Heterogeneity of $GABA_A$ Receptors: Revived Interest in the Development of Subtype-selective Drugs

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Abstract: Gamma-aminobutyric acid (GABA) is the most important inhibitory transmitter in the central nervous system. Most of the actions of GABA are mediated by $GABA_A$ receptors. These are choride ion channels that can be opened by GABA and can be modulated by a variety of pharmacologically and clinically important drugs. $GABA_A$ receptors are composed of five subunits that can belong to different subunit classes. So far, 19 different subunits have been identified in mammalian brain, exhibiting a distinct but overlapping regional and cellular distribution and giving rise to an enormous heterogeneity of $GABA_A$ receptors. Depending on the subunit composition these receptors exhibit distinct electrophysiological and pharmacological properties. Drugs in clinical use are only weakly receptor subtype selective, explaining their similar and broad pharmacological effects. Investigations of mice with a point mutation in a $GABA_A$ receptor subunit that eliminates the actions of drugs on certain receptors, only, have indicated that different receptor subtypes mediate distinct actions of GABAergic drugs. This conclusion was supported by experiments with newly developed compounds exhibiting a significantly increased receptor subtype selectivity, suggesting a tremendous clinical potential of drugs with high selectivity for certain receptor subtypes. In addition, the recent availability of structural information on $GABA_A$ receptors from homology modeling studies will stimulate experiments leading to the identification of the binding sites of the various $GABA_A$ receptor ligands. Accumulating structural information on receptor subtypes will finally lead to more precise pharmacophore models that will provide the basis for a more rational drug design.

Keywords: $GABA_A$ receptors, subtypes, subunits, structure, distribution, function, pharmacology.

1. INTRODUCTION

γ-Aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter in the central nervous system. 17-20% of all neurons in the brain are GABAergic [1] Most of the physiological actions of GABA are generated via $GABA_A$ receptors. These receptors are chloride ion channels composed of 5 protein subunits [2] that can be opened by GABA and can be modulated by a variety of pharmacologically and clinically important drugs, such as benzodiazepines, barbiturates, steroids, anesthetics and convulsants [3, 4]. These drugs produce at least part of their clinically relevant effects by interacting with distinct allosteric binding sites on $GABA_A$ receptors [5, 6]. Based on the pharmacological action of these drugs it was concluded that $GABA_A$ receptors are involved in controlling the excitability of the brain [7, 8] in the modulation of anxiety [9, 10], of feeding and drinking behaviour [11, 12], circadian rhythms [13, 14] and cognition, vigilance, memory, and learning [15-17].

Current therapies with these drugs, however, pose several problems that limit their continued use. Thus, treatment of anxiety with benzodiazepines is often associated with sedation, tolerance development, as well as withdrawal symptoms and relapse of anxiety upon discontinuation [18, 19]. This led to the application of antidepressants and other drugs for long-term treatment of anxiety disorders [20]. Although benzodiazepines are the strongest anticonvulsants currently available, their use is mostly restricted to a short-term medication for status epilepticus due to their sedative effects and the development of tolerance [21]. Ony in some cases benzodiazepines are used for long-term treatment of specific epilepsies, such as myoclonic seizures [22]. Development of tolerance and dependence is also a problem when benzodiazepines are used as hypnotics [23]. In addition, daytime drowsiness occurring with long-acting compounds, which may even accumulate in the body with multiple use, or rebound insomnia after the use of short-acting drugs, are often reported [24]. Anterograde amnesia and psychotic states have also been related to the use of some short acting benzodiazepines like triazolam [24]. Moreover, benzodiazepines alter the sleep architecture in a typical fashion [23].

$GABA_A$ receptors are widely distributed all over the brain, and evidence for the existence of distinct receptor subtypes has accumulated during the last 25 years. If these subtypes mediate specific benzodiazepine actions, drugs selectively interacting with these subtypes should have significant therapeutic advantages and exhibit less side effects. Over time, thousands of different benzodiazepines and probably a comparable number of non-benzodiazepine compounds acting via the benzodiazepine binding site of $GABA_A$ receptors have been synthesized [25-27]. Results, however, were disappointing. Compounds identified to exhibit a weak selectivity for binding to certain $GABA_A$ receptor subtypes exhibited some

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separation between the sedative and anxiolytic properties at low concentrations. At slightly higher concentrations all these compounds again exhibited sedative effects [28]. For some time, the development of partial agonists at the benzodiazepine binding site of $GABA_A$ receptors was reinforced in the hope that these would exhibit more selective actions. But in spite of their efficacy in animal models, results of clinical trials in patients with anxiety disorders were not truly convincing, neither with regard to efficacy nor with regard to side effects [29]. In addition, even with non-benzodiazepine compounds interacting with the benzodiazepine binding site of $GABA_A$ receptors the development of tolerance and dependence was observed [29].

Thus, due to these disappointments, the interest for developing new $GABA_A$ receptor subtype-selective drugs seemed to go down during the last years. Interestingly, companies such as Hoffmann la Roche (Novartis), that made their fortune in developing and marketing the benzodiazepines, left the field at a time when new hope was rising at the horizon: molecular biological studies have indicated that there are many more $GABA_A$ receptor subunits than expected, opening the possibility of an enormous heterogeneity of $GABA_A$ receptors in the brain [4, 30]. These conclusions were supported by studies investigating the regional, cellular and subcellular distribution of the various subunits in the brain and others investigating the subunit composition of native $GABA_A$ receptors [31]. Recombinant receptor studies enabled researchers to characterize the properties of the individual receptor subtypes in more detail and to determine their pharmacology. Development of novel receptor subtype-selective compounds became thus much more straightforward. A combination of molecular biological and pharmacological studies for the first time allowed to identify receptor subtypes mediating certain effects of benzodiazepines or other compounds *in vivo* [32]. And finally, novel information on the structure of $GABA_A$ receptors raises the hope that detailed structural information on the various pharmacological binding pockets of these receptors soon will be available thus allowing a more rational drug design. In this article all these new approaches will be discussed and their potential for future drug development will be indicated.

2. HETEROGENEITY OF $GABA_A$ RECEPTORS INDICATES THE POTENTIAL FOR A SELECTIVE MODULATION OF NEURONAL SYSTEMS VIA GABAERGIC DRUGS

2.1. $GABA_A$ Receptors can be Composed of up to Five Different Subunits

2.1.1. $GABA_A$ Receptor Subunits

So far, a total of 6α, 3β, 3γ, one δ, one ε, one π, one θ and 3ρ subunits of $GABA_A$ receptors as well as alternatively spliced isoforms of several of these subunits [30, 31] have been cloned and sequenced from the mammalian nervous system. This set of 19 different subunits is the largest of any among the mammalian ion channel receptors. At least for the human brain this subunit set seems to be final. By applying search algorithms designed to recognize sequences of all known $GABA_A$ receptor type subunits in species from man down to nematodes, in a recent study no new $GABA_A$ receptor subunits were detectable in the human genome [33]. In non-mammalian species, however, additional subunit homologues have been identified [30] [34, 35]

2.1.2. Homo-oligomeric Receptors

Recombinant receptor studies have indicated that depending on the subunits used for transfection of the cells, receptors with distinct pharmacological and electrophysiological properties do arise [4]. Thus, some [36-39] but not all [40-42] $GABA_A$ receptor subunits can form homo-oligomers. The extent of formation of these homo-oligomers varies dramatically. In some cases data on the formation of homo-oligomers are contradictory [43-45] but a robust expression of GABA-activated homo-oligomeric chloride channels was observed with ρ subunits [46, 47] or with β1 or β3 subunits [48, 49].

2.1.3. $GABA_A$ Receptors Composed of Two Different Subunits

In most heterologous expression systems, channels consisting of two different subunits formed more efficiently than homo-oligomeric channels and could be activated by lower GABA concentrations. In addition, the chloride ion flux induced in hetero-oligomeric channels was higher than that in homo-oligomeric channels [43]. The efficiency of receptor formation, however, again seems to depend on the subunit combination and on the expression system used [31, 39, 44, 50, 51]

ρ subunits can form homo- as well as hetero-oligomeric channels with other ρ subunits. Channels formed exhibit properties of the previously characterized $GABA_C$ receptors [52]. Since ρ subunits are structurally part of the family of $GABA_A$ receptor subunits, it was recommended that ρ-containing receptors should be classified as a specialized set of the $GABA_A$ receptors [30]. Originally, ρ subunits were assumed not to co-assemble with other classes of $GABA_A$ receptor subunits [46, 47]. Recent studies, however, indicated that ρ subunits can assemble with γ2 subunits and possibly also with glycine receptor subunits, and form functional receptors with properties found in certain cell types of the retina [53, 54], brainstem [55], hippocampus [56] or other brain regions [57-59].

No information is available on the possible formation of $GABA_A$ receptors composed of αδ, βδ, or γδ subunits, and no functional channels were formed on co-transfection of α1ε or β1ε or of α1π or β1π subunit combinations [31].

2.1.4. $GABA_A$ Receptors Composed of Three Different Subunits

Robust $GABA_A$ receptor expression was obtained when α, β, and γ subunits were co-expressed in various heterologous systems [4, 60], and only α, β, and γ subunits produce $GABA_A$ receptors with a pharmacology resembling that of most native receptors [4]. This indicates that the majority of native receptors is composed of αβγ subunits. In a minority of receptors, the δ, ε, and π subunits seem to be able to replace the γ subunit in $GABA_A$ receptors, whereas the θ subunit might be able to replace a β subunit in these receptors [31].

Several groups have investigated the subunit stoichiometry of αβγ receptors. Most of these studies agree that these receptors contain two α, two β, and one γ subunit [50, 61-63]. Studies on the subunit arrangement indicated that a total of four alternating α and β subunits are connected by a γ subunit (Fig. **1**) [50]. These studies, however, could not distinguish between two mirror images of the same subunit arrangement. The absolute subunit arrangement could be determined when a pentameric $GABA_A$ receptor was modeled according to the structure of the acetylcholine binding protein [64], a remote homologue of the extracellular part of the nicotinic acetylcholine receptor (nAChR) and $GABA_A$ receptor. Modeling a pentameric receptor's extracellular domain consisting of two α, two β and one γ subunit [65] results in a single (absolute) subunit arrangement (Fig. **1**) in which amino acid residues known to contribute to ligand binding sites and interfaces are correctly positioned in the respective subunits. This absolute subunit arrangement was also independently determined using concatenated $GABA_A$ receptor subunits [66]. There were several combinations of concatenated dimers and trimers that resulted in functional $GABA_A$ receptors on expression in Xenopus oocytes. All these combinations resulted in an identical pentameric receptor exhibiting a subunit arrangement as suggested by the homology model of GABA receptors [66]. Whether all receptors composed of αβγ subunits or those composed of αβδ, αβε, or αβπ subunits exhibit the same subunit stoichiometry and subunit arrangement, presently is not known.

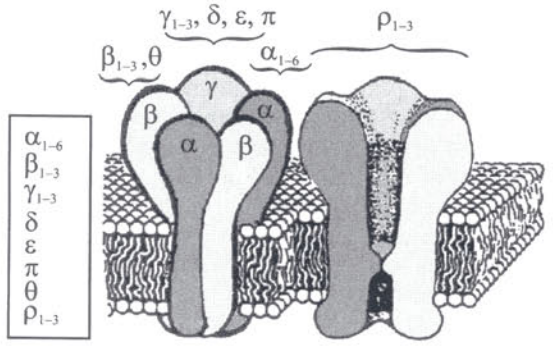


Fig. (1). Stoichiometry and subunit arrangement of $GABA_A$ receptors. A total of 6α, 3β, 3γ, one δ, one ε, one π, one θ and 3ρ subunits of $GABA_A$ receptors have been cloned and sequenced. These possibly give rise to >500 different $GABA_A$ receptor subtypes. The majority of $GABA_A$ receptors is composed of 2α, 2β and 1γ subunit. In a minority of receptors, the δ, ε, and π subunits seem to be able to replace the γ subunit in $GABA_A$ receptors, whereas the θ subunit might be able to replace a β subunit in these receptors. ρ subunits predominantly form homo-oligomeric receptors or hetero-oligomeric receptors with each other. But other subunit-combinations containing ρ subunits seem also to be possible.

2.1.5. $GABA_A$ Receptors Composed of Four Different Subunits

In agreement with the determined subunit stoichiometry of $GABA_A$ receptors, recombinant receptor studies have also indicated that receptors containing two different α subunits as well as a β and a γ subunit can assemble and exhibit properties that are distinct from those of receptors containing only a single type of α subunit [31]. Other studies have indicated that dependent on which α subunit is neighbouring the γ subunit, receptors with distinct properties do arise [67]

Similarly, it has been demonstrated that receptors containing two different types of β subunits together with one type of α and γ subunit are able to assemble and to exhibit properties different from receptors that contain only a single β subunit subtype [68]. Finally, it has been demonstrated that recombinant receptors composed of α1, β1, the long splice variant of γ2, and δ (α1β1γ2Lδ) subunits can also be formed and exhibit properties distinct from those of α1β1γ2L or α1β1δ receptors [69, 70].

2.1.6. $GABA_A$ Receptors Composed of Five Different Subunits

Although experiments investigating the coexpression of five different subunits have been performed in *Xenopus* oocytes, the results obtained were difficult to interpret [43]. This is not surprising because from the five different subunits simultaneously expressed in the oocytes a variety of different receptor subtypes composed of 3, 4, or 5 different subunits could have been formed, that all could have contributed to the chloride current measured in these cells. Since many different types of recombinant receptors can be formed and since the efficiency of formation of these receptors depends on the expression system used, it currently is not clear whether all receptors that can be formed in heterologous expression systems are actually formed in the brain.

2.2. Distribution of Subunits in the Brain Suggests Many $GABA_A$ Receptor Subtypes with Specific Function

2.2.1. Regional Distribution of $GABA_A$ Receptor Subunits in the Brain

"In situ" hybridization [71-73] and immunohistochemical studies [74-76] have indicated that the individual subunits exhibit a distinct but overlapping regional and cellular distribution. Thus, subunits α1, β1, β2, β3, and γ2 are found throughout the brain, although differences in their distribution were observed. Subunits α2, α3, α4, α5, α6, γ1, and δ are more confined to certain brain areas [31] and in some brain regions, a complementary distribution of α2, α4, β3, and δ versus α1, β2, and γ2 subunits was detected [31].

The subunit γ1 is a minor subunit and exhibits a quite specific brain distribution. It is preferentially located in the central and medial amygdaloid nuclei, in pallidal areas, the substantia nigra pars reticulata and the inferior olive. In contrast, the γ3 subunit is expressed in most brain areas but with low abundancy. The δ subunit is frequently co-distributed with the α4 subunit , e.g. in the thalamus, striatum, outer layers of the cortex and in the dentate molecular layer. In the cerebellum, however, it is co-distributed with the α6 subunit [76].

The ε subunit originally has been cloned from human tissue [42, 77, 78]. An unusually high level of divergence from their human homologs was detected in the rat tissue in one report [79] that could not be confirmed in another study [80]. Northern blots and in situ histochemistry originally were contradictory in the human brain [42, 77] as well as in

the rat brain [79, 80]. Subsequent extensive in situ hybridization studies in the rat have indicated that this subunit is expressed by neurons located in septal and preoptic areas, as well as in various hypothalamic nuclei, amygdala and thalamus [80, 81]. Interestingly, the ε mRNA was also detected in major neuronal groups with broad-range influence, such as the cholinergic (basal nucleus), dopaminergic (substantia nigra compacta), serotonergic (raphe nuclei) and noradrenergic (locus coeruleus) systems and immunohistochemistry confirmed these results [80, 81]. From these data it can be assumed that drugs selectively interacting with $GABA_A$ receptors containing ε subunits will produce quite specific effects.

The π subunit was detected in several peripheral human tissues as well as in the brain (hippocampus and temporal cortex) and was particularly abundant in the uterus [41]. So far, no study investigating the detailed regional distribution of the π subunit in the brain has been published. The π subunit protein can co-assemble with α, β, and γ subunits and possibly also replace a γ subunit, and confers unique ligand binding and electrophysiological properties to the recombinant receptors in which it combines [41, 82].

The θ subunit was the last so far identified [83] and seems to be expressed in various brain regions, including the hypothalamus, amygdala, hippocampus, substantia nigra, dorsal raphe and locus coeruleus [81]. θ subunits showed strikingly overlapping expression patterns with ε subunits throughout the brain, especially in the septum, preoptic areas, various hypothalamic nuclei, amygdala, and thalamus, as well as in monoaminergic cell groups [81]. Surprisingly, θ produced a functional receptor only when coexpressed (and probably coassembled) with an α, a β and a γ subunit [83] and due to its relatively high sequence identity with β1 subunits it was suggested that θ subunits might be able to replace one of the β subunits in $GABA_A$ receptors. As with the ε subunit, there were some discrepancies in the cDNA sequence obtained by different groups [79, 83].

The ρ subunits seem to be preferentially expressed in the retina. Immunohistochemistry in the retina using an antibody recognizing all 3 ρ subunits revealed staining restricted to the terminals of bipolar cells in the inner plexiform layer which did not overlap with $GABA_A$ α or β subunits [84, 85]. mRNA encoding ρ subunits, however, is present also in the superior colliculus, dorsal lateral geniculate nucleus and cerebellar Purkinje cells [86, 87]. In addition, pharmacological effects characteristic for ρ subunit containing receptors have been reported in the cerebellum [57, 88], superior colliculus [58, 89], amygdala [90], hippocampus [91, 92], dorsal geniculate nucleus [93], and spinal cord [94]. This indicates that ρ subunits may be present in many CNS regions and are more prevalent than previously suspected.

2.2.2. Cell-Specific Expression of $GABA_A$ Receptor Subunits in the Brain

Studies on the cellular localization of subunits indicated that α1, β2, and γ2 subunits are co-localized extensively on GABAergic interneurons in hippocampus and other brain regions [75, 76, 95], supporting the conclusion that α1β2γ2 receptors are the most abundant $GABA_A$ receptors in the brain. Using confocal laser microscopy, it was demonstrated that in the hippocampus all parvalbumin-positive interneurons and 50% of the calretinin-positive neurons contained the α1 subunits, whereas interneurons containing calbindin-D_{28k} were devoid of α1 subunit staining [96]. Similarly, most neurons positive for neuropeptide Y and a subset of somatostatin-positive cells contained α1 subunits, in contrast to cholecystokinin- and vasoactive intestinal peptide-containing cells, which lacked the α1 subunit staining. These results demonstrated cell-specific expression of $GABA_A$ receptors containing the α1 subunit among subsets of hippocampal interneurons, pointing to a pronounced functional specialization of these cells and supporting the conclusion that disinhibition may be of major functional relevance in regulating the balance between excitation and inhibition in hippocampal circuits [96].

In the raphe nuclei the vast majority of serotonergic neurons express strong α3-subunit-immunoreactivity but are devoid of α1 subunit staining. Only a small population of serotonergic neurons co-express these two subunits. In contrast, both the α1- and α3-subunit-immunoreactivities are present in glutamate decarboxylase-positive neurons [97]. These data indicated that serotonergic and GABAergic neurons selectively express distinct patterns of α subunits, suggesting that they possess distinct subtypes of $GABA_A$-receptors.

In other studies, double- and triple-immunofluorescence staining indicated that 84-95% of the cholinergic neurons in the basal forebrain expressed the α3 subunit but not the α1 subunit. In contrast, parvalbumin-positive GABAergic neurons in these brain regions were frequently co-stained with the α1-subunit antiserum, and to a lesser extent with the α3-subunit antibody [98]. Triple immunofluorescence staining revealed that 45-60% of parvalbumin-immunoreactive neurons expressed both the α1- and the α3-subunit in the various subnuclei of the medial septum-diagonal band of Broca complex, whereas most of the remaining parvalbumin-positive neurons were labeled with only the α1-subunit antiserum [98]. The α3 subunit, however, not only is associated with serotonergic or cholinergic neurons, but also with noradrenergic and dopaminergic neurons in the brainstem [98].

The different and distinct distribution of individual $GABA_A$ receptor subunits on specific cell populations indicates that a selective modulation of the respective receptors will generate quite specific effects. In addition, data indicating an overlapping distribution of α3, θ, and ε $GABA_A$ subunits in the dorsal raphe and the locus coeruleus suggest that novel $GABA_A$ receptor subtypes that so far have not been studied in detail, may regulate neuroendocrine and modulatory systems in the brain [80, 81]. Physiological and pharmacological modulation of $GABA_A$ receptors containing the α3 subunit may thus be expected to have a profound influence on brain functions under monoaminergic or cholinergic control [98].

It has to be kept in mind, however, that the extent of expression of $GABA_A$ receptor subunits and their regional and cellular distribution in rodents do not necessarily correspond to that in the human brain. Thus, evidence has been presented indicating that the α3 subunit that is virtually absent in rodent hippocampus, was strongly expressed in the

CA1 area of human hippocampus and was present to varying degrees in dentate granule cells of human brain [99]. In addition, little or no α1-subunit staining was apparent in human CA3 pyramidal cells, whereas moderate α1-subunit immunoreactivity has been reported in rodent CA3 dendritic fields [99]. Furthermore, hilar mossy cells abundantly expressed both the α1- and α2-subunits in human, whereas this has not been observed in rodents. And finally, prominent staining was observed on the basal dendrites of dentate granule cells in human brain [99]. Basal dendrites, which are usually absent in normal rat granule cells, are a common feature in human brain [100, 101]. These species differences underscore the need for caution in interpreting findings from animal models of human disease, and indicate that more complete immunohistochemical studies in human brain tissue are urgently needed.

2.2.3. Subcellular Localization of $GABA_A$ Receptor Subunits

If subunits are present in the same $GABA_A$ receptor subtype, they should be colocalized in the neuronal membrane. Immunocytochemical studies investigating the co-localization of subunits in $GABA_A$ receptor clusters on neuronal membranes [74, 102-104] indicated that the majority of $GABA_A$ receptors present in the brain are composed of α, β, and γ subunits. Electron microscopic studies confirmed this conclusion [105, 106].

Other studies indicated that individual subunits exhibit a quite distinct subcellular distribution [107-109]. For instance, cerebellar granule cells express six $GABA_A$ receptor subunits abundantly (α1, α6, β2, β3, γ2, and δ). The α1, α6, β2/3, and γ2 subunits have been found by immunogold localizations to be concentrated in GABAergic Golgi synapses and also are present in the extrasynaptic membrane at a lower concentration. In contrast, immunoparticles for the δ subunit could not be detected in synaptic junctions, although they were abundantly present in the extrasynaptic dendritic and somatic membranes [110]. Receptors containing the δ subunit seem also to contain α6 and β subunits. It has been demonstrated that α6βδ receptors exhibit a 50 fold higher affinity for GABA than the α1βγ2 receptors [111]. In contrast to α1βγ2 receptors, δ containing receptors exhibit a smaller single channel conductance, a much longer open time, and do not desensitize on the prolonged presence of GABA [69]. Together with the exclusive extrasynaptic localization of these receptors, these properties indicate that tonic inhibition observed in these cells is mediated mainly by the persistent activation of α6βδ receptors by GABA that is present in the extracellular space of glomeruli [110, 112, 113]. In contrast, phasic inhibition of granule cells is attributable to the transient activation of synaptic α6βγ2 and/or α1βγ2 receptors, that exhibit a lower affinity for GABA, a more pronounced desensitization and much shorter open times than α6βδ receptors [110].

Other studies have indicated that two receptor populations with distinct kinetics coexist also in CA1 pyramidal cells and in many other cell types: slow extrasynaptic receptors that dominate the responses of excised patches to exogenous GABA applications and fast synaptic receptors that generate rapid IPSCs [114, 115]. The charge carried by the activation of tonically active $GABA_A$ receptors can be more than three times larger than that produced by phasic inhibition, even when the frequency of phasic events is large [116-118]. It is quite possible that α4βδ receptors, that exhibit properties similar to α6βδ receptors [119] might at least partially be responsible for extrasynaptic tonic inhibition in these cells. Experiments indicating that tonic conductance sometimes can also be enhanced by benzodiazepines [120, 121] might indicate that tonic inhibition can also be produced by gamma subunit containing receptors. Overall, these and other data indicate that inputs from different neurons use different receptors that are located in distinct parts of the receptive neuron. In addition, the number and subtypes of $GABA_A$ receptors present in distinct synapse populations can be different and these factors are regulated by both pre- and postsynaptic influences [115].

2.3. Subunit Composition of Native $GABA_A$ Receptors Confirms Extensive Receptor Heterogeneity

2.3.1. Extreme Promiscuity of $GABA_A$ Receptor Subunits in the Brain

As indicated above, some cells seem to express only a few subunits whereas other cells express a large number of different subunits. When many subunits are expressed in the same cell, an extremely large number of different receptors exhibiting a distinct subunit composition and arrangement theoretically could form. Not all receptors that could be formed will actually be formed in the brain because subunits not always will be expressed at the same time or in the same part of the cell and because assembly presumably is governed by preferred subunit partnerships that lead to receptors with a defined subunit stoichiometry and arrangement. Therefore, it is of paramount importance to determine the subunit composition of receptors actually formed in the brain.

During the last years a variety of $GABA_A$ receptor subunit-specific antibodies have been generated and have been used for purifying $GABA_A$ receptor subtypes from brain membrane extracts by immunoprecipitation or immunoaffinity chromatography. The analysis of the subunit composition of the purified receptors indicated an extreme promiscuity of the various subunits [31]. Although the antibodies used were highly specific for the respective subunits, most if not all of the other subunits investigated could be co-purified with antibodies directed against an individual α or β subunit, suggesting that α and β subunits can combine with most of the other subunits to form a variety of different receptor subtypes.

These studies indicating co-purification of α with other α subunits, or β with other β subunits, of course also indicated that two different α or two different β subunits are present in at least some $GABA_A$ receptors in the brain [31]. The presence and abundance of receptors containing two different types of α subunits was recently determined in mouse brains containing point mutated α subunits [122]. Interestingly, depending on the α subunit located close to the γ subunit these receptors exhibit distinct pharmacological properties [122-127] in agreement with results obtained with recombinant receptors [67]. Whereas the existence in the brain of $GABA_A$ receptors containing two different α and/or β subunits is generally accepted, discrepant results were obtained concerning a possible colocalization of different γ

subunits, or of γ and δ subunits in the same $GABA_A$ receptor. Whereas in several studies [128-131] it was demonstrated that $GABA_A$ receptors seem to contain only a single type of γ subunit, other studies, in disagreement with the presumed subunit stoichiometry of 2α, 2β and 1γ subunit, suggested a significant colocalization of the alternatively spliced short and long form of the γ2 subunit [132] or of the γ2 and γ3 subunit [133] in the same receptor. Whereas one study concluded that γ and δ subunits might be present in the same $GABA_A$ receptor [134] in several other investigations, γ and δ subunits could not be demonstrated in the same receptors isolated from brain tissue [129, 131, 135, 136].

2.3.2. Identification, Subunit Composition and Abundance of Specific $GABA_A$ Receptor Subtypes

Given the promiscuity of α and β subunits, it is impossible to purify a single $GABA_A$ receptor subtype by a one step immunoprecipitation. Recently, however, a subtractive purification strategy has been developed that successfully allowed the determination of the subunit composition of solubilized hetero-oligomeric $GABA_A$ receptors [129]. In this strategy, immunoaffinity chromatography of receptors, elution of the retained receptors and Western blot analysis identify the subunits copurifying with a subunit to be investigated. Co-purifying subunits and thus, receptors containing them are then quantitatively removed one by one using immunoaffinity columns containing antibodies selective for the subunit to be eliminated until only one receptor subtype is left. Its subunit composition can then be determined by Western blot analysis. By measuring the total amount of receptors in the extract before and after chromatography on the individual immunoaffinity columns, this procedure can also be used to determine the percentage of receptors removed by the column, and thus, to estimate the relative abundance of individual receptor subtypes [129-131].

Using this method, the subunit composition of α6 subunit-containing $GABA_A$ receptors from cerebellum was determined [131]. Data obtained were consistent with the results from recombinant receptor studies mentioned above and indicated that most of the receptors found in the cerebellum consist of 2α, 2β and 1γ or 1δ subunits. In addition, a significant part of the receptors contains two different α and/or two different β subunits, and is thus composed of 4 or 5 different subunits [129, 131].

2.3.3. Abundance of $GABA_A$ Receptor Subtypes Containing Specific Subunits

The determination of the subunit composition of individual $GABA_A$ receptors as described above requires large amounts of subunit selective antibodies and is quite time consuming. Therefore, in most cases the abundance of $GABA_A$ receptors has been determined by quantitative immunoprecipitation of all receptor subtypes containing a certain subunit by using subunit-specific antibodies and subsequent receptor binding studies. Results of these studies have recently been summarized [31]. Overall, there is agreement that α1, β2, and γ2 subunits are the most abundant $GABA_A$ receptor subunits in the brain. Antibodies directed against these subunits precipitate 70-90%, 55-90% and 50-70% of [^{3}H]muscimol binding sites from rat or mouse brain membrane extracts, respectively. Subunits α2 or α3 seem to be present in 35% or >14% of all $GABA_A$ receptors of the brain, respectively. Whereas on average only 6% of $GABA_A$ receptors in the brain seem to contain α4 subunits, in the hippocampus about 13% and in the thalamus about 20% of $GABA_A$ receptors might contain this subunit. α5 subunits are present in only 7-8% of all $GABA_A$ receptors in the brain, but this subunit is especially enriched in hippocampus, where it might be present in up to 31% of all receptors. As mentioned above, α6 subunits are present only in cerebellar granule cells and in the cochlea. In the cerebellum about 58% of all $GABA_A$ receptors contain α6 subunits. Whereas the β2 subunit is the most abundant β subunit, β1 and β3 subunits are also quite abundant compared with some α subunits. And whereas a large part of the $GABA_A$ receptors in the brain seems to contain a γ2 subunit, receptors containing a γ1, a γ3, or a δ subunit on the average are not very abundant in the brain These receptors, however, again are enriched in certain brain regions [31] and see below. So far, the relative abundance of receptors containing the ε, π, θ, or ρ subunits in the brain has not been determined.

Although there is some evidence for the existence of αβ receptors in the brain (for review see [31]), homo-oligomeric $GABA_A$ receptors composed of α, β, γ, δ, ε, π, or θ subunits so far have not been identified in the brain. In the absence of high affinity ligands for these receptors they only can be identified using electrophysiological techniques. Homo-oligomeric receptors composed of ρ subunits, however, have been identified *in vivo* [47].

Given the promiscuity of subunits discussed above and the receptor subtypes so far identified, it was estimated that more than 500 distinct $GABA_A$ receptor subtypes might exist in the brain [31]. The number of receptors that are relatively abundant in the brain (α1β2γ2, α2β2γ2, α3β2γ2, α4β2γ2, α5β2γ2, α6β2γ2, α4β2δ or α6β2δ receptors), however, is much smaller. But due to the widespread distribution and quantitative importance of the GABAergic system even minor $GABA_A$ receptor subtypes probably exhibit an abundance comparable with that of major norepinephrine, dopamine, serotonin or peptide receptors. Further experiments investigating the composition of $GABA_A$ receptors in various brain tissues will have to confirm this conclusion.

To finally prove the existence of all these receptors in the brain, however, these receptors have to be functionally identified in situ by electrophysiological and pharmacological techniques. This is the most difficult and tedious part of the identification of receptor subtypes because it requires a distinct pharmacological or electrophysiological fingerprint of the receptors to be identified. Such fingerprints only can be established by recombinant receptor studies and it can only be hoped that the properties of the native receptors are not significantly changed due to endogenous phosphorylation [4] or to interaction with other proteins at the synapse [137]. Although some progress has been achieved recently in identifying $GABA_A$ receptors in the brain [138] in most cases an unequivocal identification of receptor subtypes in situ currently is not possible due to the lack of highly selective pharmacological tools.

3. $GABA_A$ RECEPTOR SUBTYPES MEDIATE DISTINCT BEHAVIOURAL EFFECTS

3 .1. Using Gene-knockout Techniques for Studying the Function of $GABA_A$ Receptors

Several attempts have been made to identify the function of $GABA_A$ receptor subtypes by generating mouse lines in which the genes for certain receptor subunits were inactivated. Ablation of a particular receptor subunit would be expected to perturb the structure of a defined group of $GABA_A$ receptors and cause a corresponding alteration in the physiology and pharmacology of the mutant mice, thus allowing conclusions to be made on the function of receptors containing the ablated subunit.

3.1.1. α1 Knockout Mice

A knockout of the α1 subunit, one of the major $GABA_A$ receptor subunits in the nervous system, resulted in a loss of more than 50% of all $GABA_A$ receptors in the brain of these mice [139, 140]. Judged from the overall abundance of these subunits in the brain, the loss of $GABA_A$ receptors should have been even larger. But upregulation of the α2 and α3 subunits by 37% and 39%, respectively [140, 141] and a relatively modest downregulation of γ2 subunits by only 47% [141] most likely represented compensatory changes within the GABAergic system. Such changes, as well as those outside the $GABA_A$ receptor system [142] have to be considered when interpreting the phenotype of these mice. Thus, in spite of this dramatic loss of $GABA_A$ receptors, this mutation did not result in an overt phenotype. The α1 knockout mice were viable although underrepresented in offsprings from heterozygote crosses, indicating that there was some lethality. Apart from the changes in subunit expression described above, α1 knockout mice had lower body weights until the age of at least 3 months and exhibited a tremor when handled [140]. These mice did not display any major deficits in beam balancing and swimming ability test. The level of spontaneous locomoter activity and exploration and the performance on the rotating rod were similar to wild type. The mice had no spontaneous seizures, but bicuculline-induced seizure susceptibility was increased [141]. The mice also had some changes in the effects of ethanol, anesthetics, or other drugs [143-145] but interpretation of these effects is difficult (see above).

3.1.2. α2 Knockout Mice

These mice were recently generated by a group of researchers at Merck Sharp and Dohme (Harlow, UK). Although early on there was about a 30% decrease in the number of mutants surviving to weaning, α2-subunit knock-outs now appear to breed and develop normally. It is currently not known whether genetic deletion of this subunit altered the expression of other $GABA_A$ receptor subunits [146]. These mice, however, tended to display a lower basal level of locomotion and were less active in tests for locomotor response to novelty than their wild-type counterparts. In addition, they exhibited shorter durations of ethanol-induced loss of righting reflex, suggesting a role for the α2 subunit in the mediation of ethanol´s hypnotic actions [146].

3.1.3. α3 Knockout Mice

Recently, also α3-knockout mice were generated. The α3 subunit is the main $GABA_A$ receptor subunit normally expressed by catecholaminergic and serotonergic neurons in the brainstem as well as basal forebrain cholinergic neurons (chapter 2.2.2.). α3 subunit-deficient mice are viable and fertile and are without any gross developmental defect or morphological brain abnormality. No evidence for any compensatory upregulation of other $GABA_A$ receptor α subunits was detected [147]. Mutant mice exhibit increased spontaneous locomotor activity in the open field, possibly due to enhanced dopaminergic tone, despite that their response to the motor stimulating effect of systemic amphetamine was not significantly altered in this test. At the same time, prepulse inhibition of the acoustic startle response was disrupted in the α3 subunit null mice, and this disruption was reversed by pretreatment with the antipsychotic haloperidol. These results suggest that the function of the mesolimbic dopaminergic system is impaired in the absence of α3-$GABA_A$ receptors and that the disruption of neuronal inhibition normally mediated by these receptors is associated with a psychotic-like phenotype. Hence, drugs targeted selectively to the α3-$GABA_A$ receptors may constitute an effective treatment for sensorimotor gating deficiency, which is characteristic for a number of psychiatric conditions, including schizophrenia [147]

3.1.4. α5 Knockout Mice

The α5 subunit is expressed primarily in hippocampus, including the CA1 and CA3 regions. In the α5 knockout mice, the number of benzodiazepine binding sites in the hippocampus was decreased by approximately 16%, which corresponds to the number of α5 receptors in this brain region and suggests no upregulation of other subunits [148]. α5 Subunit knockout mice display normal motor performance and coordination. The benzodiazepine chlordiazepoxide retained its anxiolytic-like action in these mice, indicating that α5-containing $GABA_A$ receptors are not involved in this action. In the Morris water maze test, a spatial learning task dependent on hippocampal function, α5 knockout mice displayed a decreased latency to find the submerged platform compared to wild-type mice, indicating that these mice show a significantly improved performance [148]. Thus, results obtained with the α5 knockout mice suggested that an inverse agonist selective for α5-containing $GABA_A$ receptors may be suitable as a drug enhancing cognitive functions.

3.1.5. α6 Knockout Mice

The expression of the α6 subunit is largely restricted to cerebellar granule cells. In the cerebellum of α6-knockout mice, the β2, β3, and γ2 subunits were reduced by approximately 50%, 20% and 40% [149, 150]. In addition, a selective post-translational loss of the δ subunit was apparent in cerebellar granule cells of these mice which indicated that the δ subunit is co-assembled with the α6 subunit [149, 150]. In cerebellar granule cells from α6 knockout mice, a tonic conductance, which is dependent on the presence of the $GABA_A$ receptor α6 subunit, is absent. Finally, the absence of α6 subunits not only triggered changes in $GABA_A$ receptor subunit expression, but also a compensatory upregulation

of a potassium channel [151]. This presumably is responsible for the fact that the response of these cells to excitatory synaptic input is unaltered. α6 Knockout mice showed the same level of exploratory activity in the open field as wild-type mice and learned the horizontal wire task [149]. However, they were more sensitive to the motor-impairing action of diazepam in an accelerating rotarod test than their wild-type counterparts [152].

3.1.6. β2 Knockout Mice

In β2 knockout mice, expression of the α subunits α1-α6 was reduced by approximately 39-69%, indicating that the β2 subunit is substantially associated with all known α subunits and that other β subunits do not substitute for the missing β2 subunits [140]. Because the β2 subunit is the most abundant of the three β subunits, it is somewhat surprising that the β2 knockout mice had normal body weights and did not display major deficits in the rotating rod, beam balancing, and swimming ability tests [140]. In a novel environment, they exhibited a higher level of locomotor activity than wild-type mice, although they habituated to a similar degree as wild-type mice.

3.1.7. β3 Knockout Mice

Interestingly, in contrast to the disruption of the much more abundant β2-subunit gene, disruption of the β3-subunit gene produced mice with an epileptic phenotype [153, 154]. This possibly could be explained by the differential localization of receptors containing these subunits: α1β2γ2 receptors preferentially seem to be located on GABAergic interneurons [76, 96-98]. By inhibiting inhibitory neurons, these receptors probably mediate disinhibition needed for normal functioning of the brain [96]. Loss of these receptors would thus strengthen the inhibition of other neurons and counteract a possible loss of inhibition in glutamatergic projection neurons [140]. In contrast, β3 subunit-containing receptors possibly are located on projection neurons. Loss of these receptors would disinhibit these neurons and cause convulsions [154]. Nevertheless, lack of the β3 subunits leads to dramatic changes in the expression of other receptor subunits: approximately half of the $GABA_A$ receptors were lost.

3.1.8. γ2 Knockout Mice

In γ2-subunit knockout mice the total number of $GABA_A$ receptors was apparently unchanged, whereas the number of benzodiazepine binding sites was reduced by approximately 90%, consistent with the γ2 subunits being necessary for benzodiazepine binding [155]. Receptors in these mice were not properly clustered and displayed a reduced single-channel conductance. These results are consistent with the conclusion that receptors in these mice were composed of αβ subunits, only, and that the γ2 subunit is required for synaptic clustering of $GABA_A$ receptors [156]. Both, the reduced conductance and the absence of clustering of receptors probably contributed to the observation that knockout of γ2 subunits was lethal [155]. By contrast, mice heterozygous for the γ2 subunit knockout developed and behaved normally. The synaptic clustering of $GABA_A$ receptors was only partially reduced (about 15-30%, depending on the brain region) and the unclustered receptors consisted of α- and β-subunits [156]. When exposed to certain fear-inducing stimuli, these animals showed a striking disease phenotype with a high anxiety response to natural and learned aversive stimuli, as well as cognitive bias for threat cues [157]. This indicates that a reduction in the number of $GABA_A$ receptors containing γ2 subunits might cause a predisposition for anxiety and is consistent with the fact that benzodiazepines produce their anxiolytic action by interacting with $GABA_A$ receptors containing a γ2 subunit [4].

3.1.9. δ Knockout Mice

Finally, disruption of the δ-subunit gene was associated with an attenuated sensitivity to neuroactive steroids and with multiple defects in behavioral responses to ethanol [158, 159]. In addition, δ knockout mice develop spontaneous seizures and are more susceptible to pentylenetetrazole-induced convulsive seizures [160] suggesting a major role of δ-containing receptors, that seem to be located extrasynaptically [110, 119], in the actions of neurosteroids and ethanol and in controlling the excitability of the brain.

3.1.10. Conclusions from $GABA_A$ Receptor Subunit-Knockout Studies

From investigating $GABA_A$ receptor subunit-knockout mice some conclusions on the function of $GABA_A$ receptor subtypes could be drawn that seem to be supported by experiments using alternative approaches (see below). Thus, these studies provided evidence for possible subunit partnerships (α6 and δ), for a connection of α5 subunit-containing receptors and learning, of β3 subunit-containing receptors and seizures, of γ2 subunits and synaptic localization of receptors, and of δ subunit-containing receptors and the action of neurosteroids and ethanol as well as seizure control. The interpretation of most results from knockout mice, however, is confounded by possible adaptive changes in the development and function of the brain caused by the lacking receptors [150, 151, 161]. Studies on subunit knockout mice, thus, in most cases did not clarify the function of the respective $GABA_A$ receptor subtypes in the brain.

3.2. Combining Molecular Genetics and Pharmacology for Studying the Function of $GABA_A$ Receptors

3.2.1. Function of $GABA_A$ Receptors Containing α1 Subunits

Recently, a novel approach was developed for studying the function of receptors containing specific α subunits [162]. This approach was based on introduction of a point mutation into the α1 subunit of $GABA_A$ receptors, by which the histidine at the position 101 was replaced by an arginine (α1His101Arg). This point mutation renders α1-containing receptors insensitive to allosteric modulation by diazepam (Fig. **3**, compound **1**) without altering their GABA-sensitivity. In the absence of significant changes in signal intensity produced by the mutated receptors, animals containing this mutation developed normally and the cellular and subcellular location of receptors was unchanged [162]. In these animals, therefore, diazepam mediated its effects through only the α2, α3, and α5 subunit-containing receptors (see chapters 4.1. and 4.2.2.). A comparison of drug-induced behavioural responses in α1(His101Arg) and wild-type mice then allowed identification of the diazepam effects that were missing in mutant mice and thus, to determine the

contribution of α1-containing receptors to the effects of diazepam. It was demonstrated [162] that α1(His101Arg) mice failed to show the sedative, anterograde amnesic and partly the anticonvulsant actions of diazepam. In contrast, the anxiolytic like, myorelaxant, and ethanol-potentiating effects were fully retained, indicating that they are produced via the non-mutated benzodiazepine-sensitive $GABA_A$ receptors containing α2, α3, or α5 subunits [162].

Using a similar approach, most of these findings were confirmed by a different group [163]. Some discrepancies between the studies concerning the sensitivity of mutants towards the ataxic effects of diazepam, or the existence of a diazepam-induced paradoxical hyperactivity in the α1(His101Arg) mutants, could later be resolved and were caused by differences in the behavioural protocols used by the two groups [164].

Hypnotic effects are classically considered to involve more pronounced depression of the central nervous system than sedation, and this can typically be achieved by increasing the dose of sedative-hypnotic drugs. Benzodiazepine hypnotics have distinct effects both on sleep and the sleep electroencephalogram (EEG). They induce dose-dependent increases of non-rapid eye movements (NREM) sleep, a reduction of REM sleep in humans and a typical benzodiazepine "fingerprint" consisting of a reduction in delta activity in humans and rats, an increase of sigma activity in humans, and high-frequency activity in rats [165]. These effects are common for agonists acting at the benzodiazepine site, irrespective of whether they are benzodiazepines or non-benzodiazepine compounds such as zolpidem and zopiclone [166]. When the effects of diazepam on sleep were investigated in α1(His101Arg) and wild-type mice, surprisingly it was demonstrated that the "benzodiazepine fingerprint" in the sleep EEG, in contrast to the sedative action [162, 163], was present in both genotypes, indicating that these changes in the sleep EEG are mediated by $GABA_A$ receptors other than α1 [165]. These results seemed to indicate that the hypnotic effect of diazepam and its EEG "fingerprint" can be dissociated from its sedative action.

3.2.2. Function of $GABA_A$ Receptors Containing α2 Subunits

In subsequent studies, the function of α2-, α3-, or α5-subunit-containing receptors in the brain was investigated. For this, point mutations were introduced into α2-, α3-, or α5-subunits at positions homologous to that of α1 (His101Arg) [α2(His101Arg); α3(His126Arg); or α5(His105Arg)] thus rendering α2-, α3-, or α5-subunit-containing receptors in these mice diazepam-insensitive.

α2-containing $GABA_A$ receptors are expressed in the limbic system in regions that are involved in emotional stimulus processing [167], e.g. amygdala and hippocampus. $GABA_A$ receptors in the limbic system and in the reticular activating system, have been postulated to mediate the therapeutically most important anxiolytic actions of benzodiazepines. In the light/dark choice test and the elevated plus maze test, diazepam increased the time the mice spent in the lit compartment or the open arms, respectively, in wild typ mice, but not in α2(His101Arg) mice [168]. This indicates that α2-containing $GABA_A$ receptors, which constitute approximately 15% of the diazepam-sensitive $GABA_A$ receptors [169] mediate the anxiolytic-like action of diazepam.

α2(His101Arg) mice displayed normal sedative and anticonvulsant responses to diazepam [168], however, the myorelaxant action as measured in the horizontal wire test was strongly impaired [170]. Appreciably higher doses of diazepam were required to induce myorelaxation compared to its anxiolytic-like effect [168]. The myorelaxant action of the $GABA_B$ receptor agonist baclofen, however, was indistinguishable in α2(His101Arg) and wild-type mice [170]. Thus, α2-containing receptors apparently mediate the anxiolytic as well as muscle relaxant action of diazepam. But these actions presumably are elicited by receptors in different brain regions and there seems to be a difference in the dose necessary to exhibit these effects. From this it can be predicted that a partial agonist selective for α2-containing $GABA_A$ receptors will exhibit anxiolytic but no sedative and muscle relaxant properties.

Other investigations indicate that benzodiazepines affect sleep and waking EEG patterns through their action on α2-containing $GABA_A$ receptors, implying that these receptors are strategically expressed in distinct neuronal circuits (hypothalamic and pontine nuclei and in the hippocampus), relevant for the modulation of these rhytmic brain activities [171].

At this point, however, a word of caution on such seemingly clearcut conclusions seems adequate. Because diazepam still acts on receptor subtypes containing the α1, α3, and α5 subunits in α2(His101Arg) mice, the experiments do not necessarily predict whether, e.g an α3- or α5-selective agonist may also display an anxiolytic-like action in the various anxiolytic tests. In addition, since most of the anxiolytic tests for mice rely on locomotor activity, marked changes in activity between two groups of mice can confound the interpretation of data and can lead to discrepant interpretations derived from different behavioural tests [172].

3.2.3. Function of $GABA_A$ Receptors Containing α3 Subunits

Neurons that express exclusively α3-containing receptors are located in the reticular activating system (i.e. noradrenergic, dopaminergic and serotonergic neurons) and in the basal forebrain (cholinergic neurons) [97, 98]. Previously, it had been suggested that the anxiolytic effect of diazepam is due to the dampening in particular of the noradrenergic neurons in the locus coeruleus and its interactions with serotonergic neurons. However, in α3(His126Arg) mice the anxiolytic activity of diazepam, as tested by the light-dark choice test and the elevated plus-maze test, was not impaired compared with wild-type mice [168]. Furthermore, the sedative and anticonvulsant activities of diazepam were unchanged [168]. However, the muscle relaxant activity was moderately reduced, indicating that α3-containing receptors also contribute to this response [170].

Despite α3 being the exclusive α subunit in the reticular nucleus of the thalamus, α3-containing $GABA_A$ receptors seem not to mediate diazepam´s effect on the sleep EEG because the diazepam-induced EEG changes did not differ between α3(His126Arg) and wild-type mice [173].

The reticular nucleus of the thalamus regulates thalamocortical oscillations. Thalamic circuits generate sleep spindles and may contribute to some forms of generalized absence epilepsy. The suppression of thalamic oscillations by the anti-absence drug clonazepam is retained in slices of α1(His101Arg) mice but not α3(His126Arg) mutant mice, indicating that this suppression is mediated exclusively by α3-containing $GABA_A$ receptors [174]. Thus, α3-containing $GABA_A$ receptors are an interesting target for novel anti-absence drugs.

3.2.4. Function of $GABA_A$ Receptors Containing α5 Subunits

In α5(His105Arg) mice, the sedative, anticonvulsant and anxiolytic-like action of diazepam were indistinguishable from wild-type. Only the muscle relaxant action of diazepam was reduced in these mutant mice [175]. Thus, the myorelaxant action of diazepam appears to be mediated largely by α2-, α3-, and α5-containing $GABA_A$ receptors. At a moderate dose of diazepam (e.g. at 10 mg/kg), the role of the α2-containing $GABA_A$ receptors appears to be essential because α2(His101Arg) mice do not display muscle relaxation at this dose in contrast to α3(His126Arg) and α5(His105arg) mice [32].

Whereas in α1(His101Arg), α2(His101Arg) and α3(His126Arg) mice the mutant subunits have been found to be expressed at normal levels, the expression of the mutant α5 subunits in α5(His105Arg) mice was reduced in hippocampal pyramidal cells by approximately 30%, but not in other brain regions. However, the laminar distribution of the remaining α5 subunit in the hippocampus was unchanged [32]. The reasons for this selective reduction are not known. In contrast to the α1, α2, and α3 subunits, the α5 subunits in the hippocampus is located largely extrasynaptically. Interestingly, another group reported that in their α5(His105Arg) mice generated using a similar, but not identical targeting strategy, no reduction of α5-containing $GABA_A$ receptors in the hippocampus or in the whole brain were found as determined by quantiative radioligand binding assays using [^{3}H]Ro15-4513 [172].

In line with the role of the hippocampus in certain forms of associative learning and the abundant location of α5 subunits in the hippocampus, α5(His105Arg) mice displayed selective changes in a hippocampus-dependent learning and memory performance test (trace fear conditioning) [175]. These findings were supported and extended by a subsequent study that demonstrated that hippocampal α5 $GABA_A$ receptors are involved in the modulation of associative learning (acquisition and retention) by the trace manipulation and in extinction learning [176]. This is consistent with the finding mentioned above that α5-subunit knockout mice showed an improved performance in a particular Morris water maze test [148] possibly indicating that α5-subunit-containing $GABA_A$ receptors are mediating the memory impairing effect of diazepam. Selective partial inverse agonists of $GABA_A$ receptors containing α5 subunits might thus represent excellent candidates for the generation of memory enhancing drugs.

Interestingly, in a recent study it was demonstrated that the manifestation of tolerance to the motor-depressant action of diazepam is associated with the chronic activation of two competitive mechanisms orchestrated by α1- and α5-$GABA_A$ receptors, respectively, [177]. Results are consistent with the assumption that chronic activation of extrasynaptic α5-containing $GABA_A$ receptors is associated with a down-regulation of these receptors in the dentate gyrus of the hippocampus. This might enhance hippocampal synaptic efficacy and NMDA receptor subunit mRNA expression in dentate gyrus, possibly opposing the α1 receptor-mediated phasic inhibition in the forebrain areas involved in motor control. Thus, chronic activation of α5 subunit-containing $GABA_A$ receptors seems to be crucial for the normal development of sedative tolerance to diazepam [177].

3.2.5. Function of $GABA_A$ Receptors Containing β2 Subunits

Recently, an approach similar to that used for unraveling the function of $GABA_A$ receptors containing different α subunits was also used for studying the function of receptors containing distinct β subunits. Thus, it has been demonstrated that the intravenous anesthetic etomidate (Fig. **4**, compound **2**) shows $GABA_A$ receptor subtype selectivity *in vitro* for β2- and β3-containing receptors when studied on recombinant receptors [178]. This drug is essentially inactive in β1 containing receptors. This selectivity is determined through a single amino acid residue Asn265 in the transmembrane 2 segment of the receptor [179]. Recently, the point mutation β2(Asn265Ser) that renders β2 subunit-containing $GABA_A$ receptors less sensitive to the intravenous anesthetic etomidate was introduced into the β2 subunit gene of a mouse [180]. As with the histidine mutations in the α subunits, this single amino acid switch is also effectively silent with regard to normal GABAergic function. In Purkinje neurons the amplitude of GABA-induced currents was similar to wild-type but, unlike wild-type, responses were not modulated by etomidate, suggesting that receptors containing β2 subunits mediate the ataxic effects of etomidate. Similarly, synaptic currents were not different but the prolongation of decay induced by etomidate was significantly reduced in the β2(Asp265Ser) mice. The anesthesia following administration of etomidate was determined by measuring the sleep time (time to recover righting reflex) and was similar to that in wild-type mice, indicating that β2-containing receptors are not involved in the anesthetic effect of etomidate. At lower doses, which produced sedation in wild-type mice, no etomidate effects were observed in the β2(Asn265Ser) mice, indicating that the sedative effect of etomidate is produced *via* β2-containing receptors [172]. In addition, these mice had a reduced hypothermic response to anesthetic doses of etomidate compared with wild-type controls and after a transient loss of righting reflex regain normothermia more rapidly compard with wild-type controls. Thus, β2 subunit-containing $GABA_A$ receptors mediate a significant porportion of the hypothermic effects of etomidate. The observation that mutant mice recovered their motor abilities much more rapidly than wild-type and exhibited less slow wave sleep after anaesthesia [180] might thus be explained by both the lack of sedation and the lack of hypothermic effects of etomidate in these mice.

3.2.6. Function of $GABA_A$ Receptors Containing β3 Subunits

The equivalent mutation β3(Asn265Met) in the β3 subunit of mice has also been generated and experiments indicated that the righting reflex after etomidate is profoundly affected in this mouse. In addition, the anaesthetic ability to prevent the paw withdrawal reflex was abolished [181]. These results suggest that the β3-containing receptors are the primary mediators of the anaesthetic effects of etomidate. Since β2-containing receptors mediate the ataxic, sedative, and hypothermic effects of etomidate, whereas the anesthetic qualities (i.e. loss of consciousness, lack of purposeful movement in response to noxious stimuli, and presence of burst-suppression activity on the electroencephalogram) of etomidate seem to be mediated by β3 subunit-containing receptors, this further indicates that anesthetics that selectively activate specific subunits may produce surgical anesthesia with improved recovery characteristics. [172].

The β3(Asn265Met) mutation not only abolishes the modulatory and direct effects of the intravenous anesthetics etomidate, but also that of propofol (Fig. **4**, compound **5**) and substantially reduced the modulatory actions of the volatile anesthetic enflurane (Fig. **4**, compound **6**). In contrast, the modulatory action of the neurosteroidal intravenous anesthetic alphaxalone is preserved [181]. In hippocampal pyramidal neurons of β3(Asn265Met) mice, the potentiation of GABA-induced chloride currents by etomidate was substantially reduced. Because β3 is the predominant, but not exclusive, β subunit in these neurons, this result indicates that β3-containing neurons have become etomidate-insensitive [181]. In addition, it was demonstrated that the immobilizing effect of etomidate and propofol is critically dependent on β3-containing $GABA_A$ receptors [181]. In contrast, the immobilizing action of enflurane and halothane seems to be only partially mediated by β3-containing $GABA_A$ receptors and be present only at higher concentrations [32]. Essentially identical results for enflurane and halothane have also been obtained when studying β3 knockout mice [182]. These data indicate that in addition to β3-containing receptors possibly providing an end point for the immobilizing response of these drugs, other targets such as β1- or β2-containing receptors, two-pore domain background potassium channels, neuronal nACh receptors, or glutamate receptors, at which volatile anesthetics have been shown to have actions *in vitro*, are likely to play a major role in mediating enflurane´s and halothane´s actions [181]. Taken together, these results show that a single molecular target is a major determinant of behavioural responses evoked by the intravenous anesthetics etomidate and propofol, whereas the volatile anesthetics appear to act via a broader spectrum of targets.

3.3. Conclusions on the Function of $GABA_A$ Receptor Subtypes in the Brain

Together, these studies for the first time have demonstrated that the various benzodiazepine actions as well as the actions of some anaesthetics are not only generated in different brain regions but are also mediated by distinct $GABA_A$ receptor subtypes. It has to be kept in mind, however, that part of the behavioural effects of diazepam mediated by one receptor subtype might be counteracted by other $GABA_A$ receptor subtypes. Thus, to clearly delineate all effects mediated by a specific receptor subtype, a strategy has to be used that allows to address a single $GABA_A$ receptor subtype, only, under conditions in which drug enhancement in all the other receptor subtypes is eliminated. The drastically reduced behavioural background effect of the drug under these conditions will then allow the study of even very small effects of the drug mediated exclusively by one $GABA_A$ receptor type. In addition, the use of benzodiazepine binding site ligands that specifically modulate receptors containing γ1 or γ3 subunits could further enhance the resolution with which the function of individual receptor subtypes can be studied in genetically modified mice. Nevertheless, results already available indicate that the development of subtype-selective drugs will offer the possibility to specifically address only a subset of $GABA_A$ receptors and thus, enhance the selectivity of action and reduce side effects.

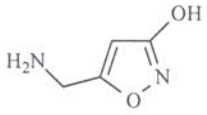
4. NOVEL SUBTYPE-SELECTIVE DRUGS ELICIT SPECIFIC BEHAVIOURAL EFFECTS

4.1. Overview on the Pharmacology of $GABA_A$ Receptors

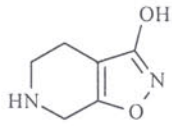
$GABA_A$ receptors not only can be directly activated or inhibited via their GABA binding site, but can also be allosterically modulated by benzodiazepines, barbiturates, steroids, anesthetics, convulsants, and many other drugs (Figs. 2-4), the number of which is constantly increasing [4, 29]. Due to the availability of selective high affinity ligands, the GABA/muscimol- (Fig. **2**, compounds **1** and **2**), the benzodiazepine- (Fig. **3**, compound **1**) and the convulsant picrotoxinin/t-butylbicyclophosphorothionate (TBPS)-binding site (Fig. **2**, compounds **8** and **9**) can be directly investigated by radioligand binding studies. Using such studies, compounds competitively interacting with the radioligands and thus, directly binding to the respective sites could be identified, forming the basis for the establishment of structure-affinity relationships that could be used as a guide for further development of site-specific ligands. Whereas only a few different classes of compounds (Fig. **2**, compounds **1**-**7**) are currently known as ligands for the GABA recognition site [183], many different classes of compounds interact with the benzodiazepine binding site of $GABA_A$ receptors [4, 26, 27, 30] (Fig. **3**). The situation is not as clear for the TBPS binding site. Although ligands have been identified that seemed to interact with [^{35}S]TBPS binding in a competitive way, due to the presumed location of the TBPS binding site within or close to the chloride ion channel, binding of TBPS is strongly influenced by structural changes induced by allosteric ligands of $GABA_A$ receptors (Fig. **4**) and it sometimes is difficult to distinguish between a competitive or allosteric interaction [4, 184].

The interaction of all the other drugs with $GABA_A$ receptors can only be investigated by electrophysiology or by studying the allosteric effects of these drugs at the [^{3}H]muscimol-, [^{3}H]benzodiazepine- or [^{35}S]TBPS-binding site. These techniques, however, in most cases don´t allow to clarify whether ligands exert their modulatory action via the same or a different allosteric binding site. Therefore, the total number of allosteric binding sites present on $GABA_A$ receptors is not known, and it is also not known whether

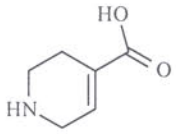
GABA (1)



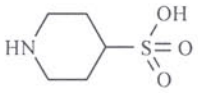
Muscimol (2)



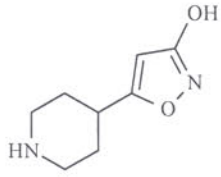
THIP (3) (Gaboxadol)



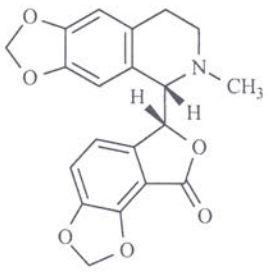
Isoguvacine (4)



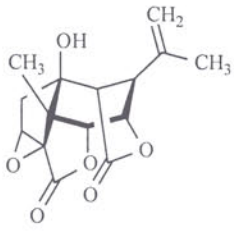
P4S (Piperidine-4-sulfonic acid) (5)



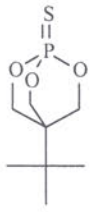
4-Piol (6)



Bicuculline (7)



Picrotoxin (8)



TBPS (9)

Fig. (2). $GABA_A$ receptor ligands. Compounds 1-6 are GABA-site agonists. Compound 7 is a GABA-site antagonist. Compounds 8 and 9 are $GABA_A$ receptor antagonists that inhibit the action of GABA *via* an allosteric site probably located within the ion channel.

different drugs act via separate or partially overlapping binding sites. Structure-activity studies for most of the allosteric modulators of $GABA_A$ receptors are thus not possible at present, preventing a structurally guided development of novel ligands for the respective binding site.

It is assumed that allosteric ligands interacting with $GABA_A$ receptors either induce or stabilize a certain structural conformation of the receptor eliciting an enhancement or a reduction of the GABA-induced chloride ion flux. This for the first time has been demonstrated for benzodiazepine binding site ligands (Fig. **3**). Compounds that enhance the actions of GABA are called allosteric "agonists". These compounds exhibit anxiolytic, anticonvulsant, muscle relaxant and sedative hypnotic effects. Compounds that allosterically reduce GABA-induced chloride flux are called "inverse agonists". These compounds have actions opposite to those of "agonists": they are anxiogenic, proconvulsant and enhance vigilance, learning and memory. A third class of compounds obviously stabilizes a conformational state that does not directly change GABA-induced chloride flux. These compounds in most cases don't elicit behavioural effects on their own, but prevent interaction of "agonists" or "inverse agonists" with these receptors. They are therefore called allosteric "antagonists" [4].

Benzodiazepine binding site agonists, inverse agonists, or antagonists have been identified in each class of compounds interacting with the benzodiazepine binding site of $GABA_A$ receptors The "agonist" or "inverse agonist" efficacy of a compound usually is distinct in different receptor subtypes. Thus, a compound can be a "full agonist" at one type of receptor and exhibit different degrees of "partial agonist" activity at other receptor subtypes [30, 60, 185, 186]. It is even possible that the efficacy of a compound reverses direction at different receptor subtypes: A compound can be a "partial agonist" at one receptor and be an "antagonist" or "partial inverse agonist" at another receptor subtype [60, 185, 186]. This explains, for instance, the different spectrum of actions of various clinically used benzodiazepines. Although compounds such as diazepam, clonazepam or bromazepam exhibit a comparable affinity for all $GABA_A$ receptor subtypes composed of $\alpha1\beta\gamma2$, $\alpha2\beta\gamma2$, $\alpha3\beta\gamma2$ or $\alpha5\beta\gamma2$ subunits, their efficacy at individual receptor subtypes is different thus generating their specific anxiolytic, anticonvulsant, muscle relaxant and sedative-hypnotic activity spectrum [60].

Agonistic, antagonistic and inverse agonistic effects are not only mediated via the benzodiazepine binding site of $GABA_A$ receptors but there is evidence that opposite effects on $GABA_A$ receptor function could also be mediated via the barbiturate- (Fig. **4**, compound **1**), steroid- (Fig. **4**, compound **9**), γ-butyrolactone- (Fig. **4**, compound **10**), or possibly also via other allosteric binding sites of $GABA_A$ receptors [4]. It is not clear, however, whether these actions are mediated via the same or via different binding sites. Benzodiazepines and compounds interacting with the benzodi-

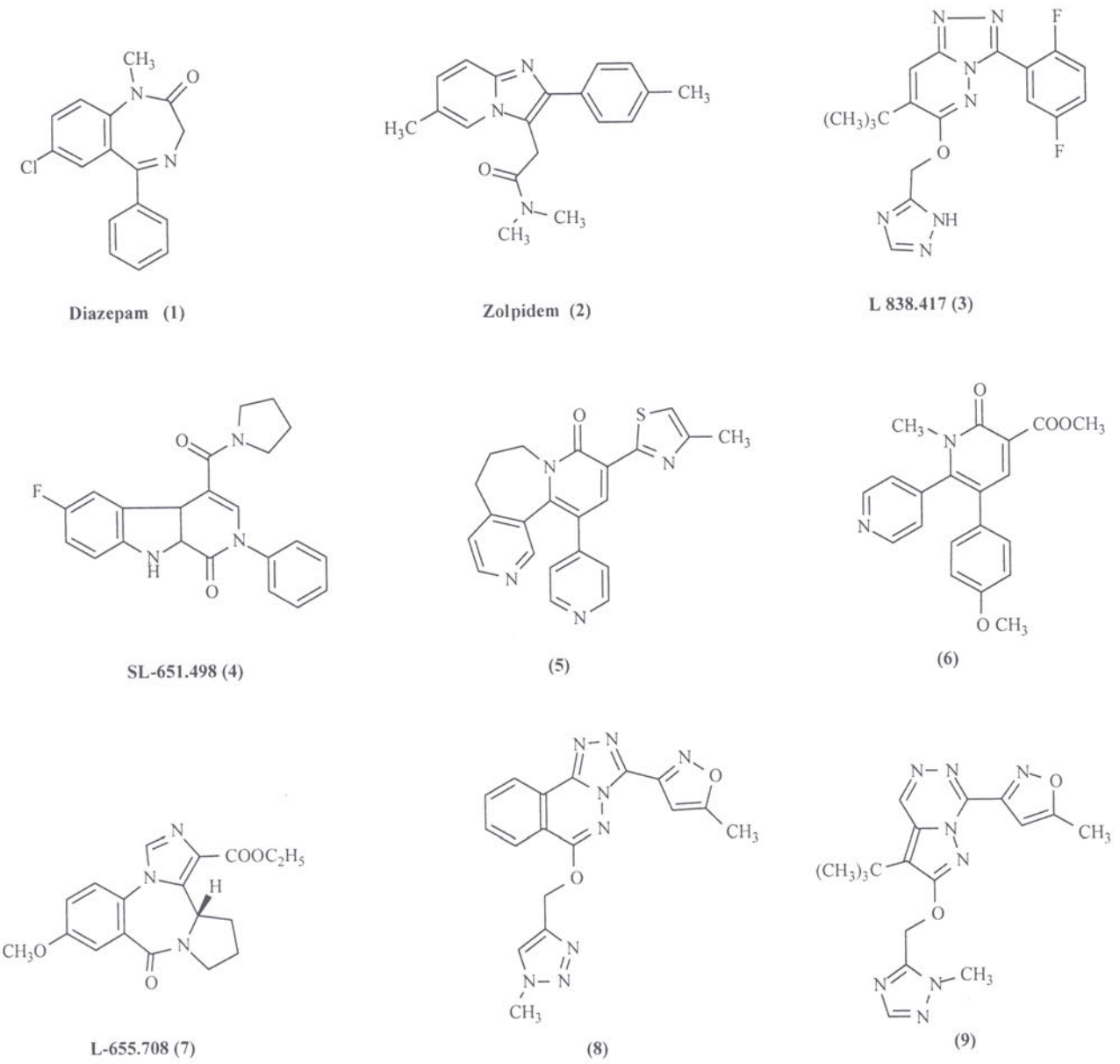


Fig. (3). Benzodiazepine binding site ligands

azepine site of GABA$_A$ receptors only can modulate ongoing GABAergic activity. These compounds cannot elicit chloride ion flux in the absence of GABA and thus exhibit an extremely low degree of toxicity. In contrast, barbiturates, steroids, volatile and intravenous anesthetics and other compounds interacting with GABA$_A$ receptors are able to modulate GABA-induced choride flux at low concentrations, but directly elicit chloride flux at these receptors in the absence of GABA at higher concentrations and thus, exhibit a much higher toxicity than benzodiazepine site ligands [4].

Such bi- or multi-phasic actions of drugs at different concentrations seem to be the rule rather than the exemption for GABA$_A$ receptor ligands, thus further complicating the pharmacology of these receptors. The same drug can elicit agonistic and inverse agonistic effects via different binding sites of the same receptor. A receptor-subtype-selectivity of a drug elicited via one binding site could be counteracted by its additional interaction with a second site that is present in a broader range of receptor subtypes [187]. And finally, some but not all effects elicited by a drug via different binding sites at the same receptor could be inhibited or enhanced by another drug interacting with one of these sites, rendering interpretation of pharmacological studies extremely complicated.

4.2. Recent Developments in the Generation of GABA$_A$ Receptor Subtype Selective Drugs

A large variety of compounds interacting with allosteric binding sites on GABA$_A$ receptors have been identified, but only a few GABA$_A$ receptor subtype selective drugs have been developed, and the selectivity of these compounds was not very high [4]. Most of the drugs exhibiting some receptor subtype-selectivity interacted with the benzodiazepine binding site of GABA$_A$ receptors. Only recently, some recep-

tor subtype-selectivity was also identified in compounds interacting with the GABA binding site, or with some so far unidentified binding sites at $GABA_A$ receptors.

4.2.1. Compounds Interacting with the GABA Binding Site of $GABA_A$ Receptors

Studies on recombinant $GABA_A$ receptors have indicated that the direct agonists (Fig. **2**, compounds **1** and **2**) or antagonists (Fig. **2**, compound **7**) at the GABA binding site of these receptors seem not to exhibit a significant receptor subtype selectivity [119, 188, 189] reflecting the very strict structural requirements for $GABA_A$ receptor recognition and activation. In addition, therapeutic use of full GABA agonists (exhibiting an efficacy comparable to that of GABA) or antagonists may be associated with severe side effects. Full GABA agonists that open all $GABA_A$ receptor associated chloride channels indiscriminately, will cause inhibition of most neuronal systems, whereas GABA antagonists are potential anxiogenics and proconvulsants. Since partial agonists at the benzodiazepine site of $GABA_A$ receptors often exhibited some receptor subtype-selectivity (see below), partial GABA binding site agonists were developed as potential therapeutics [183].

Within the series of compounds so far developed showing agonist activity at the GABA binding site of $GABA_A$ receptors, most of the ligands are structurally derived from the $GABA_A$ agonists muscimol (Fig. **2**, compound **2**), 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP, Fig. **2** compound **3**) or isoguvacine (Fig. **2** compound **4**) [183]. As observed for THIP, the heterocyclic GABA isosteres imidazole-4-acetic acid or piperidine-4-sulphonic acid (Fig. **2**, compound **5**) show characteristics of partial $GABA_A$ agonists. Similarly, the non-fused THIP analogue 5-(4-piperidyl)isoxazol-3-ol (4-PIOL, Fig. **2**, compound **6**), and its analogues are weak partial agonists of potential clinical interest [183].

Using recombinant $GABA_A$ receptors, recently some functional selectivity has been shown for a number of compounds such as the GABA binding site agonists imidazole-4-acetic acid, piperidine-4-sulphonic acid, THIP, and 4-PIOL showing highly subunit-dependent potency and maximal response and even indicate that coexpression of different α subunits in the same receptor results in novel GABA binding site pharmacology. Thus, THIP is approximately 10 times more potent at $\alpha4\beta3\delta$ receptors than at $\alpha4\beta3\gamma2S$ receptors. Furthermore, at δ-containing receptors THIP appears to have a completely different pharmacological profile than at other receptor combinations [183, 190]. The full spectrum of pharmacological actions of THIP at different $GABA_A$ receptor subtypes, however, has still not been investigated.

THIP is currently developed as Gaboxadol and appears to have potent analgesic effects [190]. Gaboxadol and morphine are approximately equipotent as analgesics, although their relative potencies are dependent on the animal species and experimental models used. Gaboxadol-induced analgesic effects were shown to be insensitive to the opioid antagonist naloxone indicating that these effects are not mediated by the opioid receptors [191]. The neuronal and synaptic mechanisms of action of the analgesic effects of Gaboxadol currently are not known, but the observation that $GABA_A$ agonist-mediated analgesia seems not to lead to dependence suggests that GABAergic drugs may play a role in future treatment of pain [190].

Gaboxadol seems also to improve the quality of sleep. The compound shows no effect on the onset of REM sleep, normalizes the sleep pattern, and patients did not experience hangovers or impaired attention as reported for benzodiazepines [183, 190]. Similar results have been obtained with muscimol, with the GABA uptake inhibitor Tiagabine and with the glia-selective GABA uptake inhibitor THPO [190] strongly suggesting that the functional consequences of a direct acting agonist or enhanced synaptic GABA concentration are different from those seen with $GABA_A$ receptor modulators.

Interestingly, $\alpha4\beta3\delta$ receptors, for which Gaboxadol seems to exhibit the highest efficacy, so far could not specifically be modulated by any other known compound. Effects mediated by this receptor subtype are thus not known. These receptors seem to be predominantly expressed in the thalamus, striatum, outer layers of the cortex and in the dentate molecular layer (see chapter 2.2.1.) and in analogy to the $\alpha6\beta\delta$ receptors presumably are located extrasynaptically, providing tonic inhibition to the respective neurons. It has been demonstrated that expression of α4-containing receptors (and δ-containing receptors) is upregulated in the course of pilocarpine- or kainic acid-induced temporal lobe epilepsy [192-194]. Since δ-subunit knockout mice have spontaneous seizures and demonstrate a greater sensitivity to pharmacologically induced seizures, these receptors might be valuable targets for treatment of epilepsy [195].

Expression of these receptors also dramatically changes in the rodent progesterone withdrawal model of the premenstrual syndrome. In this model, withdrawal of progesterone leads to an increase in anxiety [196, 197] and in the expression of the α4 subunit, and to a decrease in the expression of other receptor subunits in the hippocampus. There is a concomitant change in the pharmacology of receptors, from lorazepam-sensitive to lorazepam-insensitive and the benzodiazepine site antagonist acts as an agonist as is found at recombinant $\alpha4\beta\gamma2$ receptors. Blockade of the α4 gene transcript prevented these withdrawal properties [197]. These data suggest that newly expressed $GABA_A$ receptors containing α4 subunits are involved in the generation of the premenstrual syndrome. Drugs specifically interacting with these receptors might thus be beneficial for treating this syndrome. Interestingly, it has recently been reported that the GABA agonist THIP which has markedly higher efficacy for $\alpha4\beta\delta$ receptors than other $GABA_A$ receptor subtypes, is anxiolytic in the rodent progesterone withdrawal model. Whether $\alpha4\beta\gamma2$ or $\alpha4\beta\delta$ is the target, however, remains unclear and the relevance to the human condition is yet unproven [195].

4.2.2. Compounds Interacting with the Benzodiazepine Binding Site of $GABA_A$ Receptors

Most of the receptor subtype-selective compounds so far identified interact with the benzodiazepine binding site of $GABA_A$ receptors. Since this site is located at the interface of α and γ subunits (Fig. **1**) its binding properties are influenced by the types of the subunits forming this interface.

Since there are six different α and three different γ subunits, up to 18 different $GABA_A$ receptor-associated benzodiazepine binding sites may exist. Whether receptor subtypes containing two different α subunits exhibit benzodiazepine binding site properties different from that of receptors containing a single type of α subunit presently is not known. Evidence so far available rather indicates that one of the two α subunits (presumably that neighboring the γ subunit) predominates the benzodiazepine binding properties of these receptors [67].

Most compounds interacting with the benzodiazepine binding site are inactive or only weakly active at receptors containing γ1 subunits [60, 185]. Although there seems to be some activity of benzodiazepine ligands at receptors containing γ3 subunits [60], these receptors exhibit a very low abundance in the brain [76]. Thus, the currently prescribed benzodiazepines and most of the structurally unrelated compounds interacting with the benzodiazepine binding site of $GABA_A$ receptors mediate their effects predominantly by interacting with $GABA_A$ receptors composed of α1β2, α2β2, α3β2, or α5β2 subunits. Receptors composed of α4β2 or α6β2 subunits exhibit a drastically different pharmacology. Most of the classical benzodiazepines, such as diazepam, flunitrazepam, or clonazepam, do not interact with these receptors [4, 60, 198]. The selectivity of compounds developed for α4β2 or α6β2 receptors is only weak [199-201] and thus, they currently cannot be selectively addressed and the behavioural effects mediated by these receptors are not known. Since most of the benzodiazepine site ligands modulate α1β2, α2β2, α3β2, or α5β2 receptors to a more or less similar extent, it is no surprise that the clinical spectrum of action of these compounds is quite similar. Only some of these drugs in current use, such as zolpidem (Fig. **3**, compound **2**), exhibit a selectivity for α1-subunit containing receptors [4, 60]. Since α1-containing receptors are the major $GABA_A$ receptors in the brain and mediate the sedative effects of benzodiazepines, these compounds are predominantly used for their sedative/hypnotic properties.

The traditional approach for the development of compounds that are selective for $GABA_A$ receptor subtypes used radioligand binding assays and thus, selected for a differential affinity of the compounds for different receptors. Whereas the first compounds with some receptor subtype selectivity exhibited a higher affinity for α1-containing receptors [4], in the last years compounds were developed with a selective affinity for α2/α3-, or α5-subunit containing receptors [27, 202-206]. Unfortunately, however, it turned out that a selective affinity not necessarily corresponds with a selective efficacy for the receptor subtype. Compounds could have a high affinity but a low efficacy for a certain receptor subtype and could have a high efficacy for receptors for which they have a low affinity [60, 207-209]. Overall, the effects of a compound will be determined by a combination of affinity and efficacy for the individual receptor subtypes. In any case, functional effects of compounds as measured by electrophysiological techniques in cells expressing recombinant $GABA_A$ receptor subtypes probably provide a better idea on the action of compounds *in vivo*. Obtaining such functional data, however, requires labor-intensive studies not yet routinely performed by the various groups developing $GABA_A$ receptor subtype selective drugs, and thus, not much information is available on the efficacy of drugs at various receptor subtypes under standardized conditions [210-213]. Only recently, a high throughput approach has been developed based on the application of fluorescence imaging technologies, in particular the use of fluorescence resonance energy transfer (FRET) in conjunction with voltage-sensitive dyes [214, 215]. This technique has been applied with great success to $GABA_A$ receptors, enabling rapid quantification of the affinity and efficacy of allosteric modulators at receptor subtypes [119, 215].

By investigating the efficacy of compounds for various receptor subtypes, compounds could be identified that exhibit selectivity for $GABA_A$ receptor subtypes other than α1-containing receptors. One of the first such modulators identified was a triazolo[4,3-b]pyridazine, L-838,417 (Fig. **3**, compound **3**), a benzodiazepine site ligand with high affinity to α1-, α2-, α3-, and α5-subunit containing receptors. This compound, however, does not enhance GABA-response on α1-receptors but acts as a partial agonist on α2, α3-, and α5-containing receptors [163]. When evaluated in animal models, this compound behaved as a non-sedating anxiolytic in the elevated plus maze and fear-potentiated startle test, without causing sedation or ataxia which is in agreement with conclusions from experiments using genetically modified mice. Partial agonists at the benzodiazepine binding site have been shown previously to produce anxiolytic but no sedative actions in animal experiments, but under clinical conditions the sedative actions were still present [28]. It remains to be determined whether the anxiolytic actions of L-838,417 will be devoid of sedative activity in human patients. Recent evidence indicates that this is the case at least for nonhuman primates [216]. L-838,417 in addition was able to engender muscle relaxation in the absence of other motor effects. Studies on the clinical effects of this compound are thus eagerly awaited.

At least two different series of compounds which also have functional selectivity for α2- and α3-containing $GABA_A$ receptors, were subsequently described [210, 211, 213]. The compound SL651.498 (Fig. **3**, compound **4**) [210] exhibits high affinity for receptors containing α1 or α2 subunits, but 10 times lower affinity for receptors containing α5 subunits. But it behaves as a full agonist at recombinant $GABA_A$ receptors containing α2 or α3 subunits and as a partial agonist at recombinant $GABA_A$ receptors containing α1 or α5 subunits. SL651.498 produced anxiolytic-like and skeletal muscle relaxant effects similar to those of benzodiazepines, but with drastically reduced side effects [210, 217]. In another study, a tricyclic pyridone (Fig. **3**, compound **5**) with functional selectivity for the α3 over the α1 containing subtype has been developed that was efficacious in animal models of anxiety and showed no sedation or potentiation of ethanol effects [213]. A compound in the related 3-heteroaryl-2-pyridone class [211] was a selective inverse agonist at α3-containing receptors with minimal efficacy at the α1- and α2-containing receptors (Fig. **3**, compound **6**). When evaluated in animal models, this compound was found to be anxiogenic. This suggested an important role for α3-containing $GABA_A$ receptors in anxiety, in contrast to the data generated from the genetically modified mice [195].

Recent reports also indicate that it is possible to develop compounds with selective efficacy for α5-containing $GABA_A$ receptors. One of the first compounds developed (Fig. **3**, compound **7**; FG 8094/L-655.708) exhibited a 50 fold higher affinity for α5 subunit containing receptors as compared to α2 or α3 receptors, a 100 fold higher affinity as compared to α1, and a 200 fold selectivity compared to α6 [27]. This compound acts as a partial inverse agonist on α5 containing receptors and its tentative use for cognition enhancement has been patented. Fig. **3**, compound **8** exhibited high binding affinity for α1-, α2-, α3-, and α5-containing receptors and is a potent inverse agonist at α5-containing receptors with little or no efficacy at the other receptor subtypes [212]. Similar properties were exhibited by compound **9** (Fig. **3**). In agreement with the notion that α5-containing receptors might influence learning and memory, these compounds improved the performance of rodents in a well known model of spatial memory, the Morris water maze. Interestingly, these α5-selective inverse agonists did not appear to be convulsant and they were not pro-convulsant in the presence of the convulsant pentylenetetrazol [220]. These data clearly indicate the potential utility of these compounds as cognitive enhancers in disorders such as mild cognitive impairment and Alzheimer´s disease.

4.2.3. Compounds Interacting with so far Unidentified Binding Sites of $GABA_A$ Receptors

4.2.3.1. Compound Interactions Modulated by the Type of α-Subunit

Several compounds, such as furosemide [201, 221, 222], amiloride [223], γ-butyrolactones [224, 225], or the ROD compounds [226-228] have been identified that seem to interact with novel, so far unidentified binding sites at $GABA_A$ receptors. Efficacy of these compounds seems to depend on the type α subunit present in the receptors. Thus, for instance, the diuretic compound furosemide (Fig. **4**, compound **11**) exhibits approximately 100-fold selectivity for α6 subunit-containing receptors over α1-containing receptors [221]. This compound not only blocks α6-containing receptors but also (with less affinity) α4-containing receptors [201, 222]. Similarly, the diuretic amiloride acted as antagonist, reducing the sensitivity of the receptor to GABA without affecting the maximal current amplitude. Receptors containing an α6 subunit were about 10-fold more sensitive to amiloride than those containing other α subunits. In contrast to furosemide, that in addition showed some β-subunit dependence (see below), amiloride showed no additional dependence on the identity of the β or γ subunit, and thus, could be useful for developing drugs targeting this unique modulatory site on $GABA_A$ receptors [223].

In other experiments it was demonstrated that GABA-responses in α1β2γ2-transfected cells and early granule neurons from the cerebellum were potentiated by γ-butyrolactones (Fig. **4**, compound **10**) whereas α6β2γ2-transfected cells and mature granule neurons were not significantly altered [224]. Finally, compound (+)-ROD188 (Fig. **4**, compound **12**), that shares structural similarity with bicuculline (Fig. **2**, compound **7**), allosterically stimulated GABA-induced currents in α1β2γ2 and α1β2 receptors, indicating that the respective binding site does not require a γ subunit and this compound was to a certain extent selective for the α6 isoform of $GABA_A$ receptors [227, 228].

Recently, a structural analogue (Fig. **4**, compound **8**) of the fluoroquinolone antibiotic norfloxacin was identified that seemed to modulate $GABA_A$ receptors via a novel binding site not identical with the sites for TBPS, GABA, benzodiazepines, barbiturates, neuroactive steroids, norfloxacine or loreclezole [229]. This compound potentiated submaximal (EC_{50}) GABA currents recorded from HEK-293 cells expressing human α2β2γ2L, but not α1β2γ2L $GABA_A$ receptors and induced anxiolytic effects with a maximum efficacy comparable to the optimal effect of diazepam. Unlike diazepam, however, this compound had no central nervous system depressant effects in the range of doses tested. These data provided compelling evidence to support the selectivity of this compound for α2 subunit containing $GABA_A$ receptors and indicate that targeting these receptors with ligands not interacting with the benzodiazepine binding site is a viable means of achieving robust anxiolytic activity without sedation [229].

4.2.3.2. Compound Interactions Modulated by the Type of β-Subunit

It is widely accepted that the type of the β subunit present in a $GABA_A$ receptor does not significantly influence the GABA, benzodiazepine, barbiturate, propofol, or steroid site pharmacologies of human $GABA_A$ receptor subtypes composed of αβγ subunits [230, 231], although another study indicated that the β variant had some effect on the benzodiazepine site pharmacology [232]. Since the benzodiazepine site is located at the αγ interface of $GABA_A$ receptors, the type of the β subunits presumably influenced the coupling of the benzodiazepine site to channel opening.

In recent years, a number of modulators of the $GABA_A$ receptor, for example loreclezole (Fig. **4**, compound **3**) [233, 234], etomidate (Fig. **4**, compound **2**) [179], or furosemide (Fig. **4**, compound **11**) [221, 235], have been identified that demonstrate β2/β3 selectivity over β1. In all cases, the potency of the modulator was reduced or abolished when an asparagine at the position 289 in human β2 and 290 in human β3, that is located within the TM2 region of the β subunit, was replaced by serine, (the homologous residue in β1). The replacement of the β1 subunits serine 290 by asparagine produced the converse effect. Salicylidene salicylhydrazide was one of the first compounds with a selectivity for receptors containing the β1 subunits [236]. This compound partially and selectively inhibited GABA-activated chloride ion channels of β1-containing receptors and it was demonstrated that mutation of either threonine 255 located within the TM1, or isoleucine 308 located extracellularly just prior to TM3 within the β1 subunit to the β2 counterpart was sufficient to abolish the inhibition [236]. However, the converse individual mutations within the β2 subunit did not introduce any inhibition. Thus, different amino acid residues are important for conferring the β2/β3 and β1 selectivity of these compounds. Currently, it is not clear whether these residues are located close to the binding sites of these compounds or whether they only are important for transduction of the drug effects.

In a subsequent study, various anti-inflammatory agents including mefenamic acid, flufenamic acid (Fig. **4**, compound **7**), meclofenamic acid, tolfenamic acid, niflumic acid and diflunisal were investigated [231]. These compounds exhibited varying levels of efficacy and potency at β2 or β3 subunit-containing receptors, while having antagonist or weak inverse agonist profiles at β1-containing receptors. The relatively high therapeutic plasma concentrations of these nonsteroidal anti-inflammatories that are achieved, suggest that appreciable brain levels might also be reached. Indeed, observations of antiepileptogenic effects and adverse events associated with anti-inflammatory overdose are consistent with activity at central $GABA_A$ receptors. Given that low micromolar concentrations are sufficient to potentiate β2/β3-containing $GABA_A$ receptors, this suggests that modulatory effects could occur at clinically relevant concentrations [231]. The β1 subunits are also widely distributed in the brain, making it difficult to speculate as to what therapeutic use a β1-selective inhibitor would have. So far, the influence of different α subunits on the effects of these anti-inflammatory agents has not yet been investigated. If such an influence is identified, compounds interacting with the respective binding site might very well be able to address certain $GABA_A$ receptor subtypes. But in any case, the fact that there are compounds exhibiting β subtype-selectivity offers the possibility to develop more selective compounds with higher affinity and efficacy and thus, study the function of these receptors in the brain.

4.2.3.3. Compound Interactions Modulated by Other Subunits

The pyrazolopyridine tracazolate (Fig. **4**, compound **4**) exhibits anxiolytic and anticonvulsant activity. Compared with the standard benzodiazepine chlordiazepoxide, it was 2 to 20 times less potent as an anxiolytic, but interestingly displayed a much larger window of separation between the anxiolytic effect and potential side effects (sedation, motor incoordination, and its interaction with ethanol and barbital) [237]. Recently, it was demonstrated that tracazolate has a unique pharmacological profile on recombinant $GABA_A$ receptors: its potency (EC_{50}) is influenced by the nature of the β subunit, but more importantly, its intrinsic efficacy, potentiation, or inhibition is determined by the nature of the third subunit (γ1-3, δ, or ε) within the receptor complex. [238]. The allosteric modulation induced by the binding site mediating the effects of tracazolate seems thus to be especially sensitive to the receptor subunit composition. In turn it is possible that depending on the structure of the compound interacting with this binding site different receptor subtypes are selectively modulated. Such compounds, thus, might have a substantial therapeutic potential.

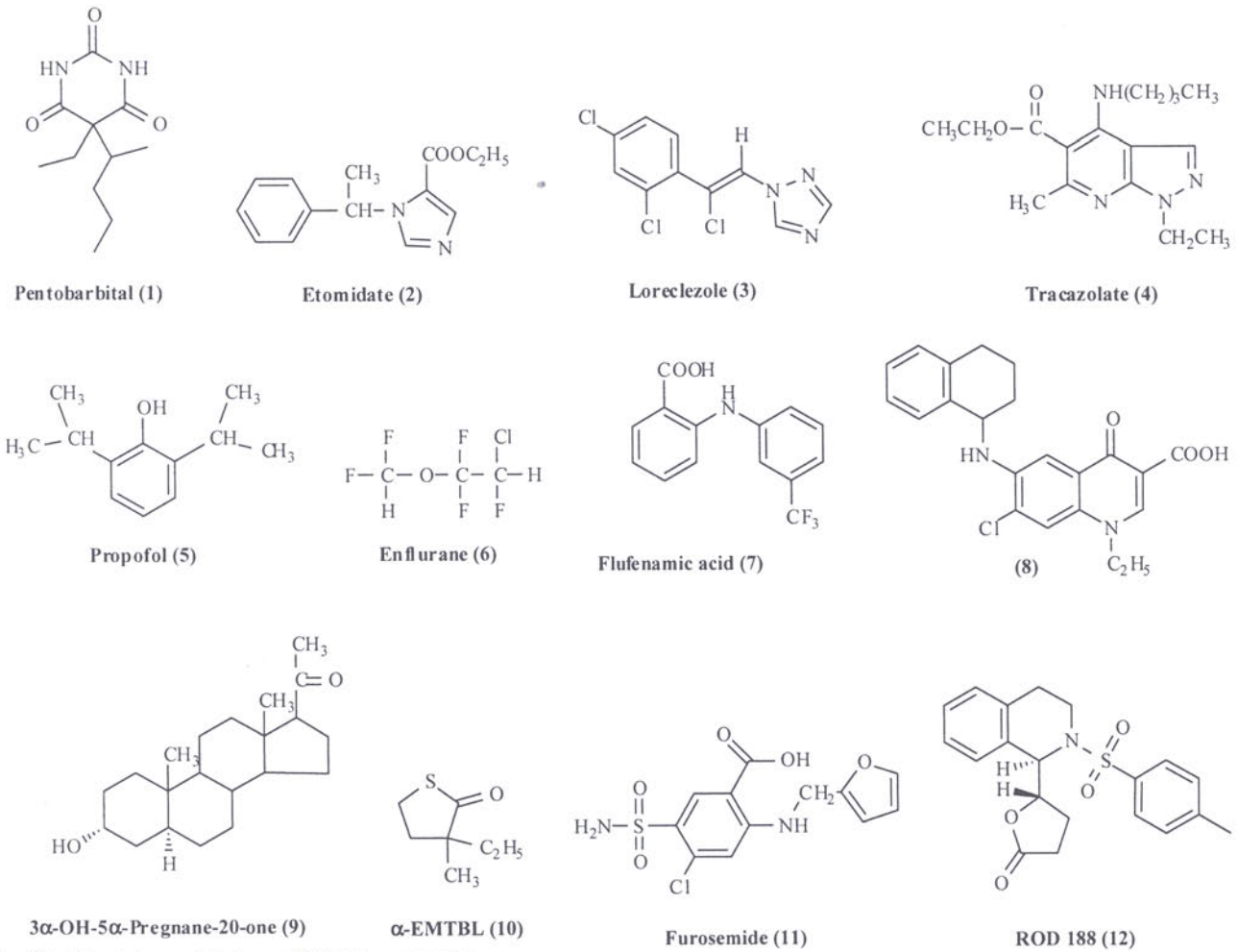


Fig. (4). Allosteric modulators of $GABA_A$ receptors

Neuroactive steroids are endogenous metabolites of progesterone and deoxycorticosterone and exhibit anxiolytic, anticonvulsant, analgesic, sedative, and, at relatively high doses, anesthetic actions [239]. A variety of experiments has indicated that these compounds are highly selective and extremely potent modulators of the $GABA_A$ receptor [240]. However, most studies have indicated that neither potency nor efficacy of these compounds appear to depend significantly on the subunit composition of receptors [240]. This conclusion is supported by the finding that neither α nor γ subunits seem to be necessary for the interaction of steroids with $GABA_A$ receptors, since neuroactive steroids can modulate homo-oligomeric receptors composed of β subunits [4]. Nevertheless, whole-cell clamp electrophysiological studies have demonstrated conclusively that neurosteroids such as 3α-OH-5α-pregnane-20-one (Fig. **4**, compound **9**) act differentially at synaptic $GABA_A$ receptors in different brain regions. Whether this heterogeneity is the result of the expression of distinct $GABA_A$ receptor subtypes or is caused by other factors, such as phosphorylation or local steroid metabolism [240, 241] is not clear. Recent evidence, however, seems to indicate that neurosteroids [242] and possibly also etomidate and some anesthetics [243], especially stimulate $GABA_A$ receptors such as extrasynaptic δ-containing receptors for which GABA is only a partial agonist [244]. Thus, steroids exhibited a strong stimulation when the low efficacy agonist β-alanine was used for stimulation of recombinant receptors, and exhibited a weak stimulation when the higher efficacy agonist taurine was used for stimulation. It is thus also conceivable that a differential activation of extrasynaptic receptors by endogenously released β-alanine or taurine could influence the efficacy of endogenous steroids in different brain regions [242]. A major role of δ subunit containing receptors for steroid action is also supported by the observation that the effects of neuroactive steroids are greatly reduced in mice lacking the δ subunit [158].

Despite the fact that ethanol is the most widely used psychoactive agent, its actions on brain functions are poorly understood. Several types of receptors and channels have been shown to be functionally altered by ethanol, which include glutamate-, serotonin-, glycine-, and $GABA_A$-receptors and G protein-coupled inwardly rectifying K^+ channels [243]. Ethanol effects on these targets are seen only at fairly high concentrations (above 60 mM). Recently, it was demonstrated that recombinant α4β3δ and α6β3δ $GABA_A$ receptors are reproducibly enhanced at 3 mM ethanol, a concentration six times lower than the legal blood-alcohol intoxication (driving) limit in most states (0.08% wt/vol or 17.4 mM). In contrast, ethanol required a more than 15 fold higher concentration for activation of α4β3γ2, α6β3γ2 or α1β2γ2 receptors [243]. It thus seems unlikely that γ2-containing synaptic receptors are primary ethanol responders, but they might contribute to ethanol toxicity at high concentrations. Surprisingly, ethanol was 10 fold more effective on β3- than on β2-containing α4βδ- and α6βδ-receptors. Since these receptors presumably are located extrasynaptically, it is possible that ethanol primarily acts via extrasynaptic receptors. It has to be admitted, however, that over the years many effects of ethanol on $GABA_A$ receptors have been described that partially could not be reproduced by other groups. Also in this case there are conflicting data [245] indicating that α4β2δ receptors could be activated by surprisingly low concentrations of ethanol that could not be confirmed by [243]. It remains to be determined what accounts for these discrepancies. But if the high efficacy of ethanol for certain $GABA_A$ receptor subtypes proves to be true, this might offer the development of a completely new pharmacology for these receptors with the aim of reducing ethanol addiction and dependence and ameliorating withdrawal symptoms.

5. $GABA_A$ RECEPTOR STRUCTURE SUGGESTS NEW AVENUES FOR THE DEVELOPMENT OF SUBTYPE SELECTIVE DRUGS

The $GABA_A$ receptor is a member of the superfamily of pentameric ligand-gated ion channels that also includes the nAChR, the 5-hydroxytryptamine type 3 receptor and the glycine receptor. So far, no receptor belonging to this superfamily has been characterized structurally by x-ray crystallography. However, cryo-EM images of the nAChR in the open and closed state at modest resolution were published as early as 1995 [246]. A pentameric acetylcholine binding protein (AChBP), that turned out to be homologous to the extracellular domain of nAChR, has then been sucessfully crystallized [64] and subsequently, this structure has been used to refine the cryo-EM images of the nAChR in the two functional states [247]. Finally, a cryo-EM atomic structure of the transmembrane domain of the nAChR consisting of 5 so-called 4 α-helix bundles, one bundle per subunit, was released in 2003 [248].

Since anion and cation channels belong to a common superfamily, insight in the structure of anion channels can be gained on the basis of the structural data available from the nAChR family by means of "comparative" or "homology" modeling. Sequence similarity between cation and anion conducting receptors is rather low. Thus, topology and architecture of receptors will be conserved, but structural details are expected to be variable, leading to model uncertainties. Additional model errors can come from incorrect or ambiguous sequence alignments and from intrinsic limits of the different methods that can be used. It should be kept in mind that interpretation of homology models must account for these caveats. Nevertheless, since AChBP coordinates have been released (1I9B, http://www.rcsb.org/pdb/), $GABA_A$ receptor extracellular domain models have turned into popular and usefuls tools to describe the approximate structural properties of the extracellular binding sites [65].

One of the most interesting uses of structural models is the study of putative drug binding sites. The pockets that are found in the extracellular domain at the interface between subunits of $GABA_A$ receptors have been described in [65]. The agonist binding site has been confirmed to consist of amino acid residues belonging to the so-called "loops" A, B and C from the "principal" part of the binding site, and the so-called "loops" D and E of the "complementary" part of the binding site (Fig. **5a**). In the case of the $GABA_A$ receptor, possible pairs for the principal and complementary subunits can be beta-alpha, theta-alpha, or rho-rho, respectively. The same picture of a pocket framed by the "loops" also emerged for the binding site of benzodiazepine ligands, which is localized at the alpha-gamma interface, and thus consists of loops A, B and C of the alpha subunit and loops D and E of

the gamma subunit. These three-dimensional models of the binding sites have nicely confirmed what has been suspected on the basis of mutagenesis experiments, and have been used to some degree to attempt docking studies of selected ligands, see for example [249]. The homology between $GABA_A$ receptors and AChBP is too low, however, to expect that subtype differences in the binding sites will be modeled properly, but the models can be used as good guides for the overall architecture of the binding sites. For instance, the 3D arrangement of the "loops" narrows down on choices for possible subsites of agonistic and antagonistic substances [65].

Of course, the structure of the transmembrane fragment of the nAChR [248] has also raised much interest for modeling purposes. Presently, the structural fragments provided by the AChBP for the extracellular domain and by the nAChR transmembrane domain provide a first glimpse on the three dimensional organization of the superfamily, which places different protein segments, that have been shown to be of functional importance, into a "region in space" and into defined neighbouring relations. Fig. **5** shows ribbon models of what a $GABA_A$ receptor is thought to look like (Ernst *et al.*, manuscript in preparation). For example, the interface between two subunits in the transmembrane domain is now known to be made up not only by TM2 helices of different subunits, but also of the TM3 of one, and the TM1 of the other subunit (Fig. **5**). Which amino acid residues precisely make the intersubunit contacts cannot be determined accurately, though. While the majority of investigations appears to be in support of the helical TM1-4 model, there is still some debate as to whether this helical dominated model for the transmembrane domains is correct [250].

The 4 Å structure (1OED; PDB, http://www.rcsb.org/pdb/) of the nAChR transmembrane fragments in the resting state [248] is loosely packed and thus suggests that the transmembrane domain of this receptor family contains additional "cavities" beyond the ones found extracellularly. One set of putative pockets is located between subunits at the junctions between the extracellular and "transmembrane" domain (the latter is not entirely inserted in the membrane), and extend into the subunit junctions inside the lipid bilayer (Fig. **5a** and **5b**). Thus, each interface between subunits may contain multiple independent binding sites that could be distributed anywhere between the extracellular part of the receptor and the inside of the lipid bilayer. Another type of cavity is found to be contained inside each of the subunits, surrounded by the four helices that make up the transmembrane domain (Fig. **5a** and **5c**). These latter cavities are also found in models based on unrelated proteins [251], and those formed by the alpha subunits are thought to correspond to the long-proposed "anaesthetic pockets" for the volatile anaesthetics that are defined by a serine residue in the TM2 of the alpha subunit [252]. Altogether, the qualitative features of this structure go well with what is known about the structure of this domain in various receptors. Due to alignment ambiguities in two of the helical segments, however, certain specific interpretations of homology models based on the nAChR structure are still subject to intense debate [253]. In favour of the model can be said that the loose packing of the 4-helix bundle seen in 1OED also explains the high accessibility of individual amino acid residues in the putative transmembrane helices that was observed in studies using the substituted cysteine accessibility method (SCAM) and was originally interpreted as evidence against a helical transmembrane motif [250]. In addition, the observed conformational flexibility in this domain also is very consistent with a loosely packed highly mobile structure.

The occurrence of multiple pockets at subunit interfaces as well as "inside" of the individual subunits themselves (see Fig. **5**) explains, at least in principle, the large assortment of proposed "separate" allosterically interacting modulatory sites that are described in the literature [4]. Mutagenesis studies have already identified several segments that are essential for the action of certain modulatory drugs and that can now be examined in the light of three dimensional models. For instance, the TM2 segment of the beta subunit, that is homologous to that of the alpha subunit which contributes a serine residue to the putative volatile anaesthetics pocket in $GABA_A$ receptors, is known to be responsible for the beta-subtype selectivity of loreclezole action [231]. Loss or change of drug effect upon mutation in a segment that mediates subtype specific drug action could be due to drug binding, or due to the segment being crucial for the transduction of the drug effect. This question can, at least in principle, be adressed by a combined approach of identifying pocket forming segments in structural models and subsequent mutagenesis and SCAM studies. For instance, β2Met286C has been shown to be protected by propofol from covalent modification by cystein reagents in a concentration dependent manner, a strong hint towards a binding site near this residue [254]. In homology models of $GABA_A$ receptors, this residue indeed is part of a putative pocket (Ernst *et al.*, manuscript in preparation).

This example illustrates how comparative models can contribute to the identification of binding sites. Once a binding site is identified and sequence differences between subunits indicate structural differences in receptor subtypes, a more rational search for subtype selective drugs on the basis of structural pharmacophores can be undertaken. The intrinsic limits of model accuracy can be overcome by indirect structure mapping, for example utilizing SCAM techniques [255] in order to improve the model of the pockets in question.

More accurate models can come only from direct structure determination, for example my means of x-ray crystallography. It can be expected that within the next few years crystallization of $GABA_A$ receptors or their fragments will be successful and atomic resolution structures of one or the other subtype of $GABA_A$ receptors will become available. If only the structure of one $GABA_A$ receptor subtype would be determined successfully, more subtypes can be modeled with much higher accuracy than at the present time. Computational drug screening would then be possible in x-ray crystallographic structures and accurate structural models derived from close homologues. First success to grow crystals of nAChRs has been reported [256]. Some success has also been reported in overexpressing extracellular fragments of glycine receptors [257] and of $GABA_A$ receptors [258] in amounts that would be useful for crystallization experiments. In addition, fragments of the helical domain of

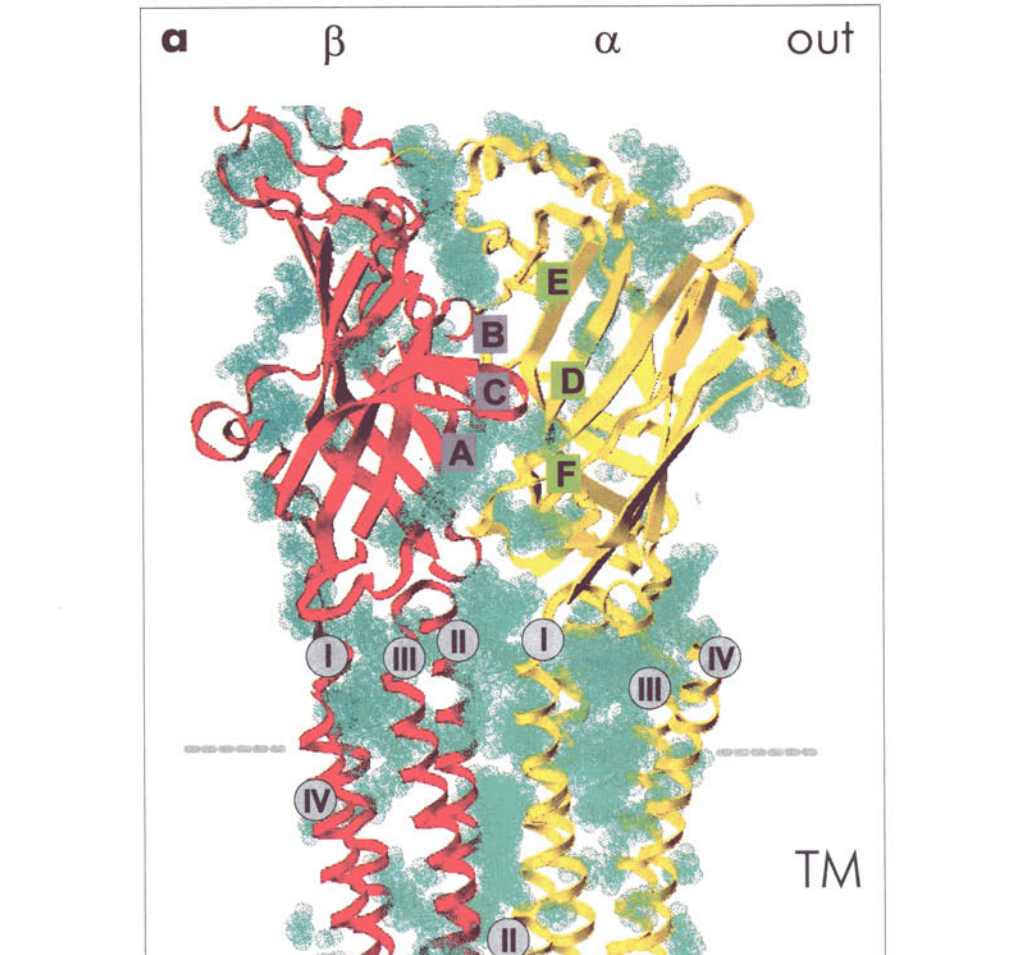


Fig. (5). Model structures of the $GABA_A$ receptor. The protein is shown in ribbon representation. The putative pockets are represented as space filling pseudo-solvent, coloured in cyan. **(a)** Beta-alpha "dimer" of a pentameric receptor seen from the outside, showing the extracellular and transmembrane domain. The remaining 3 subunits are not shown for clarity, the cytoplasmic loop is missing due to lacking structural information. So-called "loops" A thru F (see text), forming the putative GABA pocket, are labeled. Roman numerals I to IV label the four transmembrane helices of each subunit, the approximate part that is inserted into the membrane is indicated by the horizontal dotted lines. "Out" and "in" indicate "cytoplasmic" and "intracellular" side of the lipid bilayer, respectively. Note that the view of some helices is obscured by the pseudo-solvent that fills the putative pockets. **(b)** Perspective (foreshortened) view of the five subunits' transmembrane domain, the 4 helices that comprise each subunit are labeld on two of the subunits. The putative pockets contained at the interface between subunits are pictured in cyan. **(c)** Orthographic (projected) view corresponding to (a), but with the putative pockets located inside of each subunit's 4-helix bundle visualized in cyan.

Table 1. Brain Activities and Behavioural Phenotypes Modulated by $GABA_A$ Receptor Subtypes

Receptors	modulate	animal model
α1βγ2	sedation, anterograde amnesia, seizure susceptibility but seem not to modulate sleep seizure susceptibility, tremor	knockin [162, 163, 165] knockin [165] knockout [140, 141, 261]
α2βγ2	anxiety muscle tone sleep locomotion	knockin [168], drugs [163, 211, 229] knockin [168, 170] knockin [171, 173] knockout [146]
α3βγ2	muscle tone seem not to modulate sleep absence seizures accoustic startle response, psychosis?	knockin [168, 170] knockin [173] knockin [174] knockout, [147]
α5βγ2	learning, memory muscle tone tolerance to sedative action of diazepam	knockin [175, 176] knockout [148] drugs [218-220] knockin [175] knockin [177]
α4β3δ	sleep, seizures, pain, ethanol action	drugs [183, 190] drugs [243]
α4 receptors	premenstrual syndrome seizures	drugs [196, 197] drugs [192, 193]
δ receptors	steroid actions ethanol actions seizures	knockout [158] knockout [159] knockout [160]
β2 receptors	ataxia, sedation, hypothermia, locomotion	knockin [180] knockout [140]
β3 receptors	anesthesia, seizures	knockin [181] knockout [182] knockout [153, 154]
γ2 receptors	anxiety	knockout [157]

glycine receptors [259], as well as extracellular domain fragments of nicotinic receptors [260] have been studied with nmr.

Beyond the exciting prospect to embark on structure guided drug development leading to drugs with high subtype specificity, knowledge of the molecular structure of $GABA_A$ receptors has additional implications on basic research of the nervous system. The identification of residues which affect the *in vitro* potentiation by various drugs offers the possibility to selectively affect the function of these compounds in living animals by introducing the appropriate point mutation into the genome of mice [162, 163, 180, 181]. This will allow us to learn much more about the mechanism of action of many GABAergic drugs, and to identify those parts of the central nervous system and the respective ion channels which mediate specific behaviour. Hopefully, this will open up a whole new area of study in the field of ion channels and enable a much clearer understanding of how psychoactive drugs mediate their effects on the central nervous system.

6. THERAPEUTIC POTENTIAL OF $GABA_A$ RECEPTOR SUBTYPE-SELECTIVE DRUGS

Due to the fact that GABA is the major inhibitory transmitter in the central nervous system and the observation of a widespread distribution of $GABA_A$ receptors in the brain, it is clear that these receptors directly or indirectly modulate most if not all brain functions. At the behavioural level, however, many of these effects cannot be observed because they are counteracted by other effects of this transmitter at the same or at a different level of network organization, or are covered by the dominant effects of GABA on locomotion and vigilance. Recent evidence for the existence of >500 different $GABA_A$ receptor subtypes with distinct regional, cellular and subcellular distribution in the brain (chapter 2) as well as evidence indicating that receptor subtypes containing different α, β, or γ subunits mediate different functions in the brain (chapter 3), point to a tremendous therapeutic potential of $GABA_A$ receptors subtype-selective drugs.

Whereas drugs interacting with $\alpha1\beta\gamma2$ receptors probably will exhibit effects such as sedation, anterograde amnesia, and anticonvulsant properties more or less similar to those of classical benzodiazepines (chapter 3.2.1.), partial agonists selectively addressing $\alpha2\beta\gamma2$ receptors should produce quite selective anxiolytic actions with less side effects. Higher doses of these drugs then should be able to produce muscle relaxation and induce sleep (chapter 3.2.2.). Whereas partial agonists selectively interacting with $\alpha3\beta\gamma2$ receptors might have a potential as anti-absence drugs (chapter 3.2.3.), they also might be suitable as antipsychotics (chapter 3.1.3.) and have additional so far unidentified actions due to the very specific regional distribution of these receptors in the brain. Evidence indicates that partial inverse agonists at $\alpha5\beta\gamma2$ receptors might be able to enhance learning and memory and thus, possibly can be used to improve the cognitive function of patients suffering from Alzheimer´s disease (chapter 3.2.4.). Interestingly, drugs selectively interacting with extrasynaptic $\alpha4\beta\delta$ receptors might have a tremendous potential as non-opioid analgesics, as hypnotics with a new spectrum of actions, and as antiepileptics, and might also be useful for treating the premenstrual syndrome (chapter 4.2.1.). So far, the function of receptors containing $\gamma1$ subunits is not known. But given their very specific regional distribution in the amygdala, pallidum, substantia nigra and inferior olive (chapter 2.2.1.), it can be expected that such drugs will produce highly interesting and quite selective effects. Similarly, no information is available on the function of receptors containing ε, θ, or π subunits. The possible existence of receptors composed of $\alpha3$, θ, and ε subunits and their quite specific location in the dopaminergic, noradrenergic, serotonergic and cholinergic modulatory system (chapters 2.2.1. and 2.2.2.), however, offers the possibility to exert highly selective effects by addressing this completely new type of receptors. Due to the selective location of these receptors, it can be speculated that they might exhibit mood stabilizing and/or antipsychotic effects useful for treatment of depression and schizophrenia. Since development of tolerance and dependence might depend on cooperative actions of different receptor subtypes [177], subtype-selective drugs might also exhibit less tolerance and depencence liability.

Of course, it is the question whether it will be possible to selectively address these various receptor subtypes. However, recent success in the development of drugs exhibiting some selectivity for $\alpha2\beta\gamma2$, $\alpha5\beta\gamma2$, or $\alpha4\beta\delta$ receptors, indicates that further efforts in improving their selectivity will be successful and worthwile (chapter 4). Finally, recent informations on the structure of $GABA_A$ receptors as well as continuously improving techniques for the expression and crystallization of membrane proteins offer the hope that structures of $GABA_A$ receptor subtypes with or without their ligands bound will become available in the near future, thus tremendously enhancing the possibilities for the application of structure-guided drug design. Such information not only will help to develop receptor subtype-selective drugs interacting with the benzodiazepine binding site, but also to develop more potent drugs interacting with so far unidentified drug binding sites at these receptors that also might exhibit some receptor subtype selectivity (chapter 4.2.3.). For instance drugs selectively interacting with the $\alpha+\beta-$ interface of $GABA_A$ receptors should be influenced by the type of the α as well as the β subunit isoform present in these receptors and thus, offer the possibility to differentiate also for receptors containing different β subunits. It is of course difficult to predict the action of such drugs. But since receptors containing $\beta2$ subunits mediate the ataxic, sedative and hypothermic effects of etomidate, whereas the anesthetic qualities of this drugs as well as the anticonvulsant properties seem to mediated via $\beta3$ subunit containing receptors, such a differentiation could be quite helpful.

Overall, these new developments should cause a revival of $GABA_A$ receptor research and strongly stimulate the development of drugs with a higher $\alpha2$-, $\alpha3$-, or $\alpha5$-receptor subtype selectivity. Targeting drugs to $GABA_A$ receptor subtypes holds the promise of increased clinical specificity compared with the classical benzodiazepines, which act more or less indiscriminately on all diazepam-sensitive $GABA_A$ receptors. In addition, therapeutic indications beyond those of the classical benzodiazepine drugs may emerge from drugs specifically enhancing or reducing the activity of minor $GABA_A$ receptor subtypes located at certain neurons only.

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ABBREVIATIONS

GABA	=	γ-Aminobutyric acid
$GABA_A$ receptor	=	γ-Aminobutyric acid type A receptor
TM	=	transmembrane
TBPS	=	Tert. butyl-bicyclophosphorothionate
nAChR	=	Nicotinic acetylcholine receptor
AChBP	=	Acetylcholine binding protein
SCAM	=	Substituted cysteine accessibility method
THIP	=	4,5,6,7-Tetrahydroisoxazolo[5,4-c]pyridin-3-ol
4-PIOL	=	5-(4-Piperidyl)isoxazol-3-ol;

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