Structure, Pharmacology, and Function of GABA\textsubscript{A} Receptor Subtypes

I. Chapter Overview

Gamma-aminobutyric acid type A (GABA\textsubscript{A}) receptors are the most important inhibitory transmitter receptors in the central nervous system (CNS). They are chloride channels that can be opened by GABA and modulated by a variety of different drugs such as benzodiazepines, barbiturates, neuroactive steroids, anesthetics, and convulsants. These receptors are composed of five subunits that can belong to different subunit classes, giving rise to a large variety of distinct receptor subtypes. Depending on their subunit composition, these receptor subtypes exhibit distinct pharmacological and electrophysiological properties. In this chapter, the pharmacology of GABA\textsubscript{A} receptors is reviewed, new compounds interacting with these receptors are described, and novel receptor subtype-selective compounds are discussed. In addition, evidence for the function of distinct GABA\textsubscript{A} receptor subtypes in the brain is summarized. Finally, information
on the molecular structure of the extracellular and transmembrane domain of GABA<sub>A</sub> receptors based on the X-ray crystallographic structure of the acetylcholine binding protein and on the cryo-electronmicroscopic structure of the nicotinic acetylcholine receptor is provided. This structure contains multiple solvent accessible cavities that possibly are used by a variety of allosteric modulators for their interaction with GABA<sub>A</sub> receptors, thus explaining the rich pharmacology of these important receptors.

II. Introduction

GABA is the most abundant inhibitory neurotransmitter in the CNS. In the brain, 17–20% of all neurons are GABAergic (Somogyi et al., 1998). Most of the physiological actions of GABA are generated via GABA<sub>A</sub> receptors. These receptors are chloride ion channels 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 (Sieghart, 1995). These drugs produce at least part of their clinically relevant effects by interacting with distinct allosteric binding sites on GABA<sub>A</sub> receptors (Sigel and Buhr, 1997; Smith and Olsen, 1995). Based on the pharmacological action of these drugs, it was concluded that GABA<sub>A</sub> receptors are involved in controlling the excitability of the brain (Fritschy et al., 1999; Olsen and Avoli, 1997), in the modulation of anxiety (Nutt et al., 1990; Pratt, 1992), of feeding and drinking behavior (Berridge and Pecina, 1995; Cooper, 1989), circadian rhythms (Turek and Van Reeth, 1988; Wagner et al., 1997), cognition, vigilance, memory, and learning (Izquierdo and Medina, 1991; Paulsen and Moser, 1998; Sarter et al., 1988).

III. Heterogeneity of GABA<sub>A</sub> Receptors

GABA<sub>A</sub> receptors are composed of five subunits that consist of a large N-terminal extracellular domain, four transmembrane (TM) domains, and a large intracellular loop between TM3 and TM4 (Nayem et al., 1994; Schofield et al., 1987; Tretter et al., 1997). So far, a total of six α, three β, three γ, one δ, one ε, one π, one θ, and three ρ subunits of GABA<sub>A</sub> receptors have been cloned and sequenced from the mammalian nervous system, and for several of these subunits splice variants have been identified (Barnard et al., 1998; Sieghart and Sperk, 2002). 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<sub>A</sub> receptor
type subunits in species from man down to nematodes, in a study no new GABA_{A} receptor subunits were detectable in the human genome (Simon et al., 2004). In nonmammalian species, however, additional subunit homologs have been identified (Barnard et al., 1998; Hosie et al., 1997; Schuske et al., 2004).

GABA_{A} receptors are widely distributed all over the brain, and evidence has accumulated indicating an enormous heterogeneity of these receptors (Sieghart and Sperk, 2002). Thus, in situ hybridization studies (Laurie et al., 1992; Persohn et al., 1992; Wisden et al., 1992) and immunohistochemical studies (Fritschy et al., 1992; Pirker et al., 2000; Sperk et al., 1997) have demonstrated that each one of the subunits has a distinct regional and cellular distribution in the brain. Whereas some cell types contain only a few GABA_{A} receptor subunits, others express most, if not all GABA_{A} receptor subunits. If all these subunits could randomly coassemble with each other, more than 150,000 GABA_{A} receptor subtypes with different subunit composition and arrangement could be formed (Burt and Kamatchi, 1991). GABA_{A} receptors can be, and sometimes are, composed of up to five different subunits. However, due to restrictions imposed during assembly of GABA_{A} receptors, not all receptors that can be formed theoretically are actually formed in the brain. Nevertheless, from the number of different receptor subtypes so far isolated using subunit-specific antibodies, it was estimated that more than 500 distinct GABA_{A} receptors probably do exist in the brain (Sieghart and Sperk, 2002). The number of receptors that are relatively abundant, however, is much smaller. Thus, the majority of native receptors are composed of \( \alpha \), \( \beta \), and \( \gamma \) subunits. In minor receptor subtypes, the \( \delta \), \( \epsilon \), and \( \pi \) subunits seem to be able to replace the \( \gamma \) subunit in GABA_{A} receptors, whereas the \( \theta \) subunit might be able to replace a \( \beta \) subunit. But systematic studies on the composition of GABA_{A} receptors containing \( \epsilon \), \( \pi \), or \( \theta \) subunits so far are not available (Sieghart and Sperk, 2002).

Evidence is now convincing that receptors composed of \( \alpha \), \( \beta \), and \( \gamma \) subunits contain two \( \alpha \), two \( \beta \), and one \( \gamma \) subunit (Chang et al., 1996; Farrar et al., 1999; Im et al., 1995; Tretter et al., 1997) and that in these receptors a total of four alternating \( \alpha \) and \( \beta \) subunits are connected by a \( \gamma \) subunit (Baumann et al., 2002; Ernst et al., 2003; Tretter et al., 1997). Whether all receptors composed of \( \alpha \beta \gamma \) subunits or those composed of \( \alpha \beta \delta \), \( \alpha \beta \epsilon \), or \( \alpha \beta \pi \) subunits exhibit the same subunit stoichiometry and subunit arrangement, presently is not known.

\( \rho \) Subunits originally were assumed not to coassemble with other classes of GABA_{A} receptor subunits (Cutting et al., 1991; Enz and Cutting, 1998). However, it has been demonstrated that these subunits also can assemble with GABA_{A} \( \gamma 2 \) or glycine receptor subunits and form functional receptors with properties found in certain cell types of the retina (Pan et al., 2000; Qian and Rips, 1999), brain stem (Milligan et al., 2004), hippocampus
(Hartmann et al., 2004), or other brain regions (Arakawa and Okada, 1988; Drew et al., 1984; Strata and Cherubini, 1994). In addition, $\rho$ subunits can form homo- as well as hetero-oligomeric channels with other $\rho$ subunits that exhibit properties of the previously characterized GABA$_C$ receptors (Bormann, 2000). Since $\rho$ subunits are structurally part of the family of GABA$\textsubscript{A}$ receptor subunits, it was recommended that $\rho$-containing receptors should be classified as a specialized set of the GABA$\textsubscript{A}$ receptors (Barnard et al., 1998).

IV. Pharmacology of GABA$_\text{A}$ Receptors

GABA$\textsubscript{A}$ 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, the number of which is constantly increasing (Korpi et al., 2002; Sieghart, 1995). Currently, only three distinct binding sites present on GABA$\textsubscript{A}$ receptors can be directly investigated by appropriate radioligand binding studies: the GABA/muscle-, the benzodiazepine-, and the $\text{r}$-butyl-bicyclophosphorothionate (TBPS)/picrotoxinin-binding site (Sieghart, 1995). Using such studies, compounds competitively interacting with the radioligands and thus, directly binding to the respective sites could be identified. The interaction of all the other drugs with GABA$\textsubscript{A}$ receptors can only be investigated by electrophysiology or by studying the allosteric effects of these drugs at the [$^3$H]muscle-, [$^3$H]benzodiazepine-, or [$^{35}$S]TBPS-binding site. These techniques, however, in most cases do not allow to clarify whether the allosteric effects of different ligands are mediated via the same or distinct binding sites. Therefore, the total number of allosteric binding sites present on GABA$\textsubscript{A}$ receptors is not known. Structure–activity studies for most of the allosteric modulators of GABA$\textsubscript{A}$ receptors are thus not possible at present, preventing a structurally guided development of novel ligands for the respective binding site.

The aim of this chapter is to provide a short overview on the pharmacology of GABA$\textsubscript{A}$ receptors and their subtypes as well as on new developments in this field. A complete coverage of all interactions of drugs with GABA$\textsubscript{A}$ receptor is out of the scope of this article. More detailed descriptions on the complex interactions of different drugs with GABA$\textsubscript{A}$ receptors have been published previously and are referred to in the text.

A. GABA Binding Site of GABA$\textsubscript{A}$ Receptors

Mutagenesis studies on recombinant GABA$\textsubscript{A}$ receptors have identified several amino acid residues on $\alpha$ and $\beta$ subunits that seem to be important for binding of GABA (Fig. 1, compound 1) or muscimol (Fig. 1, compound 2)
FIGURE 1. GABA<sub>α</sub> receptor ligands. Compounds 1–3 are GABA site agonists. Compounds 4 and 5 are benzodiazepine site agonists, and compound 6 is a GABA<sub>α</sub> receptor antagonist that inhibits the action of GABA via an allosteric site probably located within the ion channel.

(Smith and Olsen, 1995). It thus was concluded that the GABA binding site of GABA<sub>α</sub> receptors is located at the interface of an α and a β subunit. Currently, only a few different classes of compounds are known as ligands for the GABA binding site (Frolund et al., 2002). Studies on recombinant GABA<sub>α</sub> receptors have indicated that the currently known full agonists (exhibiting an efficacy comparable to GABA) or antagonists at the GABA binding site of these receptors seem not to exhibit a significant receptor subtype selectivity (Adkins et al., 2001; Ebert et al., 1994; Luddens and Korpi, 1995). In addition, the use of these compounds is associated with severe side effects. Full GABA agonists that open all GABA<sub>α</sub> receptor-associated chloride channels indiscriminately, cause inhibition of most neuronal systems, thus severely interfering with the function of the brain, whereas GABA antagonists precipitate anxiety and convulsions.

However, several partial agonists at the GABA binding site of GABA<sub>α</sub> receptors, such as imidazole-4-acetic acid, piperidine-4-sulfonic acid, THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol), or 4-PIOL (5-(4-piperidyl)isoxazol-3-ol), have been developed that exhibit some receptor subtype-dependent potency and efficacy (Frolund et al., 2002). Thus, THIP (Fig. 1, compound 3) is approximately 10 times more potent at α4β3δ than at α4β3γ2S receptors. This compound, which is currently developed as Gaboxadol, also seems to exhibit a highly interesting spectrum of in vivo actions. For instance, it seems to have potent analgesic effects comparable to that of morphine and seems to improve the quality of sleep (Krogsgaard-Larsen...
et al., 2004). The full spectrum of pharmacological actions of THIP at different GABA\(_A\) receptor subtypes, however, has still not been investigated.

B. Benzodiazepine Binding Site of GABA\(_A\) Receptors

Benzodiazepines, such as diazepam (Fig. 1, compound 4), are the strongest anticonvulsive, muscle relaxant, sedative-hypnotic, and anxiolytic compounds in clinical use (Woods et al., 1992). They enhance the action of GABA on GABA\(_A\) receptors by increasing the GABA-induced frequency of opening of the chloride channels (Study and Barker, 1981) and thus, allosterically modulate these receptors. Benzodiazepines and compounds interacting with the benzodiazepine site of GABA\(_A\) receptors only can modulate ongoing GABAergic activity. These compounds cannot elicit chloride ion flux in the absence of GABA (Macdonald and Olsen, 1994; Study and Barker, 1981) and thus, exhibit an extremely low degree of toxicity.

Mutagenesis studies have identified several amino acid residues on \(\alpha\) and \(\gamma2\) subunits of recombinant GABA\(_A\) receptors that seem to be important for binding of benzodiazepines and compounds interacting with the benzodiazepine binding site (Sigel, 2002). It thus was concluded that the benzodiazepine binding site of GABA\(_A\) receptors is located at the interface of an \(\alpha\) and a \(\gamma2\) subunit.

I. Agonists, Antagonists, Inverse Agonists

Many different classes of compounds interact with the benzodiazepine binding site of GABA\(_A\) receptors (Adkins et al., 2001; Atack, 2005; Huang et al., 2000; Korpi et al., 2002; Sieghart, 1995; Teuber et al., 1999). In each of these classes, compounds could be identified that enhanced or reduced the action of GABA on GABA\(_A\) receptors. 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, enhance vigilance, learning, and memory and are called “inverse agonists.” A third class of compounds obviously stabilizes a conformational state that does not directly change GABA-induced chloride flux. These compounds in most cases do not elicit behavioral effects on their own but prevent interaction of “agonists” or “inverse agonists” with these receptors. They are therefore called allosteric “antagonists” (Sieghart, 1995).

The efficacy of compounds for eliciting such effects can be different. Thus, in addition to full agonists or full inverse agonists exhibiting a maximum enhancement or reduction of GABAergic currents, respectively, there are compounds with weaker actions (partial agonists or partial inverse agonists).
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 (Barnard et al., 1998; Hevers and Luddens, 1998; Puia et al., 1991; Wafford et al., 1993). 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 (Hevers and Luddens, 1998; Puia et al., 1991; Wafford et al., 1993). 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 $\alpha_1\beta_2$, $\alpha_2\beta_2$, $\alpha_3\beta_2$, or $\alpha_5\beta_2$ subunits, their efficacy at individual receptor subtypes is different (Hevers and Luddens, 1998), thus generating their specific anxiolytic, anticonvulsant, muscle relaxant, and sedative-hypnotic activity spectrum (Woods et al., 1992).

2. Heterogeneity of 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 $\alpha$ and $\gamma$ subunits, its binding properties are influenced by the types of the subunits forming this interface. Since there are 6 different $\alpha$ and 3 different $\gamma$ subunits in the mammalian nervous system, up to 18 different GABA_A receptor-associated benzodiazepine binding sites may exist. Most compounds interacting with the benzodiazepine binding site are inactive or only weakly active at receptors containing $\gamma_1$ subunits (Hevers and Luddens, 1998; Puia et al., 1991). Although there seems to be some activity of benzodiazepine ligands at receptors containing $\gamma_3$ subunits (Hevers and Luddens, 1998), these receptors exhibit a very low abundance in the brain (Pirker et al., 2000). 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 $\alpha_1\beta_2$, $\alpha_2\beta_2$, $\alpha_3\beta_2$, or $\alpha_5\beta_2$ subunits.

Receptors composed of $\alpha_4\beta_2$ or $\alpha_6\beta_2$ subunits exhibit a drastically different pharmacology. Most of the classical benzodiazepines, such as diazepam, flunitrazepam, or clonazepam, do not interact with these receptors (Hevers and Luddens, 1998; Sieghart, 1995; Wafford et al., 1996). Imidazobenzodiazepines, such as Ro15-4513 or flumazenil, however, interact with these receptors but also with $\alpha_1\beta_2$, $\alpha_2\beta_2$, $\alpha_3\beta_2$, or $\alpha_5\beta_2$ receptors (Huang et al., 2000; Sieghart, 1995; Zhang et al., 1995). Since the selectivity of new compounds aimed to address $\alpha_4\beta_2$ or $\alpha_6\beta_2$ receptors is only weak (Gu et al., 1993; Huang et al., 2000; Knoflach et al., 1996;
Wong et al., 1993; Zhang et al., 1995), the behavioral effects mediated by these receptors are not known.

3. Benzodiazepine Site Ligands with Some GABA<sub>A</sub> Receptor Subtype Selectivity

Since most of the benzodiazepine site ligands modulate \( \alpha_1 \beta_2 \gamma_2 \), \( \alpha_2 \beta_2 \gamma_2 \), \( \alpha_3 \beta_2 \gamma_2 \), or \( \alpha_5 \beta_2 \gamma_2 \) receptors to a more or less similar extent, it is no surprise that the clinical spectrum of action of these compounds is quite similar (Woods et al., 1992). Only some of the drugs in current use, such as the sedative/hypnotic compound zolpidem (Fig. 1, compound 3), exhibit a selectivity for \( \alpha_1 \) subunit-containing receptors (Hevers and Luddens, 1998; Sieghart, 1995).

Due to the clinical importance of benzodiazepine-type drugs, a tremendous effort was put into the development of receptor subtype-selective drugs. During the last few years, compounds were developed with a preferential affinity for \( \alpha_2 \beta_2 \gamma_3 \) or \( \alpha_5 \) subunit-containing receptors (Huang et al., 1999, 2000; Li et al., 2003; Liu et al., 1996; Quirk et al., 1996; Teuber et al., 1999; Zhang et al., 1993). However, drugs with a selective affinity do not necessarily exhibit a selective efficacy for the respective receptor subtype. Therefore, functional effects of compounds as measured by electrophysiological techniques or fluorescence imaging technologies using voltage-sensitive dyes (Adkins et al., 2001; Gonzalez and Tsien, 1997; Gonzalez et al., 1999) in cells expressing recombinant GABA<sub>A</sub> receptor subtypes are currently used for developing of compounds with a receptor subtype-selective action.

Such approaches have led to the identification of compounds such as the triazolo[4,3-b]pyridazine L-838,417 (Fig. 2, compound 1), a benzodiazepine site ligand with high affinity to \( \alpha_1 \), \( \alpha_2 \), \( \alpha_3 \), and \( \alpha_5 \) subunit-containing receptors. This compound, however, acts as a partial agonist on \( \alpha_2 \), \( \alpha_3 \), and \( \alpha_5 \)-containing receptors and does not enhance the GABA response on \( \alpha_1 \) receptors (McKernan et al., 2000). In animal models, this compound exhibited nonsedating anxiolytic properties, and this also seems to hold true for studies in nonhuman primates (Rowlett et al., 2005).

The compound SL651.498 (Fig. 2, compound 2) exhibits high affinity for receptors containing \( \alpha_1 \) or \( \alpha_2 \) subunits but 10 times lower affinity for receptors containing \( \alpha_3 \) subunits (Griebel et al., 2003). Nevertheless, it behaves as a full agonist at recombinant GABA<sub>A</sub> receptors containing \( \alpha_2 \) or \( \alpha_3 \) subunits and as a partial agonist at recombinant GABA<sub>A</sub> receptors containing \( \alpha_1 \) or \( \alpha_3 \) subunits. SL651.498 produced anxiolytic-like and skeletal muscle relaxant effects similar to those of benzodiazepines but with drastically reduced side effects (Griebel et al., 2003; Licata et al., 2005).

In another attempt, a tricyclic pyridone (Fig. 2, compound 3) with functional selectivity for the \( \alpha_3 \) over the \( \alpha_1 \) containing subtype has been
FIGURE 2 Novel benzodiazepine binding site ligands with selective efficacy for certain GABA\(_A\) receptor subtypes.

developed that was efficacious in animal models of anxiety and showed no sedation or potentiation of ethanol effects (Crawforth et al., 2004). A compound in the related 3-heteroaryl-2-pyridone class (Fig. 2, compound 4) (Collins et al., 2002) was a selective inverse agonist at \(\alpha_3\)-containing receptors with minimal efficacy at the \(\alpha_1\)- and \(\alpha_2\)-containing receptors. When evaluated in animal models, this compound was found to be anxiogenic, suggesting an important role for \(\alpha_3\)-containing GABA\(_A\) receptors in anxiety (Atack et al., 2005; Whiting, 2003).

Evidence indicated that it is possible to also develop compounds with selective efficacy for \(\alpha_5\)-containing GABA\(_A\) receptors. FG 8094/L-655.708 (Fig. 2, compound 5) was one of the first such compounds developed. It exhibited an affinity for \(\alpha_5\) receptors that was 50-fold higher than that to \(\alpha_2\) or \(\alpha_3\) receptors, 100-fold higher than that to \(\alpha_1\), and 200-fold higher than that to \(\alpha_6\) receptors (Teuber et al., 1999). This compound acts as a partial inverse agonist on \(\alpha_5\)-containing receptors (Sternfeld et al., 2004) and its tentative use for cognition enhancement (see later) has been patented. Two other compounds, one of them compound 6 (Fig. 21) (Chambers et al., 2002, 2003; Sternfeld et al., 2004), have been developed with partial inverse agonist properties for \(\alpha_5\) receptors but little or no efficacy at other receptor subtypes (Chambers et al., 2002, 2003; Sternfeld et al., 2004). In agreement
with the notion that z5-containing receptors might influence learning and memory (see in a later section) these compounds enhance cognition in animals without anxiogenic and convulsive effects (Chambers et al., 2002, 2003; Sternfeld et al., 2004). These data clearly indicate the potential utility of such compounds as cognitive enhancers in disorders such as mild cognitive impairment and Alzheimer’s disease.

4. Interaction of Benzodiazepine Site Ligands with Other GABA<sub>A</sub> Receptor Binding Sites

In addition to their interaction with the benzodiazepine binding site located at the α1/γ interface of GABA<sub>A</sub> receptors, at least some benzodiazepines or benzodiazepine site ligands can also interact with binding sites present on receptors composed of α and β subunits, only (Im et al., 1995, 1998; Khom et al., 2006; Thomet et al., 1999; Walters et al., 2000). Such interactions so far have not been studied extensively and seem to be of low affinity. It can be expected, however, that more thorough investigation of these sites will identify benzodiazepine site ligands with high affinity for these sites and that the properties of the respective binding sites will differ depending on the type of α and β subunits present in the receptors. In addition, receptors composed of α5ω (Hanchar et al., 2005) also seem to be able to bind certain benzodiazepine binding site ligands with high affinity.

C. TBPS-Binding Site of GABA<sub>A</sub> Receptors

TBPS (Fig. 1, compound 6) and picrotoxinin are convulsants that non-competitively block GABA-gated chloride flux by binding to one or more sites located within or close to the chloride channel (Korpi et al., 2002). The majority of electrophysiological experiments have been performed with picrotoxinin, an equimolar mixture of the inactive picrotin and the active compound picrotoxinin, because picrotoxinin exhibits a rapid onset of action (Yoon et al., 1993). TBPS exhibits a slow onset of action in electrophysiological experiments but is much better suited for receptor binding studies, where it exhibits a high affinity for the picrotoxinin binding site (Squires et al., 1983). The fast onset of picrotoxinin inhibition on repetitive GABA application and the slow onset in the absence of GABA suggest that an open channel facilitates the actions of this compound (Inoue and Akaike, 1988). Binding of [35S]TBPS can be competitively inhibited by picrotoxinin, pentylentetrazole, and convulsant barbiturates (Maksay and Simonyi, 1985; Maksay and Ticku, 1985a, b) and presumably also by a variety of insecticides, such as lindane and dieldrin, or by bicyclic cage compounds such as the 4-propyl-4' -ethynylbicycloorthobenzoate EBOB (Korpi et al., 2002).

In addition, binding of [35S]TBPS can also be allosterically inhibited by GABA or GABA binding site agonists (Korpi et al., 2002; Sieghart, 1993), in
line with the assumption that not only $[^{35}S]$TBPS binding but also its unbinding is facilitated when the channel is open. GABA_A receptor antagonists, such as bicuculline and SR 95531, inhibit these effects of GABA on $[^{35}S]$TBPS binding. Furthermore, compounds that are able to allosterically open the GABA_A receptor associated chloride channel (e.g., barbiturates, etozolate, etomidate, and steroids, see later) are also able to reduce binding of TBPS (Sieghart, 1995). Benzodiazepines inhibited the binding of TBPS only in the presence of micromolar GABA concentrations, in line with the observation that benzodiazepines affect chloride ion conductance only in the presence of GABA. On the other hand, compounds reducing the efficacy of GABA at GABA_A receptors (by reducing the frequency of opening of GABA-induced chloride channels), such as some convulsant β-carbolines, enhanced TBPS binding (Korpi et al., 2002; Sieghart, 1995). These results are in line with the assumption that the high affinity TBPS binding site might be associated with the “closed” conformation of the chloride channel and might represent TBPS “trapped” in its binding site. In any case, the degree of TBPS binding in the presence of GABA seems to closely reflect the functional state of GABA_A receptors.

D. Interaction of Barbiturates with GABA_A Receptors

Sedative-hypnotic barbiturates, such as pentobarbital (Fig. 3A, compound 1), phenobarbital, or secobarbital in electrophysiological studies, enhance the actions of GABA by increasing the average channel open duration but have no effect on channel conductance or opening frequency (Study and Barker, 1981). The effects of barbiturates on GABA_A receptors rank in order with their potency as anesthetics (Olsen, 1982) indicating that GABA_A receptors are prime candidates in mediating these effects. At concentrations > 50 μM barbiturates are able to directly open GABA_A receptor-associated chloride channels in the absence of GABA (Hevers and Luddens, 1998; Korpi et al., 2002; Sieghart, 1995), and at still higher concentrations they change desensitization of receptors, suggesting the existence of several sites of interaction of barbiturates with GABA_A receptors (Sieghart, 1995). Due to the low affinity of barbiturates, the respective binding sites cannot be investigated directly by receptor binding studies. But sedative-hypnotic barbiturates allosterically enhance $[^{3}H]$GABA, $[^{3}H]$muscimol, or $[^{3}H]$flunitrazepam binding. In contrast to convulsant barbiturates, which allosterically enhance $[^{35}S]$TBPS binding, the sedative-hypnotic barbiturates and some related compounds, such as etomidate (Fig. 3A, compound 2) and etozolate (Fig. 3A, compound 3), seem to allosterically reduce $[^{35}S]$TBPS binding (Sieghart, 1995). These results indicate that the site of action of sedative-hypnotic barbiturates is different from that of GABA, benzodiazepines, or TBPS. Since the modulatory action of pentobarbital can be observed already in homo-oligomeric GABA_A receptors composed of either α, β, γ, or δ
subunits (Sieghart, 1995), the binding site(s) probably are highly conserved between subunits. Whether etomidate or etazolate exhibit a similar mechanism of action currently is not known.

E. Interaction of Steroids with GABA<sub>A</sub> Receptors

Several steroids, such as the anesthetic alphaxalone (Fig. 3A, compound 4) or the sedative-hypnotic, anxiolytic, and anticonvulsant 3α-hydroxylated, 5α-, or 5β-reduced metabolites of progesterone (Fig. 3A, compound 5) and deoxycorticosterone at nM concentrations enhance GABA-stimulated chloride conductance, whereas at >1 μM concentrations these compounds, like barbiturates, produce direct opening of the GABA<sub>A</sub>
receptor-associated chloride channel (Belelli and Lambert, 2005; Lambert et al., 1995, 2001; Sieghart, 1995). This points to the existence of at least two different steroid binding sites on GABA$_A$ receptors. Steroids active at the GABA$_A$ receptor increase both the frequency and the duration of chloride channel opening (Peters et al., 1988). These steroids enhance $[^3]H$muscimol and $[^3]H$flunitrazepam binding and allosterically reduce $[^3]S$TBPS binding. Other experiments indicate that steroids interact with barbiturates in a manner inconsistent with competition with a common binding site (Sieghart, 1995). Together, these experiments provided evidence for a site of action of steroids distinct from the binding sites for GABA, benzodiazepines, barbiturates, and TBPS. The stereoselective action and nM affinity of steroids suggest the existence of a high affinity steroid binding site rather than an unspecific membrane interaction of steroids (Belelli and Lambert, 2005; Lambert et al., 2001; Sieghart, 1995).
The location of the steroid binding sites on GABA<sub>A</sub> receptors currently is not known.

In addition to steroids that enhance the actions of GABA on GABA<sub>A</sub> receptors, other steroids, such as pregnenolone sulfate (Fig. 3A, compound 6) and dehydroepiandrosterone (DHEAS), act as noncompetitive antagonist at these receptors. These compounds inhibit GABA-induced currents and exhibit excitatory actions on neurons (Lambert et al., 2001; Sieghart, 1995). It is not clear whether the site of action of these two compounds is identical and whether they are interacting with the same binding site(s) as steroids that enhance the action of GABA on GABA<sub>A</sub> receptors.

Most studies have indicated that neither potency nor efficacy of neuroactive steroids appear to depend significantly on the subunit composition of receptors (Belelli and Lambert, 2005; Lambert et al., 2001). This conclusion is supported by the finding that neuroactive steroids can modulate homooligomeric receptors composed of β subunits (Sieghart, 1995) and thus, neither α nor γ subunits seem to be necessary for the interaction of steroids with GABA<sub>A</sub> receptors. Nevertheless, electrophysiological studies have demonstrated that neurosteroids act differentially at synaptic GABA<sub>A</sub> receptors in different brain regions. Whether this heterogeneity is the result of the expression of distinct GABA<sub>A</sub> receptor subtypes or is caused by other factors, such as phosphorylation or local steroid metabolism (Belelli and Lambert, 2003; Lambert et al., 2001; Pinna et al., 2000), is not clear.

Evidence, however, seems to indicate that neurosteroids especially stimulate GABA<sub>A</sub> receptors such as extrasynaptic δ-containing receptors (Stell et al., 2003). 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 (Mihalek et al., 1999).

F. Interaction of Anesthetics with GABA<sub>A</sub> Receptors

In addition to the anesthetic steroids, barbiturates, and related compounds, such as etomidate and etazolate mentioned previously, a variety of volatile and other intravenous anesthetics from different chemical classes modulate the GABA<sub>A</sub> receptor (Franks and Lieb, 1994; Rudolph and Antkowiak, 2004; Sieghart, 1995). Thus, the volatile anesthetics isoflurane, enflurane (Fig. 3B, compound 1), or halothane at high micromolar or low millimolar concentrations, or the general anesthetics chlorromethiazole or propofol (Fig. 3B, compound 2) at low micromolar concentrations enhance GABA-gated chloride currents. At high concentrations these compounds, like barbiturates and steroids, directly open GABA<sub>A</sub> receptor-associated chloride channels, and these currents can be blocked by picrotoxinin and are sensitive to the competitive GABA antagonist bicuculline (Hevers and
Luddens, 1998; Sieghart, 1995). The anesthetic binding site of GABA\(_A\) receptors seems to depend on the type of \(\beta\) subunit present in these receptors. Its location, however, has not been unequivocally identified (see in a later section), and it is not clear whether the various anesthetics including barbiturates and steroids mediate their effects via the same or different binding sites. In addition to their interaction with GABA\(_A\) receptors, they also affect glutamate and nACH receptors (Franks and Lieb, 1994; Hevers and Luddens, 1998). But GABA\(_A\) receptors are the prime candidates for mediating the anesthetic effects of these substances in vivo.

G. Interaction of Other Compounds with GABA\(_A\) Receptors

1. Interactions Influenced by the Type of \(\alpha\) Subunits

   a. Furosemide and Amiloride  Several compounds have been identified that seem to interact with novel, so far unidentified binding sites that are modulated by the type of \(\alpha\) subunit present in GABA\(_A\) receptors. Thus, for instance, the diuretic furosemide (Fig. 3B, compound 3) exhibits approximately a 100-fold selectivity for \(z6\)- over \(z1\)-containing receptors (Korpi et al., 1995). This compound not only blocks \(z6\) receptors but also (with less affinity) \(z4\) receptors (Knoll et al., 1996; Korpi and Luddens, 1997). Similarly, the diuretic amiloride (Fisher, 2002) acted as antagonist of GABA\(_A\) receptors in an \(z\) subunit dependent way by reducing the sensitivity of the receptor to GABA without affecting the maximal current amplitude. In contrast to furosemide, which in addition showed some \(\beta\)-subunit dependence (see in a later section), amiloride showed no additional dependence on the identity of \(\beta\) or \(\gamma\) subunits (Fisher, 2002). Its structure could thus be useful for developing drugs targeting this unique modulatory site on GABA\(_A\) receptors.

   b. \(\gamma\)-Butyrolactones  The actions of \(\gamma\)-butyrolactones, such as \(z\)-EMTBL (Fig. 3B, compound 4), also seem to be influenced by the \(z\) subunit type (El Hadri et al., 2002; Mathews et al., 1994). It was demonstrated that GABA responses in \(z1\beta2\gamma2\)-transfected cells or early granule neurons from the cerebellum were potentiated by \(\gamma\)-butyrolactones whereas those in \(z6\beta2\gamma2\) transfected cells or mature granule neurons were not significantly altered (Mathews et al., 1994).

   c. ROD 188  (\(+\))-ROD188 (Fig. 3B, compound 5) shares structural similarity with bicuculline (Razet et al., 2000; Sigel et al., 2001; Thomet et al., 2000) and allosterically stimulated GABA-induced currents in \(z1\beta2\gamma2\) and \(z1\beta2\) receptors. This indicated that the respective binding site does not require a \(\gamma\) subunit. In addition, the effect of this compound was larger in
x6 subunit-containing GABA<sub>A</sub> receptors (Sigel et al., 2001; Thomet et al., 2000).

d. Structural Analog of the Fluoroquinolone Antibiotic Norfloxacin  A structural analog of the fluoroquinolone antibiotic norfloxacin was identified (Fig. 3B, compound 6) (Johnstone et al., 2004) that potentiated submaximal GABA currents in HEK-293 cells expressing human α2β2γ2L but not α1β2γ2L GABA<sub>A</sub> receptors. This compound seemed to modulate GABA<sub>A</sub> receptors via a novel binding site not identical with the sites for TBPS, GABA, benzodiazepines, barbiturates, neuroactive steroids, norfloxacin, or lorazepole and induced anxiolytic effects with a maximum efficacy comparable to the optimal effect of diazepam. Unlike diazepam, however, this compound had no CNS depressant effects in the range of doses tested (Johnstone et al., 2004). Unfortunately, the action of this interesting compound on other receptor subtypes so far has not been investigated.

2. Interactions Influenced by the Type of β Subunits

a. Lorazepam, Etomidate, DMCM, Furosemide  It is widely accepted that the type of the β subunit present in a GABA<sub>A</sub> receptor does not significantly influence the GABA, benzodiazepine, barbiturate, propotol, or steroid site pharmacologies of human GABA<sub>A</sub> receptor subtypes composed of γ<sub>1</sub> subunits (Hadingham et al., 1993; Smith et al., 2004). However, a number of modulators of the GABA<sub>A</sub> receptor, for example lorazepam (Fig. 3B, compound 7) (Warford et al., 1994; Wingrove et al., 1994), etomidate (Belledi et al., 1997), the β-carboline DMCM (Stevenson et al., 1995), or furosemide (Thompson et al., 1999), have been identified that exhibit selectivity for β2/β3 over β1 receptors. 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, which 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.

b. Salicylidene Salicylhydrazide  Salicylidene salicylhydrazide was one of the first compounds with a selectivity for receptors containing the β1 subunit (Thompson et al., 2004). 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 (Thompson et al., 2004). 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. 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.

c. Nonsteroidal Anti-inflammatory Agents In a subsequent study, various anti-inflammatory agents including mefenamic acid, flufenamic acid (Fig. 3B, compound 8), meclofenamic acid, tolfenamic acid, nimilumic acid, and diflunisal were investigated (Smith et al., 2004). 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. 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 and might become lead compounds for the development of more selective compounds with higher affinity and efficacy.

3. Interactions Modulated by Other Subunits

a. Tracazolate The pyrazolopyridine tracazolate (Fig. 3B, compound 9) 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 barbiturals) (Patel et al., 1985). 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 (Thompson et al., 2002). The allosteric modulation induced by the binding site mediating the effects of tracazolate seems thus to be especially sensitive to the receptor subunit composition.

b. Ethanol 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 (Wallner et al., 2003). Ethanol effects on these targets are seen only at fairly high concentrations (above 60 mM). 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 (Wallner et al., 2003). 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 ten-fold more effective on β3- than on β2-containing α4β3δ and α6β3δ receptors. Since these receptors presumably are located extrasynaptically, it is possible that ethanol at low concentrations primarily acts via extrasynaptic receptors composed of α4β3δ or α6β3δ (Sundstrom-Poromaa et al., 2002; Wallner et al., 2003 see also Boehm et al., this volume).

c. Other Compounds A variety of other compounds, such as avermectin B1a, Ro5-4864, PK8165, PK9084, PK11195, melatonin, polyamines, antidepressants, clozapine, dihydrogenated ergot compounds, carbamazepin, or phenytoin, to name only a few, have been identified that interact with GABA_A receptors and are discussed in previous reviews (Hefers and Luddens, 1998; Korpi et al., 2002; Sieghart, 1995). Similarly, divalent cations, such as Zn²⁺, Cd²⁺, Mn²⁺, Mn²⁺, and Co²⁺, trivalent cations, such as La³⁺ and lanthanides, and chloride ions are able to modulate GABA_A receptors (Korpi et al., 2002; Sieghart, 1995). Several binding sites for the divalent cations were identified on GABA_A receptors (Fisher and Macdonald, 1998; Horenstein and Akabas, 1998; Hosie et al., 2003).

V. Function of GABA_A Receptor Subtypes in the Brain

The large number of different GABA_A receptor subtypes existing in the brain and the striking segregation of some of these subtypes in functionally different neuronal populations raise the possibility that a selective modulation of certain receptor subtypes will precipitate quite specific pharmacological effects and will make it possible to study the function of the respective receptors in the brain. So far, however, no pharmacological tools are available that can address a certain receptor subtype with a sufficiently high selectivity.

Attempts to investigate the function of GABA_A receptor subtypes by generating mouse lines in which the genes for certain receptor subunits were inactivated did not yield clear-cut results due to adaptive changes in development and function of the brain caused by the lacking receptors (Rudolph and Mohler, 2004; Sieghart and Ernst, 2005). A combined molecular genetic and pharmacological approach (Rudolph et al., 1999), however, for the first time provided information on the function of some major GABA_A receptor subtypes. This approach was based on the introduction of a point mutation into specific α subunit-types of GABA_A receptors that rendered the respective receptors insensitive to allosteric modulation by diazepam without significantly changing the function and distribution of these receptors. In these animals, therefore, those effects of diazepam normally mediated by the respective receptors are lost and can be identified by comparing diazepam effects in wild-type and mutated animals. Using this approach, it was
demonstrated that GABA_A receptors containing z1 subunits mediate the sedative, anterograde amnesic, and partly the anticonvulsant actions of diazepam (Crestani et al., 2000; McKernan et al., 2000; Rudolph et al., 1999). These data are in line with the predominantly sedative effects of z1-selective compounds such as zolpidem (Crestani et al., 2000).

Using a similar approach, it was demonstrated that receptors containing z2 subunits seem to mediate the anxiolytic, muscle relaxant, and hypnotic effects of diazepam, whereas z3-containing receptors seem to have a weak function in muscle relaxation (Rudolph and Mohler, 2004; Sieghart and Ernst, 2005). However, the observation that an inverse agonist selective for z3-containing receptors precipitates anxiety (Atack et al., 2005) and that an agonist possibly selective for z3-containing receptors (Langen et al., 2005) exhibits anxiolytic effects, strongly argues for an involvement of these receptors in anxiety (Wafford et al., 2004; Whiting, 2003). Further experiments will have to clarify this discrepancy.

A point mutation that eliminates the interaction of diazepam with receptors containing z5 subunits seems to eliminate the memory impairing effects of diazepam (Crestani et al., 2002; Yee et al., 2004). Similarly, it was demonstrated that z5 subunit-deficient mice exhibit increased abilities in learning and memory tasks (Collinson et al., 2002). This is in line with the observation that a selective partial inverse agonists of GABA_A receptors containing z5 subunits exhibited cognition enhancing properties without exhibiting convulsant, proconvulsant, or anxiogenic activity (Chambers et al., 2004).

An approach similar to that used for unraveling the function of GABA_A receptors containing different z subunits was also used for studying the function of receptors containing different b subunits. Thus, the point mutation b2(Asn265Ser) that renders b2 subunit-containing GABA_A receptors less selective to the intravenous anesthetic etomidate (Belelli et al., 1997; Hill-Venning et al., 1997) was introduced into the b2 subunit gene of a mouse (Reynolds et al., 2003). As with the histidine mutations in the z subunits, this single amino acid switch is also effectively silent with regards to normal GABAergic function, but receptors containing this mutation no longer were sensitive to the sedative, ataxic, and hypothermic effects of etomidate. This indicates that these effects are mediated by receptors containing the b2 subunit. This point mutation, however, did not impair the anesthetic effects of etomidate indicating that these effects are mediated via receptors containing other b subunits.

The equivalent mutation b3(Asn263Met) in the b3 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 anesthetic effect of etomidate was abolished by this point mutation (Jurd et al., 2003). These results indicate that the b3-containing receptors are the primary mediators of the anesthetic effects of etomidate.
VI. GABA<sub>A</sub> Receptor Structure

The GABA<sub>A</sub> receptor is a member of the superfamily of pentameric ligand-gated ion channels that also includes the nACh receptor, 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. In 2001, however, the X-ray crystallographic structure of a soluble remote homolog of the N-terminal domain of nACh receptor subunits, the acetylcholine binding protein (AChBP), has been published (Brejc et al., 2001). This protein forms homopentamers that resemble the nACh receptor extracellular domain. Its crystal structure, featuring a novel fold of modified immunoglobuline-like topology, was then used to construct comparative models of the extracellular domain of other superfamily members including the GABA<sub>A</sub> receptor (Ernst et al., 2003). Modeling a pentameric receptor extracellular domain consisting of two α, two β, and one γ subunit results in a single (absolute) subunit arrangement (Fig. 4) in which amino acid residues known to contribute to ligand binding sites and interfaces are correctly positioned in the respective subunits (Ernst et al., 2003).

As has been established previously, there are two binding pockets for GABA in GABA<sub>A</sub> receptors, formed at the extracellular interface between adjacent α and β subunits. The pockets are formed by six so-called “loops,” termed loop A, B, and C of the β subunit at the plus (principle) side and D, E,

![Diagram of GABA<sub>A</sub> receptor extracellular domains.](image-url)

FIGURE 4 Model structure of GABA<sub>A</sub> receptor extracellular domains. The absolute arrangement for α1, β2, and γ2 containing GABA<sub>A</sub> receptors is shown, view from the extracellular site. The + and − sides of the subunits are identified on the inner circumference of the channel. Labels indicate the interfaces at which the benzodiazepine binding site or the two GABA binding sites are located. Taken from Ernst et al. (2003), with permission. (See Color Insert.)
and F of the α subunit at the minus (complementary) side (Ernst et al., 2003). It should be noted that this terminology has been established for the ligand-binding segments of pentameric ligand-gated ion channel prior to the publication of the AChBP crystal structure, and not all of them are loops in the structural sense.

The same picture of a pocket framed by the "loops" also emerged for the binding site of benzodiazepine ligands, which is localized in the extracellular domain at the α-γ interface, and thus consists of loops A, B, and C of the α subunit and loops D and E of the γ subunit (Ernst et al., 2003). These three-dimensional (3D) 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 (Kuc et al., 2003). The homology between GABA<sub>A</sub> 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 (Ernst et al., 2003).

Following the release of the AChBP structure, cryo-EM images of the electric fish organ nACh receptor in the open and closed state (Unwin, 1995) have been analyzed by fitting the core of the AChBP X-ray structure into the two sets of EM-density maps (Unwin et al., 2002). The ACh-bound state turned out to be pseudosymmetrical, with all subunits in the conformation that corresponds with the HEPES bound conformation of AChBP subunits. The ACh-free (resting) state, on the other hand, was found to be conformationally asymmetrical. The extracellular domain of the two α subunits, which form the plus sides of the ACh pockets is in a conformation distinct from the β, γ, and δ subunits extracellular domain (Unwin et al., 2002).

The cryo-EM atomic structure of the transmembrane domain of the nACh receptor in the resting state was published in 2003 (Miyazawa et al., 2003) consisting of five bundles of four α-helices. Shortly thereafter, a first model combining the extracellular and transmembrane domains of the nACh receptor has been discussed (Unwin, 2003), which later was published in a refined version and released with the protein data bank identifier 2BG9 (Unwin, 2005). Thus, the structure of the combined extracellular and transmembrane domains of the nACh receptors can now also be used as a template to model the corresponding structures of the GABA<sub>A</sub> receptor.

Such studies have been performed (Ernst et al., 2005), providing important information on the overall organization of the extracellular and transmembrane domain of GABA<sub>A</sub> receptors. The structure (1OED; PDB, http://www.rcsb.org/pdb/) of the nAChR transmembrane fragments in the resting state, and thus, also that of the GABA<sub>A</sub> receptor and of other members of the superfamily, is loosely packed suggesting that the interface between subunits of this receptor family contains additional "cavities"
beyond the ones found extracellularly (Ernst et al., 2005; Sieghart and Ernst, 2005). These are located at the junction between the extracellular and “transmembrane” domain (the latter is not entirely inserted in the membrane) and extend into the subunit junction inside the lipid bilayer (Fig. 5). In some models, these cavities of the helical domain appear to communicate with their extracellular counterparts (Ernst et al., 2005). Thus, it might be that the interface between subunits contains a continuous groove that might allow conformational mobility of the receptor but could also provide multiple independent binding sites.

Another type of cavity is found to be contained inside each of the subunits (Fig. 5), surrounded by the four helices that make up the transmembrane domain (Ernst et al., 2005; Sieghart and Ernst, 2005). These latter

![Figure 5](image_url)

**FIGURE 5** Solvent-accessible space contained in GABA<sub>A</sub> receptor models. Two views of a GABA<sub>A</sub> receptor model are shown to illustrate the pockets found by pocket-finding algorithms. The left view shows a dimer from the outside of the pore, the right view is from extracellular, with the β-folded domain invisible. The protein is shown in ribbon representation, the putative pockets identified with PASS are shown in dotted space filling representation. Clusters of connected solvent accessible volumes that may correspond to drug binding pockets are highlighted by colors: pink for the space associated with the subunit-interface of the β-folded domain, purple for the large cavity present at the subunit interface at the junction between the β-folded and the helical domain that extends into the interface of the helical domains, and green for the cavity that is present inside each of the four-helix bundles that form the helical domain of each subunit. Additional smaller clusters are shown in pale gray. It should be noted that because of the high uncertainty in side-chain positions, the total volume, shape, and electrostatic properties of the pockets varies considerably among models; in some models some of the pockets may even be missing. Within the uncertainty of the method, it is also possible that there is significant communication between “pink” and “purple” as well as between “green” and “purple pockets.” For this illustration, a representative model was used whose properties correspond to what the majority of models display. Figure was taken from Ernst et al. (2005), *Mol. Pharmacol.*, 68, 1291–1300, with permission. (See Color Insert.)
cavities are also found in models based on unrelated proteins (Bertaccini and Trudell, 2001), and those formed by the $\alpha$ subunits are thought to correspond to the long-proposed "anesthetic pockets" for the volatile anesthetics that are defined by a serine residue in the TM2 of the $\alpha$ subunit (Nishikawa et al., 2002).

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 debate (Lobo et al., 2004). Using multiple alignments and proper interpretation of chemical restraints, such as hydrophobic packing, however, a favored alignment could be selected that agrees with a number of additional experimental observations (Ernst et al., 2005). The loose packing of the four-helix bundle seen in structures of the transmembrane domain of the nACh receptor (Unwin, 2005) also explains the high accessibility of individual amino acid residues in the putative transmembrane helices (Akabas, 2004; Williams and Akabas, 2002) that was observed in studies using the substituted cysteine accessibility method (SCAM) and was originally interpreted as evidence against a helical transmembrane motif (Cascio, 2004). In addition, the observed conformational flexibility (Akabas, 2004) 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 four-helix bundle of individual subunits explains the large number of proposed "separate" allosterically interacting modulatory sites discussed previously. Depending on the functional state of the receptors, drugs might be able to bind into one or several of these pockets and by that stabilize or induce distinct conformations of the receptor that finally cause a change in chloride flux. 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 3D 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 anesthetics pocket in GABA$_A$ receptors, is known to be responsible for the $\beta$-subtype selectivity of loreclezole, etomidate, and $\beta$-carboline action (see in an earlier section).

Loss or change of a drug effect on 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 addressed by a combined approach of identifying pocket forming segments in structural models and subsequent mutagenesis and SCAM studies. For instance, $\beta$2Met286C has been shown to be protected by propofol from covalent modification by cystein reagents in a concentration-dependent manner, a strong hint toward a binding site near
this residue (Bali and Akabas, 2004). In homology models of GABA\(_A\) receptors, this residue indeed is part of a putative pocket (Ernst et al., 2005).

The models of the GABA\(_A\) receptor, thus, are not only consistent with most experimental data but could also explain experimental observations and propose the location of putative drug binding sites. These models can now be used to design new experiments for clarification of pharmacological and structural questions as well as for shedding light on conformational changes during binding of agonists, gating and allosteric modulation of these receptors. Overall, these experiments will lead to an improvement in the accuracy of the models and finally pave the way for a structure-based drug design.

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CHAPTER 10, FIGURE 4  Model structure of GABA_A receptor extracellular domains. The absolute arrangement for x1, β2, and γ2-containing GABA_A receptors is shown, view is from extracellular. The + and – sides of the subunits are identified on the inner circumference of the channel. Labels indicate the interfaces at which the benzodiazepine binding site or the two GABA binding sites are located. Taken from Ernst et al. (2003), with permission.
CHAPTER 10, FIGURE 5  Solvent-accessible space contained in GABA_A receptor models. Two views of a GABA_A receptor model are shown to illustrate the pockets found by pocket-finding algorithms. The left view shows a dimer from the outside of the pore, the right view is from extracellular, with the β-folded domain invisible. The protein is shown in ribbon representation, the putative pockets identified with PASS are shown in dotted space filling representation. Clusters of connected solvent accessible volumes that may correspond to drug binding pockets are highlighted by colors: pink for the space associated with the subunit-interface of the β-folded domain, purple for the large cavity present at the subunit interface at the junction between the β-folded and the helical domain that extends into the interface of the helical domains, and green for the cavity that is present inside each of the four-helix bundles that form the helical domain of each subunit. Additional smaller clusters are shown in pale gray. It should be noted that because of the high uncertainty in side-chain positions, the total volume, shape, and electrostatic properties of the pockets varies considerably among models; in some models some of the pockets may even be missing. Within the uncertainty of the method, it is also possible that there is significant communication between “pink” and “purple” as well as between “green” and “purple pockets.” For this illustration, a representative model was used whose properties correspond to what the majority of models display. Figure was taken from Ernst et al., Mol. Pharmacol., reprinted ahead of print, August 13 2005, with permission.