Drugs of the Future 2006, 31(8): 685-694

CCC: 0377-8282/2006
DOI: 10.1358/dof.2006.031.08.1008656

**Review Article**

# $GABA_A$ receptors as targets for different classes of drugs

***Werner Sieghart***
*Division of Biochemistry and Molecular Biology, Center for Brain Research, Medical University Vienna, Spitalgasse 4, 1090 Vienna, Austria; e-mail: Werner.Sieghart@meduniwien.ac.at*

**CONTENTS**



**Abstract**

$GABA_A$ receptors are the major inhibitory transmitter receptors in the brain and are the site of action of many clinically important drugs that modulate anxiety, excitability of the brain, feeding and drinking behavior, circadian rhythms, cognition, vigilance, learning and memory. Evidence has accumulated for the existence of a multiplicity of $GABA_A$ receptor subtypes with distinct regional, cellular and subcellular distribution in the brain. Each of these receptors has a distinct structure and thus also exhibits distinct pharmacological properties. Drugs selectively interacting with these receptor subtypes should therefore exert highly selective actions. This conclusion was recently supported by evidence indicating that different receptor subtypes mediate different actions of drugs. This review discusses recent progress in the development of $GABA_A$ receptor subtype-selective drugs, as well as recent structural information indicating that these receptors contain a multiplicity of possible binding pockets that could become targets for the development of novel drugs with subtype-specific actions. Targeting drugs to $GABA_A$ receptor subtypes holds promise for the treatment of various diseases with a reduced incidence of side effects. In addition, therapeutic applications beyond those of classical benzodiazepines may emerge for drugs specifically enhancing or reducing the activity of minor $GABA_A$ receptor subtypes located only at certain neurons.

## Introduction

γ-Aminobutyric acid (GABA) is the most abundant inhibitory transmitter in the central nervous system (CNS), with 17-20% of all neurons in the brain being GABAergic (1). Most of the physiological actions of GABA are generated via $GABA_A$ receptors. These receptors are chloride ion channels comprised of five protein subunits 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 (2). These drugs produce at least part of their clinically relevant effects by interacting with distinct allosteric binding sites on $GABA_A$ receptors. Based on the pharmacological action of these drugs, it was concluded that $GABA_A$ receptors are involved in controlling the excitability of the brain and in the modulation of anxiety, feeding and drinking behavior, circadian rhythms, cognition, vigilance, memory and learning (3).

## Multiplicity of $GABA_A$ receptor subtypes

$GABA_A$ receptors are comprised of five subunits that form the central chloride ion channel and belong to different subunit classes. A total of six α, three β, three γ, one δ, one ε, one π, one θ and three ρ subunits of $GABA_A$ receptors, as well as alternatively spliced isoforms of several of these subunits, have been cloned and sequenced from the mammalian nervous system (4, 5), and all these subunits have a distinct regional, cellular and subcellular distribution (3, 6-8). There are neurons expressing only a few $GABA_A$ receptor subunits and others that appear to express most, if not all, of these subunits, giving rise to the formation of a large variety of distinct receptor subtypes. Depending on their subunit composition, these receptor subtypes exhibit distinct pharmacological and electrophysiological properties (2, 9).

The majority of $GABA_A$ receptors are comprised of two α, two β and one γ subunit. The two α or two β subunits are not necessarily identical. In a minority of $GABA_A$ receptors, the δ, ε and π subunits appear to be able to replace the γ subunit, whereas the θ subunit may be able to replace a β subunit in these receptors (3). However, little data are currently available on the subunit composition of $GABA_A$ receptors containing ε, π or θ subunits. ρ

Subunits predominantly form homo-oligomeric receptors or hetero-oligomeric receptors with each other, but other subunit combinations containing $\rho$ subunits also appear to be possible (10).

It has been estimated that more than 500 distinct GABA$_A$ receptor subtypes exist in the brain (3). Most of these receptors are present in minor amounts. Nevertheless, due to the ubiquitous presence and quantitative importance of the GABAergic system in the brain, even receptors present at low levels might be as abundant as some norepinephrine, dopamine or 5-HT receptor subtypes. The existence of a large number of receptor subtypes with distinct regional and cellular distribution thus offers the possibility to develop subtype-selective compounds with highly specific actions.

## GABA$_A$ receptor subtypes mediate distinct drug effects

The actual proof that different GABA$_A$ receptor subtypes mediate different effects of drugs was provided recently using a combined molecular genetic and pharmacological approach (11). This approach was based on the introduction of a point mutation into an $\alpha$ subunit of GABA$_A$ receptors, which renders receptors containing the mutated $\alpha$ subunit insensitive to allosteric modulation by diazepam. Most of the actions of diazepam are mediated via receptors comprised of $\alpha1\beta\gamma2$, $\alpha2\beta\gamma2$, $\alpha3\beta\gamma2$ or $\alpha5\beta\gamma2$ subunits (see section on the benzodiazepine binding site of GABA$_A$ receptors). When the point mutation was introduced into the $\alpha1$ subunit, diazepam was no longer able to interact with $\alpha1$-containing receptors and mediated its effects through only $\alpha2$-, $\alpha3$- or $\alpha5$-containing receptors. A comparison of drug-induced behavioral responses in the mutated and wild-type mice then allowed the identification of the diazepam effects that were missing in mutant mice, which in turn allowed for the determination of the contribution of $\alpha1$-containing receptors to the effects of diazepam. Using this approach, it was demonstrated that receptors comprised of $\alpha1$, $\beta$ and $\gamma2$ subunits mediate the sedative, anterograde amnesic and, in part, the anticonvulsant actions of diazepam (11, 12). After similar mutations were introduced into the genes of $\alpha2$, $\alpha3$ or $\alpha5$ subunits, it was demonstrated that receptors comprised of $\alpha2$, $\beta$ and $\gamma2$ subunits appear to mediate the anxiolytic and, at appreciably higher doses, the muscle relaxant actions of diazepam (13, 14). In addition, diazepam appears to affect the sleep and waking EEG pattern via these receptors (15). Receptors comprised of $\alpha3$, $\beta$ and $\gamma2$ subunits were reported to mediate some of the muscle relaxant activities of diazepam and possibly the antiabsence effects of clonazepam (14, 16), and receptors comprised of $\alpha5$, $\beta$ and $\gamma2$ subunits appear to influence learning and memory (17). The latter conclusion was supported by studies with $\alpha5$ subunit knockout mice which indicated that these mice exhibit significantly improved performance in learning and memory tasks (18). Selective inverse agonists at $\alpha5$ receptors should therefore enhance learning and memory.

Using a similar molecular genetic and pharmacological approach for receptors containing different $\beta$ subunits, it was demonstrated that receptors comprised of $\alpha$, $\beta2$ and $\gamma2$ subunits mediate the sedative, ataxic and hypothermic effects of the general anesthetic etomidate (19). In contrast, receptors comprised of $\alpha$, $\beta3$ and $\gamma2$ subunits are primarily responsible for mediating the anesthetic effects of etomidate (20). Recently, using $\alpha5$ subunit knockout mice, it was demonstrated that $\alpha5$-containing receptors mediate the amnesic but not the sedative/hypnotic effects of etomidate. Drugs that increase the function of $\alpha5$-containing receptors may thus cause amnesia without sedation or unconsciousness, and may be of use to suppress the intraoperative awareness occurring in 1 or 2 cases per 1,000 anesthetized patients (21). Thus, in summary, different receptor subtypes mediate different effects of drugs, strongly suggesting that GABA$_A$ receptor subtype-selective drugs should exhibit selective actions with significantly reduced side effects.

## Pharmacology of GABA$_A$ receptors

GABA$_A$ receptors can not only be directly activated or inhibited via their GABA binding site, but they can also be allosterically modulated by a variety of different drugs, the number of which is constantly increasing (2, 10, 22). Currently, only three distinct binding sites present on GABA$_A$ receptors can be directly investigated by appropriate radioligand binding studies: the GABA/muscimol, benzodiazepine and *tert*-butylbicyclophosphorothionate (TBPS)/picrotoxin binding sites. Using such studies, compounds competitively interacting with the radioligands, and thus directly binding to the respective sites, can be identified (2). The interaction of all 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. However, in most cases these techniques do not allow us 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$_A$ receptors is not known. In addition, closer investigation of the effects observed indicated that compounds in many cases concentration-dependently interact with several allosteric binding sites, giving rise to sometimes opposite effects on GABA-induced chloride ion flux at different concentrations (2, 10). Furthermore, many compounds have been identified that can not only modulate GABA-induced chloride flux, but at higher concentrations can also directly activate the GABA$_A$ receptor-associated chloride channel in the absence of GABA (2, 23).

All these effects contribute to the high degree of complexity of the pharmacology of GABA$_A$ receptors, which is further increased by the fact that the pharmacological properties depend on the GABA$_A$ receptor subtype investigated. Since a large number of receptor subtypes contribute to receptor binding studies in brain membranes or

whole-cell patch clamp studies in slices, a mixture of effects is observed that can not easily be attributed to a single receptor subtype. In order to clarify the pharmacology of drugs, it is therefore imperative to investigate drug actions on individual recombinant receptor subtypes. However, thorough functional studies on the pharmacology of recombinant receptors using different drugs have only recently been published, and therefore no detailed information on the efficacy of interaction with different receptor subtypes is available for most drugs (10). Moreover, even using recombinant receptors, more than one receptor subtype could be spontaneously formed upon co-transfection of several $GABA_A$ receptor subunits into heterologous expression systems and the mixture of receptors again can confound the pharmacological results (24).

### *Drugs interacting with the GABA binding site of $GABA_A$ receptors*

Currently, only a few different classes of compounds are known to be ligands for the GABA binding site (25). Studies on recombinant $GABA_A$ receptors have indicated that the currently known full agonists (exhibiting efficacy comparable to GABA) or antagonists at the GABA binding site of these receptors appear not to exhibit significant receptor subtype selectivity (26-28). Consequently, due to the ubiquitous distribution of $GABA_A$ receptors, these compounds cause inhibition of most neuronal systems, strongly interfering with the function of the brain, causing sedation, movement disturbances and other severe side effects upon systemic application. On the other hand, $GABA_A$ receptor antagonists precipitate anxiety and convulsions.

However, several partial agonists at the GABA binding site of $GABA_A$ receptors, such as imidazole-4-acetic acid, piperidine-4-sulfonic acid, THIP (4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol, gaboxadol) or 4-PIOL (5-[4-piperidyl]isoxazol-3-ol), have been developed which exhibit some receptor subtype-dependent potency and efficacy (25, 29). Thus, THIP is approximately 10 times more potent at $\alpha4\beta3\delta$ than at $\alpha4\beta3\gamma2S$ receptors. This compound, which is currently in clinical development, also appears to exhibit a highly interesting spectrum of *in vivo* actions. For instance, it appears to have potent analgesic effects comparable to morphine and to improve the quality of sleep (25, 29).

### *Drugs interacting with the benzodiazepine binding site of $GABA_A$ receptors*

Benzodiazepines, such as diazepam, are the strongest anticonvulsant, muscle relaxant, sedative/hypnotic and anxiolytic compounds in clinical use. They enhance the action of GABA on $GABA_A$ receptors by increasing the GABA-induced frequency of opening of chloride channels and thus allosterically modulating these receptors (23). Benzodiazepines and compounds interacting with the benzodiazepine binding site of $GABA_A$ receptors can only modulate ongoing GABAergic activity. These compounds cannot elicit chloride ion flux in the absence of GABA, and thus exhibit extremely low toxicity.

Many different classes of compounds have been identified to interact with the benzodiazepine binding site of $GABA_A$ receptors (2, 5, 30, 31). In each of these classes, compounds have been identified that enhance or reduce 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 those of "agonists": they are anxiogenic and proconvulsant and enhance vigilance, learning and memory. A third class of compounds binding to the receptor via the benzodiazepine binding site do not directly alter GABA-induced chloride flux. These compounds in most cases do not elicit behavioral effects on their own, but prevent the interaction of "agonists" or "inverse agonists" with these receptors. They are therefore called allosteric "antagonists" (2, 5).

The efficacy of compounds for eliciting such effects can vary. Thus, in addition to full agonists or full inverse agonists exhibiting a maximum enhancement or reduction of GABAergic currents, respectively, some compounds show weaker actions (partial agonists or partial inverse agonists). In addition, the agonist or inverse agonist efficacy of a compound is usually distinct for different receptor subtypes. Thus, a compound can be a full agonist at one type of receptor and exhibit partial agonist or even inverse agonist activity at other receptor subtypes (5, 9).

Since the properties of compounds interacting with the benzodiazepine site are dependent on the type of $\alpha$ and $\gamma$ subunit, but not the $\beta$ subunit, present in the recombinant receptors, it is assumed that the benzodiazepine binding site is located at the $\alpha/\gamma$ interface of $GABA_A$ receptors (32). This conclusion is also supported by mutagenesis studies (33), as well as recent modeling studies of the $GABA_A$ receptor (34, 35). Since $GABA_A$ receptors can contain any of the 6 different $\alpha$ and 3 different $\gamma$ subunits, up to 18 different $GABA_A$ receptor subtypes with distinct benzodiazepine binding sites may exist. These distinct benzodiazepine binding sites could be used to selectively modulate these receptor subtypes, provided that drugs can be found that selectively interact with these sites.

Classical benzodiazepines, such as diazepam, flunitrazepam or clonazepam, as well as other compounds interacting with the benzodiazepine binding site of $GABA_A$ receptors, strongly interact with receptors comprised of $\alpha$, $\beta$ and $\gamma2$ subunits. The majority of these compounds, however, are inactive or only weakly active at receptors containing $\gamma1$ subunits (36). Although some benzodiazepine ligands can modulate receptors containing $\gamma3$ subunits (9), these receptors are present in very low amounts in the brain (3). 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 containing $\gamma$2 subunits.

Receptors composed of $\alpha$4$\beta\gamma$2 or $\alpha$6$\beta\gamma$2 subunits exhibit markedly different pharmacology (2, 9). Most of the classical benzodiazepines, such as diazepam, flunitrazepam or clonazepam, do not interact with these receptors. Imidazobenzodiazepines, such as Ro-15-4513 or flumazenil, however, interact not only with these receptors but also with receptors containing $\alpha$1,2,3,5$\beta\gamma$2 subunits. Since the selectivity of compounds targeting $\alpha$4$\beta\gamma$2 or $\alpha$6$\beta\gamma$2 receptors is only weak (37-39), the behavioral effects mediated by $\alpha$4- or $\alpha$6-containing receptors are presently not known. Given this information, it is clear that most of the effects of diazepam or other benzodiazepine binding site ligands are mediated via GABA$_A$ receptors comprised of $\alpha$1$\beta\gamma$2, $\alpha$2$\beta\gamma$2, $\alpha$3$\beta\gamma$2 or $\alpha$5$\beta\gamma$2 subunits. Since the classical benzodiazepines exhibit relatively similar efficacy for modulating all these receptors, it is not surprising that the clinical spectrum of action of these compounds is quite similar. Only some drugs in current use, such as the sedative/hypnotic compound zolpidem, exhibit some selectivity for $\alpha$1 subunit-containing receptors (2, 9).

## 1. Partial benzodiazepine-site agonists exhibit selective actions

Several years ago the concept emerged that anxiolytic effects can be elicited by full agonists at low receptor occupation, whereas partial agonists require full receptor occupation to elicit the same effect. For eliciting sedative/hypnotic actions, however, full agonists require high receptor occupancy (40, 41). It was therefore expected that partial agonists could never achieve a sufficiently high receptor occupancy and therefore might maintain the beneficial anxiolytic properties but have a lower propensity to cause unwanted sedative/hypnotic side effects. Indeed, in preclinical animal assays, it appeared that partial agonists retained their anxiolytic activity but had decreased sedation, withdrawal and abuse liability. Examples of such compounds are bretazenil (42) and abecarnil (43). Both compounds were evaluated in the clinic, but unfortunately neither proved successful. Abecarnil did not achieve the required level of anxiolytic activity, although it was not sedative, whereas bretazenil was reported to lack sufficient separation between anxiolytic and sedative effects (22). Results from animal studies can not therefore be directly translated to the human situation.

Another compound which reached phase III development for generalized anxiety disorder, however, appeared to come close to the expectations linked with partial agonists. Ocinaplon is a low-potency compound (micromolar EC$_{50}$ at GABA$_A$ receptors) with full agonist properties at $\alpha$1$\beta$2$\gamma$2 receptors and partial agonist properties at $\alpha$2$\beta$2$\gamma$2, $\alpha$2$\beta$2$\gamma$2 and $\alpha$5$\beta$2$\gamma$2 receptors (22). This compound was reported to exhibit a significant anxiolytic effect in patients and only 1% of the patients treated reported somnolence or sedation (44). The clinical profile of this compound is thus surprisingly different from the sedative and hypnotic properties expected from its significant activity at $\alpha$1 subunit-containing receptors. The reason for this interesting effect is currently not clear, but it is possible that the low potency of ocinaplon leads to only a small activation of the respective receptors, causing beneficial effects. If this is true, it is possible that the partial agonists developed so far are still too strong and that drugs with low efficacy should be developed in the future. Alternatively, however, it is possible that the beneficial effects of ocinaplon are caused by an active metabolite, as suspected for pagoclone, another benzodiazepine-site ligand (45). Further experiments will be needed to clarify the mechanism of action of this type of compound. Although the clinical development of ocinaplon is on hold owing to potential hepatic toxicity (http://www.dovpharm.com), it could have provided proof of principle for the development of partial agonists as anxiolytic drugs. Among the thousands of benzodiazepine-site ligands of different structural classes synthesized with the aim of developing better anxiolytics, low-potency weak partial agonists have never been thoroughly investigated because they were not considered of interest. Suitable compounds are thus probably available at a number of pharmaceutical companies. If they are derived from a basic structure with known low toxicity, such as the classical benzodiazepines, the clinical development of such compounds might be straightforward and possibly successful.

## 2. GABA$_A$ receptor subtype-selective drugs acting via the benzodiazepine site

Much effort has been put into the development of compounds with selective affinity for the benzodiazepine binding site of certain GABA$_A$ receptor subtypes. Most of the compounds developed preferentially interact with the benzodiazepine binding site of $\alpha$1-containing receptors. Thus, the triazolopyridazine CL-218872, the benzodiazepines oxazepam and cinolazepam, or the imidazopyridine zolpidem exhibit approximately 10 times higher affinity for $\alpha$1- than for $\alpha$2-, $\alpha$3- or $\alpha$5-containing receptors (46, 47). Zolpidem, in addition, exhibits practically no affinity for $\alpha$5-containing receptors (2). In agreement with the results indicating that compounds preferentially interacting with $\alpha$1-containing receptors should exhibit sedative actions, zolpidem appears to predominantly exert sedative/hypnotic properties. So far, however, no compounds with selective binding affinity for $\alpha$2-, $\alpha$3- or $\alpha$5-containing receptors have been reported.

Although the affinity of a compound for a certain receptor subtype is important, it is the efficacy that determines drug action. Recently, several compounds have been developed that exhibit subtype-selective efficacy. The first of these was L-838417 (12). This compound exhibits comparable nanomolar affinities for recombinant

human α1-, α2-, α3- or α5-containing receptors, but significant efficacy selectivity: the maximum potentiation of an $EC_{20}$ concentration of GABA was essentially zero at α1-containing receptors, but was 34%, 39% and 36% of that of the nonselective full agonist chlordiazepoxide, respectively, at α2-, α3- and α5-containing receptors (22). Thus, different receptors, although exhibiting similar affinity for ligands, show structural differences in the receptor domains mediating the allosteric coupling of the benzodiazepine binding site, GABA site and the intrinsic ion channel, thus leading to different efficacies of compounds.

L-838417 was found to be a robust anxiolytic agent with a 30-fold separation over doses needed to elicit a sedative effect, and it was found to be an effective nonsedating anxiolytic agent in primates, also exhibiting a much reduced potential for abuse compared with both full nonselective benzodiazepine agonists and zolpidem (48). These data supported the conclusion that the α1-containing $GABA_A$ receptors primarily mediate the sedative effects of benzodiazepines, whereas the α2-, α3- or α5-containing $GABA_A$ receptors mediate the anxiolytic effects.

This was further supported by results for SL-651498, which appears to be a full agonist at α2- and α3-containing receptors and a partial agonist at α1- and α5-containing receptors (49). In preclinical models, the compound appeared to have a significant separation between anxiolytic and sedative activity. Similarly, in primate studies, SL-651498 was found to be an effective anxiolytic and muscle relaxant, but did not induce ataxia (50). The development status of this compound is unknown, because it does not appear in the sanofi-aventis pipeline (http://en.sanofi-aventis.com), although in the past it has been forwarded as a treatment for both anxiety and muscle spasms (22).

Another compound exhibits a quinoline structure derived from the fluoroquinolone antibiotics (*e.g.,* norfloxacin) and appears to have no efficacy at recombinant human α1β2γ2 receptors, but significant efficacy at α2β2γ2 receptors (51). No data were provided regarding the effects at α3- or α5-containing receptors. This compound was shown to have significant anxiolytic activity in the absence of sedative activity.

Pagoclone is a cyclopyrrolone compound that is well tolerated in humans and effective in panic attacks and generalized anxiety disorder. It is a full agonist at α3-containing $GABA_A$ receptors and a partial agonist at α1-, α2- and α5-containing receptors (52). Due to lack of robust efficacy, however, this compound is now being developed by Indevus (http://www.indevus.com) for the treatment of stuttering (45).

The possible involvement of α3-containing receptors in the anxiolytic effects of benzodiazepine-site ligands was supported by the preclinical effects of the inverse agonist compound α3IA (53). This compound not only exhibited selective affinity ($EC_{50}$ = 1300 nM at α1-, 185 nM at α2- and 70 nM at α3-containing receptors), but also some functional selectivity (maximum inhibition of $EC_{20}$ concentrations of GABA: 31% at α1-, 24% at α2-, 45% at α3- and 14% at α5-containing receptors). When tested in rats, α3IA was anxiogenic, possibly supporting a role for α3-containing receptors in mediating the anxiolytic responses of benzodiazepines.

Similar results were reported from a compound of the 3-heteroaryl-2-pyridone class (54), which was anxiogenic in animal experiments and acted as a selective inverse agonist at α3-containing receptors with minimal efficacy at α1- or α2-containing receptors.

A possible role for α3-containing receptors in the anxiolytic effects of benzodiazepine-site ligands is also weakly supported by results for ELB-139, which is described as a low-affinity partial agonist at α3-containing receptors (55). However, no data were presented indicating that it is subtype-selective. The compound has an anxiolytic profile but no data on its sedative properties were reported.

The strongest support for a role for α3-containing receptors in mediating the anxiolytic responses of benzodiazepines comes from data generated with the imidazopyridine TP-003 (56). This compound exhibits an equivalent high affinity at recombinant α1-, α2-, α3- and α5-containing and minimal affinity at α4- and α6-containing $GABA_A$ receptors. Interestingly, however, this compound exhibited significant efficacy only at α3-containing receptors, acting as a full agonist. In rodents, TP-003 produced significant anxiolytic but no sedative activity, strongly suggesting an important role for α3-containing $GABA_A$ receptors in the anxiolytic response to benzodiazepines. However, this conclusion was not supported by data from genetically modified mice, which indicated that α2- and not α3-containing receptors mediate the anxiolytic effects of benzodiazepines. The reason for this apparent discrepancy remains unclear. However, since TP-003 requires a rather high 75% receptor occupancy for anxiolysis, in contrast to chlordiazepoxide which requires 25% receptor occupancy for its anxiolytic effect, it cannot be excluded that a weak interaction with other $GABA_A$ receptor subtypes, such as α2-containing receptors, might contribute to the anxiolytic effect of TP-003 (56)

Recent reports also indicate that it is possible to develop compounds with selective efficacy at α5-containing $GABA_A$ receptors. One of the first compounds developed was FG-8094/L-655708, which exhibited 50-fold higher affinity for α5 subunit-containing receptors as compared to α2- or α3-containing receptors and 100-fold higher affinity as compared to α1-containing receptors (31). This compound acts as a partial inverse agonist at α5-containing receptors and its tentative use for cognition enhancement has been patented. Similar properties were exhibited by two other compounds (57, 58) which are potent inverse agonists at α5-containing receptors with little or no efficacy at other receptor subtypes. Both 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 convulsive and they were not proconvulsive in the presence of pentylenetetrazol. These data clearly indicate the potential utility of these compounds as cognition enhancers in

disorders such as mild cognitive impairment and Alzheimer's disease.

*Subtype selectivity of drugs interacting with unidentified binding sites on GABA$_A$ receptors*

Several compounds, such as furosemide (39, 59), amiloride (60), γ-butyrolactones (61, 62), ROD compounds (63-65) or a structural analogue of the fluoroquinolone antibiotic norfloxacin (51), have been identified which appear to interact with novel, as yet unidentified binding sites on GABA$_A$ receptors. The efficacy of these compounds appears to depend on the type of α subunit present on the receptors. Other compounds, such as loreclezole (66, 67) or etomidate (68), preferentially act on GABA$_A$ receptors containing a β2 or β3 subunit, and have no effect on receptors containing β1 subunits.

Salicylidene salicylhydrazide (69) was one of the first compounds with selectivity for receptors containing the β1 subunit. In addition, various antiinflammatory agents (70), including mefenamic acid, flufenamic acid, meclofenamic acid, tolfenamic acid, niflumic acid and diflunisal, exhibited varying levels of efficacy and potency at β2- or β3-containing receptors, while having antagonist or weak inverse agonist profiles at β1-containing receptors. Although amino acid residues have been identified on GABA$_A$ receptors which appear to be involved in the action of loreclezole (66, 67) and etomidate (68), or of salicylidene salicylhydrazide (69), it is not yet clear whether these residues are located close to the binding sites of these compounds or whether they are only important for transduction of the drug effects. 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 in order to study the function of receptors containing different β subunits in the brain.

The action of tracazolate (71) also appears to depend on the type of β subunit on the receptor, and is strongly influenced by the nature of the third subunit (γ, δ or ε) (72). This compound is a low-potency anxiolytic with a large window of separation between the anxiolytic effect and potential side effects. It will be interesting to identify the site of interaction of this compound on GABA$_A$ receptors because it could be a prototype drug with a highly interesting clinical profile.

Neuroactive steroids are endogenous metabolites of progesterone and deoxycorticosterone that exhibit anxiolytic, anticonvulsant, analgesic, sedative and, at relatively high doses, anesthetic actions (73). A variety of experiments have indicated that these compounds are highly selective and extremely potent modulators of the GABA$_A$ receptor (74). For a long time it was assumed that neither the potency nor the efficacy of steroids depends on the subunit composition of GABA$_A$ receptors. Recent evidence, however, indicates that neurosteroids (75), and possibly also etomidate and certain anesthetics (76), stimulate GABA$_A$ receptors such as extrasynaptic δ-containing receptors.

It has been suggested that at least some of the actions of ethanol are mediated via GABA$_A$ receptors, but it has proved difficult to pinpoint the interaction of ethanol with these receptors. According to the vast literature on ethanol and GABA$_A$ receptors, it emerges that the effects of ethanol at these receptors, if they can be observed at all, are seen only at fairly high concentrations (above 60 mM) (76). Recently, it was demonstrated that 3 mM ethanol reproducibly enhances GABA action at α4β3δ and α6β3δ GABA$_A$ receptors (76). This concentration is 6 times lower than the legal blood alcohol intoxication (driving) limit in most states (0.08% wt/vol or 17.4 mM). Effects seen at such low concentrations of ethanol might therefore be responsible for the anxiolytic actions of ethanol seen after the first sips from a glass of wine. This was recently supported by the finding that Ro-15-4513, which has been demonstrated to specifically antagonize the ataxic effects of ethanol, binds with nanomolar affinity to α4β3δ and α6β3δ GABA$_A$ receptors. Moreover, its binding can be selectively and competitively inhibited by low micromolar concentrations of ethanol, suggesting that Ro-15-4513 and ethanol interact with the same binding site (77). The pharmacological properties of this novel binding site on α4β3δ and α6β3δ GABA$_A$ receptors are completely different from those of the benzodiazepine binding site on αβγ2 receptors. Given the anxiolytic effects of low concentrations of ethanol, these data indicate that anxiolytic effects may not only be mediated by α2βγ2 receptors, but also by other types of GABA$_A$ receptors, for instance α4β3δ receptors. Thus, the Ro-15-4513 binding site on α4β3δ GABA$_A$ receptors may be a target for the development of compounds with anxiolytic activity.

## Structure of GABA$_A$ receptors suggests new targets 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 nicotinic acetylcholine (nACh), 5-HT$_3$ and glycine receptors. Recently, structural information on this receptor superfamily became available using cryo-EM images of the nACh receptor (78-81), as well as from the crystal structure of the pentameric acetylcholine binding protein (AChBP), which turned out to be homologous to the extracellular domain of the nACh receptor (82). Using the AChBP, and later on the structural information from the nACh receptor extracellular and transmembrane domains as a template, homology models of the extracellular and transmembrane domains of GABA$_A$ receptors have been generated (34, 35). The resulting structural information was not only consistent with the available experimental data, but also for the first time allowed the visualization of the receptor in three dimensions and the identification of its functional parts.

Interestingly, the analysis of solvent-accessible space in these models indicated the presence of at least 15 different pockets, which appear to be necessary for conformational changes of GABA$_A$ receptors and may also be used as possible drug binding sites (35). Five of these

pockets are located in the extracellular domain at the five interfaces between subunits. Two of the extracellular pockets form the two GABA binding sites, and one forms the benzodiazepine binding site of $GABA_A$ receptors. In the remaining two extracellular pockets, no drug binding site has yet been identified. Five additional pockets are located at the five interfaces of the helical transmembrane domains, and five pockets are located within the four helix bundles that form the transmembrane domain of each subunit (35).

The occurrence of multiple pockets at subunit interfaces, as well as "inside" of the individual subunits themselves, explains, at least in principle, the large number of proposed "separate" allosterically interacting modulatory sites described in the literature. Mutagenesis studies have already identified several segments that are essential for the action of certain drugs and that can now be examined in the light of three-dimensional models. For instance, amino acid residues in $\alpha$ subunits demonstrated to be important for the action of volatile anesthetics (83) have been found to point into the intrahelical pocket of $\alpha$ subunits using the current structural models (35), thus providing strong support for an "anesthetic" pocket in $GABA_A$ receptors. In addition, the TM2 segment of the $\beta$ subunit, which is homologous to that of the $\alpha$ subunit forming the volatile anesthetic pocket, is known to be responsible for the $\beta$ subtype selectivity of loreclezole (70). Another residue looking into the intrahelical pocket of $\beta$ subunits when mutated to a cysteine has been demonstrated to be protected by propofol from covalent modification by cysteine reagents (84), a strong indication of a binding site near this residue. Since an intrahelical pocket is present in each of the two $\alpha$ and two $\beta$ subunits of a receptor comprised of two $\alpha$, two $\beta$ and one $\gamma$ subunit, more than one binding site for these drugs is present on $GABA_A$ receptors. It is therefore possible that, depending on the conformational state of the receptor, drugs can enter one or more of these pockets and either stabilize or induce a certain conformation and modulate the function of the receptor.

In conclusion, there are many more potential drug binding sites on $GABA_A$ receptors than previously thought and several of them are formed by amino acid sequences that are different in different receptor subtypes. It can thus be assumed that the respective pockets exhibit receptor subtype-specific structures, offering the possibility of developing receptor subtype-selective compounds with novel mechanisms of action. The availability of structural models of $GABA_A$ receptors now allows us to visualize possible drug binding sites, conceive experiments for refining their structure and use this information, as well as that on structural differences in receptor subtypes, for a more rational search for subtype-selective drugs. Targeting drugs to $GABA_A$ receptor subtypes holds the promise of increased clinical specificity compared with the classical benzodiazepines. In addition, therapeutic applications beyond those of the classical benzodiazepines may emerge from drugs specifically enhancing or reducing the activity of minor $GABA_A$ receptor subtypes located only at certain neurons.

**Acknowledgements**

Work from the authors' laboratory was supported by the Austrian Science Fund.

**References**

1. Somogyi, P., Tamas, G., Lujan, R., Buhl, E.H. *Salient features of synaptic organisation in the cerebral cortex*. Brain Res Rev 1998, 26: 113-35.

2. Sieghart, W. *Structure and pharmacology of $\gamma$-aminobutyric acid$_A$ receptor subtypes*. Pharmacol Rev 1995, 47: 181-234.

3. Sieghart, W., Sperk, G. *Subunit composition, distribution and function of GABA$_A$ receptor subtypes*. Curr Top Med Chem 2002, 2: 795-816.

4. Simon, J., Wakimoto, H., Fujita, N., Lalande, M., Barnard, E.A. *Analysis of the set of GABA(A) receptor genes in the human genome*. J Biol Chem 2004, 279: 41422-35.

5. Barnard, E.A., Skolnick, P., Olsen, R.W. et al. *International Union of Pharmacology. XV. Subtypes of $\gamma$-aminobutyric acid$_A$ receptors: Classification on the basis of subunit structure and receptor function*. Pharmacol Rev 1998, 50: 291-313.

6. Pirker, S., Schwarzer, C., Wieselthaler, A., Sieghart, W., Sperk, G. *GABA(A) receptors: Immunocytochemical distribution of 13 subunits in the adult rat brain*. Neuroscience 2000, 101: 815-50.

7. Wisden, W., Laurie, D.J., Monyer, H., Seeburg, P.H. *The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon*. J Neurosci 1992, 12: 1040-62.

8. Persohn, E., Malherbe, P., Richards, J.G. *Comparative molecular neuroanatomy of cloned GABAA receptor subunits in the rat CNS*. J Comp Neurol 1992, 326: 193-216.

9. Hevers, W., Luddens, H. *The diversity of GABA$_A$ receptors. Pharmacological and electrophysiological properties of GABA$_A$ channel subtypes*. Mol Neurobiol 1998, 18: 35-86.

10. Sieghart, W., Ernst, M. *Heterogeneity of GABAA receptors: Revived interest in the development of subtype-selective drugs.* Curr Med Chem - Central Nervous System Agents 2005, 5: 217-42.

11. Rudolph, U., Crestani, F., Benke, D. et al. *Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes*. Nature 1999, 401: 796-800.

12. McKernan, R.M., Rosahl, T.W., Reynolds, D.S. et al. *Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor alpha1 subtype*. Nat Neurosci 2000, 3: 587-92.

13. Low, K., Crestani, F., Keist, R. et al. *Molecular and neuronal substrate for the selective attenuation of anxiety*. Science 2000, 290: 131-4.

14. Crestani, F., Low, K., Keist, R., Mandelli, M., Mohler, H., Rudolph, U. *Molecular targets for the myorelaxant action of diazepam*. Mol Pharmacol 2001, 59: 442-5.

15. Kopp, C., Rudolph, U., Low, K., Tobler, I. *Modulation of rhythmic brain activity by diazepam: GABA(A) receptor subtype and state specificity*. Proc Natl Acad Sci USA 2004, 101: 3674-9.

16. Sohal, V.S., Keist, R., Rudolph, U., Huguenard, J.R. *Dynamic GABA(A) receptor subtype-specific modulation of the synchrony and duration of thalamic oscillations*. J Neurosci 2003, 23: 3649-57.

17. Crestani, F., Keist, R., Fritschy, J.-M. et al. *Trace fear conditioning involves hippocampal alpha5 GABA(A) receptors*. Proc Natl Acad Sci USA 2002, 99: 8980-5.

18. Collinson, N., Kuenzi, F.M., Jarolimek, W. et al. *Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the GABAA receptor*. J Neurosci 2002, 22: 5572-80.

19. Reynolds, D.S., Rosahl, T.W., Cirone, J. et al. *Sedation and anesthesia mediated by distinct GABA(A) receptor isoforms*. J Neurosci 2003, 23: 8608-17.

20. Jurd, R., Arras, M., Lambert, S. et al. *General anesthetic actions in vivo strongly attenuated by a point mutation in the GABA(A) receptor beta3 subunit*. FASEB J 2003, 17: 250-2.

21. Cheng, V.Y., Martin, L.J., Elliott, E.M. et al. *Alpha5 GABAA receptors mediate the amnestic but not sedative-hypnotic effects of the general anesthetic etomidate*. J Neurosci 2006, 26: 3713-20.

22. Whiting, P.J. *GABAA receptors: A viable target for novel anxiolytics?* Curr Opin Pharmacol 2006, 6: 24-9.

23. Macdonald, R.L., Olsen, R.W. *GABAA receptor channels*. Annu Rev Neurosci 1994, 17: 569-602.

24. Boileau, A.J., Baur, R., Sharkey, L.M., Sigel, E., Czajkowski, C. *The relative amount of cRNA coding for gamma2 subunits affects stimulation by benzodiazepines in GABA(A) receptors expressed in Xenopus oocytes*. Neuropharmacology 2002, 43: 695-700.

25. Frolund, B., Ebert, B., Kristiansen, U., Liljefors, T., Krogsgaard-Larsen, P. *GABA(A) receptor ligands and their therapeutic potentials*. Curr Top Med Chem 2002, 2: 817-32.

26. Adkins, C.E., Pillai, G.V., Kerby, J. et al. *alpha4beta3delta GABA(A) receptors characterized by fluorescence resonance energy transfer-derived measurements of membrane potential*. J Biol Chem 2001, 276: 38934-9.

27. Ebert, B., Wafford, K.A., Whiting, P.J., Krogsgaard-Larsen, P., Kemp, J.A. *Molecular pharmacology of gamma-aminobutyric acid type A receptor agonists and partial agonists in oocytes injected with different alpha, beta, and gamma receptor subunit combinations*. Mol Pharmacol 1994, 46: 957-63.

28. Luddens, H., Korpi, E.R. *GABA antagonists differentiate between recombinant GABAA/benzodiazepine receptor subtypes*. J Neurosci 1995, 15: 6957-62.

29. Krogsgaard-Larsen, P., Frolund, B., Liljefors, T., Ebert, B. *GABA(A) agonists and partial agonists: THIP (gaboxadol) as a non-opioid analgesic and a novel type of hypnotic*. Biochem Pharmacol 2004, 68: 1573-80.

30. Gardner, C.R., Tully, W.R., Hedgecock, C.J. *The rapidly expanding range of neuronal benzodiazepine receptor ligands*. Prog Neurobiol 1993, 40: 1-61.

31. Teuber, L., Watjens, F., Jensen, L H. *Ligands for the benzodiazepine binding site—A survey*. Curr Pharm Des 1999, 5: 317-43.

32. Sigel, E., Buhr, A. *The benzodiazepine binding site of GABA$_A$ receptors*. Trends Pharmacol Sci 1997, 18: 425-9.

33. Sigel, E. *Mapping of the benzodiazepine recognition site on GABA$_A$ receptors*. Curr Top Med Chem 2002, 2: 833-9.

34. Ernst, M., Brauchart, D., Boresch, S., Sieghart, W. *Comparative modeling of GABA$_A$ receptors: Limits, insights, future developments*. Neuroscience 2003, 119: 933-43.

35. Ernst, M., Bruckner, S., Boresch, S., Sieghart, W. *Comparative models of GABAA receptor extracellular and transmembrane domains: Important insights in pharmacology and function.* Mol Pharmacol 2005, 68: 1291-300.

36. Khom, S., Baburin, I., Timin, E.N., Hohaus, A., Sieghart, W., Hering, S. *Pharmacological properties of GABAA receptors containing gamma1 subunits*. Mol Pharmacol 2006, 69: 640-9.

37. Gu, Z.Q., Wong, G., Dominguez, C., de Costa, B.R., Rice, K.C., Skolnick, P. *Synthesis and evaluation of imidazo[1,5-a][1,4]benzodiazepine esters with high affinities and selectivities at "diazepam-insensitive" benzodiazepine receptors*. J Med Chem 1993, 36: 1001-6.

38. Wong, G., Koehler, K.F., Skolnick, P. et al. *Synthetic and computer-assisted analysis of the structural requirements for selective, high-affinity ligand binding to diazepam-insensitive benzodiazepine receptors*. J Med Chem 1993, 36: 1820-30.

39. Knoflach, F., Benke, D., Wang, Y. et al. *Pharmacological modulation of the diazepam-insensitive recombinant gamma-aminobutyric acidA receptors alpha 4 beta 2 gamma 2 and alpha 6 beta 2 gamma 2*. Mol Pharmacol 1996, 50: 1253-61.

40. Facklam, M., Schoch, P., Bonetti, E.P. et al. *Relationship between benzodiazepine receptor occupancy and functional effects in vivo of four ligands of differing intrinsic efficacies*. J Pharmacol Exp Ther 1992, 261: 1113-21.

41. Martin, J.R., Schoch, P., Jenck, F., Moreau, J.L., Haefely, W.E. *Pharmacological characterization of benzodiazepine receptor ligands with intrinsic efficacies ranging from high to zero*. Psychopharmacology (Berlin) 1993, 111: 415-22.

42. van Steveninck, A.L., Gieschke, R., Schoemaker, R.C. et al. *Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol.* Br J Clin Pharmacol 1996, 41: 565-72.

43. Rickels, K., DeMartinis, N., Aufdembrinke, B. *A double-blind, placebo-controlled trial of abecarnil and diazepam in the treatment of patients with generalized anxiety disorder.* J Clin Psychopharmacol 2000, 20: 12-8.

44. Lippa, A., Czobor, P., Stark, J. et al. *Selective anxiolysis produced by ocinaplon, a GABAA receptor modulator.* Proc Natl Acad Sci USA 2005, 102: 7380-5.

45. Atack, J.R., Pike, A., Marshall, G. et al. *The in vivo properties of pagoclone in rat are most likely mediated by 5'-hydroxy pagoclone*. Neuropharmacology 2006, 50: 677-89.

46. Sieghart, W. *Multiplicity of GABAA-benzodiazepine receptors.* Trends Pharmacol Sci 1989, 10: 407-11.

47. Sieghart, W., Schuster, A. *Affinity of various ligands for benzodiazepine receptors in rat cerebellum and hippocampus*. Biochem Pharmacol 1984, 33: 4033-8.

48. Rowlett, J.K., Platt, D.M., Lelas, S., Atack, J.R., Dawson, G.R. *Different GABAA receptor subtypes mediate the anxiolytic,*

*abuse-related, and motor effects of benzodiazepine-like drugs in primates*. Proc Natl Acad Sci USA 2005, 102: 915-20.

49. Griebel, G., Perrault, G., Simiand, J. et al. *SL651498, a GABAA receptor agonist with subtype-selective efficacy, as a potential treatment for generalized anxiety disorder and muscle spasms*. CNS Drug Rev 2003, 9: 3-20.

50. Licata, S.C., Platt, D.M., Cook, J.M., Sarma, P.V., Griebel, G., Rowlett, J.K. *Contribution of GABAA receptor subtypes to the anxiolytic-like, motor, and discriminative stimulus effects of benzodiazepines: Studies with the functionally-selective ligand SL651498*. J Pharmacol Exp Ther 2005, 313: 1118-25.

51. Johnstone, T.B., Hogenkamp, D.J., Coyne, L. et al. *Modifying quinolone antibiotics yields new anxiolytics*. Nat Med 2004, 10: 31-2.

52. Sandford, J.J., Forshall, S., Bell, C. et al. *Crossover trial of pagoclone and placebo in patients with DSM-IV panic disorder.* J Psychopharmacol 2001, 15: 205-8.

53. Atack, J.R., Hutson, P.H., Collinson, N. et al. *Anxiogenic properties of an inverse agonist selective for alpha3 subunit-containing GABA A receptors*. Br J Pharmacol 2005, 144: 357-66.

54. Collins, I., Moyes, C., Davey, W.B. et al. *3-Heteroaryl-2-pyridones: Benzodiazepine site ligands with functional selectivity for alpha 2/alpha 3-subtypes of human GABA(A) receptor-ion channels*. J Med Chem 2002, 45: 1887-900.

55. Langen, B., Egerland, U., Bernoster, K., Dost, R., Unverferth, K., Rundfeldt, C. *Characterization in rats of the anxiolytic potential of ELB139 [1-(4-chlorophenyl)-4-piperidin-1-yl-1,5-dihydroimidazol-2-on], a new agonist at the benzodiazepine binding site of the GABAA receptor*. J Pharmacol Exp Ther 2005, 314: 717-24.

56. Dias, R., Sheppard, W.F., Fradley, R.L. et al. *Evidence for a significant role of alpha 3-containing GABAA receptors in mediating the anxiolytic effects of benzodiazepines*. J Neurosci 2005, 25: 10682-8.

57. Sternfeld, F., Carling, R.W., Jelley, R.A. et al. *Selective, orally active gamma-aminobutyric acidA alpha5 receptor inverse agonists as cognition enhancers*. J Med Chem 2004, 47: 2176-9.

58. Chambers, M.S., Atack, J.R., Carling, R.W. et al. *An orally bioavailable, functionally selective inverse agonist at the benzodiazepine site of GABAA alpha5 receptors with cognition enhancing properties*. J Med Chem 2004, 47: 5829-32.

59. Korpi, E.R., Luddens, H. *Furosemide interactions with brain GABAA receptors*. Br J Pharmacol 1997, 120: 741-8.

60. Fisher, J.L. *Amiloride inhibition of gamma-aminobutyric acid(A) receptors depends upon the alpha subunit subtype*. Mol Pharmacol 2002, 61: 1322-8.

61. Mathews, G.C., Bolos-Sy, A.M., Holland, K.D. et al. *Developmental alteration in GABAA receptor structure and physiological properties in cultured cerebellar granule neurons*. Neuron 1994, 13: 149-58.

62. El Hadri, A., Abouabdellah, A., Thomet, U. et al. *N-Substituted 4-amino-3,3-dipropyl-2(3H)-furanones: New positive allosteric modulators of the GABA(A) receptor sharing electrophysiological properties with the anticonvulsant loreclezole*. J Med Chem 2002, 45: 2824-31.

63. Razet, R., Thomet, U., Furtmuller, R. et al. *5-[1'-(2'-N-Arylsulfonyl-1',2',3',4'-tetrahydroisoquinolyl)]-4, 5-dihydro-2(3H)-furanones: Positive allosteric modulators of the GABA(A) receptor with a new mode of action*. J Med Chem 2000, 43: 4363-6.

64. Thomet, U., Baur, R., Razet, R. et al. *A novel positive allosteric modulator of the GABA(A) receptor: The action of (+)-ROD188*. Br J Pharmacol 2000, 131: 843-50.

65. Sigel, E., Baur, R., Furtmueller, R., Razet, R., Dodd, R.H., Sieghart, W. *Differential cross talk of ROD compounds with the benzodiazepine binding site*. Mol Pharmacol 2001, 59: 1470-7.

66. Wafford, K.A., Bain, C.J., Quirk, K. et al. *A novel allosteric modulatory site on the GABAA receptor beta subunit*. Neuron 1994, 12: 775-82.

67. Wingrove, P.B., Wafford, K.A., Bain, C., Whiting, P.J. *The modulatory action of loreclezole at the gamma-aminobutyric acid type A receptor is determined by a single amino acid in the beta 2 and beta 3 subunit*. Proc Natl Acad Sci USA 1994, 91: 4569-73.

68. Belelli, D., Lambert, J.J., Peters, J.A., Wafford, K., Whiting, P.J. *The interaction of the general anesthetic etomidate with the gamma-aminobutyric acid type A receptor is influenced by a single amino acid*. Proc Natl Acad Sci USA 1997, 94: 11031-6.

69. Thompson, S.A., Wheat, L., Brown, N.A. et al. *Salicylidene salicylhydrazide, a selective inhibitor of beta 1-containing GABAA receptors*. Br J Pharmacol 2004, 142: 97-106.

70. Smith, A.J., Oxley, B., Malpas, S., Pillai, G.V., Simpson, P.B. *Compounds exhibiting selective efficacy for different beta subunits of human recombinant gamma-aminobutyric acid A receptors*. J Pharmacol Exp Ther 2004, 311: 601-9.

71. Patel, J.B., Malick, J.B., Salama, A.I., Goldberg, M.E. *Pharmacology of pyrazolopyridines*. Pharmacol Biochem Behav 1985, 23: 675-80.

72. Thompson, S.-A., Wingrove, P.B., Connelly, L., Whiting, P.J., Wafford, K.A. *Tracazolate reveals a novel type of allosteric interaction with recombinant gamma-aminobutyric acid(A) receptors*. Mol Pharmacol 2002, 61: 861-9.

73. Lambert, J.J., Belelli, D., Hill-Venning, C., Peters, J.A. *Neurosteroids and GABAA receptor function*. Trends Pharmacol Sci 1995, 16: 295-303.

74. Lambert, J.J., Harney, S.C., Belelli, D., Peters, J.A. *Neurosteroid modulation of recombinant and synaptic GABAA receptors*. Int Rev Neurobiol 2001, 46: 177-205.

75. Bianchi, M.T., Macdonald, R.L. *Neurosteroids shift partial agonist activation of GABA(A) receptor channels from low- to high-efficacy gating patterns*. J Neurosci 2003, 23: 10934-43.

76. Wallner, M., Hanchar, H.J., Olsen, R.W. *Ethanol enhances alpha 4 beta 3 delta and alpha 6 beta 3 delta gamma-aminobutyric acid type A receptors at low concentrations known to affect humans*. Proc Natl Acad Sci USA 2003, 100: 15218-23.

77. Hanchar, H.J., Chutsrinopkun, P., Meera, P. et al. *From the cover: Ethanol potently and competitively inhibits binding of the alcohol antagonist Ro15-4513 to {alpha}4/6beta3{delta} GABAA receptors*. Proc Natl Acad Sci USA 2006, 103: 8546-51.

78. Unwin, N. *Acetylcholine receptor channel imaged in the open state*. Nature 1995, 373: 37-43.

79. Unwin, N. *Structure and action of the nicotinic acetylcholine receptor explored by electron microscopy*. FEBS Lett 2003, 555: 91-5.

80. Unwin, N. *Refined structure of the nicotinic acetylcholine receptor at 4A resolution*. J Mol Biol 2005, 346: 967-89.

81. Unwin, N., Miyazawa, A., Li, J., Fujiyoshi, Y. *Activation of the nicotinic acetylcholine receptor involves a switch in conformation of the [alpha] subunits*. J Mol Biol 2002, 319: 1165-76.

82. Brejc, K., van Dijk, W.J., Klaassen, R.V. et al. *Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors*. Nature 2001, 411: 269-76.

83. Nishikawa, K., Jenkins, A., Paraskevakis, I., Harrison, N.L. *Volatile anesthetic actions on the GABAA receptors: Contrasting effects of alpha 1(S270) and beta 2(N265) point mutations*. Neuropharmacology 2002, 42: 337-45.

84. Bali, M., Akabas, M.H. *Defining the propofol binding site location on the GABAA receptor*. Mol Pharmacol 2004, 65: 68-76.