0031-6997/98/5002-0291$03.00/0
PHARMACOLOGICAL REVIEWS
Vol. 50, No. 2

Printed in U.S.A.

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

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I. Introduction

This article does not aim to review in detail the properties of γ-aminobutyric $acid_A$ ($GABA_A$)[b] 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, 1996; 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 $GABA_A$ 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.[c] 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 $GABA_A$ 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* $GABA_A$ receptors.

A. Earlier Classifications of γ-Aminobutyric Acid Receptors

1. γ-Aminobutyric $acid_A$ and γ-aminobutyric $acid_B$ 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 $GABA_A$ receptor. $GABA_B$ 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 $GABA_B$ receptor, as a 7-transmembrane domain protein, has been accomplished recently (Kaupmann *et al.*, 1997). The complete structural and functional distinction between $GABA_A$ and $GABA_B$ receptors has a clear parallel to that between nicotinic and muscarinic acetylcholine receptors, between $5\text{-}HT_3$ and metabotropic serotonin receptors, ionotropic and metabotropic glutamate receptors, or ionotropic P_{2X} and G protein-coupled P_{2Y} receptors for nucleotides.

2. γ-Aminobutyric $acid_C$ receptors. A third type of GABA receptor, insensitive to both bicuculline and baclofen, was designated $GABA_C$ (Drew *et al.*, 1984). The $GABA_C$ responses are also of the fast type associated with the opening of an anion channel; they are, however, unaffected by typical modulators of $GABA_A$ receptor

[b] Abbreviations: β-CCE, ethyl ester of β-carboline 3-carboxylate; β-CCM, methyl ester of β-carboline 3-carboxylate; β-CCP, propyl ester of β-carboline 3-carboxylate; BZ, benzodiazepine; BZp, peripheral type BZ binding sites; CACA, *cis*-4-aminocrotonic acid; CNS, central nervous system; DMCM, methyl-6,7-dimethoxyl-4-ethyl-β-carboline-3-carboxylate; DNA, deoxyribonucleic acid; GABA, γ-aminobutyric acid; HEK, human embryonic kidney; $5\text{-}HT_3$, 5-hydroxytryptamine type 3; mRNA, messenger ribonucleic acid; TM, transmembrane domain. Abbreviations of other compounds are given with their structures in fig. 2.

[c] A partial exception may appear to be the review on the purinoceptors (Fredholm *et al.*, 1994) but the structural basis was only clear at that time for the P_1 (adenosine) receptors and the P_{2Y} (ATP) receptors, both being G protein-coupled types. Neither the subtypes nor the subunits of the transmitter-gated channel P_{2X} receptors were known then.

channels such as benzodiazepines and barbiturates (Sivilotti and Nistri, 1991; Bormann and Feigenspan, 1995; Johnston, 1996). Native responses of the $GABA_C$ type have occurred in retinal bipolar or horizontal cells across vertebrate species (Feigenspan *et al*., 1993; Quian and Dowling, 1993; Lukasiewicz, 1996) and can be expressed by rat retinal messenger ribonucleic acid (mRNA) injection in the oocyte system (Polenzani *et al*., 1991).

Although the term "$GABA_C$ 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 $GABA_A$ 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 $GABA_A$ and $GABA_C$ receptors, with a metabotropic family, $GABA_B$, lying between them. Moreover, if the designation of $GABA_C$ were retained, then it would be difficult to refuse the extension to $GABA_D$, etc., types for ionotropic receptors which do not match either of the previously recognized $GABA_A$ and $GABA_C$ specifications. This would further decrease the logic of the $GABA_A$/$GABA_B$ classification scheme. Thus, Sato *et al*. (1996) have proposed such a "$GABA_D$" type, for an embryonic brainstem ionotropic GABA receptor that is insensitive to both $GABA_A$ and $GABA_B$ antagonists and is activated by both $GABA_A$ and $GABA_B$ agonists. Again, Perkins and Wong (1996) have suggested, based on an anomalous current evoked by GABA in hippocampal pyramidal neurons, that a "$GABA_D$" channel may occur there with a different ionic selectivity. We therefore *recommend* that the term $GABA_C$, 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 $GABA_A$ 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

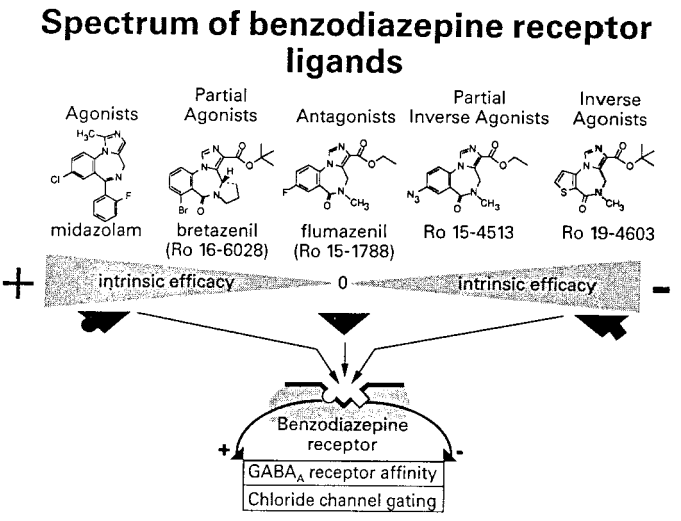


FIG. 1. A diagrammatic representation of the spectrum of ligands with different efficacies, positive or negative, at the BZ binding site, and their allosteric actions on the GABA site (Haefely, 1989). The ligand efficacy depends on subunit composition. The evidence used was based on either whole animal responses or wild-type receptors. A similar profile would be obtained in, e.g., $\alpha_1\beta_n\gamma_2$ recombinants but not at some other subtypes.

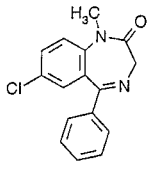
that all three elements are coupled allosterically (Chang *et al*., 1981; Olsen, 1981; Paul *et al*., 1981; Sigel and Barnard, 1984). The term "GABA/BZ receptor" came into use for this complex (and is still encountered). Progress in this field until recently was driven by the synthesis of a vast range of BZs and BZ-like drugs, all acting at brain $GABA_A$ 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 [^{3}H]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-dimethoxyl-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 [^{3}H]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_1 and BZ_2 subtypes of the GABA/BZ receptor.

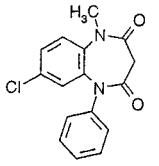
In addition to these two central BZ receptor types, diazepam binding sites with high affinity for many BZs but with pharmacological properties clearly distinct from those of the "central" BZ receptors were identified

A) CHEMICAL STRUCTURES OF BZ/ω LIGANDS

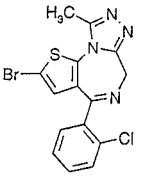
1) 1,4-benzodiazepines (and 1,5-benzodiazepines)



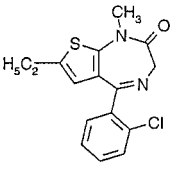
Diazepam



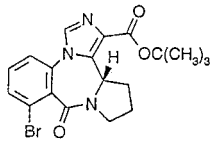
Clobazam



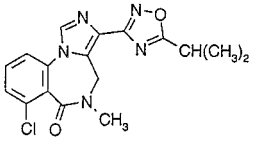
Brotizolam



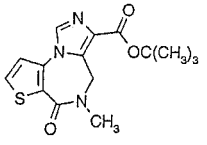
Clotiazepam



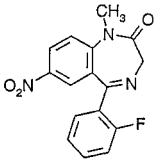
Bretazenil



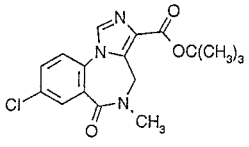
FG 8205



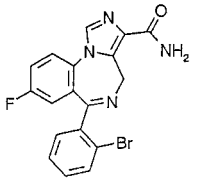
Ro 19-4603



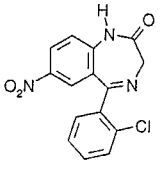
Flunitrazepam



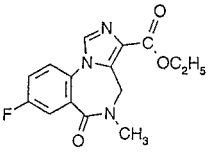
ZG-63



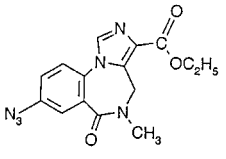
Imidazenil



Clonazepam



Flumazenil



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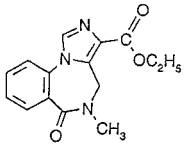

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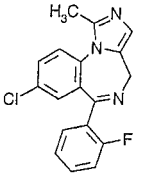
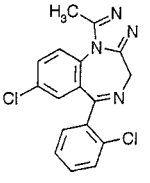
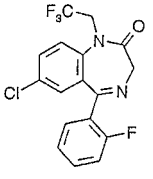


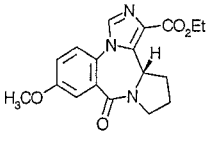
Midazolam



Triazolam

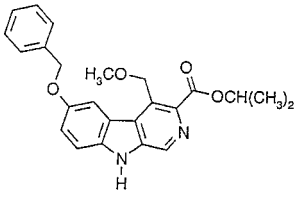


2'-Oxoquazepam

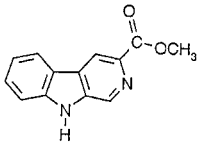


L-655,708

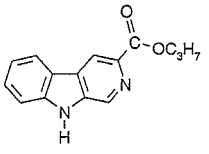
2) β-carbolines



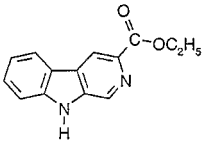
Abecarnil



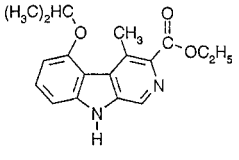
β-CCM



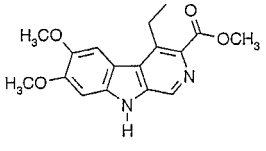
β-CCP



β-CCE



ZK 93426

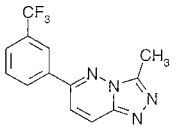


DMCM

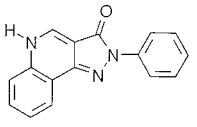
FIG. 2. Structures of ligands discussed in the text. *A*, acting at the BZ site; *B*, *C*, and *D* acting at other sites.

A) CHEMICAL STRUCTURES OF BZ/ω LIGANDS (continued)

3) Other structural types



CL 218872



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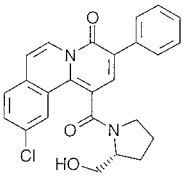
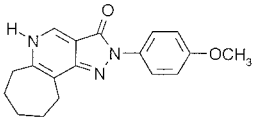


Ro 19-8022



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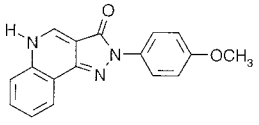

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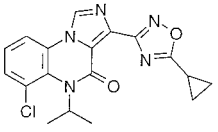

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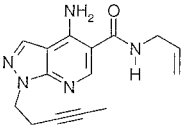

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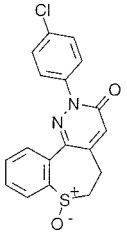

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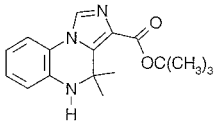

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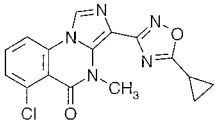

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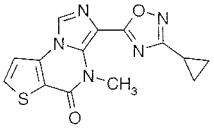

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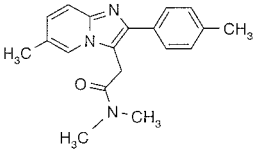
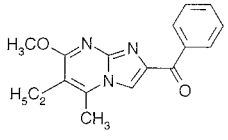
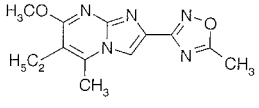


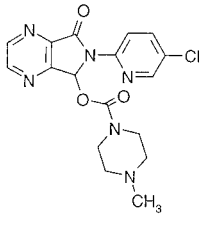
Zolpidem



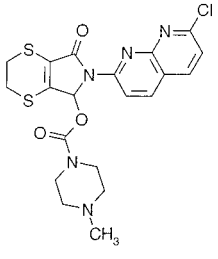
Divaplon



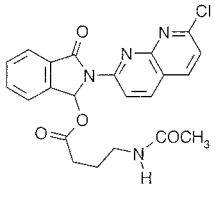
Ru 33-203



Zopiclone



Suriclone



RP 60503

FIG. 2. Continued

B) CHEMICAL STRUCTURES OF GABA AGONISTS OR ANTAGONISTS

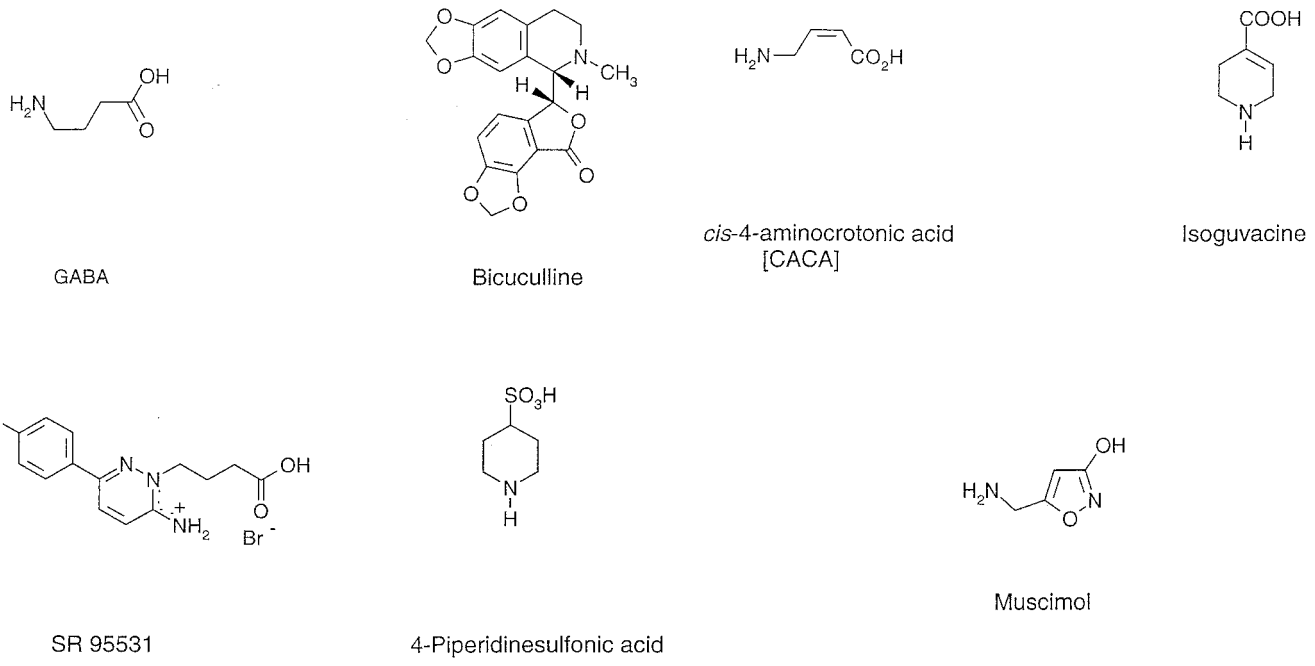


FIG. 2. Continued

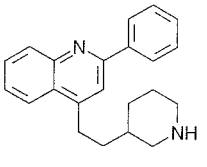
in several peripheral tissues (Braestrup and Squires, 1977). These were designated "peripheral type BZ binding sites" (BZp) (Basile and Skolnick, 1986; Verma and Snyder, 1989) and frequently became referred to as "peripheral BZ receptors." These BZp receptors can be distinguished because they can be 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.

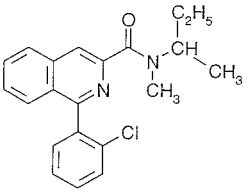
Likewise, the term "GABA/BZ receptor," although useful for two decades, now may be considered obsolete, because (*a*) a binding site of some form for BZs is not specific to $GABA_A$ receptors, as just noted; (*b*) the BZ site is only one of a set of regulatory sites now known on $GABA_A$ receptors, as defined below, so it does not uniquely define these receptors; and (*c*) as will be discussed below, some $GABA_A$ receptors are insensitive to BZs because of one of several distinct molecular causes. Thus "GABA/BZ receptors" is neither synonymous with "$GABA_A$ receptors" nor does it define precisely a single receptor subset. Similarly, the terms BZ_1 and BZ_2 for subtypes of the $GABA_A$ receptor no longer are recommended. As described below, evidence on recombinant subunits now indicates that there are many more than two subtypes of $GABA_A$ receptors. This is paralleled by biochemical evidence on brain $GABA_A$ receptor proteins, e.g., using photoaffinity labeling of their BZ sites by irreversible reaction (Mohler *et al*., 1980) with [^{3}H]flunitrazepam, which showed that multiple subunits carry BZ sites (reviewed by Olsen *et al*., 1996). BZ_2, as the term has been used in the literature, does not equate to any one molecular subtype alone.

4. Excitatory γ-aminobutyric acid$_A$ 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

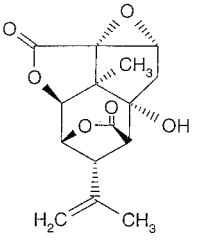
C) CHEMICAL STRUCTURES OF COMPOUNDS ACTING AT OTHER SITES ON $GABA_A$ RECEPTORS



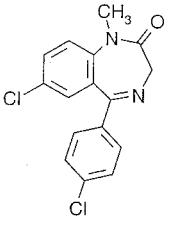
PK 9084



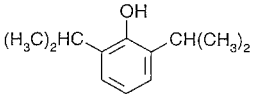
PK 11195



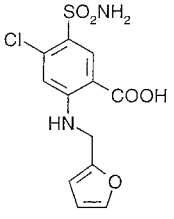
Picrotoxinin



Ro 5-4864

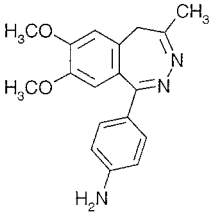


Propofol

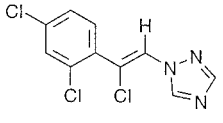


Furosemide

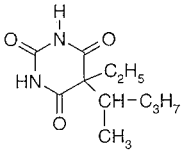
D) BENZODIAZEPINES NOT ACTING AT $GABA_A$ RECEPTORS



GYKI 52 322



Loreclezole



Pentobarbital

FIG. 2. Continued

receptors indicates that they are GABA-activated anion channels, in general similar to the inhibitory $GABA_A$ receptors. $GABA_A$ 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 $GABA_A$ receptor activity. The relative bicarbonate permeability of the channel rarely has been measured for any identified $GABA_A$ receptor subtype, but the possibility that it is increased in a particular case has been supported by Staley *et al*. (1995) and Perkins and Wong (1996). It need not be assumed that this would involve a receptor outside the range of $GABA_A$ receptor subtypes. Indeed, Kaila *et al*. (1997) have shown that activity-induced changes in intracellular chloride and bicarbonate and extracellular potassium, along with normal $GABA_A$ 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 *et al*., 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 $GABA_A$ 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 $GABA_A$

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., ionotropic (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$_A$ 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, Angelotti 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 α_6 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 pyrazinones, 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-

fication, a topic which now requires a fuller discussion below.

III. The Structures of the γ-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 6α, 4β, 3γ, 1δ, 1ϵ, 1π, and 3ρ mammalian types [for references see Burt and Kamatchi, 1991; plus (ϵ) Davies *et al*., 1997 and Whiting *et al*., 1997; (π) Heblom and Kirkness, 1997; (ρ_{1-3}), see Section I.A.2.; for database accession numbers see fig. 3]. These polypeptides are all ~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 γ_4 subunit (Harvey *et al*., 1993) has not yet been isolated by cDNA cloning and so is not included here. However, the β_4 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 γ_2 subunit from one gene (Whiting *et al*., 1990; Kofuji *et al*., 1991), which can be distributed differently in the brain (Glencorse *et al*., 1992). Two such forms are also known for the β_2 and β_4 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 β_3 subunit (Kirkness and Fraser, 1993). Three potential forms of the α_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 α_6 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 ρ) to form a $GABA_A$ receptor, we must consider in a given mammalian species, including the splice variants, at least 7 α forms, 7 β forms, 4 γ forms, 1 δ, 1 π, and 1 ϵ form. The recently discovered ϵ and π subunits in each case can combine with α and β subunits to form a functional, BZ-insensitive receptor (Davies *et al*., 1997; Heblom and Kirkness, 1997; Whiting *et al*., 1997). The π 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 α and β subunits with one or more of the γ, δ or ϵ subunit types (or possibly, exceptionally, of α and β types alone). In addition there are three known ρ subunits that occur in the retina: ρ_1 (Cutting *et al*., 1991); ρ_2

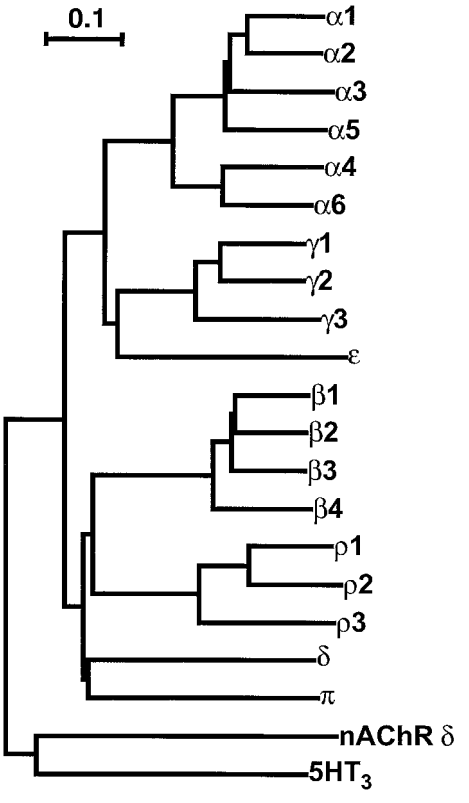


FIG. 3. Dendrogram depicting the relatedness of amino acid sequences between $GABA_A$ receptor subunits. Amino acid sequences were retrieved from the SWISS-PROT or NCBI databases. Most $GABA_A$ receptor sequences are available from the rat and so were used for the analysis, except for the β_4 and ϵ subunits. The chicken β_4 subunit sequence was incorporated because the human homologue has been identified recently (Levin *et al*., 1996), although only a partial human sequence has yet been published. Similarly, the human ϵ subunit has been identified (Davies *et al*., 1997), but the rat sequence has yet to be published. Thus, accession numbers of $GABA_A$ receptor subunit sequences used were: α_1, P18504; α_2, P23576; α_3, P20236; α_4, P28471; α_5, P19969; α_6, P30191; β_1, P15431; β_2, P15432; β_3, P15433; δ, P18506; ϵ, U66661; γ_1, P23574; γ_2, P18508; γ_3, P28473; π, U95368; ρ_1, P50572; ρ_2, P47742 and ρ_3, P50573. Outgroup sequences (see below) were all from the rat: nicotinic acetylcholine receptor δ (nAChR δ), P25110; nicotinic acetylcholine receptor α_2, P12389; and 5-hydroxytryptamine type 3 receptor, P35563. The predicted signal-peptide cleavage sites of all subunits were determined by the method of Nielsen *et al*. (1997). These did not always correspond to those previously indicated in the corresponding database entries and in such cases the newly determined cleavage sites were taken to be the more accurate. These were (signal peptide length in parentheses): α_5 (25), β_3 (25), δ (21), and ρ_3 (25). A multiple alignment of the predicted mature peptides and the consequent phylogenetic tree descriptive file were created using CLUSTAL W version 1.7 (Thompson *et al*., 1994) under the default parameters. NJPLOT (Perriere and Gouy, 1996) was used to generate the graphic output of the gene tree. The branch root was determined by including in the analysis sequences of two non-$GABA_A$ receptor subunits from the same superfamily (Barnard, 1996b). Shown in this figure is the tree generated from the rat nicotinic acetylcholine receptor δ subunit and the 5-hydroxytryptamine type 3 ($5HT_3$) receptor subunit as outgroup representatives. No significant differences were found when either of these were substituted with the rat nicotinic acetylcholine receptor α_2 subunit sequence. The sum of the horizontal branch lengths connecting any two sequences represents the fractional divergence in their amino acid sequence, the scale bar corresponding to 10% sequence divergence. Vertical branches connecting groups are presented only for clarity, and their lengths do not infer differences between separate sequences, or groups of sequences, on the tree.

(Cutting *et al*., 1992; Kusama *et al*., 1993b); ρ_3 (Ogurusu and Shingai, 1996; Shingai *et al*., 1996). In co-expressions, evidence was not obtained to show that a ρ subunit can participate in combinations with the aforementioned α, β, or γ 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-ρ $GABA_A$ receptors and of ρ-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 ρ 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 (~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 $\alpha_2\beta\gamma\delta$).

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 $GABA_A$ receptors from pig brain cortex and image analysis in the electron microscope, dispersed single receptor molecules can be visualized and analyzed (fig. 4). This method yields a power spectrum for each particle with a peak at its dominant symmetry. Figure 4 illustrates that this symmetry is five-fold, over the population of particles analyzed (Nayeem *et al*., 1994). Further, the negatively stained images obtained for all the receptor particles indicated a central pore in the pentameric rosette. These data correspond to the images observed with negatively stained *Torpedo* receptor particles, because of a central channel in the membrane enclosed within the pentameric receptor in the latter case (Toyoshima and Unwin, 1988). The particles isolated from brain will comprise a variety of $GABA_A$ receptor subtypes. These data show that at least the majority of those receptors are pentameric; a deviating small minority with an atypical subunit number would not be distinguished from the experimental noise. Independent evidence to support the pentameric structure has been obtained in several ways. Hydrodynamic estimates of the size of $GABA_A$ receptors, either native (Mamalaki *et al*., 1989) or $\alpha_1\beta_3\gamma_2$ recombinants (Tretter *et al*., 1997), in solution are consistent with the pentameric molecular weight. Further, the integral ratios of the subunits combined in several forms of functional recombinant receptors, as determined by diverse methods, fit best in each case with a subunit total of five (Im *et al*., 1995; Chang *et al*., 1996; Tretter *et al*., 1997). For parallel evidence, a method similar to that of Nayeem *et al*. (1994) has been used for the native $5HT_3$ receptors by Boess *et al*. (1995) and there is supporting evidence by other methods for the neuronal nicotinic receptors and the glycine receptor (reviewed by Barnard,

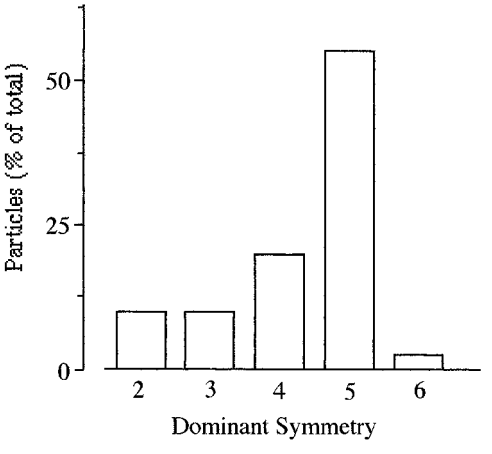


FIG. 4. Evidence for the pentameric structure of native $GABA_A$ receptors. The electron microscopic images of a population of pure $GABA_A$ receptor molecules analyzed to yield dominant symmetry for each particle were used in plotting the histogram (for details see Nayeem *et al*., 1994). Particles 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).

1996b), all of these being in the same superfamily and all being deduced to be pentameric. In view of this concurrence with other receptors in the same superfamily, it is presumed that the pentameric structure that has been observed, within experimental error, for $GABA_A$ 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) $GABA_A$ 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 ρ-containing receptors are also pentameric, but this question has not been studied as yet.

C. The Subunit Isoforms in One Receptor

The majority of $GABA_A$ 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: $\mathbf{2}\alpha.\mathbf{2}\beta.\gamma$; $\mathbf{2}\alpha.\beta.\mathbf{2}\gamma$; $\alpha.\mathbf{2}\beta.\mathbf{2}\gamma$. 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 $3\alpha.\beta.\gamma$ and $\alpha.\beta.3\gamma$ 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 $\alpha_3\beta_2\gamma_2$ or $\alpha_1\beta_2\gamma_2$ 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 $(\alpha_1\alpha_2).2\beta.\gamma$.

In the α subunits, there is a variety of evidence for such a co-occurrence of isoforms in a minority of $GABA_A$ receptors. This evidence has come first from co-precipitation of a second α isoform when a brain-derived population of $GABA_A$ receptors is treated with an antibody specific for a first α isoform. Receptors containing at least the pairs $\alpha_1\alpha_2$, $\alpha_1\alpha_3$, $\alpha_1\alpha_5$, $\alpha_2\alpha_3$, and $\alpha_3\alpha_5$ 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_6 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_1 and α_6 co-occurring in one cerebellar receptor (Pollard *et al*., 1995; Khan *et al*., 1996), although not for all the α_6 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_2 with γ_3 and also of γ_{2L} with γ_{2S} (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_1 and α_6 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_1 and α_6 was not seen (Caruncho and Costa, 1994). Third, some electrophysiological properties of a recombinant $\alpha\beta\gamma$ assembly containing two isoforms of α can be distinct from those with either isoform separately, demonstrated with $\alpha_1\alpha_3$ or $\alpha_1\alpha_5$ 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 1
Methods for recognition of γ-aminobutyric acid$_A$ receptor subtypes in situ

Method	Requirements
1. High-resolution labeling methods a. Immunofluorescence b. Immunocytochemical reaction c. Freeze-fracture/antibody labeling d. Antibody labeling in postembedding electromicroscopy[a]	Spatial separation of receptor subtypes must be adequate
2. Single cell RT-PCR,[b] combined with patch-clamp recording	a. Only one subtype is present b. Large cells are required c. All the receptor mRNAs present must give rise to the assembled receptor[c]
3. Use of an absolutely subtype-specific drug (e.g., furosemide, for $\alpha_6\ \beta_{2/3}\ \gamma_2$)[d]	a. Specific for one defined composition; cases will be rare b. Patch-clamping must be applicable, or the drug must be labeled, for in situ binding

[a] For example, using sized gold particles (Nusser *et al.*, 1996).
[b] RT-PCR, reverse transcriptase-polymerase chain reaction.
[c] Examples to the contrary are given by Williamson and Pritchett (1994).
[d] 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 $\alpha_4\ \beta_3\ \gamma_2$ receptors as well as $\alpha_6\ \beta_{2/3}\ \gamma_2$ receptors, but is 14-fold less active at the former (Wafford *et al.*, 1996).

TABLE 2
Some of the γ-aminobutyric acid$_A$ receptor subtypes in specified rat neurons[a]

Neurons	Subunits					Possible subtypes
Olfactory bulb						
Mitral cells	α_1	α_3	β_2	γ_2		
Granule cells	α_2		β_3	γ_2		A2a3
	α_5		β_3	γ_2		A5a3
Short-axon cells	α_1		β_2	γ_2		A1a2
Periglomerular cells	α_2	α_5			δ	
Hippocampus						
Pyramidal cells	α_2		β_3	γ_2		A2a3
	α_5		β_3	γ_2		A5a3
Dentate gyrus granule cells	α_2		β_3	γ_2		A2a3
Most interneurons	α_1		β_2	γ_2		A1a2
Thalamus						
Relay neurons	α_1		β_2	γ_2	δ	
Reticular nucleus neurons	α_3			γ_2		
Hypothalamus						
Supraoptic nucleus	α_1	α_2	$\beta_{2,3}$	γ_2		
Ventromedial, arcuate nuclei	α_2		β_3	γ_2		A2a3
	α_5		β_3	γ_2		A5a3
Cerebellum						
Purkinje cells	α_1		$\beta_{2,3}$	γ_2		A1a2
Granule cells	α_1	α_6	$\beta_{2,3}$	γ_2	δ	A6a2,A16a2,A06
Golgi type II cells	α_1	α_3		γ_2		
Motoneurons						
(Cranial nerve nuclei)						
Facial motor nucleus						
Hypoglossal nucleus	α_1	α_2		γ_2		
Trigeminal motor nucleus						
Ambiguus nucleus		α_2		γ_2		

[a] Co-expressed subunits were visualized immunohistochemically at the cellular level. The subunits analyzed here are α_1, α_2, α_3, α_5, α_6, β_2, β_3, γ_2, and δ (where the anti-β_2 antibody used does not distinguish between β_2 and β_3, "$\beta_{2,3}$" is noted). 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)].

in 27% of those in rat cerebellum, from which both $\alpha_6\beta_n\delta$ and $\alpha_6\beta_n\gamma_2$ 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 (Whiting *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 α_6 gene also lack the δ subunit protein in the cerebellar granule cells and the results obtained support other evidence that α_6 and δ are paired in receptors there and not α_1 and δ without α_6 (Jones *et al*., 1997). The specific pharmacology of $\alpha_6\delta$-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 $\alpha_1\alpha_6$-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 $\mathbf{2}\alpha.\beta.\mathbf{2}\gamma$ (with the γ subunits in some cases being replaceable by δ or by ϵ). Several lines of evidence support this. Thus, for the γ_2-containing receptors, there is evidence from immunoprecipitation analyses (as noted above) that the $\gamma_2\gamma_3$ 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 $\alpha_1\alpha_6.\beta.\gamma_{2S}\,\gamma_{2L}$ (Khan *et al*., 1994b, 1996). Further, Backus *et al*. (1993) have deduced, by incorporating mutant subunits with altered electrophysiological effects in the recombinant $\alpha_3\beta_2\gamma_2$ receptor [expressed in human embryonic kidney (HEK) 293 cells], that the $\mathbf{2}\alpha.\beta.\mathbf{2}\gamma$ composition best fitted the properties found.

On the other hand, Chang *et al*. (1996), using a similar principle (in oocytes and using α_1, not α_3 subunits), found that there the evidence apparently favors the $\mathbf{2}\alpha.\mathbf{2}\beta.\gamma$ composition. The same stoichiometry also was derived for $\alpha_1\beta_3\gamma_2$ 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 β_1 with β_3, and of β_2 with β_3 (but not β_1 with β_2), 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 β_1, β_2, or β_3 subunits by immunoprecipitation and also excluded the $\beta_1\beta_2$ combination; however, in contrast to the findings just noted, they found that the $\beta_1\beta_3$ or $\beta_2\beta_3$ pairings also were absent. Overall, it is desirable to allow for possible $\mathbf{2}\alpha.\mathbf{2}\beta.\gamma$ forms in the nomenclature. In view of this situation and of the evidence for $\mathbf{2}\alpha.\beta.\mathbf{2}\gamma$ 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 $\alpha_i\,\beta_j\,\gamma_k$ type tested so far can yield a functional receptor in the membrane (e.g., Kirsch *et al*., 1995). The limit to

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 assembles (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 γ/δ position. Moreover, the δ subunit has a more restricted expression in the brain than γ subunits (Wisden and Seeburg, 1992) and has fewer co-occurrences with other subunits; the same is true for ϵ (Whiting *et al*., 1997), whereas π 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, $\alpha.,\gamma.,\alpha.,\beta.,\delta$, 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 $\alpha_1\beta_1\gamma_{2S}$), 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 α 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 ρ 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 at 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 (Polc *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
Modulators acting at the benzodiazepine (or Bz/ω) site[a]

Chemical class	Examples
1,4-Benzodiazepines	Diazepam
1,5-Benzodiazepines	Clobazam
2,3-Benzodiazepines	GYKI-52322
Imidazobenzodiazepinones	Bretazenil; FG 8205; ZG-63[b]
Imidazobenzodiazepine carboxamides	Imidazenil
Heterocyclic, annelated 1,4-diazepines	Brotizolam; clotiazepam; Ro 19-4603
Triazolopyridazines	CL 218872
Pyrazoloquinolines	CGS 8216; CGS 9895
Quinolines	PK 9084
Imidazoquinolines	NNC 14-0578[b]; U-93631
Imidazoquinazolines	NNC 14-8198[b]
Benzoquinolizones	Ro 19-8022
Pyrazolopyridines	CGS 20625; ICI 190622
Benzothiepinopyridazinones	Y-23684[b]
Thienopyrimidines	NNC 14-0590
Imidazopyridines	Zolpidem
Imidazopyrimidines	Divaplon; Ru 33-203
Cyclopyrrolones	Zopiclone, suriclone
β-Carbolines	Abecarnil

[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-23684 (Yasumatsu *et al*., 1994).

TABLE 4
Classification of some of the γ-aminobutyric acid$_A$ receptors

$GABA_A$ receptor subtype	Composition[a]	Characteristic properties
A1a	$\alpha_1\ \beta_n\ \gamma_2$	High affinities and efficacies for classical BZ agonists,[b] CL 218872 (partial agonist), zolpidem, 2′-oxoquazepam[c]
A1b	$\alpha_1\ \beta_n\ \gamma_3$	Same as for 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[d]
A1c	$\alpha_1\ \beta_n\ \gamma_1$	Same as for 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[e]
A2a	$\alpha_2\ \beta_n\ \gamma_2$	Similar to A3 for the ligands noted there, but other properties not yet defined
A2c	$\alpha_2\ \beta_n\ \gamma_1$	BZ/ω agonists have 2- to 20-fold lower potency than on A2a, with FG8205 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[f]
A3a	$\alpha_3\ \beta_n\ \gamma_2$	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 A1a[g]
A4a	$\alpha_4\ \beta_n\ \gamma_2$	Insensitive to classical BZ agonists, zolpidem and many other BZ/ω agonists. Notable exceptions are bretazenil, CGS 20625, and some pyrazoloquinolines. Intermediate affinities for most β-carboline inverse agonists (~10 times higher than at α6 βn γ2), but high affinity for DMCM. Flumazenil and Ro 15-4513 are agonists. The direct activation by propofol or pentobarbital is absent[h]
A5a1	$\alpha_5\ \beta_{1/3}\ \gamma_2$	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 (BZ/ω agonist) are highly selective
A5b3	$\alpha_5\ \beta_3\ \gamma_3$	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
A5a2	$\alpha_5\ \beta_2\ \gamma_2$	A5a1 differs from A5a2 in its outward rectification and its slower desensitization at depolarized voltages[i]
A6a1	$\alpha_6\ \beta_1\ \gamma_2$	Insensitive to all BZ/ω ligands except bretazenil and some other partial agonists; flumazenil and Ro 15-4513 become partial agonists and DMCM an antagonist (fig. 5)
A6a2	$\alpha_6\ \beta_{2/3}\ \gamma_2$	Same as for A6a1, but A6a2 is antagonized selectively by furosemide (see notes to table 1)
A16a2	$\alpha_6\alpha_1\ \beta_{2/3}\ \gamma_2$	Combines the binding sites of A1a and A6a2[j]
A0r		A0r: insensitive to all BZ/ω ligands, but also to bicuculline and pentobarbital. Not activated by isoguvacine
A0r1	ρ_1	
A0r2	ρ_2	
A0r12	$\rho_1\rho_2$	A0r12 (alone) has very low sensitivity to picrotoxin (in the rat) (for references: see Sections I.A. and IV.D.)
A0r3	ρ_3	
A01, A02		Insensitive to all BZ/ω ligands, not because of ρ, but (e.g.) α + β + δ or α + β + ϵ. Sensitive to bicuculline
A01	$\alpha_1\ \beta_n\ \delta$	Same as above, and highly sensitive to zinc
A04	$\alpha_4\ \beta_n\ \delta$	Generally similar to A01
A06	$\alpha_6\ \beta_n\ \delta$	Same as for A01; in cerebellar granule cells only[k]
A01e	$\alpha_1\ \beta_n\ \epsilon$	Generally similar to A01

[a] This means that, e.g., the $GABA_{A2a}$ receptor has a pharmacology which mimics that of the co-expressed recombinants $\alpha_2\beta_n\gamma_2$, where n = 1–3 (in tests so far), unless distinctions are known because of the β isoform present; $\beta_{1/3}$ means β_1 or β_3. 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 isoform 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.

[b] 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.

[c] Likewise for most inverse agonists at the BZ site, for example β-carboline, ethylcarboxylate, and Ro 19-4603.

[d] Herb *et al.* (1992); Luddens *et al.* (1994); Hadingham *et al.* (1995).

[e] Ymer *et al.* (1990); Puia (1991); Giusti *et al.* (1993).

[f] Wisden and Seeburg (1992); Hadingham *et al.* (1993); Wafford *et al.* (1993). Apart from possible locations on some brain neurons, A2c is the subtype on cerebellar Bergmann glia and on α cells of the pancreas.

[g] For example, "high affinity" for zolpidem would cover reported K_i values (for the rat receptors), for $\alpha_1\ \beta_{(1-3)}\ \gamma_2$ of 19 to 30 nM and "intermediate affinity" would cover 688 nM K_i, or 650 nM EC_{50} in potentiation of the GABA-evoked current (Faure-Halley *et al.*, 1993; Lüddens *et al.*, 1994).

[h] Yang *et al.* (1995); Huh *et al.* (1996); Knoflach *et al.* (1996); Scholze *et al.* (1996); Wafford *et al.* (1996).

[i] A5: Pritchett and Seeburg (1990); Faure-Halley *et al.* (1993); Hadingham *et al.* (1993). A5a1 (which, in the γ_{2L} form, resembles a native receptor in CA1 pyramidal neurons): Burgard *et al.* (1996). A5b3: Luddens *et al.* (1994); Hadingham *et al.* (1995). A5a2: Burgard *et al.* (1996). 8-Acetylenic imidazobenzodiazepines and L-665,708 (tested so far on $\alpha_n\ \beta_2\ \gamma_2$ receptors): Liu *et al.* (1996); Quirk *et al.* (1996).

[j] A6: On A6a1/A6a2, partial agonists bretazenil and CGS-9895 and the antagonist flumazenil show 44 to 270 nM K_i values (human, rat). A16a2 has high-affinity flumazenil and Ro 15-4513 binding sites, diazepam-sensitive. References: A6a1, A6a2, Lüddens *et al.* (1990); Korpi *et al.* (1995); Yang *et al.* (1995); Hadingham *et al.* (1996); Huh *et al.* (1996); Nusser *et al.* (1996); Wafford *et al.* (1996). A16a2: Pollard *et al.* (1995); Khan *et al.* (1996).

[k] A01: Saxena and Macdonald (1994); Ducic *et al.* (1995). A06: Quirk *et al.* (1995); Saxena and Macdonald (1996); Jones *et al.* (1997).

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 $GABA_{A122,}$ 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 γ_1 subunit always introduces atypical modulatory effects of BZs, acts as a constraint and must be kept in view when assigning the pharmacologies. 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 γ-aminobutyric acid receptor subtypes. To obtain receptors expressing properties most closely resembling those of BZ-responsive $GABA_A$ receptors on neurons, recombinant α, β, and γ 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 α_4 and α_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). β-Carboline inverse agonists can be active on these subunit combinations but usually with much lower affinity, especially for the $\alpha_6\beta_2\gamma_2$ combination (Kleingoor *et al*., 1991; Yang *et al*., 1995; Knoflach *et al*., 1996). From a wide range of recombinant studies of that kind, several

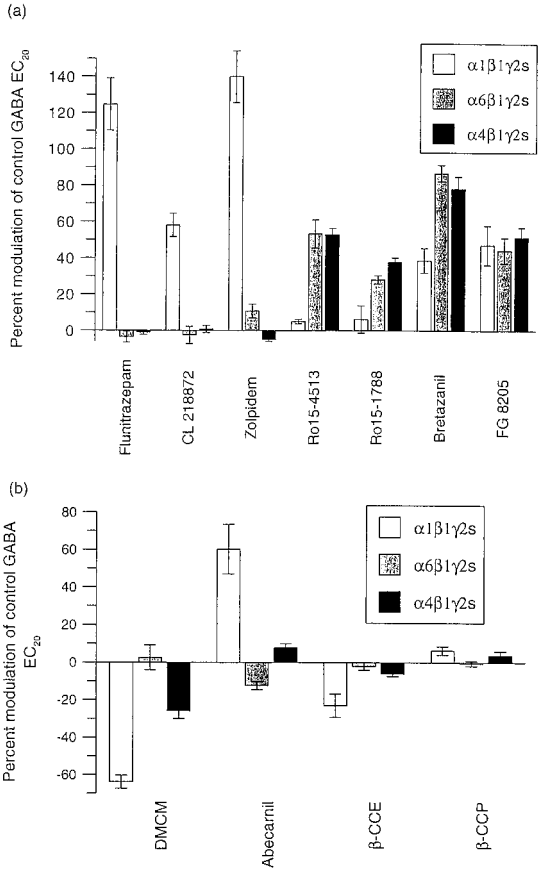


FIG. 5. Modulation of EC_{20} responses to GABA by BZ and β-carboline ligands. **a**, Modulation of GABA EC_{20} responses by BZ ligands in oocytes injected with $\alpha_1\beta_1\gamma_{2S}$, $\alpha_6\beta_1\gamma_{2S}$, and $\alpha_4\beta_1\gamma_{2S}$. **b**, Modulation of GABA EC_{20} responses by β-carboline ligands in oocytes injected with $\alpha_1\beta_1\gamma_{2S}$, $\alpha_6\beta_1\gamma_{2S}$, and $\alpha_4\beta_1\gamma_{2S}$. All compounds were examined at a concentration of 1 μM, and the data shown are the mean ± 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.

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, pyrazoloquinolines, 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 isoform 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_5-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., $\beta_{1/3}$), 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 $\gamma_2\gamma_3$. 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 $GABA_A$ 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 A0r 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 A0r 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 α 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 immunocoprecipitations, that the receptor subtype noted occurs in vivo. For example, a general finding from such studies is that $\alpha_1\beta_2\gamma_{2L/S}$ and $\alpha_1\beta_1\gamma_{2L/S}$ 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 $GABA_A$ 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_1 or α_6 (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_2), $\alpha_6\beta\delta$ and $\alpha_1\beta\gamma_2$ are expressed strongly, but $\alpha_1\beta\delta$ 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 "$\alpha_2\beta_n\gamma_1$-like $GABA_A$ receptor" instead of "$GABA_{A2c}$ 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., $\alpha_1\alpha_3\beta_n\gamma_2$. 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 $GABA_A$ 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 $GABA_A$ 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 $GABA_A$ receptors.

(c) There are other types of BZ binding sites, apart from BZp, of pharmacological significance, not on GABA 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 $GABA_A$ 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 $GABA_A$ 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 $GABA_A$ 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 not hitherto used for receptor subtypes, "omega (ω) site," 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 $GABA_A$ 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 $GABA_{A1}$ to $GABA_{A6}$ (etc.) 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 $GABA_A$ 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 ρ_1, ρ_2, or ρ_3 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-enoic acid [*cis*-4-aminocrotonic acid (CACA)] (Kusama *et al*., 1993a). CACA is essentially inactive at a wide range of the other $GABA_A$ 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) coassemble with other $GABA_A$ 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 $\rho_1\rho_2$ heteromeric receptors (Zhang *et al*., 1995) and not by rat or human ρ_1 or ρ_2 homomers (Kusama *et al*., 1993b; Wang *et al*., 1994; Zhang *et al*., 1995) nor by ρ_3 homomers (Shingai *et al*., 1996). Hence one can define, on present knowledge, A0r1, A0r2, A0r3, and A0r12 as subtypes in this series.

Pharmacologies corresponding to some of these recombinant oligomers have been observed in retinal rod bipolar cells (Bormann and Feigenspan, 1995). Further ρ-containing subtypes, if established as native receptors, would be numbered on the same principle. Whether A0r1, A0r2, and A0r3 occur in situ as homomeric functional receptors (and whether the $\rho_1\rho_3$ combination occurs) is not yet known.

V. Chromosomal Localization of γ-Aminobutyric $Acid_A$ 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 α_1, α_2, α_3, and β_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: α_4, McLean *et al*., 1995; α_5, Knoll *et al*., 1993; α_6, Hicks *et al*., 1994; β_2, Russek and Farb, 1994; β_3, Wagstaff *et al*., 1991 and Glatt *et al*., 1997; β_4, ϵ_1, Levin *et al*., 1996 and Whiting *et al*., 1997; γ_1 and γ_2, Wilcox *et al*., 1992; γ_3, Gregor *et al*., 1995).

Each cluster contains genes of the α, β, and γ/ϵ 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 γ/ϵ-α-β. 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 α genes can co-localize (fig. 6), in line with the co-occurrence of two α isoforms in one receptor that has been noted frequently (as described above).

The δ subunit has become separated from these clusters, on chromosome 1 (Sommer *et al*., 1990). The two ρ subunit genes that have been mapped, ρ_1 and ρ_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 BZ/ω ligands that can discriminate receptor subtypes are found, as differential effects of non-α 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 A1 to A6 series should come from criteria based on ligands recognizing particular β or γ isoforms. Loreclezole is a GABA-potentiating drug which does not act at the BZ site but which recognizes the presence of both β_2 and β_3 but not β_1 subunits (Wafford *et al*., 1994). Several anesthetic drugs and neurosteroids (Macdonald and Olsen, 1994; Sieghart, 1995) also act as potentiators of 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 β_2 or β_3 over a β_1 subunit, which a residue in the β TM2 domain affects (Belelli *et al*., 1997). The propofol and pentobarbital sites for *direct* activation of the receptor are diagnostic (in the γ_2-containing series) for receptors containing α_4; only the presence of α_4 abolishes that effect (Wafford *et al*., 1996). Recombinant $\alpha_1\beta_n\epsilon$ or $\alpha_2\beta_n\epsilon$ receptors lack the GABA potentiation response to these drugs (Davies *et al*., 1997) and the BZ modulation. Because there is no information on the ϵ combinations existing in situ, they are omitted from table 4.

Modulatory binding sites which differ from any of those recognized previously in the $GABA_A$ receptors have been discovered more recently. These include a site for certain pyrazinones that potentiate GABA action (Im *et al*., 1993a) and a site for some dihydroimidazoquinoxalines (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 $\alpha_6\beta_{2/3}\gamma_2$ (Korpi *et al*., 1995) and a somewhat lower selectivity at $\alpha_4\beta_{2/3}\gamma_2$ receptors (Wafford *et al*., 1996). Thus, in oocyte expression the IC_{50} values were 6 μM and 160 μM, respectively, but >5 mM 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.

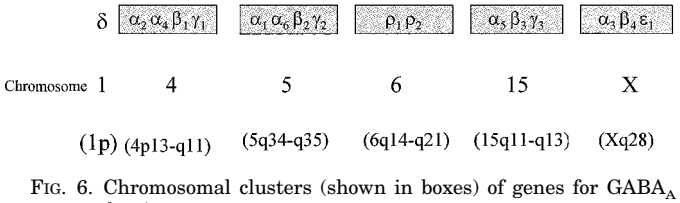


FIG. 6. Chromosomal clusters (shown in boxes) of genes for $GABA_A$ receptor subunits.

B. The γ-Aminobutyric Acid Recognition Site

Selectivity among subtypes for the 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 GABA agonists in recombinant $\alpha\beta\gamma$ receptors, varying the α, β, and γ subunit isoforms. Effects of the α and γ isoform and (less) of the β isoform were found, but these were not primarily of diagnostic value. The largest difference was that with α_4 (but not α_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 co-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.

Acknowledgments. John Alexander (Synthélabo Recherche, Paris) gave expert and invaluable help in assembling and checking the final version of our text and all of its references. IUPHAR is grateful to UNESCO for financial support toward the work of NC-IUPHAR.

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