ABSTRACT

Classical benzodiazepine drugs are in wide clinical use as anxiolytics, hypnotics, anticonvulsants, and muscle relaxants. They act by enhancing the \( \gamma \)-aminobutyric acid (GABA) receptor function in the central nervous system. The pharmacological relevance of the multitude of structurally diverse GABA receptor subtypes has only recently been identified. Based on an in vivo point mutation strategy, \( \alpha_1 \)-GABA receptors were found to mediate sedation, anterograde amnesia, and part of the seizure protection, whereas \( \alpha_2 \)-GABA receptors, but not \( \alpha_3 \)-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.

Synaptic Action of Benzodiazepines

At synapses, GABA 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 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 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).
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, ρ<sub>1</sub>-ρ<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 α-β-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 β<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 soma (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 tailor-made for tonic inhibition, due to their high affinity for GABA and slow desensitization kinetics (Brickley 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>2</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 glycinergic) 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>3</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-carbolin, CL 218,872, and zolpidem (Table 1).

Receptors containing the α<sub>5</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>5</sub>-receptors are differentiated from α<sub>2</sub>β<sub>2</sub>γ<sub>2</sub>, α<sub>2</sub>β<sub>2</sub>γ<sub>2</sub>, and α<sub>2</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 \( \gamma_z \)-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 (Möhler et al., 2000; Whiting et al., 2000; Möhler, 2001).

**Diazepam-Insensitive GABA\( _\alpha \) Receptors.** GABA\( _\alpha \) 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\( _\alpha \) 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 (Möhler et al., 2000; Whiting et al., 2000; Möhler, 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\( _\alpha \) receptors (Bormann, 2000), although they can also be considered as a homomeric class of GABA\( _\alpha \) 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 \( \gamma_z \)-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 \( \gamma_z \)-subunit. If there is no preference as to which subunit holds the position adjacent to the \( \gamma_z \)-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 \( \alpha_1 \) 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

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Fig. 2. The four classes of diazepam-sensitive GABA\( _\alpha \) receptors are distinguished by the type of \( \alpha \)-subunit (\( \alpha_1 \), \( \alpha_2 \), \( \alpha_3 \), \( \alpha_6 \)) and their largely distinct neuronal localizations as demonstrated immunohistochemically in mouse brain sections. The major known pharmacological actions mediated via the respective receptor subtypes are indicated.
### TABLE 1

**GABA<sub>A</sub> Receptor Subtypes**

<table>
<thead>
<tr>
<th>Composition</th>
<th>Pharmacological Characteristics&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Neuronal Localization&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td>α&lt;sub&gt;1&lt;/sub&gt;β&lt;sub&gt;2&lt;/sub&gt;γ&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Major subtype (60% of all GABA&lt;sub&gt;A&lt;/sub&gt; 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 Cerebral cortex (Somogyi, 1989; J.M. Fritschy, unpublished data) Hippocampus, dentate gyrus (interneurons and principal cells) (Nusser et al., 1995) Striatum (interneurons) (J.M. Fritschy, unpublished data) Thalamic relay nuclei (Somogyi, 1989; J.M. Fritschy, unpublished data) Olfactory bulb (mitral cells and interneurons) (Giustetto et al., 1998) Cerebellum (Purkinje cells, stellate, basket cells, and granule cells) (Somogyi et al., 1996; Nusser et al., 1997, 1998, 1999; Sassoe-Pognetto et al., 2000) Deep cerebellar nuclei (Sassoe-Pognetto et al., 2000)</td>
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<td>α&lt;sub&gt;1&lt;/sub&gt;β&lt;sub&gt;2&lt;/sub&gt;γ&lt;sub&gt;2&lt;/sub&gt;</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 Cerebral cortex (Fritschy et al., 1998a) Hippocampus, dentate gyrus (principal cells mainly on the axon initial segment) (Sassoe-Pognetto et al., 2000; Fritschy et al., 1998a; Nusser et al., 1994b) Olfactory bulb (granule cells) (Sassoe-Pognetto et al., 2000) Striatum (spiny stellate cells) (Fritschy et al., 1998a) Inferior olivary neurons (mainly on dendrites) (Devor et al., 2001)</td>
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<td>α&lt;sub&gt;1&lt;/sub&gt;β&lt;sub&gt;2&lt;/sub&gt;γ&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Minor subtype (10–15%). High affinity for classical benzodiazepine agonists, and the antagonist flumazenil. Intermediate affinity for zolpidem.</td>
<td>Synaptic Cerebral cortex (principal cells in particular in layers V and VI; some axon initial segments) (Fritschy et al., 1998a) Hippocampus (some hilar cells) (J.M. Fritschy, unpublished data) Olfactory bulb (tufted cells) (Giustetto et al., 1998) Thalamic reticular nucleus (Fritschy et al., 1998a) Cerebellum (Golgi cells) (Sassoe-Pognetto et al., 2000) Medullary reticular formation (Fritschy et al., 1998a)</td>
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<td>α&lt;sub&gt;1&lt;/sub&gt;β&lt;sub&gt;2&lt;/sub&gt;γ&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Less than 5% of all receptors. Insensitive to classical benzodiazepine agonists and zolpidem.</td>
<td>Extrasynaptic Inferior olivary neurons (Devor et al., 2001)</td>
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<td>α&lt;sub&gt;1&lt;/sub&gt;β&lt;sub&gt;2&lt;/sub&gt;γ&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Less than 5% of all receptors; high affinity for classical benzodiazepine agonists and the antagonist flumazenil. Very low affinity for zolpidem.</td>
<td>Synaptic Spinal trigeminal nucleus, superior olivary neurons (J.M. Fritschy, unpublished data) Cerebral cortex (J.M. Fritschy, unpublished data) Hippocampus (pyramidal cells) (Fritschy et al., 1998b) Olfactory bulb (granule cells) (Fritschy et al., 1998b)</td>
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<td>α&lt;sub&gt;1&lt;/sub&gt;β&lt;sub&gt;2&lt;/sub&gt;γ&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Less than 5% of all receptors. Insensitive to classical benzodiazepine agonists and zolpidem.</td>
<td>Extrasynaptic Cerebellum (granule cells) (Nusser et al., 1998b, 1998, 1999, 1999; Sassoe-Pognetto et al., 2000)</td>
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<td>α&lt;sub&gt;1&lt;/sub&gt;β&lt;sub&gt;2&lt;/sub&gt;γ&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Minor population. Lacks benzodiazepine site.</td>
<td>Extrasynaptic Cerebellum (granule cells) (Nusser et al., 1998b, 1998, 1999; Sassoe-Pognetto et al., 2000)</td>
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<td>α&lt;sub&gt;1&lt;/sub&gt;β&lt;sub&gt;2&lt;/sub&gt;γ&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Homomeric receptors: insensitive to bicuculline, barbiturates, baclofen, and all benzodiazepine site ligands. Also termed GABA&lt;sub&gt;A&lt;/sub&gt; receptor; for nomenclature see Barnard et al., 1998.</td>
<td>Synaptic Retina (Kaulen et al., 1998)</td>
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<sup>a</sup> Reviewed in Macdonald and Olsen, 1994; Barnard et al., 1998; Rudolph et al., 1999; Löw et al., 2000; Möller et al., 2000; Whiting et al., 2000; Möller, 2001. The term classical benzodiazepines refers to diazepam and structurally related agonists in clinical use.

<sup>b</sup> Synaptic localization is based mainly on ultrastructural evidence or on the colocalization with gephyrin and refers to the respective type of β-subunit.
of hippocampal α5 receptors, only the α5 pharmacology was apparent (Araujo et al., 1999), although the receptors contain an additional α1γ, α2γ, or α5γ subunit (Sur et al., 1998; Araujo et al., 1999). Thus, the impact of distinct α-subunits on the receptor pharmacology remains to be defined.

Recently, a novel, low affinity benzodiazepine site was identified on recombinant GABA_A receptors (α1β2γ2) (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 γ2-subunit was not a prerequisite for the low affinity site since it is also present on α1β1-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 α subunits by replacing a histidine residue with an arginine residue [α1(H101R), α2(H101R), α3(H126R), and α5(H105R)] (Wieland et al., 1992; Benson et al., 1998). Mouse lines were generated in which the α1γ, α2γ, or α3-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 α1γ, α2γ, and α3-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 α1-receptors. Among α1γ, α2γ, and α5γ-point-mutated mice only the α1γ(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 α1γ(H101R) mice as in wild-type mice in reducing motor activity. An α1γ(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 α1γ(H101R) mice generated by Rudolph et al. (1999) under comparable test conditions (Crestani et al., 2000b).

**Receptors Providing Protection Against Seizures.** The anticonvulsant activity of diazepam, assessed by its protection against pentylentetrazole-induced tonic convulsions, was reduced in α1γ(H101R) mice compared with wild-type mice (Rudolph et al., 1999). The anticonvulsant effect of diazepam, which remained in the α1γ(H101R) mice, was due to GABA_A receptors other than α1, since it was antagonized by flumazenil. Sodium phenobarbital remained fully effective as an anticonvulsant in α1γ(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 α1-receptors. The anticonvulsant action of zolpidem is exclusively mediated by α1-receptors, since its anticonvulsant action is completely absent in α1γ(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 α2-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 α2γ(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 α2γ-GABA_A receptors. Thus, the α2γ-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 dampening 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 \( \alpha_2(\text{H}126\text{R}) \) indicated that the anxiolytic effect of benzo diazepine drugs, measured as described above, is not mediated by \( \alpha_2 \)-receptors (Löw et al., 2000). The reticular activating system therefore does not appear to be a major contributor to anxiolysis. 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\( _A \)-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 anxiolysis.

**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 (Bohlhalter 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 \( \alpha_5 \)-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 (pentylenetetrazole, 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 \( \alpha_2 \)-, \( \alpha_3 \)-, and \( \alpha_5 \)-receptors from \( \alpha_1 \)-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 \( \alpha_1 \)-, \( \alpha_2 \)-, and \( \alpha_3 \)-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 \( \alpha_3 \)-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 \( \alpha_1 \)-receptors (\( \alpha_2\beta_3\gamma_2 \)) 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 Lüdendes, 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.**

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