Perspectives in Pharmacology

A New Benzodiazepine Pharmacology

H. MÖHLER, J. M. FRITSCHY, and U. RUDOLPH
Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland (H.M., J.M.F., U.R.); and Department of Applied Biosciences, Swiss Federal Institute of Technology, Zurich, Switzerland (H.M.)

Received March 5, 2001; accepted July 5, 2001 This paper is available online at http://jpet.aspetjournals.org

ABSTRACT
Classical benzodiazepine drugs are in wide clinical use as anxiolytics, hypnotics, anticonvulsants, and muscle relaxants. They act by enhancing the γ-aminobutyric acidA (GABA_A) receptor function in the central nervous system. The pharmacological relevance of the multitude of structurally diverse GABA_A receptor subtypes has only recently been identified. Based on an in vivo point mutation strategy, GABA_A receptors were found to mediate sedation, anterograde amnesia, and part of the seizure protection, whereas GABA_A receptors, but not GABA_A receptors, mediate anxiolysis. Rational drug targeting to specific receptor subtypes has now become possible. Only restricted neuronal networks will be modulated by the new subtype-selective drugs. Promising new anxiolytics have already been developed. A new pharmacology of the benzodiazepine site is on the horizon.

GABAergic inhibition is one of the most rapidly developing topics in neuropharmacology. New therapeutic opportunities arise due to increasing insights into the molecular architecture and diversity of the components involved in signal transduction such as GABA_A receptors, GABA_B receptors, and GABA transporters. GABA_A receptors are important drug targets representing the sites of action of benzodiazepines, barbiturates, and neurosteroids. The present article focuses on the pharmacological distinction of GABA_A receptor subtypes as a basis for the development of new drugs that target restricted neuronal networks. In particular, new ligands of the benzodiazepine site acting selectively on GABA_A receptor subtypes are expected to dissect the pharmacological spectrum of classical benzodiazepines and display a minimum of side effects. For further information on GABA_A receptor subtypes other recent reviews may be consulted (Barnard et al., 1998; Möhler et al., 2000; Olsen and Homanics, 2000; Whiting et al., 2000; Möhler, 2001).

Synaptic Action of Benzodiazepines
At synapses, GABA_A receptors are activated by a brief nonequilibrium exposure to high concentrations of GABA. Consistent with an increase in the affinity of the receptors for GABA, therapeutically active benzodiazepines prolonged the decay of spontaneous miniature inhibitory postsynaptic currents (mIPSC). Similarly, the amplitude of mIPSC was found to be enhanced by a benzodiazepine agonist in various neuronal systems (Perrais and Ropert, 1999; Hajos et al., 2000), suggesting that the drug-induced increase of the affinity for GABA resulted in the recruitment of more receptors for activation by GABA. However, in other neuronal systems the amplitude of the mIPSC remained unaltered by a benzodiazepine agonist (Mody et al., 1994; Poncer et al., 1996; Hajos et al., 2000), which has been interpreted to indicate that the release of a single quantum of GABA saturates all of the available GABA_A receptors in the respective synapses inducing a maximal peak response without further enhancement by the drug. Thus, at synapses that generate mIPSCs, the postsynaptic receptor occupancy by GABA appears to be cell- and synapse-specific, reflecting local differences in the number of receptors or the GABA concentration in the cleft. Accordingly, the influence of benzodiazepine agonists on the amplitude of mIPSCs appears to vary with the operational configuration of the GABAergic synapse (Hajos et al., 2000). In summary, the enhancement of a GABAergic inhibitory response by a benzodiazepine agonist is based on the prolonged decay of the mIPSC and a potential increase of the mIPSC amplitude. Even if the peak mIPSC amplitude is not enhanced per se, the drug-induced prolongation of individual mIPSCs will be reflected in an increased peak amplitude of the compound inhibitory response caused by the summation of several miniature currents (Mody et al., 1994).

ABBREVIATIONS: GABA, γ-aminobutyric acid; mIPSC, miniature inhibitory postsynaptic currents.

0022-3565/02/3001-2–8$3.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS Vol. 300, No. 1
Copyright © 2002 by The American Society for Pharmacology and Experimental Therapeutics
JPET 300:2–8, 2002
Printed in U.S.A.
GABA<sub>A</sub> Receptors and Their Multiplicity

Based on the presence of 7 subunit families comprising at least 18 subunits in the central nervous system (α<sub>1</sub>–α<sub>6</sub>, β<sub>1</sub>–β<sub>3</sub>, γ<sub>1</sub>–γ<sub>3</sub>, δ, ε, θ<sub>1</sub>–θ<sub>3</sub>) the GABA<sub>A</sub> receptors display an extraordinary structural heterogeneity. Most GABA<sub>A</sub> receptor subtypes in vivo are believed to be composed of α-, β-, and γ-subunits. The role of the δ-, ε-, and θ-subunits, which have a very limited expression pattern in the brain, remains to be determined, but it is possible that they substitute for the γ-subunit in αβ-γ combinations. The physiological significance of the structural diversity of GABA<sub>A</sub> receptors lies in the provision of receptors that differ in their channel kinetics, affinity for GABA, rate of desensitization, and subcellular positioning. In addition, the GABA<sub>A</sub> receptor subtypes can be distinguished by their pharmacology.

Synaptic and Extrasynaptic GABA<sub>A</sub> Receptors. The first electron microscopic studies of GABA<sub>A</sub> receptors revealed the ubiquitous presence of extrasynaptic GABA<sub>A</sub> receptors in the cerebellum, thalamus, and cerebral cortex (Somogyi, 1989). In fact, synaptic GABA<sub>A</sub> receptors are best seen using postembedding electron microscopy, or by immunofluorescence staining using weakly fixed brain sections (Fritschy et al., 1998a), showing pronounced enrichment in the postsynaptic density of GABAergic synapses compared with extrasynaptic sites (Nusser et al., 1995). These studies also revealed differential targeting of GABA<sub>A</sub> receptor subtypes to different types of synapses. For instance, the α<sub>2</sub> subunit in hippocampal pyramidal cells is concentrated in synapses on the axon-initial segment (Nusser et al., 1996a; Fritschy et al., 1998a), as well as in synapses formed by cholecystokinin-positive basket cells on the somata (Nyiri et al., 2001). The α<sub>6</sub> subunit, which represents diazepam-insensitive GABA<sub>A</sub> receptors in the cerebellum can be found both in excitatory and inhibitory synapses in glomeruli of the granule cell layer, as well as extrasynaptically (Nusser et al., 1996b,1999; Sassoe-Pognetto et al., 2000). Finally, GABA<sub>A</sub> receptors containing the δ-subunit in the cerebellum are exclusively found at extrasynaptic sites (Nusser et al., 1998).

Both extrasynaptic receptor types mediate tonic inhibition of neuronal activity (Brickley et al., 1996, 2001).

Synaptic and extrasynaptic GABA<sub>A</sub> receptors differ in their kinetic properties in line with their distinct functional roles. For instance, extrasynaptic GABA<sub>A</sub> receptors containing the δ-subunit in dentate gyrus and cerebellum are tailored for tonic inhibition, due to their high affinity for GABA and slow desensitization kinetics (Brickey et al., 1996; Mody and Nusser, 2000). Marked differences in desensitization kinetics have also been reported for synaptic and extrasynaptic receptors in inferior olivary neurons. GABA<sub>A</sub> receptors containing the α<sub>2</sub>-subunit are postsynaptic and characterized by rapid desensitization kinetics, whereas extrasynaptic GABA<sub>A</sub> receptor containing the α<sub>6</sub>-subunit desensitize very slowly (Devor et al., 2001). Interestingly, such differences are not evident in recombinant expression systems, suggesting the contribution of additional factors regulating GABA<sub>A</sub> receptor function at synaptic and extrasynaptic sites. A further identification of the molecular composition of GABAergic postsynaptic densities is expected to shed light on the functional regulation of synaptic GABA<sub>A</sub> receptors (Moss and Smart, 2001).

The demonstration that gephyrin, a synaptic clustering protein initially isolated with glycine receptors, is present in a subset of GABAergic synapses in the retina (Sassoe-Pognetto et al., 1995) prompted the analysis of its role in relation to GABA<sub>A</sub> receptors. Gephyrin was shown to be required, along with the γ<sub>2</sub>-subunit, for postsynaptic clustering of major GABA<sub>A</sub> receptor subtypes (Essrich et al., 1998; Kneussel et al., 1999). A recent study demonstrated by immunoelectron microscopy and double-immunofluorescence staining that gephyrin is colocalized with the vast majority of postsynaptic GABA<sub>A</sub> receptors throughout the central nervous system (Sassoe-Pognetto et al., 2000), indicating that it represents a useful marker of GABAergic (and glycineric) synapses.

Diazepam-Sensitive GABA<sub>A</sub> Receptors. Receptors containing the α<sub>1</sub>, α<sub>2</sub>, α<sub>3</sub>, or α<sub>5</sub>-subunits in combination with any of the β-subunits and the γ<sub>2</sub>-subunit are most prevalent in the brain (Fig. 2). These receptors are sensitive to benzodiazepine modulation. The major receptor subtype is assembled from the subunits α<sub>2</sub>β<sub>2</sub>γ<sub>2</sub>, with only a few brain regions lacking this receptor (granule cell layer of the olfactory bulb, reticular nucleus of the thalamus, spinal cord motoneurons) (Fritschy and Möhler, 1995; Pirker et al., 2000) (Table 1).

Receptors containing the α<sub>2</sub>- or α<sub>5</sub>-subunit are considerably less abundant and are highly expressed in brain areas where the α<sub>1</sub>-subunit is absent or present at low levels (Table 1). The α<sub>2</sub>- and α<sub>5</sub>-subunits are frequently coexpressed with the β<sub>2</sub>- and γ<sub>2</sub>-subunits, which is particularly evident in hippocampal pyramidal neurons (α<sub>2</sub>β<sub>2</sub>γ<sub>2</sub>) and in cholinergic neurons of the basal forebrain (α<sub>2</sub>β<sub>2</sub>γ<sub>2</sub>). The ligand-binding profile of these receptors differs from that of α<sub>2</sub>β<sub>2</sub>γ<sub>2</sub> by having a considerably lower displacing potency for ligands such as 3-carboxymethoxy-β-carboline, CL 218,872, and zolpidem (Table 1).

Receptors containing the α<sub>3</sub>-subunit are of minor abundance in the whole brain (Table 1) but are expressed to a significant extent in the hippocampus, where they comprise 15 to 20% of the diazepam-sensitive GABA<sub>A</sub> receptor population, predominantly coassembled with the β<sub>2</sub>-and γ<sub>2</sub>-subunits. The α<sub>3</sub>-receptors are differentiated from α<sub>2</sub>β<sub>2</sub>γ<sub>2</sub>, α<sub>5</sub>β<sub>2</sub>γ<sub>2</sub>, and α<sub>6</sub>β<sub>2</sub>γ<sub>2</sub> receptors by a lower affinity to CL 218,872 and near insensitivity to zolpidem (Table 1).
The $\gamma_1$ and $\gamma_2$ subunits characterize a small population of receptors that contain various types of $\alpha$- and $\beta$-subunits. Due to their reduced affinity for the classical benzodiazepines, they do not appear to contribute to any great extent to their pharmacology in vivo (Mohler et al., 2000; Whiting et al., 2000; Mohler, 2001).

**Diazepam-Insensitive GABA$_\Lambda$ Receptors.** GABA$_\Lambda$ receptors that do not respond to clinically used ligands, such as diazepam, flunitrazepam, clonazepam, and zolpidem, are of low abundance in the brain and are largely characterized by the $\alpha_4$- and $\alpha_6$-subunits (Table 1). Receptors containing the $\alpha_4$-subunit are generally expressed at very low abundance but more prominently in thalamus and dentate gyrus (Pirker et al., 2000); those containing the $\alpha_6$-subunit are restricted to the granule cell layer of the cerebellum (about 30% of all GABA$_\Lambda$ receptors in the cerebellum). Both receptor populations are structurally heterogeneous, and the majority of the $\alpha_6$-containing receptors are of the $\alpha_6\beta_2\gamma_2$ combination (Table 1). The benzodiazepine site profile of $\alpha_4$- and $\alpha_6$-receptors is characterized by a low affinity for flumazenil and bretazenil and a switch in the efficacy of Ro 15-4513 (ethyl 8-acido-5,6-dihydro-5-methyl-6-oxo-4H-imidazol[1,5-α][1,4]benzodiazepine-3-carboxylate) from an inverse agonist to an agonist. The $\delta$-subunit is frequently coassembled with the $\alpha_4$- or the $\alpha_6$-subunit in benzodiazepine insensitive receptors (Mohler et al., 2000; Whiting et al., 2000; Mohler, 2001).

In the retina, homomeric receptors consisting of the $\rho$-subunit represent a particular class of GABA-gated chloride channels. Their GABA site is insensitive to bicuculline and baclofen, and they are not modulated by barbiturates or benzodiazepines (Table 1). Due to these distinctive features the receptors are frequently termed GABA$_c$ receptors (Bormann, 2000), although they can also be considered as a homomeric class of GABA$_\Lambda$ receptors (Barnard et al., 1998).

**Distinct Benzodiazepine Sites Per Receptor**

The benzodiazepine site is thought to be located at the interface of the respective $\alpha$-subunit ($\alpha_1$, $\alpha_2$, $\alpha_3$, $\alpha_6$) and the $\gamma_2$-subunit. About 25% of $\alpha_1$-receptors in rat brain contain a second type of $\alpha$-subunit, i.e., the $\alpha_6$-subunit is coassembled with the $\alpha_2$-subunit. If there is no preference as to which subunit holds the position adjacent to the $\gamma_2$-subunit, a mixed pharmacology for the benzodiazepine site would be expected. Receptors with $\alpha_1$ and $\alpha_6$ subunits displayed a ligand-binding profile characteristic of both $\alpha_6$ and $\alpha_2$ subunits with the latter being predominant (Araujo et al., 1996). Therefore, each $\alpha$ subunit appears to contribute its own binding characteristics. For $\alpha_1\alpha_6\beta\gamma_2$ receptors, which represent about 40% of all $\alpha_6$ receptors (Pollard et al., 1995; Khan et al., 1996; Jechlinger et al., 1998), conflicting results were reported with either each $\alpha$-subunit contributing its typical pharmacology (Khan et al., 1996) or a single pharmacology typical for $\alpha_6$ (Pollard et al., 1995). Similarly, in radioligand binding assays
<table>
<thead>
<tr>
<th>Composition</th>
<th>Pharmacological Characteristics</th>
<th>Neuronal Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_1\beta_2\gamma_2$</td>
<td>Major subtype (60% of all $\text{GABA}_A$ receptors). Mediates the sedative, amnestic, and—to a large extent—the anticonvulsant action of benzodiazepine site agonists. High affinity for classical benzodiazepines, zolpidem, and the antagonist flumazenil.</td>
<td>Synaptic and extrasynaptic</td>
</tr>
<tr>
<td>$\alpha_3\beta_2\gamma_2$</td>
<td>Minor subtype (15–20%). Mediates anxiolytic action of benzodiazepine site agonists. High affinity for classical benzodiazepine agonists and the antagonist flumazenil. Intermediate affinity for zolpidem.</td>
<td>Synaptic</td>
</tr>
<tr>
<td>$\alpha_6\beta_2\gamma_2$</td>
<td>Minor subtype (10–15%). High affinity for classical benzodiazepine agonists, and the antagonist flumazenil. Intermediate affinity for zolpidem.</td>
<td>Synaptic</td>
</tr>
<tr>
<td>$\alpha_4\beta_2\gamma_2$</td>
<td>Less than 5% of all receptors. Insensitive to classical benzodiazepine agonists and zolpidem.</td>
<td>Extrasynaptic</td>
</tr>
<tr>
<td>$\alpha_6\beta_2\gamma_2$</td>
<td>Minor population. Lacks benzodiazepine site.</td>
<td>Homomeric receptors: insensitive to bicuculline, barbiturates, baclofen, and all benzodiazepine site ligands. Also termed $\text{GABA}_A$ receptor; for nomenclature see Barnard et al., 1998.</td>
</tr>
<tr>
<td>$\alpha_6\beta_2\delta$</td>
<td>Less than 5% of all receptors. Insensitive to classical benzodiazepine agonists and zolpidem.</td>
<td>Extrasynaptic</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Homomeric receptors: insensitive to bicuculline, barbiturates, baclofen, and all benzodiazepine site ligands. Also termed $\text{GABA}_A$ receptor; for nomenclature see Barnard et al., 1998.</td>
<td>Synaptic</td>
</tr>
</tbody>
</table>
of hippocampal $\alpha_5$ receptors, only the $\alpha_2$ pharmacology was apparent (Araujo et al., 1999), although the receptors contain an additional $\alpha_1\gamma$, $\alpha_2\gamma$, or $\alpha_3\gamma$ subunit (Sur et al., 1998; Araujo et al., 1999). Thus, the impact of distinct $\alpha$-subunits on the receptor pharmacology remains to be defined.

Recently, a novel, low affinity benzodiazepine site was identified on recombinant GABA$_A$ receptors ($\alpha_1\beta_2\gamma_2$) (Walters et al., 2000). It displayed micromolar affinity for diazepam and was insensitive to flumazenil. This site is not of therapeutic relevance since practically all clinically relevant effects of benzodiazepine drugs can be blocked by flumazenil. In addition, the presence of the $\gamma_2$-subunit was not a prerequisite for the low affinity site since it is also present on $\alpha_1\beta_1$-receptors (Walters et al., 2000). At present it cannot be excluded that the low-affinity site represents the so-called peripheral benzodiazepine site, which is known to occur also on GABA$_A$ receptors (Haefely, 1994).

**Pharmacology of GABA$_A$ Receptor Subtypes in Vivo**

In the search for benzodiazepine site ligands with higher therapeutic selectivity and a reduced side effect profile, GABA$_A$ receptor subtypes have long been considered to be promising targets. However, it was only recently that the pharmacological relevance of GABA$_A$ receptor subtypes was identified based on a gene knock-in strategy (Rudolph et al., 1999; Löw et al., 2000). The benzodiazepine site was rendered diazepam-insensitive by a point mutation in the respective $\alpha$ subunits by replacing a histidine residue with an arginine residue $\alpha_1$H101R, $\alpha_2$H101R, $\alpha_3$H126R, and $\alpha_5$H105R) (Wieland et al., 1992; Benson et al., 1998). Mouse lines were generated in which the $\alpha_1\gamma$, $\alpha_2\gamma$, or $\alpha_3$-receptor subtypes were diazepam-insensitive. In these mice certain benzodiazepine effects were expected to be blunted, which was attributed to the respective point-mutated receptor. This strategy permitted the allocation of the benzodiazepine drug actions to identified GABA$_A$ receptor subtypes. In addition, it implicated the neuronal networks expressing the particular receptor in mediating the corresponding drug actions.

The $\alpha_1\gamma$, $\alpha_2\gamma$, and $\alpha_3\gamma$-point-mutated receptors displayed a level of expression and a regional and cellular distribution that was indistinguishable from those in wild-type mice. In addition, the gating of the point-mutated receptors by GABA remained unaltered. Furthermore, the neomycin resistance marker introduced by the replacement vector was eliminated from the genome of all mouse lines generated by cre-loxP-mediated recombination. The pharmacological analysis of the point-mutated mice was therefore free of any potential interference, which may have resulted from the presence of the neomycin resistance marker. Thus, a deficit in the behavioral response to diazepam in the point-mutated mouse lines is attributed to the pharmacological role of the respective receptor subtype (Rudolph et al., 1999; Löw et al., 2000).

**Receptors Mediating Sedation.** Sedation is a major property of many benzodiazepine site ligands and has now been shown to be mediated via $\alpha_1$-receptors. Among $\alpha_1\gamma$, $\alpha_2\gamma$, and $\alpha_3\gamma$-point-mutated mice only the $\alpha_1$H101R mutants were resistant to the depression of motor activity by diazepam and zolpidem (Rudolph et al., 1999; Crestani et al., 2000a; Löw et al., 2000). This effect was specific for ligands of the benzodiazepine site since pentobarbital or a neurosteroid remained as effective in $\alpha_1$H101R mice as in wild-type mice in reducing motor activity. An $\alpha_1$H101R mouse line was also generated by McKernan et al. (2000). When measured under novelty (“stress”) conditions, diazepam increased locomotion compared with wild-type (McKernan et al., 2000). This effect is apparently dependent on the test procedure and can be induced also in the $\alpha_1$H101R mice generated by Rudolph et al. (1999) under comparable test conditions (Crestani et al., 2000b).

**Receptors Mediating Amnesia.** Anterograde amnesia is a classical side effect of benzodiazepine drugs. The memory-impairing effect of diazepam, analyzed in a step-through passive avoidance paradigm, was strongly reduced in the $\alpha_1$H101R mice compared with wild-type mice as shown by the increased latency for reentering the dark compartment 24 h after training (Rudolph et al., 1999). This effect was not due to a potential nonspecific impairment since the ability of a muscarinic antagonist to induce amnesia was retained in the $\alpha_1$H101R mice. These results demonstrate that the diazepam-induced anterograde amnesia is mediated by $\alpha_1$-receptors.

**Receptors Providing Protection Against Seizures.** The anticonvulsant activity of diazepam, assessed by its protection against pentylentetrazole-induced tonic convulsions, was reduced in $\alpha_1$H101R mice compared with wild-type mice (Rudolph et al., 1999). The anticonvulsant effect of diazepam, which remained in the $\alpha_1$H101R mice, was due to GABA$_A$ receptors other than $\alpha_1$, since it was antagonized by flumazenil. Sodium phenobarbital remained fully effective as an anticonvulsant in $\alpha_1$H101R mice with a dose response intensity similar to that of wild-type mice. These results show that the anticonvulsant activity of benzodiazepines is partially but not fully mediated by $\alpha_1$-receptors. The anticonvulsant action of zolpidem is exclusively mediated by $\alpha_1$-receptors, since its anticonvulsant action is completely absent in $\alpha_1$H101R mice (Crestani et al., 2000a).

**Receptors for Anxiolysis.** New strategies for the development of daytime anxiolytics that are devoid of drowsiness are of high priority. The particular receptor subtype that mediates the anxiolytic activity of benzodiazepine drugs was recently identified (Löw et al., 2000). When the reactivity to naturally aversive stimuli is measured in wild-type mice, diazepam increases the time spent in the lit area of the light/dark choice test and on the open arms of the elevated plus maze, respectively. In contrast, the $\alpha_2$-point-mutated mice were resistant to the effect of diazepam in these test paradigms (Löw et al., 2000). This lack of response was specific for ligands of the benzodiazepine site since $\alpha_2$H101R mice retained the ability to display an anxiolytic-like response to sodium phenobarbital. Thus, the anxiolytic-like action of diazepam is selectively mediated by the enhancement of GABAergic transmission in a population of neurons expressing the $\alpha_2$-GABA$_A$ receptors. Thus, the $\alpha_2$-GABA$_A$ receptors are highly specific targets for the development of future selective anxiolytic drugs. They represent only about 15% of all diazepam-sensitive GABA$_A$ receptors. Selective ligands are therefore expected to be devoid of the major side effects that afflict the classical benzodiazepine anxiolytics.

It had previously been assumed that the anxiolytic action of diazepam is based on the damping of the reticular activating system. It is mainly represented by noradrenergic and...
serotonergic neurons of the brain stem, which express exclusively $\alpha_2$-receptors. The analysis of the $\alpha_2$-point-mutated mice $\alpha_2(\text{H126R})$ indicated that the anxiolytic effect of benzodiazepines, drugs as described above, is not mediated by $\alpha_1$-receptors (Löwik et al., 2000). The reticular activating system therefore does not appear to be a major contributor to axiolyysis. In contrast, the $\alpha_2$-GABA$A$ receptors are highly expressed in cells in the cerebral cortex and the hippocampus, including their pyramidal cells, which display particularly high densities of $\alpha_2$-GABA receptors on their axon initial segment (Nusser et al., 1996a; Fritschy et al., 1998a). Thus, controlling the output of these principal neurons may contribute to axiolyysis.

**Receptors Mediating Myorelaxation.** The degree of muscle tone can be assessed in the horizontal wire test, in which the ability of the animals to grasp and hang onto a wire is measured. The muscle relaxant effect of diazepam is largely mediated by $\alpha_2$-GABA$A$ receptors, as shown by the failure of diazepam to induce changes in muscle tone in the $\alpha_2$-mutated mouse line (Crestani et al., 2001). It was only at high doses that $\alpha_2$-receptors were also implicated. Besides the limbic system (see above), $\alpha_2$-receptors are highly specifically expressed in the spinal cord, notably in the superficial layer of the dorsal horn and in motor neurons (Bohilhalter et al., 1996) with the latter being most clearly implicated in muscle relaxation. It is important to note that the muscle relaxant effect requires higher doses of diazepam than its anxiolytic activity. This is attributed to a higher receptor occupancy required for muscle relaxation.  

**Novel Subtype-Selective Benzodiazepine Site Ligands.** Among the clinically used ligands of the benzodiazepine site only the hypnotic zolpidem displays a pronounced preferential subtype selectivity (Langer et al., 1992) (Table 1). Additional benzodiazepine site ligands with subtype selectivity are under experimental or clinical investigation (Hood et al., 2000) and include the following agents.

L-838,417. The benzodiazepine site ligand L-838,417 interacts with comparable affinity with $\alpha_1$, $\alpha_2$, and $\alpha_3$-receptors and with only 3-fold lower affinity with $\alpha_2$-receptors. However, it displays a dramatic subtype-selective efficacy. L-838,417 fails to modulate the GABA response at $\alpha_1$-receptors but enhances the GABA response at $\alpha_2$, $\alpha_3$, and $\alpha_5$-receptors (McKernan et al., 2000). In line with a lack of $\alpha_1$-receptor activation, L-838,417 showed a high potency in anxiolytic tests (elevated plus maze and fear-potentiated startle) and in anticonvulsant tests (pentylentetrazole, audiogenic seizures). However, even at doses that occupied 95% of benzodiazepine sites, L-838,417 failed to impair motor performance (rotarod test, chain-pulling test) (McKernan et al., 2000). These findings are a major step forward. Ligands with subtype-selective efficacy, which distinguish $\alpha_2$, $\alpha_5$, and $\alpha_3$-receptors from $\alpha_2$-receptors, provide a new way to develop selective anxiolytics without a sedative component. Further improvement may be achieved by focusing the ligand affinity or efficacy more specifically on $\alpha_2$-receptors (see above).

SL65.1498. The pyrido-indole-4-carboxamide derivative SL65.1498 (6-fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1H-pyrido[3,4-b]indol-1-one) shows higher affinity for $\alpha_3$, $\alpha_5$, and $\alpha_2$-GABA$A$ receptors compared with $\alpha_2$-receptors. In addition, it acts as full agonist at $\alpha_2$- and $\alpha_5$-receptors but as a partial agonist at $\alpha_1$-GABA$A$ receptors. In line with its selectivity for the activation of $\alpha_2$- and $\alpha_5$-receptors, the compound showed potent anxiolytic action in animal models (punished lever pressing, punished drinking, elevated plus maze, light/dark test) but did not impair motor coordination (e.g., rotarod) or working memory (Morris water maze) (Scatton et al., 2000).

**Zaleplon.** Zaleplon (CL 284,846) is a pyrazolopyrimidine developed for the treatment of insomnia (Sanger et al., 1996). At recombinant receptors, zaleplon binds preferentially to $\alpha_2$-receptors (1$\beta_2$,2$\gamma_2$s) and to receptors containing the $\gamma_2$-subunit but binds 8- to 20-fold less to $\alpha_2$, $\alpha_3$, and $\alpha_5$-receptors (Dämgen and Lüddens, 1999). Thus, zaleplon is largely a ligand with preference for $\alpha_1$-receptors, which is in keeping with its preponderant hypnotic activity. The contribution of its interaction with $\gamma_2$-receptors is unclear since these receptors are of low abundance in the brain.

**Conclusions.** The genetic dissection of the pharmacological functions of GABA$A$ receptor subtypes has opened up a new strategy in drug development. The heuristic search for novel ligands at the benzodiazepine site will be replaced by the rational targeting of specific receptor subtypes. The ligand selectivity can be achieved either by a preferential ligand affinity, preferential ligand efficacy, or a mixture of both. Acting at small subpopulations of GABA$A$ receptors, these ligands are expected to lack the major side effects of the classical benzodiazepine drugs. The vision of specific anxiolytics with a reduced side-effect profile may become a reality. In addition, therapeutic indications beyond those of the classical benzodiazepine drugs may emerge from subtype-specific drugs.

**References.**

Araujo F, Ruano D, and Vitorica J (1999) Native $\gamma$-aminobutyric acid type A receptor tors from rat hippocampus, containing both $\alpha_1$ and $\alpha_5$ subunits exhibit a single benzodiazepine binding site with $\alpha_3$ pharmacological properties. *J Pharmacol Exp Ther* 290:589–597.


