4. This alternative notation can be used, but it should be made clear that this is not a statement that the receptor has this absolute composition, but denotes a recombinant set of subunits which mimics the properties of a native receptor, so far as has been tested. Because not all receptor properties necessarily can be compared and because a multiplicity of native receptors occurs at most locations, this correspondence does not establish that a single native GABA<sub>A</sub> receptor has that precise composition. In one or two favorable cases this can become very probable. In some others a distinctive pharmacology may be definable with a set of recombinant subunits assembling in a foreign cell type, but with no certainty that it occurs in situ. As one example, the δ subunit readily assembles functionally with α<sub>1</sub> or α<sub>6</sub> (plus β) subunits in transfected mammalian cells (Saxena and Macdonald, 1994, 1996; Krishek et al., 1996), but in the granule cells in the cerebellum, which contains all these subunits (and γ<sub>2</sub>), α<sub>6</sub>βδ and α<sub>1</sub>βγ<sub>2</sub> are expressed strongly, but α<sub>1</sub>βδ is undetectable, as shown both by co-immunoprecipitation (Quirk et al., 1994b) and by gene knock-out manipulations (Jones et al., 1997). If this alternative usage is applied to a native receptor, the term “like” must be added, as in “α<sub>2</sub>β<sub>1</sub>γ<sub>1</sub>–like GABA<sub>A</sub> receptor” instead of “GABA<sub>A</sub>2<sub>b</sub> receptor.” The former terminology is itself a simplification and cannot readily be used universally. Thus, additional notations would be needed to denote the subunit types that occur in pairs (when these become known), and further to denote those which occur in nonidentical pairs or as splice variants, and to allow for the combinations containing subunits other than the α, β, and γ types. Also, the functional species present after co-expression may vary with the proportions of the different cDNAs (or RNAs) used, which would have to be specified (using the coding region lengths) unless independence thereof has been documented. In some cases, mixtures of active products may be generated; it is particularly difficult to use this approach for a receptor containing two isoforms of one subunit type, e.g., α<sub>1</sub>β<sub>3</sub>δ<sub>1</sub>γ<sub>2</sub>. Further, several cases have been found in which the host cell used for the co-expression influences the properties observed; the disparities are usually between expression in the oocyte and in a mammalian cell line, but even different cell lines may affect the outcome, perhaps because posttranslational changes. Examples of these disparities are given in Section III.B.7. of Sieghart (1995).

All these limitations should be considered before it is asserted explicitly or by implication in the terminology that a given co-expression in a nonneural cell of subunit cDNAs (or RNAs) is equivalent to a specific native GABA<sub>A</sub> receptor.

C. Nomenclature for the Site Binding Benzodiazepines and Modulators with Similar Activity

Discussion of this modulatory site and its ligands is necessarily so frequent in the classification of GABA<sub>A</sub> receptors that a succinct name is clearly a necessity. It would be ideal to name this site for a natural modulatory ligand. However, whereas several endogenous potential ligands have been identified, the physiological relevance of these compounds is presently unclear. Synthetic ligands active at this site can be defined operationally as those whose binding is inhibited competitively at this site by a known BZ antagonist or partial inverse agonist such as flumazenil, CGS 8216, Ro15–4513, or RP60503.

Initially this site was recognized by the action of many 1,4-BZs (figs. 1, 2) and hitherto it has been referred to as “the BZ site,” but this nomenclature now requires fur-
ther consideration. It raises the following issues, among others:

(a) Discrimination of subtypes through this site, in practice, usually is made by ligands that are not BZs, but represent a diverse range of chemical structures, as named in table 3.

(b) BZ binding sites occur in other, entirely different, receptors, which also are commonly named for that drug class, i.e., the BZp discussed above. The “BZ site” on those receptors has no relation to the site on GABAA receptors.

(c) There are other types of BZ binding sites, apart from BZp, of pharmacological significance, not on GABAA receptors. These include, among others, the sites for 2,3-BZs, such as GYKI-52322, which are abundant in certain brain regions, in a pattern very distinct from that of 1,4-BZ binding (Horvath et al., 1994). Some of these sites are on non-N-methyl-D-aspartate glutamate receptor channels and there is no activity on GABAA receptors (Bleakman et al., 1996). Hence, when “BZ sites” in the CNS are discussed, one ought to distinguish between sites for 1,4-BZs, for 1,5-BZs (which affect likewise some GABAA receptor subtypes), and those for 2,3-BZs, all of which include drugs in current pharmacological use. At least some qualification is needed to exclude the ligands for receptors other than GABAA receptors.

It has been argued, however, that the term “BZ site” is used so widely, even when other structures are being used for studying or exploiting it, that it would be confusing to replace it by an unfamiliar term. There is no chemical class which will encompass all of the diverse ligand types for this site which are now in use, so no self-explanatory term can be substituted. A neutral term “BZ/ω” has been proposed for it (Langer and Arbilla, 1988) but this has the drawback of not being at once recognizable in this context. It could be made so by retaining “BZ” with it, as “BZ/ω.”

Therefore, the following recommendation is made: Sites of GABAA receptors at which modulation of GABA activation occurs by BZs and other ligands of related activity (even if different in chemical structure) should continue to be termed “the BZ site (or sites)” or, as an acceptable option, the “BZ/ω site.” It is not recommended to add numbers to the BZ or BZ/ω terminology, but if this is done, then the numbering should follow the GABAA numbering system. The terms “BZ receptor” or “GABA/BZ receptor” or “omega receptor” should no longer be used.

In this review the term “BZ site” will be used, but also “BZ/ω ligands” for clarity where non-BZ modulators at this site are being included. The latter option seems to be a clearer term for these ligands in that instance.

**D. Benzodiazepine Insensitivity**

A minority of GABAA receptors detected in the nervous system are not modulated at all at the BZ site (Unnerstall et al., 1981; Polenzani et al., 1991; Rovira and Ben-Ari, 1991; Sivilotti and Nistri, 1991; Quian and Dowling, 1993; Quirk et al., 1995). The nomenclature proposed here includes an additional GABA A0 class for these receptors. Because insensitivity to BZ/ω ligands can arise by different molecular mechanisms, we would need GABA A01, GABA A02, etc. However, because one form of insensitivity at this site arises from the presence of a δ subunit or of an ε subunit in some situations, whereas another could potentially arise from the presence of α and β subunits alone, it seems more self-consistent to include these as shown in table 4. More information is needed on the presence of these various pharmacologies in native receptors before we can decide on detailed A0 series assignments.

The π subunit also produces BZ-insensitive receptors with α and β subunits in vitro (Heblom and Kirkness, 1997) and could therefore yield another subset of the A0 class. However, study of the π subunit has only just begun and as yet there are no clues as to its partners in vivo. Because its expression apparently does not occur in the brain but is in a few peripheral nonneuronal locations, potential combinations of π there with subunits exclusive to the brain can be ignored pending further information and it is not included in table 4.

One class of receptors insensitive to BZ/ω ligands clearly needs to be numbered separately, because its structural basis is so different. These are the ionotropic GABA receptors which are also insensitive to bicuculline and to barbiturates (Bormann and Feigenspan, 1995; Johnston, 1996), described previously as “GABA C receptors,” as discussed earlier in this review. This receptor type contains the ρ1, ρ2, or ρ3 subunits (Section III.A.), all three of these subunits being highly expressed in the retina (Cutting et al., 1991; Enz et al., 1995; Ogurusu et al., 1997).

These receptors are distinguished also by their insensitivity to the GABA agonist isoguvacine (Woodward et al., 1993) and by their activation by cis-4-aminopent-2-enolic acid [cis-4-aminocrotonic acid (CACA)] (Kusama et al., 1993a). CACA is essentially inactive at a wide range of the other GABAA receptors (Johnston, 1996); however, CACA may not be fully diagnostic for the ρ-containing receptors because it is active on certain bicuculline-sensitive GABA receptors in hippocampal cells (Strata and Cherubini, 1994). Because, as noted above, the ρ subunits do not (so far as is known) co-assemble with other GABAA receptor subunits, we designate the ρ-containing receptors as a separate series, A0r. In the rat retina the bicuculline-resistant form of the receptor is also picrotoxin-resistant and is mimicked by expressed recombinant ρ1ρ2 heteromeric receptors (Zhang et al., 1995) and not by rat or human ρ1 or ρ2 homomers (Kusama et al., 1993b; Wang et al., 1994; Zhang et al., 1995) nor by ρ3 homomers (Shingai et al., 1996). Hence one can define, on present knowledge, A0r1, A0r2, A0r3, and A0r12 as subtypes in this series.
Pharmacalogies corresponding to some of these recombinant oligomers have been observed in retinal rod bipolar cells (Bormann and Feigenspan, 1995). Further \( \rho \)-containing subtypes, if established as native receptors, would be numbered on the same principle. Whether \( \alpha 0r1, \alpha 0r2, \) and \( \alpha 0r3 \) occur in situ as homomeric functional receptors (and whether the \( \rho_1\rho_3 \) combination occurs) is not yet known.

V. Chromosomal Localization of \( \gamma \)-Aminobutyric Acid \( \text{A} \) Receptor Genes

This topic is relevant to the subunit composition of the receptor subtypes, because it has been found that the genes for the considerable number of subunits involved occur in clusters, three or four at each locus, on human chromosomes. The first of these genes to be localized (Buckle et al., 1989) were those for the \( \alpha_1, \alpha_2, \alpha_3, \) and \( \beta_1 \) subunits, which are spread among three chromosomes. However, subsequent localizations of other subunit genes show that the genes actually occur in groups (fig. 6). (References are: \( \alpha_4, \) McLean et al., 1995; \( \alpha_6, \) Knoll et al., 1993; \( \alpha_6, \) Hicks et al., 1994; \( \beta_3, \) Russel and Farb, 1994; \( \beta_3, \) Wagstaff et al., 1991 and Glatt et al., 1997; \( \beta_4, \) \( \epsilon_2, \) Levin et al., 1996 and Whiting et al., 1997; \( \gamma_1 \) and \( \gamma_2, \) Wilcox et al., 1992; \( \gamma_3, \) Gregor et al., 1995).

Each cluster contains genes of the \( \alpha, \beta, \) and \( \gamma/\epsilon \) classes, in line with the composition of most of the receptor subtypes. Selective gene clustering on this scale is unprecedented for a receptor. Where the gene order has been determined so far, it is \( \gamma/\epsilon-\alpha-\beta \). These phenomena suggest a possible relationship to subunit co-expression. McLean et al. (1995) have proposed that these clusters are derived by a series of gene duplication events from a single ancestral \( \alpha\beta\gamma \) gene cluster. Also, two isoforms of \( \alpha \) genes can co-localize (fig. 6), in line with the co-occurrence of two \( \alpha \) isoforms in one receptor that has been noted frequently (as described above).

The \( \delta \) subunit has become separated from these clusters, on chromosome 1 (Sommer et al., 1990). The two \( \rho \) subunit genes that have been mapped, \( \rho_1 \) and \( \rho_2 \) (subunits which co-assemble), lie together on human chromosome 6 or mouse chromosome 4 (Cutting et al., 1992).

VI. Other Binding Sites in Relation to the Receptor Classification

A. Other Modulatory Sites

It must be emphasized that all the classifications of table 4 are provisional. As more \( \text{BZ/} \omega \) ligands that can discriminate receptor subtypes are found, as differential effects of non-\( \alpha \) subunits are explored further, and as the other modulatory sites are compared on many subtypes, extensions and revisions certainly will be needed. Thus, assistance in subclassifying the \( \text{A1} \) to \( \text{A6} \) series should come from criteria based on ligands recognizing particular \( \beta \) or \( \gamma \) isoforms. Loreclezole is a \( \text{GABA-potentiating} \) drug which does not act at the \( \text{BZ} \) site but which recognizes the presence of both \( \beta_2 \) and \( \beta_3 \) but not \( \beta_1 \) subunits (Wafford et al., 1994). Several anesthetic drugs and neurosteroids (Macdonald and Olsen, 1994; Sieghart, 1995) also act as potentiators of \( \text{GABA} \) (and in some cases as direct activators), but high subunit selectivity has not been found as yet. The anesthetic etomidate has a very strong preference for direct activation, for a \( \beta_3 \) over a \( \beta_1 \) subunit, which a residue in the \( \beta \) TM2 domain affects (Belelli et al., 1997). The propofol and pentobarbital sites for direct activation of the receptor are diagnostic (in the \( \gamma_2 \)-containing series) for receptors containing \( \alpha_4 \); only the presence of \( \alpha_4 \) abolishes that effect (Wafford et al., 1996). Recombinant \( \alpha_1\beta_2\alpha_4 \) or \( \alpha_2\beta_2\alpha_4 \) receptors lack the \( \text{GABA-potentiation} \) response to these drugs (Davies et al., 1997) and the \( \text{BZ} \) modulation. Because there is no information on the \( \epsilon \) combinations existing in situ, they are omitted from table 4.

Modulatory binding sites which differ from any of those recognized previously in the \( \text{GABA} \) receptors have been discovered more recently. These include a site for certain pyrazinones that potentiate \( \text{GABA action} \) (Im et al., 1993a) and a site for some dihydroimidazoxinones (Im et al., 1993b). The subunit specificity in these series has yet to be established. Furosemide is a more specific example; it is an antagonist (at a separate site) with a high selectivity at \( \alpha_6\beta_2\gamma_2 \) (Korpi et al., 1995) and a somewhat lower selectivity at \( \alpha_4\beta_2\gamma_2 \) receptors (Wafford et al., 1996). Thus, in oocyte expression the IC50 values were 6 \( \mu \)M and 160 \( \mu \)M, respectively, but >5 \( \mu \)M with all other combinations tested. Drugs of this character could provide a tool for recognizing such subunit combinations as subtypes in the CNS. Other such cases can be expected to be found as medicinal chemistry is applied systematically, using expressed subunit combinations as screening systems.

B. The \( \gamma \)-Aminobutyric Acid Recognition Site

Selectivity among subtypes for the \( \text{GABA} \) agonists available to date has not been high. Ebert et al. (1994) and Wafford et al. (1996) have described the concentration-response curves for four \( \text{GABA} \) agonists in recombinant \( \alpha\beta\gamma \) receptors, varying the \( \alpha, \beta, \) and \( \gamma \) subunit isoforms. Effects of the \( \alpha \) and \( \gamma \) isoform and (less) of the \( \beta \) isoform were found, but these were not primarily of diagnostic value. The largest difference was that with \( \alpha_4 \) (but not \( \alpha_6 \)) present, where the intrinsic activity with piperidine-4-sulfonic acid was (with \( \beta\gamma_2 \)) exceptionally low (6% at the maximum).
Large differences between the A0r receptors and the others are seen with GABA agonists and with GABA antagonists. The A0r receptor subtypes known so far are all highly resistant to bicuculline, an antagonist for all other subtypes. Some of the typical GABA_A agonists such as isoguvacine and piperidine-4-sulfonic acid are antagonists or inactive at the A0r receptors. Conversely, CACA is an agonist at A0r receptors but is usually inactive at other GABA receptors (Johnston, 1996); although, as noted above, some bicuculline-insensitive responses of GABA_A receptors to CACA also have been observed in situ.

From the experience with other receptor classes, progress in obtaining and screening new series of GABA site agonists and antagonists can be expected to be a future important aid in the classification.

VII. Conclusions

A classification can be erected for GABA_A receptors (table 4) which accounts for their combinatorial multisubunit structure; however, in the present state of our knowledge, its limitations are considerable. We have addressed the task of that classification from the broader perspective of the IUPHAR Committee on Receptor Nomenclature, in which it is important for progress in pharmacology to make a start on classifying all types of receptor, no matter how difficult this is initially. It can be argued that even the first stages of such classification will serve to order and systematize our existing knowledge of the receptor, bring uniformity to the terminology, and begin to allow rational distinctions between subtypes to be made in practical operations.

It is important to keep in mind, therefore, the special limitations that presently exist in a case such as that of the GABA_A receptors:

1. It is very difficult to equate a subtype recognized from recombinant co-expression with an in vivo subtype. This arises from the complexity and inaccessibility of the CNS and from the occurrence of multiple subtypes of GABA receptors in small regions, or even on a single neuron. The most successful earlier classifications of other receptor types were possible because of the availability of accessible tissues with responses mediated by only one or a very few subtypes of a given receptor.

2. Many more receptor subtypes can (in this case but not in most others) be created in vitro than are likely to occur in vivo. Hence the classification cannot be based solely on the varieties of response that come from recombinant systems.

3. Many of the pharmacological observations are in fields such as psychopharmacology, neuropathology, anesthesia, etc., so that their interpretation in terms of molecular subtypes is far from direct. Further, the behavioral models used for testing (e.g., for anxiolytic effects) are intrinsically ambiguous, especially because of their control by multiple GABA-ergic circuits.

However, none of these constitutes an absolute limitation in principle. We can envisage, with the powerful advance of the technologies involved, the development of a high-resolution identification and pharmacology of native GABA_A receptors, along with a comprehensive chemical exploitation of the sequence differences between each of the multisubunit structures. We regard this classification as a necessary preliminary stage in rationalizing the multiplicity of GABA_A receptors.

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SUBTYPES OF GABA\textsubscript{A} RECEPTORS


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