Pharmacology of Benzodiazepine Receptors: An Update

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Benzodiazepine receptors are allosteric modulatory sites on $GABA_A$ receptors. $GABA_A$ receptors are probably composed of five protein subunits, at least some of which belong to different subunit classes. So far six α-, four β-, three γ-, one δ- and two rho = p subunits of $GABA_A$ receptors have been identified. A large number of different subunit combinations, each of which will result in a $GABA_A$ receptor with distinct electrophysiological and pharmacological properties, are therefore possible. Many compounds from different chemical classes which are able to bind to benzodiazepine receptors have been identified. Depending on their individual efficacy, binding of these compounds either enhances, reduces or does not influence GABAergic transmission. However, the individual efficacy of the benzodiazepine receptor ligands changes with the subunit composition of the $GABA_A$ receptor. The investigation of the regional distribution, subunit composition and pharmacology of $GABA_A$ receptors will result in the development of new and more selective compounds for psychiatry.

Key Words: $GABA_A$ receptors, benzodiazepine receptors, subunit composition, pharmacology

INTRODUCTION

Benzodiazepines, such as diazepam, flunitrazepam or bromazepam, possess anxiolytic, anticonvulsant, muscle relaxant and sedative/hypnotic properties and are among the most widely prescribed drugs in current therapeutic use. From a large number of electrophysiological investigations, it is known that benzodiazepines enhance the actions of the neurotransmitter γ-aminobutyric acid (GABA) on its receptor (Polc 1988). Since this GABA-enhancing effect has been found in many different biological systems, it is now believed that benzodiazepines produce their overt effects by modulating the GABA system in the brain (Haefely et al 1985).

Biochemical investigations have indicated that there are high-affinity binding sites for benzodiazepines in brain membranes and that these binding sites exhibit many properties which one would expect from pharmacological receptors for these compounds (Braestrup and Nielsen 1983). Thus, the binding of benzodiazepines to these sites occurs rapidly, is stereospecific and saturable, and there is a strong correlation between the clinical potency of benzodiazepines and their ability to displace radiolabeled diazepam or flunitrazepam from its binding site. Therefore, it is now generally assumed that these high-affinity benzodiazepine binding sites are the receptors by which benzodiazepines exert their pharmacologically and clinically relevant actions (Haefely et al 1985).

Other investigations have indicated that these so-called "central benzodiazepine receptors" are closely associated with a $GABA_A$ receptor. Thus, binding of benzodiazepines to the central benzodiazepine receptors is stimulated in the presence of GABA and the $GABA_A$ receptor agonists muscimol, 4,5,6,7-tetrahydroisoxazolo-[5,4-C]pyridin-3-ol (THIP) and isoguvacine (Karobath et al 1981). This stimulation is inhibited by the $GABA_A$ receptor antagonist bicuculline. Reciprocally, benzodiazepines increased the binding of GABA to $GABA_A$ receptors (Skerritt et al 1982; Bristow

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et al 1990). These results are in agreement with electrophysiological studies which indicate that interaction of GABA with $GABA_A$ receptors opens chloride ion channels and that benzodiazepines enhance the frequency of chloride channel opening by their binding to the benzodiazepine receptor and thus the GABA action (Polc 1988). The central benzodiazepine receptors are thus allosteric modulatory sites of the $GABA_A$ receptors.

In addition to the $GABA_A$ receptor-associated "central" benzodiazepine receptors, two other types of benzodiazepine binding sites are present in the brain which are the so-called "peripheral" benzodiazepine binding sites, localized on the outer mitochondrial membrane of many tissues, including brain (Verma and Snyder 1989) and the "micromolar" binding sites (Bowling and De Lorenzo 1982). However, these sites are pharmacologically distinct from and unrelated to the $GABA_A$ receptor-associated benzodiazepine receptors.

Over the last several years, increasing evidence has shown that the $GABA_A$ receptors are associated not only with benzodiazepine receptors but contain several additional drug binding sites. Thus, in addition to the $GABA_A$ and benzodiazepine binding sites, separate and non-overlapping binding sites for some sedative/hypnotic barbiturates (such as pentobarbital or secobarbital), for some convulsants (such as t-butylbicyclophosphorothionate (TBPT) or picrotoxinin), for some anxiolytic, anticonvulsant and hypnotic steroids and for the anthelmintic avermectin B_{1a} have been demonstrated at the $GABA_A$ receptor (Sieghart 1992a). The various drug binding sites interact with each other in a complicated manner and the interaction of a drug with any one of these binding sites allosterically enhances or reduces GABAergic transmission. It is therefore clear that the $GABA_A$-benzodiazepine receptor is a highly regulated, complex molecular structure.

Modulation of GABAergic transmission by benzodiazepine receptor ligands

In the search for compounds with a more selective action than that of the classical benzodiazepines, many ligands with a benzodiazepine or non-benzodiazepine structure were identified which exhibited a high affinity for the $GABA_A$ receptor-associated benzodiazepine receptor (Braestrup and Nielsen 1983; Haefely at al 1985). Some of these ligands, the "benzodiazepine receptor agonists," exhibited a positive intrinsic efficacy and enhanced GABA-induced chloride ion flux. These compounds have anxiolytic, anticonvulsant, muscle-relaxant and sedative-hypnotic properties. Other ligands, the "inverse benzodiazepine receptor agonists," exhibited a negative intrinsic efficacy and reduced GABA-induced chloride flux. These compounds have convulsant, stimulant and anxiogenic effects (Polc et al 1982). A third group of high-affinity ligands, the "benzodiazepine receptor antagonists," have no or only a weak intrinsic efficacy for changing the GABAergic transmission. Therefore, these compounds have no or only weak effects when given to animals or humans but are able to inhibit the effects of both benzodiazepine receptor agonists or inverse benzodiazepine receptor agonists (Polc et al 1982). Between these extreme actions, compounds have been identified (partial agonists or partial inverse agonists) with intermediate actions. Such compounds have less positive or negative intrinsic efficacy than full agonists or inverse agonists (Haefely et al 1985).

It has been demonstrated that for the induction of an anxiolytic, anticonvulsant, muscle-relaxant or sedative/hypnotic effect, a different degree of $GABA_A$ receptor activation is necessary (Duka et al 1979; Petersen and Buus-Lassen 1981; Haefely et al 1992). Thus, for instance, full agonists are able to elicit an anxiolytic or anticonvulsant effect at a low overall receptor occupation. Partial agonists, because of their lower intrinsic efficacy for enhancement of GABAergic transmission, need a higher receptor occupation to produce the same effect. However, the weak enhancement of GABAergic transmission by partial agonists is not sufficient to induce sedative/hypnotic and muscle-relaxant actions since even full agonists need a rather high receptor occupation in order to produce these effects. Partial agonists thus exhibit fewer side-effects than full agonists (Haefely et al 1992). This of course can be used clinically and several partial agonists are currently being investigated in clinical trials.

Several recent reports indicate that partial inverse benzodiazepine receptor agonists may have activating and memory-enhancing effects (Venault et al 1986; Sarter et al 1988; Izquierdo and Medina 1991), and thus, may also be of clinical interest. However, the complete elimination of the unwanted convulsant and anxiogenic properties of full inverse agonists may be difficult to achieve and the development of safe partial inverse agonists will require a major research effort.

Heterogeneity of $GABA_A$ receptors

It is currently assumed that the various effects of benzodiazepines are elicited by $GABA_A$ receptors in different regions of the brain. Another possibility for reducing unwanted side-effects is therefore to specifically address only those receptors which mediate, for instance, the anxiolytic actions of benzodiazepines. This is feasible only if these receptors are different from those mediating sedative/hypnotic or muscle-relaxant effects. There is no evidence at present that this is the case (Doble and Martin 1992). Since the classical benzodiazepines have a similar affinity for benzodiazepine receptors in all investigated regions of the brain (Braestrup and Nielsen 1983), it was originally assumed that there is no heterogeneity of $GABA_A$-benzodiazepine receptors. However, in the last couple of years, several compounds with distinct chemical structures have been identified which seem to differentially interact with "central" benzodiazepine receptors in various brain

regions. Thus, it has been demonstrated that the triazolopyridazine Cl 218872, some benzodiazepines, such as quazepam or cinolazepam and their metabolites, some β-carbolines and the imidazopyridines zolpidem or alpidem exhibit affinities for benzodiazepine receptors in cerebellum several times higher than for those in the hippocampus and other regions of the brain (Sieghart 1989). These and other results support the existence of at least two benzodiazepine receptor subtypes: a BZ_1 receptor enriched in cerebellum and exhibiting a high affinity for the compounds mentioned above; and a BZ_2 receptor enriched in hippocampus and some other brain regions and exhibiting a low affinity for these compounds (Sieghart 1989). Since benzodiazepine receptors are allosteric modulatory sites on $GABA_A$ receptors, a heterogeneity of benzodiazepine receptors reflects a heterogeneity of $GABA_A$ receptors.

The existence of distinct $GABA_A$-benzodiazepine receptors is supported by biochemical and molecular biological studies. $GABA_A$ receptors were purified from brain membranes by affinity chromatography (Sigel and Barnard 1984), and the purified proteins were partially sequenced. The screening of brain cDNA libraries with oligonucleotide probes constructed according to the sequence information obtained led to the identification of a variety of structurally related $GABA_A$ receptor subunits (Schofield 1989). So far, a total of six α-, four β-, three γ-, one δ- and two rho = p subunits of the $GABA_A$ receptor have been cloned and sequenced. In addition, alternatively spliced forms of the γ_2- and β_4-subunits have been identified. Each of these protein subunits consists of a large extracellular part with several possible glycosylation sites, four putative transmembrane domains and a large intracellular loop (Burt and Kamatchi 1991). Subunits belonging to the same group (for instance all α- subunits) exhibit a 70% to 80% homology. Subunits belonging to different groups (for instance α- and β-, α- and γ-, or β- and γ- subunits) exhibit 30% to 40% homology. When these subunits, either alone or in combination with other subunits, were expressed in Xenopus oocytes or in human embryonic kidney cells, it was demonstrated that only the coexpression of α-, β- and γ- subunits resulted in $GABA_A$ receptors which were modulated by benzodiazepines (Burt and Kamatchi 1991; Sieghart 1992a). Analogous to the structurally related nicotinic acetylcholine (nACh) receptor, $GABA_A$ receptors probably contain a total of five subunits, although at present no information is available concerning the subunit composition of any $GABA_A$ receptor *in vivo*.

Properties of recombinant $GABA_A$ receptors

In a series of experiments, Seeburg et al have demonstrated that the type or the α-subunit predominantly determines the benzodiazepine binding properties of recombinant $GABA_A$ receptors (Wieland et al 1992). Receptors containing the α_1- subunit (together with an arbitrary β- and a γ_2-subunit) exhibit benzodiazepine binding properties corresponding to the BZ_1 receptor, whereas receptors containing α_2- or α_3-subunits exhibit properties corresponding to BZ_2 benzodiazepine receptors. Interestingly, however, receptors containing the α_5-subunit together with a β- and γ_2-subunit exhibit an extremely low affinity for the BZ_1 receptor-selective imidazopyridine zolpidem but binding properties similar to BZ_2 receptors for other BZ_1 receptor-selective ligands. Thus, zolpidem is able to distinguish between $GABA_A$ receptors with a high affinity (BZ_1), intermediate affinity (BZ_2) or extremely low affinity (BZ_3) for this compound (Wieland et al 1992; Sieghart 1992b). Receptors containing α_4- or α_6- subunits exhibit no affinity for benzodiazepine receptor agonists but a high affinity for the partial inverse agonist Ro 15-4513 and a reduced affinity for the benzodiazepine receptor antagonist Ro 15-1788 (Wieland et al 1992; Sieghart 1992b).

The information available so far indicates that the type of β-subunit influences only slightly the pharmacological properties of recombinant receptors. However, depending on the γ- subunit used, recombinant receptors with distinct properties arise. Most of the studies conducted so far have used the short (γ_{2S}-) form of the γ_2-subunit for the construction of recombinant receptors. Recent evidence seems to indicate that $GABA_A$ receptors containing the alternatively spliced long (γ_{2L}-) form of the γ_2-subunit, in contrast to those containing the γ_{2S}-subunit, could be modulated by ethanol (Wafford et al 1991). This observation and the different regional distribution of the γ_{2S}- and γ_{2L}-subunits (Glencorse et al 1992) may explain discrepant results indicating an enhancement of GABAergic function by ethanol in some, but not all, species and brain tissues investigated. In addition, these results support previous suggestions that at least some of the actions of low concentrations of ethanol are mediated by $GABA_A$ receptors (Ticku 1990).

Receptors containing a γ_1- rather than a γ_2-subunit exhibit a reduced affinity for benzodiazepines and no affinity for the benzodiazepine receptor antagonist Ro 15-1788 (Burt and Kamatchi 1991; Sieghart 1992b). In addition, most but not all of the benzodiazepine receptor agonists investigated exhibited a lower efficacy for the enhancement of GABAergic transmission than γ_2-subunit-containing receptors. The β-carboline methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM), which exhibits inverse benzodiazepine receptor agonist properties in γ_2-subunit-containing receptors, even exhibited partial benzodiazepine receptor agonist properties in receptors containing γ_1-subunits (Puia et al 1991). These observations were extended to other $GABA_A$ receptor subunit combinations, and evidence indicates that to enhance or reduce GABAergic transmission, the efficacy of benzodiazepine receptor ligands changes with receptor composition (Puia et al 1991; 1992). Thus, a compound could act as a full agonist at one receptor and as a partial agonist at another receptor. This finding opens another avenue of research for the development of drugs with fewer side-effects.

Only a few subunit combinations have so far been used in the course of investigating the properties of recombinant receptors (Sieghart 1992a; 1992b). The large number of $GABA_A$ receptor subunits that have now been identified and the hypothesis that five subunits are necessary to form an intact GABA receptor implicate an enormous number of possible subunit combinations. Depending on the structural model used several hundred to several thousand subunit combinations are possible (Burt and Kamatchi 1991), each of which will lead to pharmacologically and electrophysiologically distinct $GABA_A$ receptors. Not all these receptors necessarily exist. The identification of the subunit combinations actually existing in the brain is therefore one of the major goals of current research into $GABA_A$ receptors.

Evidence for the actual existence of $GABA_A$ receptor subtypes with properties resembling those of recombinant receptors

Different strategies have been applied to identify possible subunit combinations of $GABA_A$ receptors. *In situ* hybridization studies have indicated that the various subunits have a different but overlapping regional distribution. In addition, these studies indicated that a single cell can express many different $GABA_A$ receptor subunits (Vicini 1991; Wisden et al 1992; Laurie et al 1992). The few immunocytochemical investigations with subunit specific antibodies available to date have largely supported the results of *in situ* hybridization studies (Benke et al 1991a; Zimprich et al 1991; Killisch et al 1991; Fritschy et al 1992; Thompson et al 1992). Combined with immuno-precipitation studies, the tentative simultaneous occurrence of the most abundant subunits (α_1, β_2/β_3, γ_2, and α_3, β_2/β_3, γ_2) in the same receptor complex was proposed (Benke et al 1991; Fritschy et al 1992). Pharmacological studies have supported the existence of $GABA_A$ receptors associated with BZ_1- or BZ_2-benzodiazepine receptors (Sieghart 1989). In agreement with results of the investigation of recombinant receptors are biochemical and immunological studies, which have indicated that these receptors are associated with α_1- or α_2-/ α_3-subunits, respectively (Zezula and Sieghart 1991; Sieghart 1992b). Recent studies of the potency of various BZ_1 receptor-selective ligands for the inhibition of [^{3}H]flunitrazepam binding in various brain tissues have indicated the existence of benzodiazepine receptors with an unusually low affinity for zolpidem but not for other BZ_1 receptor-selective compounds in the brain of three- to five-day old rats (Sieghart and Schlerka 1991). These and other studies aimed at isolating $GABA_A$ receptors containing α_5-subunits (McKernan et al 1991) indicate that receptors with a BZ_3 type of pharmacology actually exist in the brain. Other studies using subunit-specific antibodies or investigating the photolabeling of benzodiazepine receptors by [^{3}H]flunitrazepam and [^{3}H] Ro 15-4513 support the existence of α_6-subunit-containing receptors in the brain (Sieghart 1992b). Interestingly, these latter receptors have properties quite similar to those of recombinant receptors containing the α_6-subunit.

Future developments

However, the exact subunit composition of even a single $GABA_A$ receptor subtype has not yet been identified. Many more experiments have to be performed in order to reach this goal. If a receptor composition existing in the brain had been identified, a recombinant receptor with the same composition could be expressed in a cell culture system. In the absence of other interfering $GABA_A$ receptors, the affinity and/or efficacy of compounds for this receptor could then be easily determined. This compares favorably with the classical investigation of receptors in brain membranes since in brain membranes a mixture of a variety of different receptor subtypes is always investigated. Using a set of different recombinant $GABA_A$ receptors, the specificity of compounds could easily be investigated, and it is to be expected that fairly rapidly compounds could be developed which exhibit a high selectivity (affinity and/or efficacy) for the individual receptors.

Thus, the investigation of the various $GABA_A$ receptor subtypes and their composition, regional distribution in the brain and pharmacology is not only of tremendous importance for the basic neurosciences, but will also result in the rapid development of new and more selective compounds for psychiatry.

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