Molecular Neurobiology

ISSN0893-7648/98/18(1):35–86/$21.00

The Diversity of $GABA_A$ Receptors

Pharmacological and Electrophysiological Properties of $GABA_A$ Channel Subtypes

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Abstract

The amino acid γ-aminobutyric-acid (GABA) prevails in the CNS as an inhibitory neurotransmitter that mediates most of its effects through fast GABA-gated Cl^--channels ($GABA_AR$). Molecular biology uncovered the complex subunit architecture of this receptor channel, in which a pentameric assembly derived from five of at least 17 mammalian subunits, grouped in the six classes α, β, γ, δ, ε, and ρ, permits a vast number of putative receptor isoforms. The subunit composition of a particular receptor determines the specific effects of allosterical modulators of the $GABA_ARs$ like benzodiazepines (BZs), barbiturates, steroids, some convulsants, polyvalent cations, and ethanol. To understand the physiology and diversity of $GABA_ARs$, the native isoforms have to be identified by their localization in the brain and by their pharmacology. In heterologous expression systems, channels require the presence of α, β, and γ subunits in order to mimic the full repertoire of native receptor responses to drugs, with the BZ pharmacology being determined by the particular α and γ subunit variants. Little is known about the functional properties of the β, δ, and ε subunit classes and only a few receptor subtype-specific substances like loreclezole and furosemide are known that enable the identification of defined receptor subtypes. We will summarize the pharmacology of putative receptor isoforms and emphasize the characteristics of functional channels. Knowledge of the complex pharmacology of $GABA_ARs$ might eventually enable site-directed drug design to further our understanding of GABA-related disorders and of the complex interaction of excitatory and inhibitory mechanisms in neuronal processing.

Index Entries: $GABA_A$; benzodiazepines; electrophysiology; recombinant receptors.

Introduction

The amino acid γ-aminobutyric-acid (GABA) is the major inhibitory transmitter in the mammalian central nervous system (CNS; Sivilotti and Nistri, 1991). Depending on the specific brain region, GABA is estimated to be present in approx 20–50% of cerebral cortex synapses

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(Halasy and Somogyi, 1993; Bloom and Iversen, 1971). Its effects are mediated by two types of receptors: second messenger-linked $GABA_B$ receptors that are indirectly coupled to K^+- or Ca^{2+}-channels via GTP-binding proteins (Bormann, 1988; Bowery, 1993; Kerr and Ong, 1995; Misgeld et al., 1995; Kaupmann et al. 1997), and ligand-gated ion channels, the $GABA_A$ receptors, that represent fast GABA-gated chloride channels ($GABA_ARs$; e.g., Bormann, 1988; Sakmann et al., 1983; Bormann and Clapham, 1985; Bormann et al., 1987; Gray and Johnston 1985). $GABA_ARs$ have a ubiquitous distribution within the CNS (reviewed by Sivilotti and Nistri, 1991; Laurie et al., 1992; Wisden et al., 1992; DeBlas, 1996), which suggests they are major participants in neuronal processing. This role is evidently supported by a multitude of psychoactive drugs that exert their effects via $GABA_ARs$ and implicate them to participate in psychiatric disorders (Lüddens and Korpi, 1996) such as anxiety (Doble and Martin, 1992; Nutt, 1990; Skolnick et al., 1986), panic and mood disorders (Rosenbaum et al., 1995; Petty, 1995), alcoholism (Korpi, 1994; Nevo and Hamon, 1995; Tabakoff and Hoffman, 1996), insomnia (Dockhorn and Dockhorn, 1996; Tsoi, 1991), and epilepsy (Glass and Dragunow, 1995; Bradford, 1995; Fraser, 1996; DeDeyn et al., 1990).

Modern molecular biology has revealed that the manifold pharmacological effects on $GABA_ARs$ are faced by a vast diversity of possible receptor subtypes. Presumably, this diversity is permitted by the combination of five of at least 17 different mammalian subunits known at present (Lüddens and Wisden, 1991; Seeburg et al., 1990; Macdonald and Olsen, 1994; Wilke et al., 1997; Davies et al., 1997; Lüddens and Korpi, 1996; Macdonald and Olsen, Sieghart, 1995). The effects of a number of psychoactive drugs modulating the $GABA_AR$ differ dramatically with the subunit combinations (e.g., Lüddens and Korpi, 1996; Macdonald and Olsen, Sieghart, 1995). All this makes the identification of native receptor isoforms, their characteristics, and perhaps the significance of their diversity in the CNS a major task for the near future. From a clinical point of view, the complexity of $GABA_AR$ isoforms offers the opportunity and necessity for site- and cell-specific drug targeting. Such drugs should advance our understanding of specific neuronal circuits in which certain $GABA_AR$ subunits prevail as well as further our understanding of psychiatric disorders.

The Molecular Structure of the $GABA_AR$

$GABA_ARs$ represent members of the superfamily of ligand-gated ion channels (Schofield et al., 1987) that include the nicotinic acetylcholine (nAchR), the glycin, and the $5\text{-}HT_3$ receptors, (Betz, 1990; Ortells and Lunt, 1995; Karlin and Akabas, 1995). These receptors combine the ligand-binding site as well as the ion-permeating pore within the same homo- or heterooligomeric complex. Each subunit comprises a large extracellular N-terminal domain that putatively includes the ligand-binding site, four hydrophobic presumed membrane-spanning domains labeled TM1–TM4, and a small extracellular C-terminus (Fig. 1A). The N-terminal domain also contains a cysteine loop, conserved in all members of the superfamily. In some subunits, a larger intracellular loop between TM3 and TM4 contributes putative phosphorylation sites. For the $GABA_ARs$, recent evidence supports a pentameric arrangement (Unwin, 1989, 1993; Nayeem et al., 1994) with a most probable α-helical TM2 region facing the channel pore (Xu et al., 1995; Unwin, 1995; Xu and Akabas, 1996). In the early 1980s, Barnard et al. (Sigel et al., 1983; Siegel and Barnard, 1984) isolated two $GABA_AR$ channel proteins. The primary sequences obtained from those proteins enabled the cloning of the very first two $GABA_AR$ subunits from bovine brain, called α and β (Schofield et al., 1987). The presence of additional subunits was suggested shortly thereafter by Levitan et al. (1988) and Malherbe et al. (1990). Expression of α and β subunits and their combinations lacked profound pharmacological characteristics like

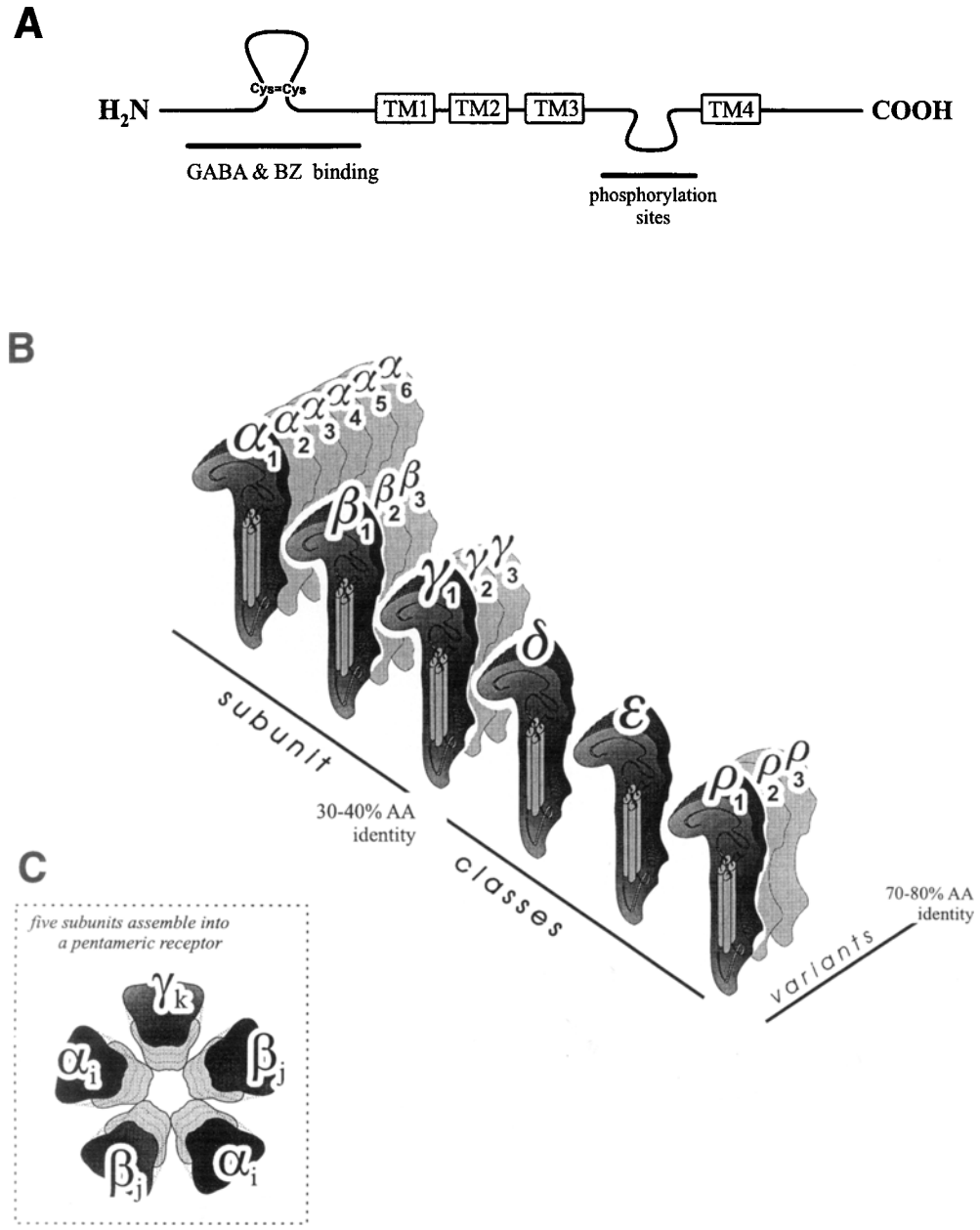

Fig. 1. Schematic representation of $GABA_A$ receptor subunits, their diversity and assembly (**A**) The putatively extracellular N-terminal domain, including the cysteine-loop conserved in all members of the superfamily, is linked to the four membrane-spanning domains TM1–TM4, ending in the extracellular C-terminus. The TM2 region is proposed to face the channel pore. In some subunits the large cytosolic loop between TM3 and TM4 contributes phosphorylation consensus sites. The N-terminal domain is thought to be involved in neurotransmitter and BZ receptor interactions. (**B**) The grouping of the six classes α, β, γ, δ, ε, and ρ is based on a sequence identity of 30–40%. Within each class between one and six variants exist that share 70–80% amino acid identity. The recently described ε subunit has been suggested to coassemble with α and β subunits, however, little information is available yet. ρ subunits are almost restricted to the retina, do not require to coassemble with other $GABA_AR$ subunits but can form functional homopentameric receptors. (**C**) As indicated in the inset, five of such subunits assemble into a heteropentameric receptor with a combination of two αi (i: 1–6), two βj (j: 1–3), and one γk (k: 1–3) subunit representing the most commonly suggested — but not finally proven — stoichiometry. The depicted subunit arrangement follows the suggestion of Tretter et al. (1997) for α1β3γ2 receptors. However, this arrangement might represent the preferred configuration of α1β3γ2 receptors, but is not necessarily extendible to other subunit combinations.

benzodiazepine (BZ) sensitivity, whereas expression of mRNA from whole brains resulted in channels closely resembling native receptors described in vertebrate neurons (Akaike et al., 1990; Yakushiji et al., 1989). In the following years, further molecular cloning revealed an ever increasing number of subunit isoforms (Macdonald and Olsen, 1994; Sieghart, 1995), that have been classified according to their degree of amino acid identity as α, β, γ, δ, ρ, and, most recently ε (Wilke et al., 1997; Davies et al., 1997). Whereas the identity between these groups is approx 30–40%, subunit variants exist that share 70–80% amino acid identity between them. For mammals, these are α1–6, β1–3, γ1–3, δ, ε, and ρ1–3 (Fig. 1B). A fourth β and a fourth γ subunit were identified in chicken, but have not yet been described in mammals (Bateson et al., 1991; Harvey et al., 1994). Each subunit has a molecular mass between 40–60 kDa as predicted from sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), resulting in pentameric receptors of approx 240–290 kDa. For five subunits splice variants have been described. The best studied is the γ2 in which a long form (γ2L vs γ2S) includes an insert of eight amino acids between TM3 and TM4 (Whiting et al., 1990; Kofuji et al., 1991). This region contributes additional phosphorylation sites with suggested relevance to ethanol modulation (Wafford et al., 1991). Long and short splice variants were also described for β4 and β2 in chicken (Bateson et al., 1991; Harvey et al., 1994). In rodents an α6 splice variant, shortened in the N-terminal domain, fails to build functional receptors (Korpi et al., 1994; Rato, 1990); in humans a β3 splice variant involving exon 1 exists with yet unknown function (Kirkness and Fraser, 1993).

In Vivo Subunit Combinations and Their Localization

The pentameric arrangement of the $GABA_A$ subunits α–ε allows for a vast number of channels with various subunit compositions. Although all subunits (except ε; Davies et al., 1997) may form homomeric receptors (Pritchett et al., 1988; Blair et al., 1988; Shivers et al., 1989), in vitro studies indicate that, typically, members of at least three (α + β + γ, δ, or ε) of the six different subunit classes contribute to a GABA-gated, functional native receptor with the BZ sensitivity only being conferred by the presence of a γ variant. Because of the novelty of the ε subunit, only preliminary information on this class is available (Wilke et al., 1997; Davies et al., 1997). The ρ1–3 subunits appear not to coassemble with $GABA_AR$ α or β subunits (Shimada et al., 1992), but to form homopentameres.

An assumed stoichiometry of 2 αi, 1 βj, and 2 γk (with i = 1–6, j = 1–3, and k = 1–3) (Backus et al., 1993), or the more recently supported 2 αi, 2 βj, and 1 γk (Sieghart, 1995; Chang et al., 1996; Tretter et al., 1997) might exist in almost a thousand variations even under restricted conditions, e.g., neglecting splice variants and assuming that subunit positions within the receptor macrostructure are either determined molecularly or of minor importance, e.g. αiβjγk ≈ αiγkβj ≈ βjαiγk. Other stoichiometries like 3 αi, 1 βj, and 1 γk (Mertens et al., 1993) or the known splice variants would increase this number to several thousands; if the relative position of subunits within the macrostructure could further determine their function, as reported for cyclic-nucleotide-gated channels (Liu et al., 1996), the number of possible receptor isoforms might exceed 100,000. Existing information about possible subunit combinations has been obtained by localization studies of mRNA and immunolabelings of receptor subunits. Because of overlapping localizations, certain subunit combinations are considered as more or less likely to represent native receptor isoforms. Further data were obtained by immunoprecipitation studies in which subunit specific antibodies precipitated receptor isoforms from different brain regions. According to these data, incompatibilities as well as developmental and functional transcriptional control reduce the probable numbers drastically. Although our present knowledge sug-

gests approx 12–24 isoforms to represent the most abundant ones (Laurie et al., 1992; DeBlas, 1996; Wisden et al., 1991; Fritschy and Mohler, 1995), physiological importance does not necessarily depend on abundance. Only a few putative subunit combinations within certain cell populations have been characterized, whereas often an unambiguous identification of native receptors remains controversial.

Distribution of Specific Subunits

According to mRNA localization and immunohistochemical staining, the α1 subunit appears to be the most abundant subunit in the CNS (e.g., Laurie et al., 1992; Wisden et al., 1992; Benke et al., 1991) with only a few regions devoid of it (Fritschy et al., 1992). α1 is often colocalized with the β2 subunit according to mRNA localization and double immunofluorescence detection (Laurie et al., 1992; Wisden et al., 1992; Sequier et al., 1988; Benke et al., 1994). The γ2 subunit is found in nearly all brain regions, albeit with different intensities (Gutierrez et al., 1994), and is often described as colocalizing with α1β2 (Laurie et al., 1992; Wisden et al., 1992; Fritschy and Mohler, 1995. Benke et al., 1991; *see* also DeBlas, 1996). Accordingly, the physiologically most abundant receptor type may be presented by the subunits α1, β2, and γ2. α variants other than α1 show a more limited distribution. The α2 and α3 subunits show stronger mRNA- and immunolabeling where weak traces of α1 are found. α2β1/3 combinations as well as α5β1/3 combinations, both with unknown γ variants, are most abundant in the hippocampus with the α2β3γ2 combination supported for the hippocampal pyramidal cells (Laurie et al., 1992; Wisden et al., 1992). The α3β3γ2 subunit combination is reported for serotonergic neurons of the raphe nuclei and cholinergic neurons of the basal forebrain (Benke et al., 1994; Gao et al., 1993, 1995). It appears that the mRNA of α2 and β3 subunits often colocalize, and, similarly, α5 and β3 subunits occur as a pair (Laurie et al., 1992; Wisden et al., 1992). Interestingly, some subunits dominate during embryonic development, as suggested by mRNA hybridization, e.g., α2, α3, and α5 (Laurie et al., 1992; Poulter et al., 1992, 1993), but are reduced or even absent in defined regions of the adult brain (Fritschy et al., 1994). Although the relevance of this switch is not yet understood, the presence of specific subunits during certain developmental stages appears to be essential for normal development (e.g., Gunther et al., 1995; Culiat et al., 1995). In contrast, certain neurons, e.g., the cerebellar Purkinje cells, maintain their subunit composition of α1 β2/3γ2 throughout all pre- and postnatal stages (Laurie et al., 1992).

As long as we do not comprehend the whole circuitry of all brain regions, even minor amounts of specific subunits, e.g., the α5 subunit, cannot be ignored. This subunit, which is reported to be concentrated in the adult hippocampus and olfactory bulb (Wisden et al., 1992; Fritschy and Mohler 1995), constitutes a receptor with a low affinity for certain BZ ligands like the imidazopyridine zolpidem (Lloyd et al., 1990; Pritchett and Seeburg, 1990; Arbilla et al., 1986). As will be discussed later, such specifics might help to identify native subunits and to elucidate their role in mental disorders. Similarly, the α4 and α6 subunit distribution is relevant in terms of their distinct BZ pharmacology (diazepam insensitive, *see* below). Major amounts of the α4 subunit are found in the hippocampus and thalamus and often colocalize with the δ subunit (Wisden et al., 1991). The α6 subunit, also found to colocalize with the δ subunit, appears to be almost exclusively restricted to the cerebellar granule cells (Lüddens et al., 1990) with some traces found in the dorsal cochlear nucleus (Varecka et al., 1994), a brain area developmentally derived from cerebellar precursors. However, the α6 localization represents one of the two contentious issues between mRNA and antibody localizations. A recent immunolocalization reports α6-like immunoreactivity to occur outside the cerebellum in the olfactory bulb, retina, spinal cord, and superior colliculus (Gutierrez et al., 1996). Interestingly, using the same antibodies in an α6 knockout mouse,

Wisden et al. (personal communication) found no immunoreactivity in the cerebellum and dorsal cochlear nucleus as expected. However, immunostaining in the olfactory bulb, retina, spinal cord, and superior colliculus persisted. This suggests further studies to exclude possible cross reactivity of this antibody, especially since Wisden et al. (Jones et al., 1997) recently suggested the presence of α6 subunits in the inferior colliculi and minor cell populations in the substantia nigra and geniculate nuclei of the thalamus (Jones et al., 1997) based on studies with a lacZ gene driven by the α6 promoter.

The role and distribution of the β subunits have been studied in less detail. Immunohistochemical studies have been hampered by the lack of specific antibodies since the prevalently used MAb Bd17 does not differentiate between β2 and β3. β1 mRNA signals were strongest in the hippocampus, less pronounced in parts of the basal nuclei (claustrum) and septum (bed nucleus of the stria terminale), and only weak in the amygdala and hypothalamus (Wisden et al., 1992; Zhang et al., 1991; Lolait et al., 1989). Wisden also reports the localization in the olfactory bulb mitral cells (Wisden et al., 1992). As already mentioned, the β2 subunit shows a more generalized distribution. It is weaker where strong β1 and β3 concentrations are found, such as in the hypothalamus and in certain regions of the hippocampus (Wisden et al., 1992; Zhang et al., 1991; Lolait et al., 1989. The β3 subunit is strong in the hippocampus, mainly CA1 and CA2, and in the olfactory bulb, cortex, part of the basal nuclei (caudate putamen and nuclei accumbens), and hypothalamus. Weak signals were found in the amygdala and thalamus (Wisden et al., 1992; Zhang et al., 1991; Lolait et al., 1989). Studies on the differential distribution of the β1, β2, and β3 subunits during early pre- and postnatal development indicate an independent regulation of their expression in different brain regions. This suggests an essential role of these subunits during development (Laurie et al., 1992; Fritschy et al., 1994; Zdilar et al., 1992) as recently emerged for the β3 subunit from the work of Culiat et al. who reported that a mouse deletion mutant devoid of a chromosomal region encoding α5 and β3 induces a highly transmittable neonatally lethal cleft palate (Culiat et al., 1993). Upon introducing a transgene coding for β3 into homozygote mutant mice they became phenotypically normal (Culiat et al., 1995), thereby identifying the subunits responsibility for the cleft palate.

The ubiquitous presence of the γ2 subunit is contrasted by the restricted distribution of the γ1 and γ3 variants. Whereas there is some overlapping distribution of the γ2 and γ3 subunits, mRNA encoding the γ1 subunit is limited to regions of the amygdala, the septum, and hypothalamus and does not appear to coexist with γ2 or γ3 in a single receptor (Quirk et al., 1994). The γ3 subunit is scarce in cerebellum and hippocampus, but present in higher amounts in the olfactory bulb, cortex and basal nuclei as well as the medial geniculate of the thalamus (Wisden et al., 1992; Herb et al., 1992; Knoflach et al., 1991). The γ2 subunit is another subunit with differing mRNA and antibody localization; i.e., in the hippocampus prominent mRNA signals were found but only low immunoreactivity (Wisden et al., 1992; Benke et al., 1991). When investigating the role of the γ2 subunit in mice by targeted disruption, it became apparent that γ2 was not required for subunit assembly, transport, membrane insertion, subcellular targeting, or clustering of $GABA_A$Rs. Postnatally, however, $\gamma2^{-/-}$ mice showed severe growth retardation, behavioral and sensorimotor abnormalities, and a drastically reduced life span, presumably caused by functional differences of $GABA_A$ receptors devoid of the γ2 subunit (Gunther et al., 1995).

As described above, the δ subunit, which colocalizes with the α4 and α6 subunits, is concentrated in the granule cells of the cerebellum with minor amounts in parts of the cerebral cortex, the thalamus, and olfactory bulb (Laurie et al., 1992; Shivers et al., 1989; Benke et al., 1991). Cerebellar granule cells contain an unusual receptor composition since the six subunits α1, α6, β2, β3, γ2, and δ colocalize in these cells. Which of these subunits are combined within a single pentameric receptor, how-

ever, remains unclear and elucidates the present problems with identifying the native receptor isoforms of the GABA$_A$R. Colocalization of the δ with the α6 subunit in the cerebellum is apparent since mutant mice lacking α6 subunits also lack expression of the δ subunit protein (Jones et al., 1997). But a number of studies addressing a possible coassembly of α1 and α6 subunits in a single receptor (together with βjγk) reached different conclusions. Immunprecipitation studies with an α6 antibody suggested the α6 subunit is combined in a 1:1 ratio with the α1 subunit (Pollard et al., 1995). Caruncho and Costa (1994), however, concluded from double immunolabelings of freeze-fracture replicas that these two subunits do not colocalize within the same receptor complex. Similarly, an electron microscopical study reported α6 to be restricted to the dendrites with the α1 subunit present on both dendrites and somata (Baude et al., 1992), whereas a more recent ultrastructural immunogold localization suggests an even distribution of both subunits on the soma but differential localizations of α1 and α6 within different synapses (Nusser et al., 1996).

The recently described ε subunit shares approx 45% amino acid identity with the γ subunits and is indicated to coassemble with α and β subunits. Initial mRNA hybridization in Northern blots of various brain regions suggests this subunit is localized mainly in the subthalamic nuclei and, to a lesser extent, in the amygdala and thalamus (Wilke et al., 1997; Davies et al., 1997). The distribution of the ρ subunits is almost restricted to the retina (Cutting et al., 1991, 1992), although ρ2 has additionally been described in other brain areas, mainly the hippocampus and cortex (cat: Enz et al., 1995). Pharmacological effects indicate the presence of receptors composed of ρ subunits in the spinal cord (Johnston et al., 1975), optic tectum (Nistri and Sivilotti, 1985; Sivilotti and Nistri, 1989), cerebellum (Drew et al., 1984; Drew and Johnston, 1992), and hippocampus (Strata and Cherubini 1994). Little is known about the recently reported ρ3 subunit, which has been obtained from the cat retina (Ogurusu and Shingai, 1996).

Based on mRNA and protein colocalization, further subunit combinations have been suggested such as α1α4β2δ and α1α6β2δ (Wisden et al., 1992), or α1α3β2/3γ2, (e.g., DeBlas, 1996; Fritschy et al., 1992). More detailed information on the identification and distribution of subunits and their combinations can be found in DeBlas, 1996; Laurie et al., 1992; Wisden et al., 1992; Fritschy and Mohler, 1995; Fritschy et al., 1992. However, care must be taken in interpreting all these data. The presence of mRNA does not necessarily correlate with the amount of functional receptors (e.g., Jones et al., 1997; Williamson and Pritchett, 1994; Hales and Tyndale, 1994), and immunoprecipitation studies might include incompletely assembled receptors as well as receptors not inserted into the outer cell membrane (*see* also Connolly et al., 1996). In regard to immunoprecipitation studies, it has been recently discussed (Sigel and Kannenberg, 1996) that some receptor pools might be underestimated in such studies because they are not readily solubilized by conventional detergent treatments. Accordingly, immunoprecipitation studies may be only a limited tool for excluding receptor combinations as native receptor isoforms. A more conclusive identification could be achieved by studying functional, mainly pharmacological, properties of native receptors.

Pharmacology of GABA$_A$Rs

A number of psychoactive drugs exert their effects mainly or exclusively via GABA$_A$Rs (Fig. 2). Aside from the natural agonist GABA, these drugs include the clinically relevant benzodiazepines (BZ), some sedative and anesthetic barbiturates and steroids, general and volatile anesthetics, and convulsants such as picrotoxin or TBPS (*t*-butylbicyclophosphorothionate). Additional binding sites for a number of substances, e.g., loreclezole, avermectin, furosemide, zinc, or lanthanum, exist (reviewed by Sieghart, 1995). They work through at least six different binding sites, all of which, to our present knowledge, allosterically interact with

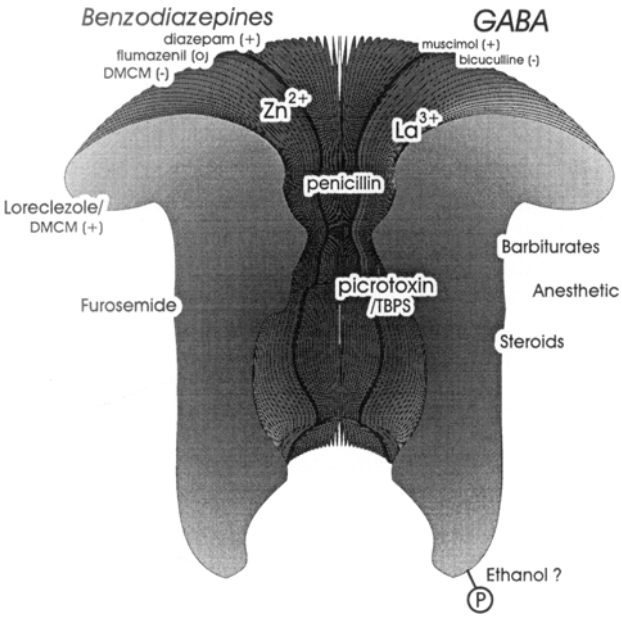

Fig. 2. The $GABA_A$ receptor-channel complex as drug target: Aside from GABA and its agonists, e.g., muscimol, or antagonists, e.g., bicuculline, various drugs exert their actions by modulating the $GABA_AR$. These include the clinically relevant benzodiazepines which, on a functional basis, can be subdivided into positive modulators like the agonist diazepam, null modulators, e.g., Ro15–1788 (antagonist) and negative modulators (inverse agonist) like the β-carboline DMCM. Further binding sites have been identified for loreclezole, furosemide, and picrotoxin. Sedative barbiturates and steroids as well as various anesthetics also work via the $GABA_AR$. Binding sites for the polycationic ions Zn^{2+} and La^{3+} exist and penicillin at high concentration supposedly is an open channel blocker. In this artistic view, the localization of the drug binding sites, though not totally arbitrary, do not suggest specific, proven localizations of these sites.

each other. The exact pharmacophores for these sites in the subunit structure is only indicated by a number of point mutations that modify pharmacological responses to some of these drugs. Modifications of putative intracellular phosphorylation sites, as suggested for ethanol (Korpi, 1994), further increase the functional diversity of $GABA_ARs$ (Moss et al., 1995; Leidenheimer et al., 1991). The complex pharmacological profiles revealed by these substances and summarized in the following paragraphs are influenced by the receptor subunit composition. The expression of subunits and subunit combinations in heterologous expression systems enabled detailed studies of the pharmacological and electrophysiological characteristics of defined receptor isoforms. Their profiles may be used to differentiate between subunit combinations in vivo and to identify native receptor isoforms.

GABA

GABA is a small amino acid derived from glutamate by glutamic acid decarboxylase. Binding of GABA to $GABA_ARs$ opens the intrinsic Cl^- channel and enables Cl^- to follow its concentration gradient. Neurons usually maintain a low intracellular Cl^- concentration and generate a chloride equilibrium potential slightly negative of the resting membrane potential (V_{mem}). Accordingly, an inward directed Cl^- conductance will stabilize V_{mem} and, as long as Cl^--channels remain open, any excitation (depolarization) will be opposed by a counteracting flow of Cl^-. $GABA_ARs$ are stimulated by muscimol and isoguvacin and inhibited by bicuculline in a competitive manner. This distinguishes them from $GABA_B$ receptors, which are stimulated by (–)baclofen and inhibited by phaclofen (Bowery, 1989, 1993; Kerr and Ong, 1995; Misgeld et al., 1995) and the $GABA_C$ receptors that are insensitive to bicuculline. Picrotoxin and TBPS inhibit $GABA_ARs$ in a noncompetitive manner (e.g., Yoon et al., 1993). Penicillin at high concentrations is known as a channel blocker (Twyman et al., 1992). Further substances are competitive antagonists at the GABA-binding site, e.g., the amidine steroid Ru 5135 (Hunt and Clements Sewery, 1981) and the arylaminopyridazines SR 95103 and SR 95531 (Chamber et al., 1985; Heaulme et al., 1987). GABA and the agonists muscimol and isoguvacin interfere allosterically with other binding sites on the receptor, e.g., they stimulate BZ binding. The synthetic GABA-

competitive analogs THIP (4,5,6,7-tetrahydroisoxazolopyridin-3-ol), 3-aminopropanesulfonate, or piperidine-4-sulfonate, which have weaker allosterical effects on the other binding sites, have been classified as partial agonists at the GABA site (Krogsgaard Larsen et al., 1994).

Concentration response curves for GABA are sigmoidal with Hill coefficients between one and two (e.g., Sakmann et al., 1983; Bormann and Clapham, 1985; Hattori et al., 1984; White, 1992), suggesting that the binding of at least two GABA molecules is required to open the channel. Binding studies with [^{3}H]bicuculline and [^{3}H]GABA (Olsen and Snowman, 1983; Olsen et al 1981) revealed high and low affinity sites with K_D values in the low and high n*M* range, respectively, which could be seen in support of two different binding sites. Still, almost µ*M* concentrations are required to open the intrinsic ion channel (Barker et al., 1984; Yang and Olsen, 1987; *see* also Kardos and Cash, 1990; Maconochie et al., 1994), a fact that has led some authors to suggest additional very-low affinity binding sites (for discussion, *see* Macdonald and Olsen, 1991). At present, both the number of GABA binding sites on the GABA$_A$R and whether different affinities represent distinct sites or modifications of identical sites remain subject to intense discussion (e.g., Macdonald and Olsen, 1994; Sieghart, 1995).

GABA as Excitatory Neurotransmitter?

Some reports in the literature describe GABA as an excitatory neurotransmitter (Alkon et al., 1992; Michelson and Wong, 1991; Avoli and Pereult, 1987; Avoli, 1992). As Cl^- follows its concentration gradient, this might simply be caused by an altered chloride distribution across the membrane, e.g., an increased intracellular Cl^- concentration, a situation assumed during development (Cherubini et al., 1991; Serafini et al., 1995) or in astrocytes and oligodendrites (von Blankenfeld and Kettenmann, 1991). A recent study of spinal neurons in *Xenopus* larvae has exemplified that the intracellular Cl^- concentration $[Cl^-]_i$ can be selectively increased in some cells by an inwardly directed Na^+-dependent Cl^--contransport. Thereby, hyper- and depolarizing GABA responses can coexist during the developmental stages (Rohrbough and Spitzer, 1996). Interestingly, the low $[Cl^-]_i$ found in most neurons appears to be actively generated by voltage-dependent, slowly-activating, and inwardly rectifying Cl^--channels (Staley et al., 1996). Another explanation for GABA-induced excitation depends on the permeability of GABA$_A$R channels for other anions, mainly for HCO_3^- (Bormann et al., 1987). As shown by Staley et al., continued stimulation can reduce the chloride gradient faster than the HCO_3^- gradient resulting in an excitatory outflow of HCO_3^- (Staley et al., 1995). So far, all results indicate a change in the Cl^- gradient to be the cause of excitatory GABA responses; no evidence has arisen for another type of GABA-gated channel.

Benzodiazepines

Among the pharmacological agents that allosterically modulated GABA$_A$Rs, the benzodiazepines have gained major clinical relevance (e.g., Lüddens and Korpi, 1996; Sieghart, 1995; Rabow et al., 1995). Present evidence suggests GABA$_A$Rs represent the only effector site of BZs in the CNS; typical agonists like diazepam, flunitrazepam, or chlordiazepoxide exert their anxiolytic, anticonvulsant, sedative, and hypnotic effects by potentiating the effect of GABA (Choi et al., 1977; Macdonald and Barker, 1978). BZs do not effect Cl^- currents in the absence of GABA (Study and Barker, 1981; Polc, 1988) and BZ binding does not compete with GABA (e.g., Schofield et al., 1987). BZs bind to an additional site within the GABA$_A$R ion channel complex that allosterically modulates GABA$_A$R currents (Sigel et al., 1983; Asano et al., 1983; Mohler and Okada, 1977; Mohler et al., 1981), but BZs cannot further increase the effect obtained at maximal GABA concentrations: they only shift the GABA dose response curve to lower concentrations (Choi et al., 1981a, b; Hattori et al., 1986). This mode of action is of clinical importance as it practi-

cally excludes overdosage when used as a single therapeutic. The BZ site appears to be the most complex of all binding sites on the $GABA_AR$: In addition to agonists that enhance GABA effects (positive modulators), so-called inverse agonists exist that reduce the GABAergic effects (= negative modulators) (e.g., Macdonald et al., 1992). These substances are accordingly anxiogenic, convulsant, and they cause alertness. Prototypical compounds are Ro15–4513 and some β-carbolines. Antagonists at the BZ site, like the clinically known flumazenil (Ro15–1788), competitively displace agonists and inverse agonists but lack any intrinsic modulatory effects on the receptor ion-channel complex (= null modulators) (e.g., Polc et al., 1982; Braestrup et al., 1982). BZ pharmacology is further complicated as the full range of efficacy is covered from positive over null to negative modulators, i.e., partial agonists and partial-inverse agonists do not show a full positive and negative modulation, respectively, even at their highest concentration (e.g., Facklam et al., 1992; Braestrup et al., 1984; Haefely, 1985). Furthermore, efficacy is not correlated with potency, i.e., the respective EC_{50} value, the concentration at which 50% of the maximal potentiation is achieved (e.g., Knoflach et al., 1993; Puia et al., 1991). Again, such classifications must be considered cautiously since the intrinsic efficacies of BZs and related substances vary with receptor isoforms, (e.g., Knoflach et al., 1993; Puia et al., 1992; Wafford et al., 1993). For example, determination of inverse agonistic properties of BZ receptor ligands has mainly been established on α1-subunit containing receptors, but some or all of these compounds act differently on other receptor isoforms, e.g., Ro15–4513 is classified as an inverse agonist, but acts as a positive modulator on α4 and α6 containing receptor isoforms (Knoflach et al., 1996). The large diversity that arises from the pharmacological BZ-GABA interactions can be utilized to discriminate between different receptor isoforms; specific BZ-ligands will be discussed later in the context of their subunit specificities. Interestingly, modulation of $GABA_AR$ function appears to be always bidirectional: As BZs potentiate GABA binding, GABA enhances the affinity for BZ agonists, but decreases binding of inverse agonists, a phenomenon described as *GABA-shift*; antagonists are not affected (e.g., Tallman et al., 1978; Braestrup et al., 1983; Karobath and Sperk, 1979; Olsen, 1982). Similar bi- or multidirectional interactions between various binding sites are observed with all drugs allosterically interacting with the $GABA_AR$.

Other substance classes, such as the β-carboline-3-carboxylate esters and the anxiolytic cyclopyrrolones zopiclone and suriclone, also recognize the BZ site. Binding studies indicate competitive (Concas et al., 1994) as well as noncompetitive interactions for the cyclopyrrolones at the BZ site (Trifiletti et al., 1984; Trifiletti and Snyder, 1984). Furthermore, some characteristics and differences in the action of zopiclone vs suriclone, which could not be explained by simple interaction at one binding site, were interpreted as either partial overlapping or multiple binding sites for these substances (Concas et al., 1994; Trifiletti et al., 1984; Trifiletti and Snyder, 1984; Blanchard and Julou, 1983). Different BZ-binding sites were similarly suggested by photoaffinity labeling studies using [^{3}H]flunitrazepam covalently crosslinked to the $GABA_AR$ by UV irradiation (Thomas and Tallman, 1983). As binding sites remained for the BZ antagonist CGS-8216 (2-phenylpyrazolo [3,4-c]-quinolin-3[5H]-one) and the benzodiazepine Ro15–1788, the authors have suggested that the photoaffinity labeling revealed high- and low-affinity binding sites for BZ-ligands (Thomas and Tallman, 1983). However, unspecific modifications caused by the UV irradiation must be considered (e.g., Evoniuk et al., 1989; Herblin and Mechem, 1984). Furthermore, Duncalf and Dunn (1996) recently described differences in sites photoaffinity-labeled by [^{3}H]flunitrazepam and [^{3}H]Ro15–4513 that indicate them to be localized at distinct regions on the α1 subunit. At present, the discussion about the existence of various BZ receptor sites, their cooperativity (Nutt et al., 1992) or simple com-

petitive interactions (Prinz and Striessnig, 1993) remains controversial.

Steroids

A class of steroids, the neurosteroids, exert part of their actions through the GABA$_A$R (Gee, 1988; Majewska et al., 1986; Morrow et al., 1987; Peters et al., 1988). They are thought to be synthesized in glial cells in the brain, and are found at higher concentrations in the brain than in plasma (Majewska, 1992). Neurosteroids include metabolites of progesterone, e.g. 3α-hydroxylated or 3α,5α-reduced progesterones, metabolites of deoxycorticosterone, as well as the synthetic steroid alphaxalone (Majewska, 1992; Harrison et al., 1987; Cottrell et al., 1987; Kokate et al., 1994; Corpechot et al., 1981, 1983). The acute induction of their sedative, hypnotic, or anesthetic effects supports the assumption of a nongenomic mode of action (Majewska, 1992; McEwen and Parsons, 1982), although some might have additional genomic effects as suggested by their affinity for the progesterone receptor (Rupprecht et al., 1996). Alphaxalone, known as a general anesthetic, was shown to potentiate GABA-evoked Cl^- currents in extracellular recordings from brain slices (Harrison and Simmonds, 1984). These results where later confirmed by voltage-clamp recordings of dissociated neurons (Harrison et al., 1987; Barker et al., 1987), chromaffine cells (Cottrell et al., 1987; Callachan et al., 1987), and primary cultures of hippocampal neurons (Majewska et al., 1986; Harrison et al., 1987). The structurally related endogenous progesterone metabolites 5α-pregnan-3α-ol-20-one (THP; tetrahydroprogesterone) and 5β-pregnan-3α-o1-20-one, or the deoxycorticosterone metabolite THDOC (tetrahydrodeoxycorticosterone; 5α-pregnane-3α,21-diol-20-one), as well as the androsterone 5α-androsteron-3α-ol-17-one all enhance GABA-stimulated $^{36}Cl^-$ fluxes and binding of GABA agonists like [^{3}H]muscimol or BZ agonists like [^{3}H]flunitrazepam (for a review, *see* Gee, 1988; *see* also Majewska et al., 1986; Peters et al., 1988; Harrison and Simmonds, 1984; Callachan et al., 1987; Harrison et al., 1987; Turner et al., 1989; Im et al., 1990; Morrow et al., 1988), but decrease the affinity of picrotoxin and TBPS at their binding site (Gee, 1988; Hajewska et al., 1986; Turner et al., 1989). Other endogenous steroids, acting as noncompetitive antagonists of the GABA$_A$R, represent inverse agonists at the steroid site, e.g., pregnenolone sulfphate (PS) and DHEAS (dehydroepiandrosterone) reduce GABA-induced effects at μ*M* concentrations (e.g., Gee et al., 1988; for reviews, *see* Sieghart, 1995). Whereas DHEAS has full antagonistic properties, PS is a mixed agonist–antagonist, i.e., Majewska et al. reported bell-shaped characteristics of PS being agonistic at n*M* but antagonistic at μ*M* concentrations (Gee et al., 1988; Majeswska et al., 1985, 1988; Majewska and Schwarz, 1987). The differences between the effects of PS and DHEAS suggest complex and different interactions of these two compounds with the GABA$_A$R (*see* Majewska, 1992). Similarly, cortical neurons of rat or HEK 293 cells transiently transfected with α1/6β1γ2 or α1/6β2γ2 subunits showed a biphasic modulation of the GABA response by 3α-OH-DHP and pregnenolone sulphate (PS) being maximal at 10 n*M* and decreasing at higher concentrations (Puia et al., 1993; Hauser et al., 1995).

The highly lipophilic nature of steroids could suggest unspecific membrane interactions. Although steroids enhanced [^{3}H]muscimol binding of solubilized and purified receptors with minimal lipid contents, detergents present in such a preparation might substitute for lipid molecules associated with a native receptor. Nevertheless, steroid effects were stereoselective and their intracellular application was ineffective (for reviews, *see* Gee, 1988; Majewska, 1992; *see* also Lambert et al., 1990; Puja et al., 1990; Purdy et al., 1990). In addition, the high nanomolar affinity supports a specific binding site on the GABA$_A$R. Since the effects of barbiturates and steroids on [^{3}H]muscimol or [^{3}H]flunitrazepam binding or $^{36}Cl^-$-transport in synaptosoms were additive and synergistic, respectively, were observed in neurons and lack competitive interaction (Peters

et al., 1988; Turner et al., 1989; Im et al., 1990), a steroid-binding site is postulated that allosterically modulates the $GABA_AR$ and differs from the GABA, barbiturate or BZ sites (e.g., Gee, 1988; Callachan et al., 1987). Neurosteroids potentiate GABA currents at concentrations of approx 30–300 n*M*. At concentrations > 1 μ*M*, which might be realistic for the anesthetic alphaxalone, steroids directly gate the channel ionophore, an effect inhibited by bicuculline and assumedly involving a different binding site (Peters et al., 1988; Majewska, 1992; Lambert et al., 1990). In general, at high steroid concentrations care must be taken to differentiate between allosteric and direct effects of these substances, the latter possibly reflecting membrane perturbing properties.

Barbiturates

$GABA_AR$ responses are modulated by the barbiturates pentobarbital, phenobarbital, or secobarbital, and the related nonbarbiturates, etazolate and etomidate. They enhance the binding of GABA, increase GABA-induced Cl^- currents (Olsen, 1982; Macdonald and Barker, 1978; Macdonald et al., 1989; Twyman et al., 1989) and, accordingly, have sedative, hypnotic, and anesthetic effects. Since barbiturates only bind with low affinity, most of their effects were measured indirectly by interactions with the GABA/muscimol- or BZ-binding site. They enhance GABA and BZ binding (Olsen and Snowman, 1982; Olsen et al., 1986; Leeb Lundberg et al., 1980, 1981; Asano and Ogasawara, 1982), but inhibit binding at the picrotoxin/TBPS site (Olsen and Snowman, 1982; Ticku and Olsen, 1978; Squires et al., 1983; Leeb Lundberg et al., 1981), suggesting an allosterical modulation via a distinct site. At concentrations > 50 μ*M* as found during anesthesia with pentobarbital (Franks and Lieb, 1994), they directly gate the intrinsic ion channel (Bormann, 1988; Macdonald and Barker, 1978), a characteristic that might hint to an additional binding site (Olsen and Snowman, 1982; Harris et al., 1995). The effects of barbiturates on $GABA_ARs$ rank in order with their potency as anesthetics, indicating $GABA_ARs$ as prime candidates in mediating their in vivo effects (Olsen and Snowman, 198 Harris et al., 1995). Pentobarbital modulation of GABA currents is already observed in homooligomeric receptors which hampers the localization of the binding site on the $GABA_AR$.

Anesthetics

In addition to the anesthetic steroids and the barbiturates mentioned above, different anesthetics of various chemical classes modulate the $GABA_AR$. The general anesthetic chlormethiazole at high micromolar concentrations or the iv anesthetic propofol at low micromolar concentrations enhance GABA-gated Cl^- currents, (Concas et al., 1991; Peduto et al., 1991; Hales and Lambert, 1991). The volatile anesthetics, substances with a chemical structure different from the iv anesthetics and the anesthetics mentioned above, also modulate the $GABA_AR$. Isoflurane (EC_{50} approx 320 μ*M*), Hall et al., 1994, halothan (0.34–1.7 m*M*; Yang et al., 1992), and enflurane (0.75–1.5 m*M*; Yang et al., 1992) enhanced [^{3}H]muscimol binding in membranes of murine cerebral cortex and cerebellum (Harris et al., 1993) and potentiated GABA-gated currents (Lin et al., 1992; Wakamori et al., 1991; Nakahiro et al., 1989) as well as increased Cl^- fluxes in rat brain cortical slices (Longoni et al., 1993). Their relative potentiation is highest at low GABA concentrations (EC_{5-10}), enhancing GABA effects by 200–600% when given below or at their respective EC_{50} (Franks and Lieb, 1994; Harris et al., 1995; Lin et al., 1992). Like barbiturates, these substances directly open $GABA_A$ channels at high concentrations (3 m*M* chlormethiazole, 30–600 μ*M* propofol; Yang et al., 1992; Longoni and Olsen, 1992). These currents can be blocked by picrotoxin and are sensitive to the competitive GABA antagonist bicuculline (Yang et al., 1992; but *see* Longoni et al., 1993). Stereoselectivity (Hall et al., 1994; Jones and

Harrison, 1993) and the noncompetitive interactions indicate a specific binding site at the $GABA_AR$, although anesthetics do not act solely via the $GABA_AR$, but also effect glutamate receptors as well as muscular nAchR. Nevertheless, all anesthetics tested so far directly and indirectly affect $GABA_ARs$. Structurally related lipophilic substances lacking anesthetic properties do not interact with $GABA_ARs$ (for discussion, *see* Franks and Lieb, 1994; Harris et al., 1995). Consequently, as for barbiturates, $GABA_ARs$ are the prime candidates for mediating the anesthetic effects of these substances in vivo (Franks and Lieb, 1994).

Picrotoxin/TBPS

Whereas the substances discussed above primarily enhance GABAergic effects, one chemically inhomogeneous group of compounds mainly comprises allosteric antagonists. Prototypic compounds of this class are picrotoxin (Yoon et al., 1993), the bicyclic caged compound TBPS (*t*-butylbicyclophosphorothionate) (Squires et al., 1983; Wong et al., 1984; Supavilai and Karobath, 1983), *t*-butylbicycloorthobenzoate, pentylenetetrazol (Maksay and van Rijn, 1993), and a variety of insecticides such as dieldrin or lindane (Lawrance and Casida, 1984; Casida, 1993; Nagata et al., 1994). They all antagonize GABA-evoked Cl^- conductances (Bormann, 1988; Olsen, 1982; Casida, 1993; Nagata and Narahashi, 1994) and therefore oppose sedative effects and are convulsants. Since picrotoxin, pentylenetetrazole, and the convulsive barbiturate isomer S(+)MPPB (S(+) N-methyl-5-phenyl-5-propylbarbituric acid) induce a monophasic dissociation of [^{35}S]TBPS from $GABA_ARs$ (Squires et al., 1983), these compounds are thought to bind at a single site on $GABA_ARs$ that differs from the GABA-, BZ, Barbiturate-, or steroid-binding sites (Maksay and Simonyi; 1985; Maksay and Ticku, 1985). TBPS partially and allosterically inhibits GABA binding and that of positive modulators at $GABA_ARs$ (Olsen, 1982; Skerritt and Johnston, 1983; Karobath et al., 1981; Im and Blakeman, 1991) and facilitates the binding of negative modulators. This allosteric interaction appears to be bidirectional as GABA and its agonists, BZs or barbiturates and steroids all modulate [^{35}S]TBPS binding.

The Convulsant-Binding Site: Reflecting the Functional State of the Receptor–Channel Complex?

Convulsants differ in their onset and recovery kinetics. Whereas the onset of picrotoxin is in the ms range, that of TBPS is slow (30 min to peak inhibition; Yoon et al., 1993). Onset as well as recovery kinetics do not follow simple kinetics (*see* Maksay, 1994), but depend on the presence of GABA agonists, i.e., the rate of onset of the picrotoxin inhibition is complete within approx 5 s with repetitive GABA application (Yoon et al., 1993; Newland and Cull, 1992) but reaches only 35% after 33 s in the absence of GABA (Newland and Cull, 1992), suggesting that an open channel facilitates the actions of picrotoxin and TBPS. Similar effects are reported for the washout phase, further supporting that the binding site is located in the pore region of the ionophore itself (Gurley et al., 1995; Inoue and Akaike, 1988; Inomata et al., 1988). Although this location is disputed and the effects might be caused by allosteric interactions from a distant binding site (Yoon et al., 1993; Newland and Cull, 1992; Van Renterghem, et al., 1987), binding at the picrotoxin/TBPS site may reflect the functional state of the receptor (Im and Blakeman, 1991). As a negative allosteric modulator, TBPS inhibits GABA binding, but [^{35}S]TBPS binding itself is inhibited by higher concentrations of GABA agonists as well as other positive modulators of the $GABA_AR$, whereas its binding is facilitated by negative modulators of the $GABA_AR$ in well-washed, but not necessarily GABA-free, preparations (Maksay and Ticku, 1985; Maksay and Simonyi, 1986; Squires and Saederup, 1987). EC_{50} values obtained this

way for the interaction of GABAergic substances with [^{35}S]TBPS binding more closely resemble EC_{50} values obtained for these substances by electrophysiological recordings of GABA-gated currents than the equilibrium binding parameters (K_i, K_D) of these compounds. However, biphasic responses are reported, indicating TBPS binding to be rather complex. BZs for example are positive modulators that inhibit [^{35}S]TBPS binding but have been reported to facilitate [^{35}S]TBPS binding when residual GABA is blocked by bicuculline (Im and Blakeman, 1991). Furthermore, because of the long time course of TBPS binding, some data may represent nonequilibrium states at the TBPS/picrotoxin-binding site (*see* also Maksay and Simonyi, 1986, 1988).

Substances Recognizing Additional Binding Sites

Furosemide

Furosemide is a loop diuretic (Greger and Wangemann, 1987) also described to affect GABA responses in a number of neuronal cell populations (Zhang et al., 1991; Thompson et al., 1988; Nicoll, 1978; Gallagher et al., 1983; Inomata et al., 1988; Misgeld et al., 1986; Ballanyi and Grafe, 1985). With prolonged perfusion it affects the Cl^- gradient of cells by inhibiting the Cl^- cation cotransport (Thompson et al., 1988; Thompson and Gahwiler, 1989). Zhang et al. (1991) reported inhibitory effects on GABA responses by m*M* concentrations of furosemide only during early postnatal development (day 2–5), whereas Misgeld et al. (1986) found no effects by furosemide in hippocampal neurons. Pearce (1993) reported a specific effect of furosemide (500 μ*M*) on neuronal cell responses in the hippocampus, where it blocked a very fast component of the GABA-response but not a second slower one. Accordingly, Korpi and coworkers (1995) directly studied the effect of furosemide on $GABA_A$Rs. Whereas furosemide up to 100 μ*M* had no effects on [^{35}S]TBPS binding and the GABA-induced dissociation of [^{35}S]TBPS from $GABA_A$Rs in cerebral cortex or hippocampal membrane preparations, furosemide specifically enhanced [^{35}S]TBPS binding in the absence of GABA and reversed GABA-induced inhibition of [^{35}S]TBPS binding in cerebellar membranes. Bumetanide, another loop diuretic blocking chloride transport, remained ineffective or, at high concentrations, slightly enhanced GABA effects. Further binding studies indicated that furosemide did not interact with GABA, BZ, or diazepam-insensitive Ro15–4513 binding, suggesting a novel binding site on the $GABA_A$Rs (Korpi et al., 1995). Autoradiography on cryostat sections of the rat revealed specific interaction with [^{35}S]TBPS binding by furosemide only on cerebellar granular cells. Such specific interaction was confirmed by expression of recombinant receptors in HEK 293 cells. Furosemide inhibition could only be observed with α6-containing receptors in the form α6β2/3, irrespective of an additional γ or δ variant, and α4β2γ2 receptors. Inhibition was absent with receptor isoforms containing β1 instead of β2 or β3, and with α subunits other than α4 or α6 (Knoflach et al., 1996; Korpi and Lüddens, 1997). Expression of α6β2γ2 subunits in *Xenopus* oocytes confirmed a rapid furosemide inhibition of GABA currents with an IC_{50} at approx 10 μ*M* (Korpi et al., 1995), a value close to the IC_{50} reported for the inhibition of short circuit currents across tubules of rabbit kidneys (Greger and Wangemann, 1987; Schlatter et al., 1983).

Loreclezole and β-Carbolines

The anticonvulsant compound loreclezole ((Z)-1-[2-chloro-2-(2,4-dichlorophenyl)-ethenyl]-1,2,4-triazole), developed for the treatment of epilepsy, modulates $GABA_A$R function (Ashton et al., 1992). In vitro, its action is not affected by BZs, barbiturates, steroids, or other anesthetics, suggesting that it binds to a distinct novel site (Wafford et al., 1994; Wingrove et al., 1994). Wingrove et al. (1994) found that loreclezole was 300-fold more potent in increasing GABA currents in human β2- and

β3 than in β1 variant containing αiβj receptors expressed in oocytes. They identified the asparagin at position 289 and 290 in the TM2 regions of the β2 and β3 subunits, respectively, to be essential for modulation by loreclezole (Wingrove et al., 1994). Mutated to serine (Wingrove et al., 1994) or methionine (Stevenson et al., 1995) the β3 subunit rendered the receptor insensitive to loreclezole.

Similarly, Stevenson et al. (1995) recently reported that the potentiating action of DMCM depends on the presence of either the β2 or β3 subunit in a $GABA_AR$ and is absent in β1-containing receptors (Stevenson et al., 1995). The β-carbolines are described as inverse agonists exerting their convulsant effects via competitive interaction with the BZ-binding site since binding of the [^{3}H]-β-CCE could be deplaced by a number of benzodiazepines (Martin and Doble, 1983) and vice versa. However, DMCM (methyl-6,7-dimethoxy-4-ethyl-carboline) shows a biphasic effect being inversely agonistic at low concentrations but potentiating at higher concentrations (Herb et al., 1992; Sigel et al., 1990; Yakushiji et al., 1989; Im et al., 1995; Malatynska et al., 1992). Since this potentiation is insensitive to the BZ antagonist Ro15 -1788 and persists in heterologous expression systems in the absence of the γ2 subunit (Im et al., 1995; Taguchi and Kuriyama, 1990), a binding site other than the BZ site was suggested. Stevenson et al. (1995) identified an aspartate at position 290 in the human β3 subunit as responsible for the potentiating DMCM effect, representing the identical point mutation that is responsible for the binding of loreclezole (Wingrove et al., 1994), and indeed, the actions of DMCM and loreclezole were nonadditive in human α1β2γ2 receptors (Stevenson et al., 1995).

Ethanol

A direct effect of ethyl-alcohol on $GABA_AR$ was originally suggested based on similarities of the effects of ethanol, BZs, and barbiturates. Early electrophysiological recordings provided some evidence for the potentiating effects of ethanol in a number of preparations, (Davidoff, 1973; Celentano et al., 1988; for a detailed overview, *see* Korpi, 1994; Rabow et al., 1995), a view further supported by the alcohol-antagonizing effects of the partial inverse BZ agonist Ro15–4513 (Allan and Harris, 1986; Suzdak et al., 1986, 1988). In cerebellar Purkinje cells, the effect of locally applied ethanol could be opposed by Ro15–4513 as well as by the β-carboline FG 7142 (Palmer et al., 1988). In behavioral and neurochemical studies, the imidazobenzodiazepine, Ro15–4513, but neither CGS 8216 nor Ro15–3505 could oppose ethanol-induced motor impairment, normalize reduced cerebellar cGMP levels, or enhance reduced [^{3}H]2-deoxyglucose uptake in various brain regions (Bonetti et al., 1989). Ethanol-induced sleep times in mice were also reduced by Ro15–4513 and combinations of related imidazobenzodiazepines (Harris et al., 1995). However, the molecular mechanisms remain unclear. Ethanol has been reported to positively modulate the binding of a number of ligands at the $GABA_AR$ (e.g., Majewska, 1988), but the results are not equivocal (for discussion *see* Korpi, 1994; Rabow et al., 1995). As in many studies the observed potentiation by ethanol was not robust (Uusi Oukari and Korpi, 1989; Kleingoor et al., 1991; Korpi et al., 1993). Additional factors (e.g., Lin et al., 1993) such as second messenger systems, phosphorylation, or specific subunits were postulated.

Zolpidem binding has been suggested to be related to ethanol sensitivity (Breese et al., 1993), based on a proposed concordance of zolpidem and ethanol sensitivity in various brain regions (77–100%, Criswell et al., 1995). Although zolpidem is a potent ligand at α1-containing receptors, and its binding is reported to increase after chronic ethanol treatments (Devaud and Morrow, 1994) further evidence for molecular determinants that link zolpidem and ethanol action is required. An altered subunit composition, e.g., reduced α1 expression, is repeatedly reported after chronic ethanol treatment (e.g., Devaud et al., 1995; Mhatre et al., 1993); however, this contrasts with an increased level of zolpidem binding. One further subunit implied in ethanol action is the γ2L splice form, identi-

fied by Whiting et al. (1990) and Kofuji et al. (1991), which contains an eight-amino-acid insert in the intracellular loop between TM3 and TM4 (Wafford et al., 1991), as compared to γ2S. This insert includes a consensus sequence for a protein kinase C (PKC) phosphorylation site, suggested as essential for ethanol potentiation in *Xenopus* oocytes (Wafford and Whiting, 1992). The involvement of γ2L in the action of EtOH has been supported (Harris et al., 1995) and questioned alike (Kleingoor et al., 1991; but *see* Marszalec et al., 1994). Sigel described minimal but significant enhancement of GABA currents in rat α1β1/2γ2 receptors by ethanol but observed no differences between the γ2L- or γ2S-containing receptor isoforms expressed in oocytes (Sigel et al., 1993). Similar results were reported by Mihic and coworkers for anesthetic concentrations of EtOH (Mihic et al., 1994). Interestingly, it was recently reported that enhancement of GABA currents in mouse L929 fibroblasts transfected with α1β1γ2L required a PKC dependent phosphorylation at both the β1 and the γ2L subunits (Lin et al., 1996). Further reports suggested phosphorylation as a critical step for the effects of ethanol. Mutant mice lacking the γ isoform of the PKC were insensitive to ethanol when challenged in a number of behavioral experiments (Harris et al., 1995). A GABAergic depression of the firing rate of cerebellar purkinje neurones facilitated by ethanol is also facilitated by 8-bromo-cAMP, (Lin et al., 1993; Freund and Palmer, 1996). The afore mentioned reduction of α1 subunit expression after chronic ethanol treatment (e.g., Devaud et al., 1995; Mhatre et al., 1993) is reminiscent of a downregulation of the α1 subunit after chronic flunitrazepam treatment which is inhibited by the protein kinase inhibitor staurosporine (Brown and Bristow, 1996). These results implicate phosphorylation as a possible link between ethanol action and $GABA_AR$s (for review and detailed discussion, *see* Tabakoff and Hoffman, 1996, Macdonald, 1995; Dietrich et al., 1989; Sanna and Harris, 1993). But despite the general indication that $GABA_AR$s mediate some of the effects of ethanol, no direct evidence is reported for a specific binding site on the $GABA_AR$.

Carbamazepine/Phenytoin

Carbamazepine and phenytoin represent two widely used antiepiletic drugs, recently described as modulating the $GABA_AR$ by potentiating GABA-induced currents in cultured rat cortical neurons as well as in HEK 293 cells transiently expressing the $GABA_AR$ α1 β2 γ2S subtype. Both compounds potentiated currents by approx 45–90% with EC_{50}s of 24.5 and 19.6 n*M* (Granger et al., 1995). Since the modulation was absent in α1β2 receptors and was minuscule in α3β2γ2 and α5β2γ2 receptor isoforms, a new site of action was suggested that depends on the presence of a γ and the α1 subunit.

Ro5–4864

The 4′-chloro-derivative of diazepam, Ro5–4864, is a prototypic ligand at the mitochondrial peripheral BZ receptor (PBR). It binds in n*M* concentrations to the PBR but effects the central $GABA_AR$ only at μ*M* concentrations. Its convulsive activity is caused by an inhibition of GABA currents (Weissman et al., 1983) and is antagonized by barbiturates and BZ (Rastogi and Ticku, 1985). Gee et al. (Gee, 1987; Gee et al., 1987) reported no effect on GABA or BZ-binding sites but a reduced [^{35}S]TBPS binding in the absence of GABA and enhanced TBPS binding in its presence. Compounds like the phenylquinolines PK 8165, PK 9084, and the isoquinoline carboxamide derivative PK 11195 have been described as differentially modulating the $GABA_AR$ by a similar binding site (Gee, 1987). Ro5–4864 was also reported as influencing the spontaneous activity of Purkinje neurons in rat cerebellar slices. Ro5–4864 induced a biphasic effect first increasing the spontaneous firing rate (EC_{50} approx 490 n*M*, +68%) followed by a depression within several minutes (IC_{50} approx 450 n*M*; –91%). The peripheral BZ receptor antagonist PK11195 influenced only the depressive effects of Ro5–4864, increasing the IC_{50} to approx 1–5 μ*M*. This suggested the depressive effects as mediated by PBR-like binding sites and the stimulatory effects as associated with an inhibition of

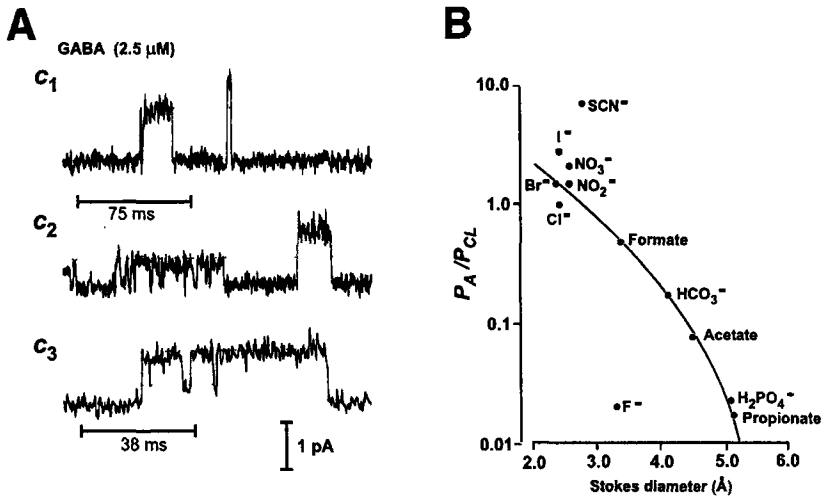

Fig. 3. Single-channel characteristics of native GABA$_A$R channels: (**A**) In response to GABA, the receptor ionophore opens to multiple conductance levels. In spinal cord neurons recorded with the single-channel patch-clamp technique (cell-attached), the major level is represented by approx 0.86 pA (approx 30 pS, c_1 c_2) with fewer levels of 1.42 pA (approx 44 pS, c_1) and 0.44 pA (approx 19 pS, c_2). In c_3 a subconductance level of 0.25 pA (approx 12 pS) is resolved. E_{patch} –3mV. (**B**) By determining the relative permeability of the ionophore for various anions, a pore diameter in spinal cord neurones of ca. 5.6 Å was calculated. NB: HCO_3^- has some physiological relevance. Modified from Bormann et al., 1987.

the GABA$_A$R (Basile et al., 1989). Such inhibition of the GABA$_A$R could be confirmed with recombinant receptors expressed in HEK293 cells. Ro5–4864 inhibited GABA responses with an IC_{50} approx 3 μ*M* by approx 60% but only in γ2-containing receptor isoforms and not in binary α1β1 receptors (Puia et al., 1989).

From Molecules to Channels: Electrophysiological Characteristics of Receptor–Drug Interactions

GABA: Single Channel Characteristics of Native Receptors in Response to GABA

Many studies employed mouse spinal cord neurons for single-channel analysis of native GABA$_A$Rs and their pharmacology. A pore diameter of 5.6 Å was suggested, because of the observed permeabilities for large polyatomic anions (Fig. 3B) (Bormann et al., 1987). In response to GABA, these channels open to 12, 17–20 and 27–30 pS conductance levels (Fig. 3A), the largest accounting for approx 95% of the current (Bormann et al., 1987; Macdonald et al., 1989). As will be discussed later, studies on recombinant receptors indicate these data vary little with the subunit composition. The main conductance level has been analyzed in further detail and revealed three different open states of 0.5, 2.6, and 7.6 ms duration (mouse spinal cord). These open states are not altered with increasing GABA concentrations, but the relative contributions are shifted towards the longer states (Macdonald et al., 1989; Twyman et al., 1990). The competitive antagonist, bicuculline, reduces the open frequency and mean duration of GABA-induced single channel events (Fig. 4) (Macdonald et al., 1989). Similarly, the noncompetitive antagonist picrotoxin reduces the channel-opening frequency (Fig. 4), whereas the channel conductance and other time constants remained unaltered according to noise analysis (Newland and Cull, 1992; Porter et al., 1992). The channel blocker penicillin reduces the channel-open probability in a concentration-dependent manner (Fig. 5) with-

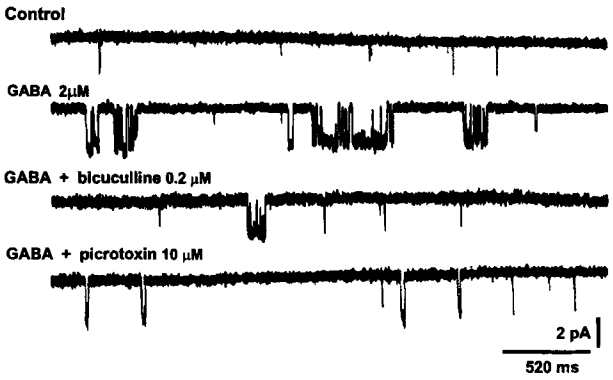

Fig. 4. Single-channel responses to GABA and its antagonists: In excised outside-out patch-clamp recordings of cultured mouse spinal cord neurons only rare and very brief openings were observed which increased in frequency after the application of GABA, concurrent with an increase of the average channel-open duration. The latter is because of a shift from short opening states to naturally occurring longer open states. Predictably, the competitive antagonist bicuculline reduced the opening frequency and open durations. The noncompetitive antagonist picrotoxin is suggested to reduce the channel open durations by a shift to the shorter states. E_{patch} –75mV. Modified from Macdonald et al., 1989.

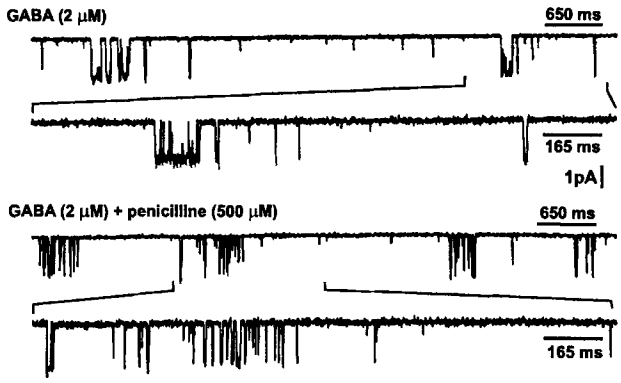

Fig. 5. The channel blocker penicillin: Although penicillin increases the channel opening frequency in excised outside-out patches of cultured mouse spinal cord neurons the channel-open durations are dramatically reduced, resulting in a reduced channel open probability of the $GABA_AR$. The kinetic analysis is in accordance with an open channel block. E_{patch} –75 mV. Modified from Twyman et al., 1992.

out altering the single-channel conductance (Twyman et al., 1992). Although penicillin increases the opening frequency, the open durations are drastically reduced by a shift to shorter open states, which is in agreement with an open-channel block (Twyman et al., 1992).

Benzodiazepines Modulate Single-Channel Characteristics

Confirming early reports by Study and Barker (1981), detailed analysis of single-channel kinetics revealed that BZs have no effect on the single-channel conductance or the average channel open durations (Macdonald and Olsen, 1994; Macdonald and Twyman, 1992; Rogers et al., 1994). Potentiation of GABA responses was caused by an increased channel-opening frequency caused by an increase in the number of bursts (increased occurrences) but not their duration (Fig. 6) (Macdonald and Olsen, 1994; Macdonald and Twyman, 1992). The inverse agonistic β-carboline DMCM reduces the channel open frequency without altering open durations channel conductances, i.e., it behaves conversely to agonistic BZs (Rogers et al., 1994). BZ potentiation is discussed as originating from an increased affinity for GABA. In this case, BZs should increase the average channel-open duration as it is observed with an increased GABA concentration (Macdonald and Olsen, 1994), but the kinetic analysis by Macdonald and Twyman (1992) did not support such a mechanism (*see* also, Rogers et al., 1994). The increased channel-opening frequency might be explained by either an increased affinity at only one of multiple binding sites, different transitions into desensitized states or altered coupling between binding site and channel, as discussed in more detail by Macdonald and Olsen (1994).

Barbiturates Alter Single-Channel Characteristics

Fluctuation analysis of neuronal intracellular recordings suggested barbiturates as

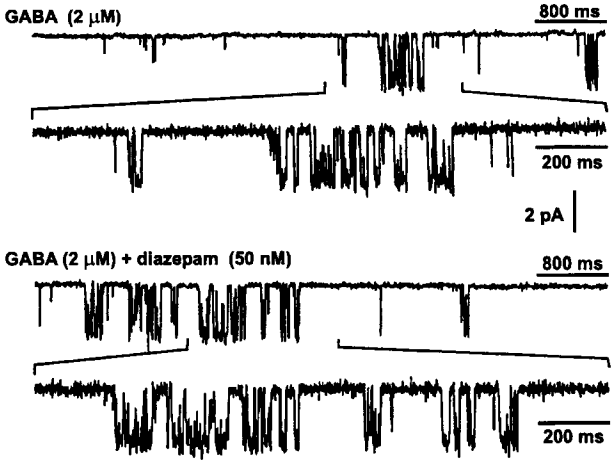

Fig. 6. Benzodiazepines increase the open frequency of the GABA$_A$R. Diazepam increases GABA-gated currents by increasing the open frequency of the channel without altering other kinetic parameters or the channel conductance when recorded in excised outside-out patches of cultured mouse spinal cord neurones. BZs applied together with saturating GABA concentrations do not further increase the currents. Accordingly, BZ effects are most pronounced at low GABA concentrations. (E_{patch} −75 mV.) Modified from Rogers et al., 1994.

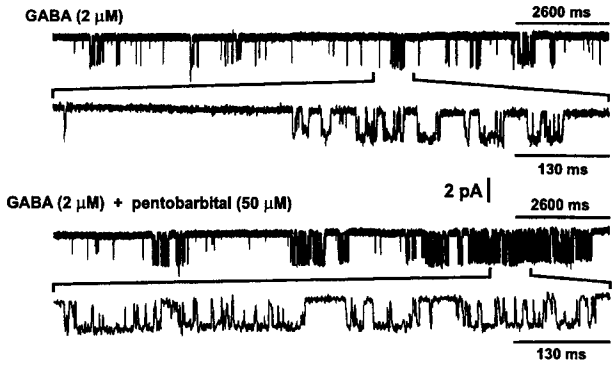

Fig. 7. Barbiturates increase the channel mean open time of the GABA$_A$R. The positive modulatory effects seen in excised outside-out patches of cultured mouse spinal cord neurons are caused by an increase of the channel mean open time but not an increase in channel opening frequency. Like BZs, the barbiturate pentobarbital does not increase the channel conductance. (E_{patch} −75 mV.) Modified from Macdonald et al., 1989.

increasing the average channel-open duration without altering the channel conductance (Study and Barker, 1981). Single-channel patch-clamp recordings confirmed such an increase of the mean open time, without changes in the single-channel conductances or frequency of openings (Fig. 7) (Study and Barker, 1981; Macdonald et al., 1989; Twyman et al., 1989; Porter et al., 1992, Jackson et al., 1982). Macdonald and coworkers (1989) suggested that this increase is caused by a shift towards the longer open states. Accordingly, the IPSP decay in neurons is reported to be markedly increased with no change in rise time or peak amplitude (Gage and Robertson, 1985; MacIver et al., 1991). Direct gating of channels by barbiturates might induce different effects. However, these effects are not well characterized (e.g., Olsen, 1982; Cash and Subbarao, 1988).

Steroids: Electrophysiological Characteristics of Steroid Modulation

In the presence of steroids, Harrison and coworkers (1987) observed that the decay time constant of spontaneous IPSPs recorded from hippocampal neurons were prolonged. They suggested that steroids increase the mean channel-open time, which was supported by fluctuation analysis of recordings from spinal cord neurons in the absence and presence of alphaxalone (Barker et al., 1987). Single-channel patch-clamp studies of bovine chromaffine cells indeed revealed an increased average channel-open duration, caused by an increased frequency of single-channel openings and a shift to longer opening states (Twyman and Macdonald, 1992). The inverse agonist PS only reduced the frequency of channel openings (Mienville and Vicini, 1989). Therefore, steroids differ from barbiturates and benzodiazepines in that they show both an increased frequency as seen with BZs and a shift towards longer durations as seen with barbiturates (*see* also Puia et al., 1990) (*see* Fig. 8).

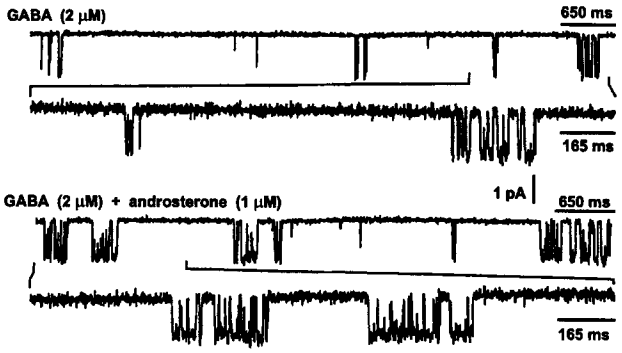

Fig. 8. Neurosteroids alter single-channel kinetics of $GABA_AR$s. The neurosteroid androsterone increases the open probability of the $GABA_AR$ in excised outside-out patches of cultured mouse spinal cord neurons by increasing both the channel opening frequency as well as the mean open duration (shift towards longer open states). (E_{patch} –75 mV.) Modified from Twyman and Macdonald, 1992.

Conductance Levels of Native and Recombinant $GABA_AR$s

Single-channel patch-clamp recordings revealed that native $GABA_AR$ channels observed in mouse spinal cord neurons open to 12, 17–20, and 27–30 pS conductance levels (Bormann et al., 1987; Macdonald et al., 1989). Properties of native $GABA_AR$s derived from other tissues have not been studied in such detail, whereas recombinant receptors have been expressed in several heterologous systems. In such expression systems, e.g., *Xenopus laevis* oocytes, HEK 293 cells, or mouse fibroblast L929 cells, channel characteristics for receptor isoforms composed of defined subunit combinations have been obtained for homooligomeric channels composed of five identical subunits, binary, ternary receptors, and receptor-channels composed of four different subunits (*see* Table 1). For homooligomeric α1, α2, or α3 channels, main conductance levels of 19 and 28 pS and minor levels of 10 and 42 pS were described (Blair et al., 1988). The main levels for β1 receptors were 10 and 18 pS with minor levels of 27 and 40 pS (Blair et al., 1988; Krishek et al., 1996). Similar conductance levels of around 10, 17, 29, and 41 pS have been described for recombinant α1β1 and α2β1 receptors in various cell lines, their main conductance levels being 15–18 pS (Levitan et al., 1988; Porter et al., 1992; Moss et al., 1990; *see* also Krishek et al., 1996). This contrasts with data obtained from the γ subunit containing α1γ2 and α1/6β2γ2 receptors with main conductance levels around 30 pS (*see* also Table 1) (Porter et al., 1992; Angelotti et al., 1992; Angelotti and Macdonald, 1993; Verdoorn et al., 1990). Accordingly, single-channel conductances might be helpful in discriminating αiβj from receptors containing a γ subunit (e.g., Gunther et al., 1995). However, they appear of limited use when identifying particular native channel subtypes. Nonetheless, this strategy is impeded by the inaccessibility of most synaptic channels to single-channel patch-clamp studies in vivo. Additional and comparative information on the pharmacological and physiological properties of native receptors and recombinant receptors expressed in vitro is needed to gain insight into the physiological consequences of the observed subunit and receptor diversity.

Properties of Recombinant $GABA_A$ Receptor Channels

Expression of Functional Homo- and Heterooligomeric $GABA_AR$s

The first paper on cloned $GABA_AR$s in 1987 stated that bovine α or β subunits, expressed separately in *Xenopus* oocytes, did not form GABA-responsive channels (Schofield et al., 1987). But it is still controversial whether expressed homooligomeric receptors are functional, and their function may well depend on the species or specific expression system used (*see* Table 2). For example, αi subunits were reported to form GABA-gated channels that could be observed by single-channel recordings (Blair et al., 1988) and whole-cell recordings (Pritchett et al., 1988; Khrestchatisky et al., 1989), or not to assemble into functional receptors (Sigel et al., 1990; Sanna et al., 1995). In

Table 1
Single-Channel Characteristics of Recombinant GABA$_A$Rs

	Conductance (pS)					
	bovine		rat		mouse	
Subunits	L929/CHO	oocytes	L 929	HEK 293	oocytes	
α1, α2, α3		19 ± 1; 28 ± 1 (10; 42) [1]				
β1		10 ± 1, 18 ± 1 (27 ± 1, 40 ± 4) [1]			18, 26 (10)	[2]
α1β1	L929: 15.3 ± 0.3 10.4 ± 0.5 [3]	10 ± 2, 18 ± 2, 27 ± 2; 41 ± 3 [4]		oocytes 16 (11) [2]		
	CHO 17; (25, 11) [5]					
α1β2				11.3 ± 0.23 (14; 18) [6]		
α2β1		10 ± 2; 18 ± 2; 27 ± 2, 41 ± 3 [4]				
α3β1		10 ± 2, 18 ± 2; 27 ± 2; 41 ± 3 [4]				
α1γ2				30.8 ± 1 (16.8) [6]		
α1β1δ			22 [7]			
α1β1γ2lδ			33 [7]			
α1β1γ2	L929· (human γ2) 27.8-30.5 19 7-21 8 few 10, 15 [8, 3]		30 [7]	32.0 ± 0.8 (17.5) [6]		
α1β2γ3				30.2 ± 3.5 [9]		

Subunits derived from the species indicated were (co)expressed in the given cells (human HEK 293, mouse L929, *Xenopus* oocytes). Single-channel patch-clamp recordings were obtained in the cell-attached or outside-out configuration. Data are in pS, *main* or (minor) conductance levels are marked when mentioned by the authors. 1. Blair et al., 1998; 2. Krishek et al., 1996; 3. Angelotti and MacDonald, 1993; 4. Levitan et al., 1988; 5. Porter et al., 1992; 6. Verdoorn et al., 1990; 7. Saxena and Macdonald, 1994; 8. Angelotti et al., 1993; 9. Herb et al., 1992.

contrast, β1 subunits are consistently reported to form functional channels (Krishek et al., 1996; Sanna et al., 1995; Joyce et al., 1993; Sigel et al., 1989), and might represent native receptors (for discussion see Krishek et al., 1996; Mathers, 1985; Taleb et al., 1987; Hamann et al., 1990). Sanna et al. (1995) reported strong GABA gating with the human β1 subunit expressed in oocytes, which, interestingly, was insensitive to 100 μ*M* bicuculline. Although they described a small amount of spontaneously active and GABA-insensitive channels, their results contrast with previous reports on spontaneously opening channels composed of rat β1 subunits, which were completely insensitive to GABA (Sigel et al., 1989). This controversy might be explained by species dependent differences. When Krishek et al. (1996) expressed murine β1 channels in oocytes or HEK 293 cells, they observed a spontaneously active chloride conductance not gated by GABA and absent in control cells. No such spontaneous activity, but a small GABA-induced inward current, was observed using bovine β1 homomeric subunits expressed in oocytes (*see* also Table 2).

The properties of other homo-oligomeric channels have not been studied in such detail. Thus far, limited and conflicting results for the β2, γ2, and δ subunits indicate their ability to form homooligomeric channels (*see* Table 2) (e.g., Shivers et al., 1989; Sigel et al., 1990; Verdoorn et al., 1990; Pritchett et al., 1989). They responded in a bicuculline- and picrotoxin-sensitive manner to high μ*M* GABA concentrations and were potentiated by pentobarbital

Table 2
Functional Expression of Homooligomeric $GABA_A$Rs

	human		Bovine		rat		murine
Subunits	HEK 293	oocytes	Sf9	Oocytes	HEK 293	oocytes	oocytes
α1	40 pA [1]	no [2]		no [3] yes, sC [4]	220 ± 30 pA [5]	occasional, small [6] no (<3 nA) [7, 8] 1 / 20 cells: 10 nA [9]	
α2		no [2]		yes, sC [4]	yes [10]		
α3				yes, sC [4]		5 nA [7]	
α5		no [2]				rare <25 nA [7, 8] occasional, small[1] [6]	
β1	40 pA [1] yes, sC [11]	EC_{50} = 123 μM[2] [2]	yes [12]	30 pA [13] EC_{50} 29.3 ± 5 μM yes, sC [4]		spontaneous[3] [8] occasional, small [6] no, 0 / 20 cells [9]	spontan sC [13] also testesd in HEK 293
β2					75 ± 18 pA [5] yes [10]	no (<3 nA) [7]	
γ2	yes [14]	no [2]			yes [10] 40 pA [15] 96 ± 40 pA [5]	no (ca 3 nA) [7, 8]	
δ					120 pA [15]	L929 cells. no 0 /18 cells [16]	
ε	no [17]						

[1]Was named α4 in their original publication.

[2]Described as bicuculline insensitive; occasionally a small, spontaneous current blocked by picrotoxin was observed.

[3]The spontaneous current could be blocked by picotoxin, resulting in an apparent outward current of + 390 nA; the spontaneous activity was abolished by coexpression of α1 or α3, but only reduced in the presence of the α2 subunit, sC: currents were observed by the single-channel patch-clamp technique.

Single subunits were expressed in the cells indicated (human HEK 293, insect Sf9 cells, or *Xenopus* oocytes). Electrophysiological recordings were obtained by the whole-cell patch-clamp technique (HEK 293 and Sf9 cells) or by two electrode voltage-clamp (oocytes). The response to GABA was observed. When available, the current amplitude to 100 μ*M* GABA (Verdoorn et al., 1990, 10 μ*M*) is given, and the fraction of responding cells is noted. 1. Pritchett et al., 1998; 2. Sanna et al., 1995; 3. Schofield et al., 1987; 4. Blair et al., 1988; 5. Verdoorn et al., 1990; 6. Khrestchaitsky et al., 1989; 7. Sigel et al., 1990; 8. Sigel et al., 1989; 9. Malherke et al., 1990; 10. Draguhn et al., 1990; 11. Puia et al., 1990; 12. Joyce et al., 1993; 13. Krishek et al., 1996; 14. Pritchett et al., 1989a; 15. Shivers et al., 1989; 16. Saxena and Macdonald, 1994; 17. Davies et al., 1997.

but not BZs. Additionally, homomeric rat β3 receptors were able to form high-affinity TBPS-binding sites when expressed in HEK 293 cells (Slany et al., 1995). Accordingly, present results suggest that α, β, γ and δ subunits can assemble into functional homooligomeric channels. However, the recently described ε subunit, expressed in HEK 293 cells, failed to assemble into functional homo-oligomeric channels (Davies et al., 1997).

When two or more different subunit classes were coexpressed, the presence of a single group of characteristics (Angelotti and Macdonald, 1993) indicated that not homomeric, but binary or oligomeric receptors, receptors, represent the preferred configuration. The small currents observed with homooligomeric channels also indicated them as expressed with a reduced efficiency as compared to coexpressed αiβj or αiγk subunit combinations (*see* Table 3) (Pritchett et al., 1988; Verdoorn et al., 1990) and both, α and β subunits appear to be required for the high-affinity GABA binding and the robust channel gating observed in

Table 3
Functional Expression Binary or δ-Containing $GABA_A$Rs

	Human	bovine		rat	
Subunits	HEK 293	oocytes (L929/CHO	L929	HEK 293	oocytes
α1β1	400 pA* [1] yes [2]	yes [3-5] [6] 347 ± 101 nA, sC[1] [7]		yes [8] 190 pA [9]	yes [10] yes, high variability [11]
		L929: yes, sC [12] CHO yes, sC [13]			150-500 nA[§] [14][2] 470 nA[#] [15] ≥3000 nA [16][3] [17]
α1β2				1500 ± 360 pA* [18] yes [8]	780 nA[#] [15]
α1β3					1640 nA[#] [15]
α2β1	yes, [19] oocytes: yes, [20]	yes [3]; sC [7]			
α3β1		yes [3] 2686±1114nA [7]		yes [8]	> 3000 nA [16] yes, high variability [11]
α4β2				<200 pA[#] [21]	
α5β1					80 ± 20 nA[§] [14][4] yes, high variability [11] > 3000 nA [16]
α5β3			3/8 cells 20-50 pA [22]		
α6β2				151 ± 28 pA [9]	
α6β3			11%: 12 ± 7 pA [23]		
α1γ1	yes [24]			yes [8]	
α1γ2				1100 ± 250 pA* [18] yes [8]	no [16]
α3γ2				yes [8]	no [16]
α4γ2				<150 pA[#] [21]	
α5γ2			no (1 / 8 cells) [22]		no [16]
β1γ2	yes [24]				16 nA [16]
β2γ2				85 ± 22 pA* [18]	155 nA [16]
β3γ2			no (0 / 6 cells) [22]		
α1γ2δ			2 / 18 cells [25]		
β1γ2δ			0 / 18 cells [25]		
α1β1δ			18% 41.1 ± 14 pA* [25]		
α1β1γ2δ			49%: 667 ± 321 pA* [25]		
α6β2δ			50%: 130 ± 70 pA [23]		
α6β3δ			63%: 371 ± 116 pA [23]		

The recently described human ε subunit showed no responses to GABA when coexpressed in combinations of human α2ε or β3ε, or rat/human α1ε, α6ε or β1ε (Davies et al., 1997). sC: Currents were observed by the single-channel patch-clamp technique.

When available data are given in pA/nA at GABA concentrations of:[#] 1 μ*M*, *10 μ*M*, or [§] 50 μ*M*, % of responding cells are given when possible.

sC: currents were observed by the single-channel patch-clamp technique.

[1]Mentioned: α1α2β1, α1α3β1, and α1α2α3β1 to be expressed with similar characteristics as α2β1.

[2]Of cells tested, 37% responded to picrotoxin with an small outward current of 9.6 ± 4.1 nA, indicating the presence of spontaneously open channels.

[3]Cells responded to picrotoxin with a small outward current of 28 nA.

[4]77% of tested cells responded to picrotoxin with an outward current of 12.2 ± 3.2 nA.

The indicated subunits of the species given on top were coexpressed in the indicated cell lines. Responses to GABA at the concentration noted in the table footnotes are given if available. Similarly the fraction of responding cells is noted when given by the authors. 1. Pritchett et al., 1988; 2. Puia et al., 1990; 3. Levitan et al., 1988; 4. Schofield et al., 1987; 5. Horne et al., 1993; 6. Angelotti et al., 1993; 7. Levitan et al., 1988; 8. Draguhn et al., 1990; 9. Ducic et al., 1995; 10. Krishek et al., 1996; 11. Malherbe et al., 1990; 12. Angelotti and Macdonald, 1993; 13. Porter et al., 1992; 14. Khrestchatisky et al., 1989; 15. Ymer et al., 1989; 16. Sigel et al., 1990; 17. Malherbe et al., 1990; 18. Verdoorn et al., 1990; 19. Jones et al., 1995; 20. Wafford et al., 1993; 21. Knoflach et al., 1996; 22. Burgard et al., 1996; 23. Saxena and Macdonald, 1996; 24. Pritchett et al., 1989; 25. Saxena and Macdonald, 1994; 26. Davies et al., 1997.

vivo. But, whereas certain subunit combinations like α1β1 or α1β2 are consistently expressed (Verdoorn et al., 1990; Draguhn et al., 1990; *see* also Angelotti et al., 1993), other subunit combinations appear to be expressed less efficiently, e.g., the α5β3 isoform (Burgard et al., 1996) or the α6β3 isoform (Saxena and Macdonald, 1996). Similar controversial results exist for binary αiγ2 receptors that either appeared to be expressed as well as α1β1 isoforms (Verdoorn et al., 1990; Draguhn et al., 1990; Im et al., 1993), or failed to respond to GABA (Sigel et al., 1990; Angelotti et al., 1993; Burgard et al., 1996; Harrison et al., 1993) (*see* also Table 3). The β2γ2 isoform is reported to show only small currents relative to other isoforms (Sigel et al., 1990; Verdoorn et al., 1990; Draguhn et al., 1990), whereas the β1γ2S or β3γ2L isoforms failed to assemble into functional channels (Sigel et al., 1990; Angelotti, 1993; Burgard et al., 1996; *see* also Harrison et al., 1993) (for details, *see* Table 3). Similarly, binary combinations of the ε subunit like αiε or βjε are nonfunctional (Davies et al., 1997).

A cellular explanation for the different expression of certain isoforms is provided by recent studies from Connolly et al. (1996), reporting that some murine subunits and subunit combinations, including homooligomeric α1 and β2 and binary α1γ2L and β2γ2L isoforms, are retained in the endoplasmatic reticulum of oocytes and HEK 293 cells and that only α1β2 and α1β2γ21 receptors were detected on the cell surface. More recently, it was reported that the β subunits were involved in the targeted distribution of $GABA_AR$: In polarized Madin-Darley canine kidney cells, the subcellular targeting of α1βj receptors differed according to the specific β subunit expressed, irrespective of the presence of γ2S subunits (Connolly et al., 1996).

Attempts to estimate the efficiency of expression for specific binary and ternary receptor isoforms from the amplitude of GABA-gated currents have to be considered critically, since parameters such as cell sizes — the keywords are membrane surface area, membrane capacitance — or different single-channel characteristics were neglected. Also, no correlation was observed between the densities of immunogold labelings and current amplitudes when probing binary and ternary receptor isoforms with antibodies towards αi, β2, or γ2 subunits in HEK 293 cells, e.g., α6β2γ2-containing ternary receptors showed smaller currents when compared to α1β2γ2 receptors, despite similarly high immunogold densities were detected with β2 and γ2 antibodies for both receptor isoforms (Ducic et al., 1995). With regard to ternary receptors of the form αiβjγk, αiβjδ, or αiβjε, to our knowledge no combination tested has failed to build functional channels. It seems that even receptor isoforms composed of four different subunits, containing at least members of the α, β, and γ subunit classes, seem to assemble consistently (Sigel et al., 1990; Verdoorn, 1989).

Subunits Determine GABA Sensitivity

In receptors consisting of binary α and β, ternary α, β and γ or more subunits, the sensitivity to GABA is influenced by the subunit composition, mainly the specific α subunit. However, few reports studied an appreciable number of subunit combinations, which would allow conclusive comparisons. Data obtained by different groups for one subunit combination are almost incomparable, because of different experimental conditions (*see* below and Table 4). Nevertheless, α6-containing receptors are consistently reported as the most sensitive binary and ternary isoforms, with EC_{50} values in the sub- to low micromolar range (0.1–2.2 μ*M*; Knoflach et al., 1996; Saxena and Macdonald, 1996; Ducic et al., 1995; *see* Table 4). The α1-containing isoforms show an intermediate sensitivity ($EC_{50} \approx$ 0.6–4.5 μ*M*; Ducic et al., 1995; 8–25 μ*M*; Ebert et al., 1994; Malherbe et al., 1990a,b) whereas α4- and α5-containing ternary receptors represent less sensitive receptors (α4: 1.4/3.9 μ*M*, α5: 2–25 μ*M*; (Knoflach et al., 1996; Sigel et al., 1990; Ducic et al., 1995; Ebert et al., 1994). The α3-containing isoforms are reported as least sensitive, with an EC_{50} approx 10-fold higher than the α1βjγk

Table 4
GABA Sensitivities of Recombinant Ternary $GABA_A$Rs

	EC_{50} GABA (μM)															
	Human				bovine				rat							
subunits	oocytes		HEK 293		oocytes		L929		oocytes		HEK 293		L929	
α1β1γ1	25	[1]			41 ± 7.3	[2]	5.2 ± 0.09	[3]	75	[4]	1.2	[5]		
α1β1γ2							5 3	[6]			4.5	[5]	6 2 (+δ· 6 9)	[7]
α1β1γ3											0 6	[5]		
α1β2γ1											0.67 (hum. γ1)	[5]		
α1β2γ2	20	[1]									12 9-19 8 4.5-7	[8] [5, 9, 10]	11	[11]
α1β2γ3											1.3	[5]		
α1β3γ1											2.1	[5]		
α1β3γ2	8	[1]									1 7	[5]	14	[11]
α2β1ε			11 2	[12]										
α2β1γ1	39 8 ± 4	[13]												
α2β1γ2	30 6 ± 11	[13]	17	[14]										
α3β1γ1	114	[1]												
α3β1γ2	208	[1]			98±19	[2]			240 ± 136	[15]	15.1 (hum. α3)	[5]		
α3β1γ3	32	[1]												
α3β2γ2	11	[1]							487 ± 42	[15]	75-130 15.1 (hum. α3)	[8, 10, 16] [5]		
α3β3γ2	28	[1]												
α4β2γ2											3.9 ± 0.7 1 4	[17] [5]		
α5β1γ2	15	[1]							17 ± 7	[15]	5.6	[5]	26	[18]
α5β2γ2									14 ± 3	[15]	5.8 ± 0.2 4.2	[16] [5]		
α5β2γ3											4.9 ± 1 2	[19]		
α5β3γ1	24	[1]												
α5β3γ2l	3	[1]											6	[18]
α5β3γ3	2	[1]												
α6β1γ2											0 5	[5]		
α6β2γ1											0 4	[5]		
α6β2γ2											1.4 ± 0.3 0 16-0 34	[17] [5, 9]	2	[11]
α6β3γ2l													2	[11]
α6β2δ											1 2	[5]	0 2	[11]
α6β3δ													0 3	[11]

Speed of GABA application: [8] <1 ms; [5] <10 ms; [11, 25] 70% <500 ms; [1 ,2] tp <30 s.

Further values were obtained for the quarternary receptors: α1α3β2γ2 26 μ*M* (human, oocyte [1]); 42.6–73.2 μ*M* (rat, HEK 293; [8]); α1α5β2γ2 18 μ*M* (human, oocyte [1]); α3α5β1β2γ2 17 ± 7 μ*M*, α3α5β2γ2 34 ± 5 μ*M*, α3α5γ2 147 ± 39 μ*M* (all rat, oocyte; [15]).

The EC_{50} in μ*M* obtained from electrophysiological recordings of the indicated cells coexpressing the listed subunits is given. Recordings were obtained by the whole-cell patch-clamp technique in HEK 293 and L929 cells and with the two electrode voltage-clamp technique in *Xenopus* oocytes.

1. Ebert et al., 1994; 2. Wafford et al., 1993; 3. Horne et al., 1993; 4. Malherbe et al., 1990; 5. Ducic et al., 1995; 6. Angelatti et al., 1993; 7. Saxena and Macdonald, 1994; 8. Kleengoor et al., 1993; 10. Gengrich et al., 1995; 11. Saxena and Macdonald, 1996; 12. Davies et al., 1997; 13. Wafford et al., 1993; 14. Jones et al., 1995; 15. Sigel et al., 1990; 16. Knoflach et al., 1993; 17. Knoflach et al., 1996; 18. Burgard et al., 1996; 19. Knoflach et al., 1991.

isoforms (Sigel et al., 1990; Verdoorn, 1994; Ebert et al., 1994). But α subunits are not the only determinants of GABA sensitivity. The β3 subunit appears to confer enhanced GABA sensitivity to α1βj, (Ymer et al., 1989), α1βjγ2, and α5βjγ2 receptors (Ducic et al., 1995; Ebert et al., 1994), and in α5β3γk receptors, sensitivity was further increased by replacement of the γ2 by the γ3 subunit (Ebert et al., 1994). The least GABA-sensitive α3β1γ2 receptors might be especially prone to substitution of the specific βj and γk subunits. An EC_{50} of the human α3β1γ2 receptor expressed in oocytes of 208 μ*M* (Ebert et al., 1994) could be decreased approx 10-fold by replacing the β1 by the β2 or β3 subunits (α3β2/3γ2: 11 μ*M*) or the γ2 by the γ3 subunit (α3β1γ3: 32 μ*M*; Ebert et al., 1994, but *see* Sigel et al., 1990; Ducic et al., 1995). But again, the available data are inconsistent (*see* Table 4): Exchange of the β2 for a β1 subunit in α3β1γ2 receptors is also reported not to alter sensitivity (EC_{50} approx 15 μ*M*; Ducic et al., 1995) or to decrease it even twofold for rat receptors (240 μ*M* ≥ 480 μ*M*; Sigel et al., 1990). Another inconsistency regards the rat α6β2δ isoform: it is described as either approx 10-fold less sensitive than the α6β2γ2 (Ducic et al., 1995) or approx 10-fold more sensitive (L929 cells, -γ2L; Saxena and Macdonald, 1996). An explanation for these differences may be that receptors from different species differ in their sensitivities or that the expression system specifically modulates the sensitivities of certain subunit combinations. Additionally, technical parameters like the speed of agonist application profoundly affects receptor desensitization, and it is well conceivable, that in some cases, receptor desensitization has contaminated evaluations of amplitude data, as suggested by Verdoorn et al. (1990). Some groups used fast-application systems to minimize these effects, whereas others describe time-to-peaks of GABA responses to be normally below 30 s, a value not accounting for submillisecond time-to-peaks and decay-time constants in the low ms range as described for spontaneous inhibitory postsynaptic potentials (sIPSPs) or with fast-application systems (e.g., Jones and Westbrook, 1995). Therefore, care has to be exerted when comparing such data. Parameters such as species origin, expression system, and experimental conditions have to be considered. Still, an increasing amount of evidence suggests the sensitivities of αiβjγk receptors as decreasing in the rank order α6<α1<≈α5≈α4<<α3, with a tendency of β2/3 and γ2/3 containing receptors representing the more sensitive isoforms when compared to γ1 or β1 containing isoforms. Similar to electrophysiological studies, the β3 and γ3 subunits were suggested to increase the GABA sensitivity in [^{35}S]TBPS binding studies. The α1/3/5βjγk-receptor isoforms containing β3 and/or γ3 subunits were more sensitive to GABA-induced alterations in [^{35}S]TBPS-binding than their β1- and γ1-containing equivalents (Lüddens et al., 1994). Korpi and Lüddens suggested in their studies (Lüddens, et al., 1994; Korpi and Lüddens, 1993) an altered coupling between the binding site and ionophore, since the binding affinity for GABA itself for the α1β2/3γ3 receptors remained unchanged.

ρ *Subunits Form Homooligomeres with a Distinct Pharmacology*

The homopentameric receptors formed by ρ1 and ρ2 subunits are more sensitive to GABA than other $GABA_AR$s in identical cells (EC_{50} approx 4 μ*M* vs 27 μ*M*; Feigenspan et al., 1993; Feigenspan and Bormann 1994), can be selectively activated by the conformationally restricted GABA stereoisomer CAMP (cis-2-aminomethyl-cyclopropane carboxylic acid), and are not modulated by BZ, barbiturates, or steroids (Shimada et al., 1992; Enz et al., 1995). They maintain the picrotoxin sensitivity typical of $GABA_A$ receptors, but are neither inhibited by the $GABA_AR$-selective drug bicuculline, nor the $GABA_B$-receptor selective drug baclofen (Shimada et al., 1992; Kusama et al., 1993a,b). Distinct single-channel properties (Feigenspan et al., 1993; Feigenspan and Bormann 1994) in addition to their homooligomeric composition, pharmacology, and restricted distribution have

led some to present them as a different class of receptors with discrete characteristics (GABA$_C$; Shimada et al., 1992; Drew et al., 1984). Although such classification as GABA$_C$ receptors remains controversial (*see* also Darlison and Albrecht, 1995), we will restrict the remainder of this review to the heteropentameric GABA$_A$Rs and refer to a recent review for details on the ρ1/2/GABA$_C$ receptors (Bormann and Feigenspan 1995).

Molecular Determinants of the GABA Site

Mutation studies indicate the GABA-binding site to be determined by both the α- and the β subunits (reviewed by Macdonald and Olsen, 1994; Mihic et al., 1995; Olsen and Tobin, 1990). A point mutation in the rat α1 subunit, exchanging the phenylalanine at position 64 to leucin (α1^{F64L}) coexpressed with β2γ2, caused a marked decrease in the affinities of GABA agonists and antagonists (Sigel et al., 1992). The homologous position in bovine α1 (F65) could be covalently modified by the photoaffinity agonist [^{3}H]muscimal and, similarly, labeled in all α, γ2, and δ, but not β subunits (Smith and Olsen, 1994). However, it remains unclear why [^{3}H]muscimol labeled binding sites in αiβj-and αiβjγk-receptor isoforms, (Pritchett et al., 1988; Zezula et al., 1996) but not or only weakly in α1γ2 or β2γ2 receptors (Zezula et al., 1996; Pregenzer et al., 1993), and why, in an earlier work, a 57-kDa GABA$_A$-receptor peptide, presumably a β subunit, but not a 52-kDa α subunit, was photoaffinity labeled with [^{3}H]muscimol (Casalotti et al., 1986; Deng et al., 1986). Other mutations in the rat β2 subunit between the disulfide bridge and TM1 (Tyr157-Thr160 and Thr202-Tyr205) reduced the binding affinities of GABA agonists and antagonists in ternary receptors, whereas equivalent mutations in the α or γ subunits had minor effects (Amin and Weiss, 1993). Homologous regions in nAchR and glycin receptors that belong to the same receptor superfamily had been previously identified as being involved in agonist/antagonist recognition (for *review, see* Smith and Olsen, 1995), indicating a conserved structural motif for ligand recognition in the superfamily of ligand-gated ion channels.

The spontaneous activity observed in homomeric rat and murine β1 receptors, in contrast to homomeric α receptors, might help to further elucidate the GABA-binding site and/or the coupling mechanism between agonist binding and channel gating in such subunits. When exchanging the leucin residues, L264 and L259, for threonine in human α1 and β1 subunits, respectively, the resulting once or twice-mutated α1β1 receptors, expressed in Sf9 cells, displayed an increased resting conductance that was insensitive to bicuculline or picrotoxin (Tierney et al., 1996). When the mutated α1^{L264T} was combined with wild-type β1, the receptors still responded to GABA though with a slower kinetic than wild-type α1β1 receptors. However, in α1β1^{L259T} receptors, the response to GABA was abolished although [^{3}H]muscimol binding was retained (Tierney et al., 1996). Still, this cannot explain the differences of the GABA-gated bovine and the non-gated murine β1 subunits mentioned earlier (Krishek et al., 1996), as these subunits differ only in a few amino acids in the intracellular TM3–TM4 loop. Nevertheless, these studies are initial steps in understanding the essential role of the β subunits for the coupling between GABA binding and channel gating.

Receptor Differentiation by Benzodiazepines

Because of their clinical relevance, the high-affinity BZ binding and BZ-induced modulation of GABA currents displayed by most native GABA$_A$Rs is of major interest (e.g., Sivilotti and Nistri, 1991; Sieghart, 1994, 1995; Olsen, 1982; Möhler et al., 1990). The presence of BZ-modulated GABA$_A$Rs in recombinant receptors was first reported for human α1 and β1 subunits coexpressed with γ2 in HEK 293 cells (Pritchett et al., 1989; *see* also Malherbe et al., 1990a). Binary receptors lacking the γ sub-

unit were either not modulated by BZs (Knoflach et al., 1991; Moss et al., 1990, 1991; Pritchett et al., 1989; Malherbe et al., 1990b; Levitan et al., 1988) or reported to show a sensitivity and specificity to BZs atypical of the hitherto described native $GABA_ARs$. The $\alpha1\beta1$ receptors are reported to be potentiated by high concentrations of the agonist diazepam (μM instead of nM) and also the null and negative modulators Ro15–1788 and DMCM (Schofield et al., 1987; Malherbe et al., 1990; but *see* Puia et al., 1989). However, accumulating evidence exists for a distinct BZ site outside of the classical BZ domain. Additive effects of Ro15–1788 and diazepam in binary receptors, and the potentiating effects of DMCM that depend on the $\beta2$ and $\beta3$ subunits (Wingrove et al., 1994; Stevenson et al., 1995), suggest an additional BZ-binding site (Malherbe et al., 1990) that might explain the atypical responses even in the absence of the classical BZ-binding site on $\alpha i\beta j$ receptors. In contrast, binary receptors containing a γ subunit consistently exhibit BZ modulation of their GABA responses, i.e., BZ-binding sites were described on $\alpha1\gamma2$ subunits (Zezula et al., 1996; Wong et al., 1992; Moss et al., 1991) and $\alpha\gamma$ or $\beta\gamma$ combinations were modulated by BZs like diazepam and triazolam and inhibited by the inverse agonist DMCM (Knoflach et al., 1993; Im et al., 1993; Wong et al., 1992). Im et al. (1993) described the $\alpha1\gamma2$ isoforms as responding similarly to $\alpha1\beta2\gamma2$ isoforms with all substances tested, whereas $\beta2\gamma2$ receptor responses to GABA could only be potentiated by subunit nonselective BZ ligands like diazepam, but not or only weakly by $\alpha1$-subunit-preferring ligands like zolpidem, alpidem, or Cl 218872 (Im et al., 1993). Furthermore, [^{3}H]flunitrazepam binding to the rat $\alpha1\gamma2$ isoform expressed in HEK293 cells was allosterically inhibited by pentobarbital, alphaxalone, propofol, and chlormethiazole (Slany et al., 1995) in contrast to $\alpha1\beta3\gamma2$ receptors, indicating clear differences in their BZ pharmacology. The presence of such binary receptors in vivo has been suggested by immunohistochemical double labelings for $\alpha3\gamma2$ isoforms (Fritschy et al., 1992) and because of similarities in [^{3}H]BZ binding between $\alpha1\gamma2$ receptors and cerebellar membranes (Wong et al., 1992; Slany et al., 1995; Wong and Skolnick, 1992). However, it remains to be further elucidated whether $\alpha i\gamma k$ receptors are associated with subunits like $\beta1$, δ or ε and, indeed, represent native receptors.

Benzodiazepines: Role of α Subunits in Ternary Receptors

The full scale of BZ modulation observed in native receptors can only be appreciated in the presence of α, β and γ subunits. In such ternary receptors the α subunits determine most of the receptor characteristics towards BZs (Pritchett and Seeburg, 1990; Puia et al., 1991; Wafford et al., 1993; Hadingham et al., 1993; Pritchett et al., 1989), which is further affected by the γ subunit (Lüddens et al., 1994; but *see* Hadingham et al., 1993, 1995; Wafford et al., 1993). Accordingly, the different pharmacological responses to BZs are at present the most useful tool to discriminate subunit compositions in vivo. Originally two classes of $GABA_A$/BZ receptors, types I and II (or BZ I and BZ II), were defined based on high and low sensitivity for certain BZ ligands, respectively. This never-precise classification (Olsen and Tobin, 1990; Lo et al., 1982; Braestrup and Nielsen, 1981) has been overcome by knowledge of the molecular receptor subunit composition. A higher affinity of type I receptors compared to type II receptors towards the 1,4 benzodiazepine 2-oxoquazepam (Olsen and Tobin, 1990; Corda et al., 1988), the triazolopyridazine Cl 218872 (Nielsen and Braestrup, 1980; Sieghart, 1983; Squires et al., 1979), the imidazopyridine zolpidem, and the inverse agonist β-carboline methyl-β-carboline-3-carboxylate (β-CCM) is now generally accepted as caused by the presence of the $\alpha1$ subunit in $\alpha i\beta j\gamma2$-receptor isoforms (Pritchett et al., 1989a,b). Type II receptors contain $\alpha2$, $\alpha3$ or $\alpha5$ subunits instead of the $\alpha1$ subunit and are characterized by a lower affinity to the aforementioned BZ ligands (Pritchett et al., 1989b). Differentiation into two

Table 5
Sensitivities of Recombinant GABA$_A$Rs to Benzodiazepine-Receptor Ligands

	EC$_{50}$ (nM)												
	α1β1γ1	α1β1γ2	α1β2γ2	α1β3γ2	α1β2γ3	α2β1γ1	α2β1γ2	α3β1γ2	α3β2γ2	α4β2γ2	α5β2γ2	α6β2γ2	α6β3δ
abecarnil						94 ± 48 [1]	19 ± 15 [1]		7 ± 2 [2]		7 ± 4 [2]		
bretazenil									15 ± 4 [2]	311 ± 87 [3]	12 ± 7 [2]	158 ± 17 [3]	
Cl 218872		68 ± 29 [4]	57 ± 6 [5]		57 ± 13 [5]	3290±860 [1]	1660±910 [1]	885±456 [4]					
diazepam		53 [6]		70 [7]			60 [6]	140 [6]			-β1- 4000 [6] -β3- 40 [8]	β3: 72 [7]	
divaplon									220 ± 107 [2]		183 ± 53 [2]		
DMCM	~200 [6]	~2 [6] 1 ± 0 4 [4]	3 7 ± 0 9 [5, 9]	20 [7]	9 8 ± 1 1 [5]			6 ± 4.7 [4]				1.7 μM [9] β3. 1 2 μM [7]	5 μM [7]
FG 8205		10 ± 1 [4]				57 ± 40 [1]	2.2 ± 0.7 [1]	10 ± 3 [4]					
flunitrazepam		29 ± 11 [4]	6 9 ± 1.4 [5]		110 ± 14 [5]	26 ± 20 [1]	3.3 ± 0.7 [1]	24 ± 10 [4]	31 ± 16 [2]		2 ± 1 [2]		
Ro15-4513										105 ± 4 [3]		168 ± 65 [3]	
triazolam		46 ± 10 [4]	5 ± 0 7 [10]					16±1 6 [4]					
zolpidem		57 ± 7 [4]	86 ± 14 [5]		> 10000 [5]	64 ± 22 [1]	300 ± 175 [1]	410 [4]					

Further values were obtained with triazolam for the combinations α1γ2: 18 ± 3 n*M* and β2γ2: 8.5 ± 0.7 n*M* [10].

The EC$_{50}$ in n*M* of BZ-receptor ligands is given. The indicated subunits were coexpressed in HEK 293, L929 cells, or *Xenopus* oocytes. Control responses were obtained at the app. EC$_{10}$– EC$_{30}$ for GABA of the particular receptor, except of Knoflach et al., 1993; Im et al., 1993, 5 μ*M* GABA; Saxena and Macdonald, 1996; 10 μ*M* GABA; and Wafford et al., 1993a, 30 μ*M* GABA. 1. Wafford et al., 1993b; 2. Knoflach et al., 1993; 3. Knoflach et al., 1996; 4. Wafford et al., 1993a; 5. Hadingham et al., 1995b; 6. Puia et al., 1991; 7. Saxena and Macdonald, 1996; 8. Burgard et al., 1996; 9. Stevenson et al., 1995; 10. Im et al., 1993.

classes, however, is reductionistic in view of the molecular complexity of the GABA$_A$R subunits. Indeed, whereas substances like 2-oxo-quazepam and the BZ inverse agonist Ro15–1788 do not discriminate α2, α3 or α5 containing receptors (Pritchett and Seeburg, 1990), a number of BZ-receptor ligands further differentiate between them. For example, α5βjγ2 receptors are characterized by a very low affinity for zolpidem (Pritchett and Seeburg, 1990) and the structurally related alpidem (Faure Halley et al., 1993) The α1-receptor-preferring ligand Cl 218872, still binds with a higher affinity to α5- than to α2- or α3-receptor isoforms (Pritchett and Seeburg, 1990; Hadingham et al., 1993), and the BZ partial-inverse agonist Ro15–4513 has an even higher affinity for α5-containing receptors than for any other receptor isoform (Lüddens et al., 1994; Hadingham et al., 1993). Like zolpidem, the affinity of β-CCM is lowest for α5-containing receptors (α5β3γ2; Pritchett and Seeburg, 1990; α5β1γ2s; Hadingham et al., 1993), whereas the affinity of DMCM is highest for α5β2γ2 and lowest for α2β2γ2 (Faure Halley et al., 1993) when compared to other α1β2γ2 receptor isoforms.

But ligand affinities obtained by binding studies rarely predict the physiological sensitivity (EC$_{50}$) or efficacy (% maximal potentiation; for details, *see* Tables 5 and 6). A prime example is zolpidem, the EC$_{50}$ of which in electrophysiological recordings is 10-fold lower for α1β1γ1 than for α3β1γ2 receptors, but the rank order of efficacy at concentrations above approx 1 μ*M* is

Table 6
Potentiation of Recombinant $GABA_A$ Rs by Benzodiazepine-Receptor Ligands

	enhancement in % of control GABA response													
	α1β1γ1	α1β1-3γ2	α1γ2	α1β1-2γ3	α2β1γ1	α2β1γ2	α3β1γ1	α3β1-2γ2	α4β2γ2	α5β1γ1	α5β1-3γ2	α5β2γ3	α6β1-3γ2	α6β3δ	β1-2γ2
abecarnil		β2 74±20 [1]		β2 96±28 [1]	32±24 [2]	64±8 [2]		β2 91±12 [3]			β2: 79 [3]				
alpidem	49±10 [4, 5]	β2 201±33 [6] β1. 344 [5]	171±42 [6]		6-20 [5] [2]	309 [5]	28 [5]	β1 212 [5]		21 [5]	β1 10 [5]				β2· 38±29 [6]
β-CCM	+50 [5]	β1 -39 [5]			+96 [5]	-38 [5]	-39 [5]	β1 -32 [5]		-2 [5]	β1 -57 [5]	-29±5 [7]			
bretazenil	61 [8]	51-94 [8]		β1 64 [8]	18.6 [2]	48 [8] 28 [2]		β1 74 [3] β2 145 [8]	215 [9]		52-58 [1, 3, 8]	57 [1]	β2 69±12 [9]		
Cl 218872		61-98 [1, 6, 10]	104±32 [6]	β2: 66±3 [1]	79±2.8% [2]	74±7.8% [2]					β3 -10 [1]	6 [1]			β2: 0±5 [6]
clonazepam	68±10 [4, 5]	β1. 135 [5]			33 [5]	222 [5]	38 [5]	β1. 304 [5]		49 [5]	β1. 68 [5]				
diazepam	58±9 [4, 5, 8]	38±19 [11] 86-135 [4-6, 8, 12, 13], 67-69 [7, 14]	110±31 [6]	25-36 [4, 7, 12] β1 57 [8]	81 [5] 41 [2]	272 [5, 8] 112.5 [2]	103 [5]	β1 345 -400 [4, 5, 8] β3 128 [7] β3γ3 110 [7]		51[5]	β1 105 -112 [4, 5, 8]		+7- (-28) [4, 9, 13] [13]	0 [6]	β2 53±18 [5]
DMCM	72 [5] -10-(-30) [15]	-30 - (-60) [1, 5, 7, 10, 12-16]	-16±8 [16]	β2 -60 [1, 7, 12]	+19-24 [2, 5]	-40 - (-51) [2, 5]	1 [5]	-70 [5] β2. -12 [7] β2γ3· -40 [7]		+18 [5] +10-60 [15]	β1 -40 - (-53) [1, 5] β1 -10-70 [15]	-52 [1]	β3 +48±20 [13] β2. -29±16 [14] β2/3: +112-146 β1 -19±8 [17]	+124±49 [13]	β1 -57±8 [16]
DMCM + Ro15-1788		0±5 [12]		2±3 [12]											
FG 8205		β1,2 54±9 [1, 10] β3· 85 [10]		β2 47±8 [1]	28±3.0% [2]	80±3.3% [2]					β3 30 [1]	78 [1]			
flunitrazepam	≈ 0 [15]	64±45 [14] 103-114 [1, 10, 12, 15]		β2 75-88 [1, 12]	81±9.6% [2]	123±3.2% [2]		β2 128±22 [3]		10-40 [15]	β2-3: 120-150 [1, 3] β1. 10-340 [15]	122 [1] 86±12 [7]	β2· 0-(-33) [14]		
Ro15-1788					21 5 [2]	9.9 [2]									
Ro15-4513		β2 -22±7 [14]			14 3 [2]	-23 3 [2]			56±6 [9]		β3 -4 [1]	-20 [1]	β2. 70±14 [9] -29±14 [14]		
triazolam	130 [4]	170-230 [4, 6]	117±43 [6]	β1 57 [4]	69 3 [2]	109 [2]		β1· 207 [4]			β1 77 [4]		β1: 40 [4]		β2: 71±16 [6]
zolpidem	59±18 [4, 5]	127-139 [6, 10] β1,2: 230- 250 [5, 12] β2 158 -165 [1, 10]	154±25 [6]	β2: 20±3 [12] β2· 165 [1]	16 -25 [2, 5]	213 [5] 132±17 [2]	0 [5]	β1· 280 [5]		14 [5]	β3 0 [1] β1: 14 [5] β3 ≈0 [18]	-14 [1]			β2. 6±6 [6]
zopiclone		117±25 [6]	154±25 [6]												β2 114±50 [6]

For the binary α2β1 receptor isoform potentiation of -1 to +15 % is described with various BZs and related substances, including DMCM [12].

For binary α1β1 receptors the following values were reported [9] diazepam: 45± 21% (+Ro15–1788 90 ± 60%); midazolam: 48% (+Ro15–1788: 90 ± 60%); flunitrazepam: 9 ± 3%; and Ro15–1788: 75% ([GABA] dependent, at 1 μ*M* only 15%). DMCM is described to show either no effect: 0–3% [16, 19] or in some cells to enhance the GABA-gated current by about +45% [19].

For α3β2 and α5β2 the following values were reported: α3β2: diazepam 6.6%, DMCM–2%; α5β2: flunitrazepam – 8 ± 5%, β-CCM–3 ± 4%; α5β3: diazepam insensitive [18].

The quarternary receptor α1β2γ2δ is enhanced with diazepam by 60 ± 21% [11].

Further data with the experimental substances can be found: AHR 14,749 [2], CGS 8216 [6], U-79089 [6], U-90167 [3], or divaplon, or imidazenil [9, 19]. 1. Hadingham et al., 1995; 2. Wafford et al., 1993b; 3. Knoflach et al., 1993; 4. Ducic et al., 1993; 5. Puia et al., 1991; 6. Im et al., 1993; 7. Knoflach et al., 1991; 8. Puia et al., 1992; 9. Knoflach et al., 1996; 10. Hadingham et al., 1993; 11. Saxena and Macdonald, 1994; 12. Herb et al., 1992; 13. Saxena and Macdonald, 1996; 14. Kleingoor et al., 1991; 15. von Blankenfeld et al., 1990; 16. Puia et al., 1989; 17. Stevenson et al., 1995; 18; Burgard et al., 1996; 19. Malherbe et al., 1990.

α3>α2>α1>>α5 (-β1γ2) (Puia et al., 1991; Wafford et al., 1993). Most BZ ligands, including the classical ligand diazepam, showed a similar rank order of efficacy being higher with the α3β1γ2 and α2β1γ2 receptors than with α1β1γ2 or especially α5β1γ2 receptors (*see* Table 6; Puia et al., 1991). Only the zolpidem analog alpidem exerted its highest efficacy in α1β1γ2 receptors with a rank order of α1>α2>α3>>α5 (Puia et al., 1991). The β-carboline DMCM showed a higher efficacy with α3-containing receptors and β-CCM with α5β1γ2 receptors (Puia et al., 1991; Wafford et al., 1993) again in contrast to their binding affinities (*see* above). Starting from the lead compound Ro15–4513 as a high affinity ligand for α5-containing receptors (Lüddens et al., 1994), imidazobenzodiazepines with a >50-fold higher selectivity for α5β2γ2 than for α1β2γ2 receptors have been recently synthesized (Liu et al., 1996). Preliminary experiments indicate their efficacy as negative modulators of α5-containing receptors expressed in oocytes to be similar or below that of DMCM. However, the above-mentioned divergence of binding studies and functional studies requires further evaluation of their functional specificity.

These results clearly indicate that not only do the affinities of most BZ ligands critically depend on the specific subunit composition of the receptor, but that the efficacy of these ligands is a function of the subunit configuration, as well (*see* Tables 5 and 6).

BZ Characteristics of α6- and α4-Containing Receptors

Ternary receptors containing α6 or α4 subunits lack the modulation by classical BZ-receptor ligands. The α6 subunit is characterized by a high sensitivity to GABA agonists and shows a unique distribution, being almost restricted to cerebellar granule cells (*see* above and Lüddens et al., 1990). Most characteristics of the α6-containing receptors seem to extend to α4 receptors, a subunit mainly localized in the thalamus. Recombinant α6βjγ2 and α4βjγ2 receptors distinguish themselves from other BZ receptors by their insensitivity to BZ ligands like diazepam. Nevertheless, they retain a normal affinity towards the inverse agonist Ro15–4513 (Lüddens et al., 1990; Knoflach et al., 1996; Korpi et al., 1995; Saxena and Macdonald, 1996; Ducic et al., 1995; Korpi and Lüddens, 1993; Wisden et al., 1991). Both receptors display a low affinity to the BZ-receptor ligands Ro15–1788 and DMCM (Lüddens et al., 1990) and display a Ro15–1788-sensitive potentiation by bretazenil (Knoflach et al., 1996). Despite earlier reports that Ro15–4513 and DMCM decrease GABA-gated currents in α1β2γ2 and α6β2γ2 receptors expressed in HEK 293 cells (Kleingoor et al., 1991), other results suggest these substances, as well as Ro15–1788, as potentiating α4- or α6-containing receptors isoforms (Knoflach et al., 1996; Saxena and Macdonald, 1996; Im et al., 1993). As described earlier, potentiating effects of Ro15–4513 and DMCM predominate in binary αiβj- or ternary αiβjγ1-receptor isoforms, e.g., in the absence of the classical BZ site, and μ*M* concentrations of DMCM are known to additionally interact and potentiate GABA$_A$Rs via the loreclezole-binding site (Wingrove et al., 1994; Stevenson et al., 1995). Photolabeling studies revealed nonidentical incorporation sites for [^{3}H] flunitrazepam and [^{3}H] Ro15–4513 on α1 (within position 1–103 and 104-carboxy-terminus, respectively) and for [^{3}H] Ro15–4513 on α1 vs α6 subunits (104-carboxy-terminus and 1–101, respectively; Duncalfe and Dunn, 1996; Duncalfe et al., 1996) and might help to elucidate the inconsistencies observed with these ligands, otherwise classified as inverse agonists.

Benzodiazepines: The Influence of Specific Subunits

The γ2 variant is used most commonly in studies with recombinant receptors, since it is the most abundant γ subunit, but exchange of the γ2 subunit with either γ1 or γ3 dramatically alters the BZ pharmacology of ternary receptors. α1β2γ3 receptors display reduced binding affinities for agonists like diazepam or 2-oxo-quazepam but unaltered affinities for antagonists or inverse agonists, as compared to γ2

receptors (Herb et al., 1992; Knoflach et al., 1991; Lüddens et al., 1994; but *see* Ymer et al., 1990a). Furthermore, the α1β3γ3 and α5β3γ3 receptors are zolpidem insensitive, probably caused by a reduced binding affinity (Herb et al., 1992). Both γ3 containing receptors display a binding affinity for Cl 218872 in the low n*M* range, as compared to 100 n*M*–10 μ*M* of γ2-containing receptors, (Lüddens et al., 1994). Electrophysiological recordings of recombinant α1β2γ2/3 receptors in oocytes confirmed the reduced sensitivity for flunitrazepam and zolpidem in the γ3 containing receptors but could demonstrate only minor differences in EC_{50} or efficacy between the γ2- and γ3-containing α1β2γk receptors for Cl 218872 (*see* Tables 5 and 6 and above; *see* also Herb et al., 1992; Hadingham et al., 1996). Exchange of the γ2 subunit with a γ1 subunit in α1β1γk receptors resulted in a reduced potentiation by diazepam, clonazepam, and bretazenil, and a slight negative modulation was observed with alpidem for α3β1γ1 and zolpidem for α2β1γ1 receptors (Wafford et al., 1993a,b). Furthermore, in α1/2/3β1γ1 receptors, the modulatory effects of Ro15–4513, β-CCM, and DMCM were no longer negative but positive (Puia et al., 1991; Wafferd et al., 1993b), an effect accompanied by a loss in binding affinity for Ro15–1788 and DMCM (Ymer et al., 1990b). A rank order of efficacy of α1>α2≈α5>α3 in αiβ1γ1 receptors was found for DMCM, which differed from that of β-CCM. This compound showed a positive modulation at α2β1γ1 and α1β1γ1 receptors, but acted as null modulator at α5β1γ1 receptors and as a negative modulator at α3β1γ1 receptors (Puia et al., 1991). Since binary αiβj and ternary α6/4βjγk receptors (j = 2, 3) are positively modulated by DMCM, the question arises whether the positive effects reported are caused by the lack of induction of a classical BZ site by the untypical γ1 subunit.

Benzodiazepines: Influence of Specific β Subunits

Exchanging the β subunit in ternary receptors did not significantly alter the BZ binding characteristics (Pritchett et al., 1989b; Hadingham et al., 1993) for flunitrazepam, DMCM, FG8205, zolpidem, or Cl 218872, nor are pentobarbital or DHEAS reported to differentiate between the β1, β2, and β3 isoforms in electrophysiological recordings of ternary α1βjγ2 receptors (human, oocytes, and HEK293; Hadingham et al., 1993). Accordingly, Puia (rat, HEK293; Puia et al., 1992) reported for diazepam or bretazenil only a tendency towards a decreased potentiation while exchanging β1 with the β2 or the β3 subunit in α1βjγ2 receptors. Sigel, however, observed a several-fold higher potentiation in α1/3/5β2γ2 receptors, as compared to α1/3/5β1γ2 (rat, oocytes; Sigel et al., 1990). The minor relevance of the β subunits in ternary receptors to BZ pharmacology was also seen when studied by [^{35}S]TBPS-binding, although, in α5βjγ2/3 and to a lesser extent in α3βjγ2/3 receptors, the β3 variant was required for high affinity [^{35}S]TBPS binding (Lüddens et al., 1994; Lüddens and Korpi, 1995). Interestingly, this correlates with the notion that α5 subunit mRNA colocalizes with β3 mRNA (Laurie et al., 1992; Wisden et al., 1992). Another study on homooligomeric β3 channels reported this subunit to be sufficient for high affinity [^{35}S]TBPS binding (rat, HEK 293 cells; Slany et al., 1995). Nevertheless, the previous results indicate the TBPS binding site to be determined by multiple subunits.

Benzodiazepines: Molecular Determinants of Ligand Recognition and Efficacy

The techniques of molecular biology have revealed structural details of the BZ-binding site that explain some of the subunit-specific characteristics of BZs. By exchanging ever-smaller regions between the α1 subunit, i.e., high-affinity binding of Cl 218872 and 2-oxo-quazepam (*see* above), and the α3 subunit, i.e., low affinity binding of these ligands, a glutamic acid position was identified. Its substitution by glycine induced a 10-fold increase in the affinity of $\alpha3^{E225G}\beta2\gamma2$ receptors for these two α1 preferring ligands (Pritchett and Seeburg, 1991) but inferred no change for the

receptor nonselective ligand diazepam. With a similar approach, an arginine at position 100 in the α6 subunit was found to be mainly responsible for the diazepam insensitivity of α6β2γ2 receptors. Interestingly, an equivalent mutation, $\alpha6^{R100E,}$ was found in a rat line bred for increased alcohol sensitivity and has been suggested to be responsible for the elevated motor impairment by diazepam observed in these rats (Korpi et al., 1993). In reverse, the diazepam-insensitivity could be transferred to the α1 subunit by exchanging the native α1 histidine to arginine (Wieland et al., 1992). Successive substitution of three additional amino acids in the α6 subunit, including Gly199, Thr161, and Val211, converted the α6β2γ2 receptor from diazepam-insensitive to diazepam-preferring (Wieland and Lüddens, 1994), identifying these amino acids as being involved in the specificity of BZ binding. When Buhr et al. studied the effects of various mutations in rat subunits expressed in *Xenopus* oocytes, they observed two point mutations in the α1 variant ($\alpha1^{V161A,}$ $\alpha1^{T206A}$) that enhance the diazepam, zolpidem, and DMCM sensitivity without altering the affinity to GABA or the potentiation by pentobarbital or steroids (Buhr et al., 1996). In contrast, the $\gamma2^{F77A}$ mutation in the rat subunit increased only the diazepam potentiation but almost completely abolished the potentiation by zolpidem (Buhr et al., 1996). The efficacy of BZ ligands is further related to position T142 in the human γ2 subunit (Mihic et al., 1994), which when exchanged for serine ($\gamma2^{T142S}$) in α1β1γ2 receptors, increased the potentiation by classical BZs such as diazepam, alprazolam, clonazepam, or flunitrazepam. Ligands with higher receptor-subtype specificity such as zolpidem, alpidem, and CI 218872 showed significantly less enhancement in receptors containing the mutated $\gamma2^{T142S}$. Similarly, the inverse agonists DMCM, FG 7142 and β-CCM show a reduced inhibition, and Ro15–4513 and the antagonist Ro15–1788 even induced a positive modulation. Thus, this mutation shifts the effects of some but not all ligands into a more positive range (Mihic et al., 1994; *see* also Nutt et al., 1992; Nutt, 1990). Since the binding affinities for flunitrazepam are not altered by this mutation, it may affect the BZ-induced conformational changes involved in the receptor-channel coupling. Whether this effect is related to the atypical potentiating effects of some inverse agonists at binary αiβj (Ro15–1788, DMCM), α6βjγk (Ro–1788; Ro15–4513, DMCM), or αiβjγ1 (Ro15–4513, β-CCM, DMCM) receptors, e.g., in the absence of the classical BZ-site, remains to be elucidated.

These data indicate that the amino acids involved in BZ effects are not clustered, but spread discontinuously over the α and γ variants. Furthermore, we are far from understanding the molecular parameters of receptor-ligand interactions at the $GABA_AR$ and are only beginning to appreciate that the potentiation of GABA effects by BZs depend on the α subunit variant, the γ variant and the ligand.

The Role of the δ Subunit

The distribution of the δ subunit suggests a colocalization mainly with the α4 and α6 subunit. As discussed previously, expression of the δ subunit in the cerebellum is closely coupled to the expression of the α6 subunit. With recombinant receptors, Saxena and Macdonald (1994) did not find functional GABA-gated channels with either δ alone, or β1γ2Lδ or α1γ2Lδ isoforms and detected only small currents with the α1β1δ channel, whereas addition of the δ subunit to the α1β1γ2L isoform reduced the currents compared to the ternary isoform and slowed the desensitization and recovery (ligand dissociation). When they studied the differential effect of the δ subunit on the suggested native cerebellar α6β3 and α6β3γ2L receptors (Saxena and Macdonald, 1996; *see* also Ragan et al., 1993; Quirk et al., 1994), the α6β3δ receptor isoform had a higher sensitivity for GABA than the α1β3γ2L or α6β3γ2L receptors (EC_{50}: 0.27 μ*M* as compared to 13.6 μ*M* and 1.9 μ*M*, respectively). Unfortunately, the corresponding α1α6 combinations suggested to exist in cerebellar granula cells by

Pollard et al., (1993) and Khan et al., (1994) were not evaluated. Recently, the neurosteroid THDOC was reported to differentially modulate δ-containing receptors (Zhu et al., 1996). Whereas the maximal potentiation in α1/6β3γ2 receptors was approx 300–400%, it was reduced to approx 100% with the coexpression of the δ subunit and fell below 50% in α6β3δ receptors. Electrophysiological recordings also revealed a differential modulation of recombinant α1β2γ2 and α6β2γ2 receptors by the negative modulatory thyroid hormones L-thyroxine (T_4) and L-triiodothyramine (T_3), indicating that the α6-containing isoforms are almost insensitive to these convulsants (Martin et al., 1996).

Additional Subunit-Differentiating Substances

In addition to BZs, a number of substances are reported to affect $GABA_A$-receptor isoforms in a subtype-specific manner, though mostly their effects have not been studied as thoroughly. For example, La^{3+} might differentially modulate binary or ternary receptors (Im et al., 1992) or receptors containing the δ subunit (Saxena and Macdonald, 1994). The recently described ε subunit is suggested as conferring insensitivity to anesthetics like pregnanolone (10 n*M*–1 μ*M*), propofol (3 μ*M*), and also pentobarbital (100 μ*M*) to α2β1ε receptors. Interestingly, at >10-fold higher concentrations, these substances still evoked direct currents in human α2β1ε or mixed rat/human α1β3ε receptors (Davies et al., 1997). The modulation by some neurosteroids like 3α-OH-DHP or PS was described as dependent on the α variant in binary or ternary receptors (Shingai et al., 1991), as well as on the γ2 or γ3 variant (Zaman et al., 1992 *see* also Puia et al., 1993). In most cases, these substances were tested only with a limited number of receptor isoforms, and their potential to discriminate between native receptors still remains to be elucidated.

A specific characteristic of α6-containing receptor isoforms is their inhibition by the diuretic furosemide. Receptors in the form α6β2/3, as well as α4β2γ2 receptors, are also furosemide-sensitive, irrespective of an additional γ or δ variant, (Knoflach et al., 1996; Korpi and Lüddens, 1997), whereas β1- or α1-containing receptors are furosemide insensitive (Korpi et al., 1995). Receptors containing a β1 subunit can also be differentiated by their insensitivity to the already discussed anticonvulsant loreclezole.

Zn^{2+} was originally reported to block GABA currents in homooligomeric α1 and β2 and binary α1β2 isoforms, but failed to inhibit currents of α1β2γ2 receptors (Draguhn et al., 1990). The presence of GABA currents antagonized by Zn^{2+} and enhanced by flurazepam in cultured superior cervical ganglion cells, (Smart and Constanti, 1990) originally suggested the presence of receptors with and without a γ subunit within the same cell. However, recent results indicate significant inhibition for α2, α3, and α5-containing αiβ2γ2 receptors as compared to α1β2γ2 receptors (Burgard et al., 1996; White and Gurley, 1995) with IC_{50} values in the low μ*M* range. Similarly, subunit combinations that may exist in cerebellar granule cells like α6βjγk and α6β3δ, are also highly sensitive to Zn^{2+} (Saxena and Macdonald, 1996). In a former study, Saxena suggested that the δ subunit confers higher Zn^{2+} sensitivity to $GABA_AR$ (Saxena and Macdonald, 1994) and reported α1β1γ2Lδ receptors to be inhibited by 10 μ*M* Zn^{2+}, a concentration which slightly enhanced currents in α1β1γ2L receptors. The small signals observed with α1β1δ receptors were inhibited by Zn^{2+} but discouraged direct comparisons. When α6-containing receptors were studied, an increased potency of Zn^{2+} in α6β3δ receptors compared to α6β3γ2L (Saxena and Macdonald, 1996) was reported. But whether this was because of the absence of the γ2L or the presence of the δ subunit has yet to be investigated. Interestingly, the aforementioned $\gamma2^{T142S}$ mutation did not only alter BZ sensitivity (Mihic et al., 1994) but also inverted the effects of Zn^{2+}, i.e., it induced significant allosteric potentiation of GABA responses in $\alpha1\beta1\gamma2^{T142S}$ receptors (*see* also Herb et al., 1992; Mihic et al., 1995).

Outview

The hitherto existing studies indicate that the large family of $GABA_A$ receptors represent a pharmacologically and functionally heterogeneous group. This diversity impedes the analysis of the physiological impact of defined receptors which is a prerequisite to understand the influence of the $GABA_A$ receptor diversity on neuronal-signal processing in defined brain regions. Thus far, no subunit combination of any native receptor in any neuronal cell has been explicitly identified, not even the combination $\alpha1\beta2\gamma2$ — one of the few receptors well characterized in vitro—which, according to all circumstantial evidence, represents a native and abundant receptor isoform. mRNA colocalization and immunoprecipitation studies support the presence of additional receptor isoforms including binary, ternary, and quaternary receptors, which might be separately localized within single cells. Although this evidence complicates the identification of the physiological role of a single-receptor subtype, it does provide a challenging task for the near future.

Recombinant receptors have been primarily used to either analyze the pharmacological properties of GABA receptors or the functional characteristics of inherent channels. Now their study indicates that differences exist between ligand-binding studies and functional receptor properties, which could be attributable to mode(s) of coupling of ligand binding and channel opening, or to interactions between intracellular events, e.g., phosphorylation, and recognition and coupling sites, all of which are largely unknown. Obviously, such studies of functional recombinant $GABA_A$ channels would be even more inspiring when combined with in vivo studies of native neurons.

A trendy, but sometimes cumbersome approach to study the functional impact of individual subunits is the generation of knockout mice lacking specific subunits. Although this method has been applied to $GABA_A$ receptors, the results are up to now quite sobering: Either the phenotype is not overtly affected, as for $\alpha5$-and $\alpha6$-knock-out mice (Jones et al., 1997; Culiat et al., 1994), or is neonatally lethal as for $\beta3$- and $\gamma2$-knock-out mice (Culiat et al., 1995; Gunther et al., 1995). Still, this approach provides one of the best means to directly address the impact of single $GABA_A$-receptor subunits on brain function. More refined approaches of this technique, i.e., the employment of brain-region specific and/or inducible promotors, with which to turn on or off transcription at will could avoid compensatory mechanisms or lethal developmental effects.

Another, still promising route that has already been proven successful in $GABA_A$ receptor research, is the development of new subunit-specific or subtype-specific drugs which — in addition to its theoretical value — could also provide lead compounds for the generation of substances that improve disorders like anxiety and epilepsy.

Thus, as with most areas of research, more questions arise as more riddles are solved. Luckily, methodological progress provides us with the tools to tackle the new challenges.

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