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Structure and Function of the Benzodiazepine Receptor**

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Willy E. Haefely: Born 1930 in Balsthal, SO. Studied medicine at the universities of Fribourg, Basel, and Vienna (1949–1955); M.D. 1956. Postdoctoral training in pathology, bacteriology and immunology, internal medicine. Since 1960 in the Pharmaceutical Research Department of Hoffmann-La Roche in Basel. 1974 habilitation and venia legendi for Pharmacology. 1981 Associate Professor of Pharmacology (Medical Faculty, Universität Basel). – Investigations and over 250 publications on the physiology and pharmacology of noradrenergic mechanisms, autonomic ganglia, aspects of sleep, GABAergic neurotransmission. Preclinical development of cardiovascular drugs, analgesics, anti-inflammatory agents, neuroleptics, antidepressants, tranquilizers, cognition improving agents. Recently, Willy E. Haefely was awarded the Roche Research and Development Prize.

Benzodiazepine tranquilizers are the most frequently therapeutically used psychotropic agents since their introduction in 1960. Since 1974 research on the mechanism of action of agents acting on the specific benzodiazepine receptors (BZR) has been one of the hot areas of research in the neurosciences. – The BZR is an allosteric modulatory site on the $GABA_A$-receptor-gated chloride channel through which γ-aminobutyric acid (GABA), the most important inhibitory neurotransmitter in the mammalian central nervous system, produces most of its effects. The uniqueness of the BZR is its ability to mediate two opposite effects (facilitating or depressing) on the $GABA_A$-receptor function which can be blocked by specific antagonists. Research on the BZR has not only revealed the mechanism of action of an important class of tranquilizers and provided the basis for the development of novel innovative drugs, it has also led to a deep insight into the molecular and synaptic aspects of the central nervous system (CNS) functions.

1. Introduction

The first benzodiazepine (BZ) derivative with tranquilizing properties, chlordiazepoxide hydrochloride (Librium®), was synthesized almost exactly 30 years ago by *Sternbach*[1]. The structure of diazepam (Valium®), the best known derivative of this class of compounds, is shown in Fig. 1. Well over 3000 compounds containing the characteristic 1,4-benzodiazepine skeleton or variations thereof have been synthesized and tested pharmacologically[2]. Approximately 50 compounds belonging to this broadly defined class are available for therapy worldwide, and are among the most frequently used prescription drugs.

The pharmacological profile of BZs is, on the one hand, rather broad, covering anxiolytic, anticonvulsant, sedative («tranquilizing», sleep improving, vigilance reducing), and muscle relaxant properties. On the other hand, this profile of activity is rather specific, clearly distinguishable from that of other agents affecting the functions of the central nervous system (CNS). Very remarkably, BZs are virtually devoid of direct actions on biological systems outside the CNS (e.g. cardiovascular and other vegetative functions). Most BZs are very potent drugs, some exerting their therapeutic effects in doses of less than one milligram. BZs are exceptionally well tolerated, toxic effects being absent up to extremely high doses and even after prolonged intake. Side effects result from their pharmacological activity and are reversible.

The high potency and the specific action on the CNS point to a highly specific interaction of the compounds with biological target structures. Indeed, BZs were found to directly affect almost exclusively a single well-defined system of chemical signal transmission in the CNS, the GABAergic system[3].

2. The GABAergic System

γ-Aminobutyric acid (GABA) is formed from glutamic acid by glutamic acid decarboxylase in a subpopulation of CNS neurons, called GABAergic (Fig. 2). GABA is stored in synaptic vesicles in the nerve endings of GABAergic neurons and released by exocytosis into the synaptic cleft when an action potential generated in the nerve cell body reaches the terminals. Inactivation of the released GABA occurs through active cellular transport (uptake) into GABAergic nerve endings and glial cells, where it can be metabolized via transamination.

GABA released into the synaptic cleft, the tiny extracellular space separating the GABAergic nerve endings from the GABA-responsive target neuron, interacts with glycoproteins, the GABA-receptors, embedded in the subsynaptic membrane (the membrane of the target neurons facing the GABAergic nerve terminal). At least two types of GABA-receptors exist, the $GABA_A$- and the $GABA_B$-receptors

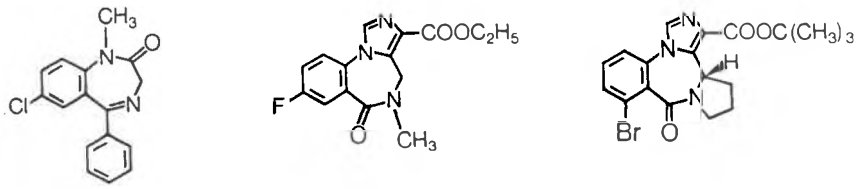

Fig. 1. Structural formulae (from the left) of diazepam, flumazenil, and Ro 16-6028, representative agonist, antagonist, and partial agonist at the benzodiazepine receptor.

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(Fig. 3). They differ by their affinity for GABA agonists (GABA mimetics) and GABA antagonists as well as by their coupling to signal transducers or effectors.

The $GABA_A$-receptor is coupled with a transmembrane ion channel (in fact, it is an integral part of this channel) selectively permeable in its open form for anions, in particular, chloride ions. Activation of the $GABA_A$-receptor, i.e. formation of a reversible complex of the receptor with GABA and the resulting conformational change in the receptor, triggers the opening of the channel, which is in a closed conformation in the absence of GABA. In the open conformation, chloride ions can move through the channel. The intensity and direction (into the neuron or the extracellular space) of the chloride flux is determined by the electrochemical gradient, generated by the equilibrium potential (transmembrane potential at which no net chloride flux would occur at a given transmembrane concentration gradient) and the actual membrane potential. Net influx of chloride increases intraneuronal negativity and, by hyperpolarizing the neuron, moves the membrane potential further away from the critical potential at which an action potential is initiated; chloride influx also short-circuits the sodium influx underlying the excitatory synaptic potential and the action potential. Net efflux of chloride reduces the membrane potential and, thereby, decreases the amplitude and slows down the conduction of the action potential. These events and the decrease of the membrane resistance (by induced chloride conductance) are responsible for the inhibitory effect of GABA on neuronal activity.

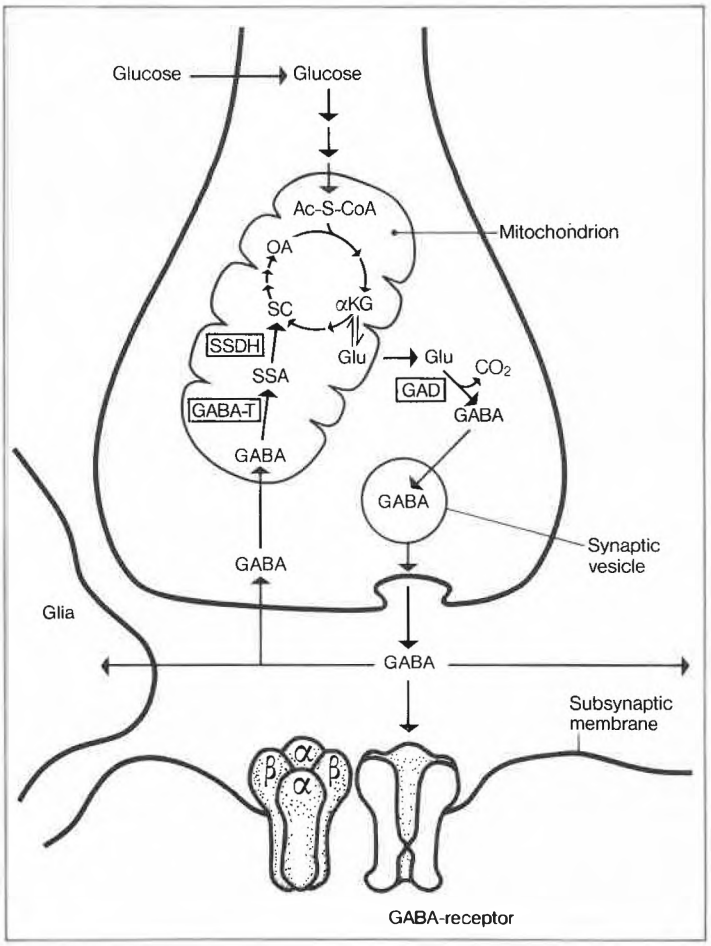

Fig. 2. Schematic diagram of a GABAergic synapse with the GABAergic nerve terminal (above) containing a mitochondrion and a synaptic vesicle, the postsynaptic GABA-sensitive neuron, the synaptic cleft, and a glial cell. Glutamate synthesized in the Krebs cycle is decarboxylated by GAD (glutamic acid decarboxylase) to GABA. After uptake from the synaptic cleft, GABA can re-enter the cycle after transamination. GABA-T = GABA transaminase; SSA = succinic acid semialdehyde; SSDH = succinic acid semialdehyde dehydrogenase; SC = succinic acid.

	$GABA_A$-RECEPTORS	$GABA_B$-RECEPTORS
COMMON AGONIST	GABA	GABA
SELECTIVE AGONIST	MUSCIMOL	BACLOFEN
SELECTIVE ANTAGONIST	BICUCCULLINE	PHACLOFEN
EFFECTOR	Cl^- CHANNEL	Ca^{2+}-CHANNEL↓ AC↓ K^+ CHANNEL↑
ALLOSTERIC MODULATION	"BENZODIAZEPINES" BARBITURATES SOME CONVULSANTS	?

Fig. 3. Characteristics of the two types of GABA-receptors.

The second type of GABA-receptors, the $GABA_B$-receptor, is not coupled to a chloride channel, but rather to a potassium channel and, perhaps, additionally affects calcium translocation and the cAMP generating system. It is not of interest in the context of the BZR.

GABA is the most important inhibitory neurotransmitter of the mammalian CNS. Approximately one third of all synapses in the brain are estimated to be GABAergic. These GABAergic synapses are vital to the function of the CNS. Depression of GABAergic synaptic function results in hyperexcitability with convulsions and death. GABAergic neurons are arranged in characteristic synaptic connections within the neuronal circuits of the CNS (Fig. 4). One type of synaptic connection mediates so-called presynaptic inhibition (Fig. 4c): a GABAergic nerve ending forms a synapse with the terminal of an excitatory neuron and the effect of GABA in this axoaxonal synapse is to reduce the amount of excitatory transmitter that is released in response to an incoming electrical signal. This type of inhibition allows distinct excitatory inputs to a neuron to be reduced without changing its responsiveness to other inputs. A second type of synaptic arrangement of GABAergic interneurons mediates feedback inhibition of an excitatory principal neuron (Fig. 4d): whenever this neuron fires an action potential, it activates the GABAergic interneuron which projects back to the principal neuron. This type of recurrent inhibition prevents excessive activation of principal neurons and acts to stabilize neuronal activity.

3. The Benzodiazepine Receptor (BZR)

The high potency of BZ tranquilizers and their specific interaction with the GABAergic system of the CNS clearly indicated from the beginning that these drugs had to interact with distinct molecules serving as receptors (recognition and transducer molecules). Identification of these BZR was achieved 1977 by the now classic radioligand binding assay[4, 5]. Incubation of brain homogenates or membrane preparations with BZs of high specific radioactivity revealed the presence of saturable binding sites for these radioligands. The reversible binding of a radioligand is competitively inhibited by non-radioactive pharmacologically active BZs. While the binding sites were found to have roughly the same affinity (K_D) for ligands throughout the CNS, their density (B_{max}) turned out to differ in the various regions.

The regional distribution of BZ binding sites can be visualized in autoradiographs of brain slices incubated with a radioligand (Fig. 5b). The distribution of BZ binding sites is virtually identical with that of $GABA_A$-receptors visualized by their binding of radioactive muscimol, a selective $GABA_A$-receptor agonist (Fig. 5c). Subcellular distribution and electron microscopic studies show BZ binding sites to be located on neuronal cell membranes. Due to the low non-specific binding of selected BZ radioligands, autoradiographs of slices of animal CNS can be obtained after injection of tracer amounts of radioligands into the living animal. When injecting the radioligand at different times after various doses of non-radioactive BZs, the inhibition of radioligand binding allows the determination of the dose- and time-dependence of BZR occupation by pharmacologically active doses of a given ligand and, thereby, to correlate receptor occupancy with pharmacological actions. This method allows the study of pharmacokinetics at the receptor level, a tremendous progress over pharmacokinetics of drug concentrations in the blood. Moreover, the correlation between receptor occupancy by a ligand and the intensity of its pharmacological effects helps to assess the intrinsic efficacy (see Section 5.3) of BZR ligands. Occupation of BZRs in the CNS can even be monitored in the living animal and human by using ligands labeled with positron emitting isotopes (^{11}C, ^{75}Br) of short half-life and positron emission tomography (PET) scanning[7]. In addition to pharmacokinetic studies, the PET-scan technique offers the possibility of searching for possible abnormalities of BZRs in the living brain.

BZRs can be irreversibly photo-affinity labeled using suitable BZR ligands and UV irradiation[8]. This technique was very useful in initial stages of BZR isolation.

There are at least two types of BZ binding sites[2]. The type discussed so far clearly mediates the well-known effects of BZ in the CNS and can, therefore, be considered the relevant receptors. A second type of high-affinity binding site occurs on non-neuronal cells both within the CNS (glial cells and other elements) and in extra-cerebral tissues. No function other than binding has so far been conclusively found for these sites, and these so-called peripheral binding sites are unlikely to be pharmacological receptors, in contrast to the central BZRs. They will not be considered further in this review.

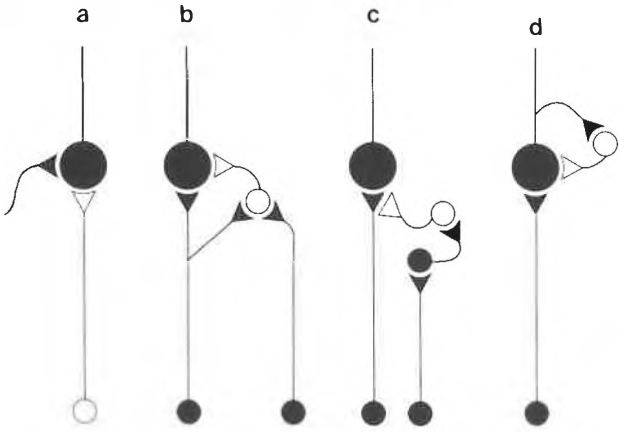

Fig. 4. Four characteristic synaptic connections of GABAergic neurons (white). Excitatory neurons are shown in black (cf. text).

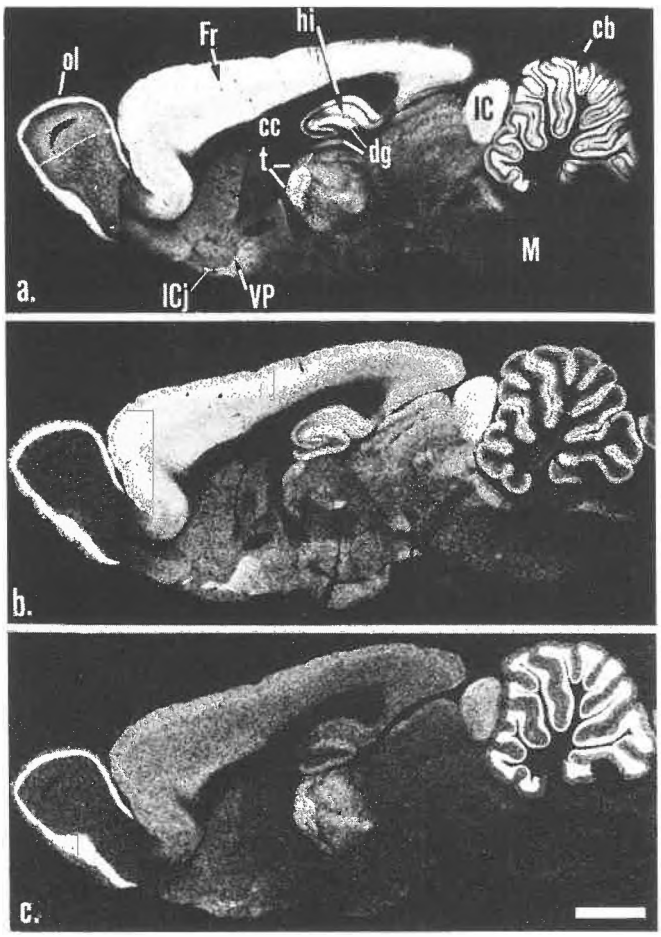

Fig. 5. Benzodiazepine receptor visualized on adjacent parasagital sections of rat brain: a) by immunohistochemistry with a monoclonal antibody directed against bovine BZR; b, c) by autoradiography with a BZR ligand, [^{3}H]-flumazenil (b) and a specific $GABA_A$-receptor agonist, [^{3}H]-muscimol (c). – Abbreviations: cb = cerebellum, cc = corpus callosum, Fr = frontal cerebral cortex, dg = dentate gyrus, hi = hippocampus, ICj = islands of Calleja, IC = inferior colliculus, M = medulla, ol = olfactory bulb, t = thalamus, VP = ventral pallidum; scale bar ≙ 2 mm (from[6]).

4. The $GABA_A$-BZR-Chloride Channel Complex

When BZ tranquilizers were found to enhance GABAergic synaptic transmission[9], it was not evident which of the various steps in the transmission process was affected directly. The observations that GABA and other $GABA_A$-receptor agonists enhanced the binding of BZ tranquilizers to their receptor[10] and that the latter compounds increased the binding of GABA to the $GABA_A$-receptor[11], strongly suggested a mutual allosteric interaction between the two binding sites and, hence, the physical and functional coupling of the two sites. Subsequent investigations, indeed, showed the two sites to be part of the same supramolecular complex forming the $GABA_A$-receptor and the associated chloride channel as well as allosteric modulatory sites.

As an integral membrane glycoprotein, the complex has to be extracted from the lipid membrane using detergents. Solubilization in detergents is a critical step as it easily destroys the functional integrity of the complex. The solubilized protein-lipid mixture is purified on an affinity chromotography column containing an immobilized BZ and eluted by rinsing with a soluble BZ ligand[2]. Purified material reveals two major subunits on gel electrophoresis. An α-subunit with a molecular weight of about 53 kD is strongly labeled after subjecting the native membranes to the photoaffinity procedure and, hence, carries the BZ binding site(s). The β-subunit (about 57 kD) is weakly, if at all, labeled with a BZ photo-affinity ligand, but can be affinity-labeled with the GABA-agonist muscimol, indicating that it carries the GABA binding site(s). Monoclonal antibodies raised against the purified complex coprecipitate a protein from solubilized material that binds GABA and BZs as well as a third category of barbiturates and convulsive ligands thought to alter the function of the chloride channel directly[12]. Molecular mass determination suggests the complex containing the three mentioned binding sites to be a heterotetramer with an $\alpha_2\beta_2$ stoichiometry. The model proposed on the basis of these findings (Fig. 6) has very recently been beautifully substantiated by investigators from the University of Cambridge, U.K., and from Genentech, California. They have cloned and sequenced cDNAs encoding the two subunits α and β in the bovine brain[13]. The deduced amino acid sequence of the α-subunit is 456 units long, that of the β-subunit has 474 units. The two subunits show partial sequence homology and each subunit has four potential transmembrane segments of approximately 20 amino acids. Both the large N-terminal part with potential glycosylation sites and the small C-terminal part would be extracellular. A small cytosolic part of the β-subunit contains a potential phosphorylation site. The overall structure of the $GABA_A$-receptor-complex (Fig. 6) shows obvious similarities with two other

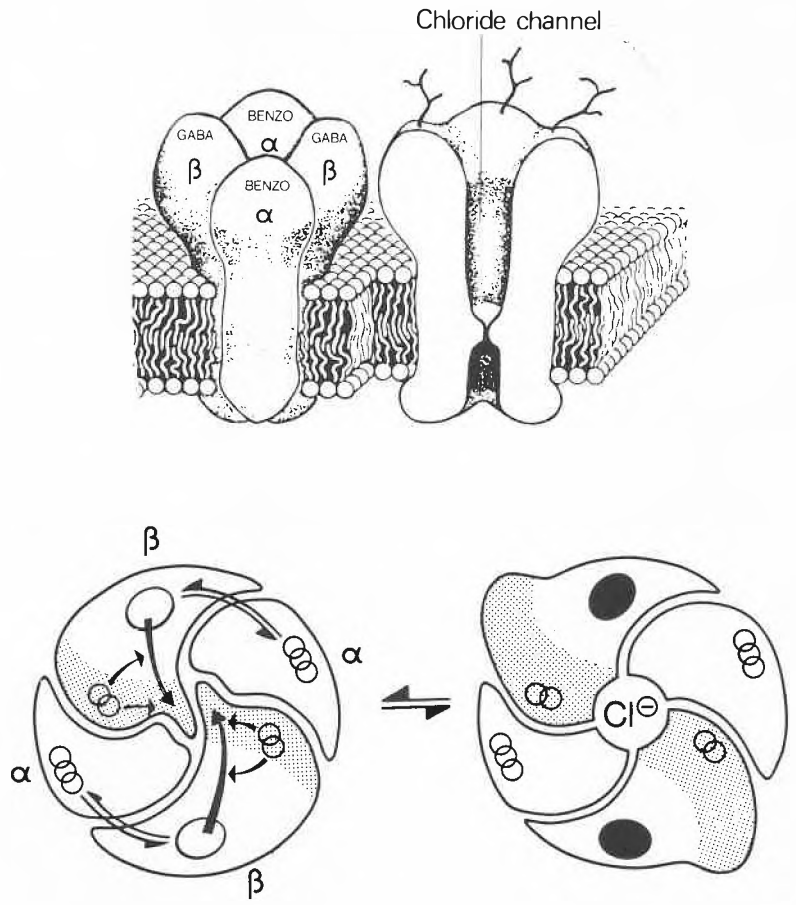

Fig. 6. Hypothetical model of the $GABA_A$-receptor-chloride channel complex with allosteric modulatory sites. Above a view on the complex in the neuronal cell membrane, below a view on the complex from the extracellular space. The β-subunit contains the GABA binding site (large oval) and the allosteric sites for barbiturates and convulsants (the two overlapping small circles). The α-subunit carries the binding site for the 3 prototypes of BZR ligands (the three overlapping small circles). The two large arrows represent the main function of the complex, opening of the channel by the GABA-induced change of the conformational state. The small arrows indicate the allosteric modulation of the gating process.

ligand-operated ion channels, the nicotinic cholinoceptor-cation channel and the glycine receptor-chloride channel. Injection into the frog oocyte of the two native mRNAs from the bovine brain or of the RNA transcripts from the cDNAs led to the synthesis and membrane incorporation of fully functioning $GABA_A$-receptor-chloride channel complexes, modulated like the native complex in neurons by ligands of the BZR and the convulsant receptor. Frog oocytes do not normally express the complex.

Interesting questions about the complex remain to be answered. As an example, there is the issue of the possible existence of structural subtypes (heterogeneity) of the complex in the various areas of the CNS. Some classes of BZR ligands have been reported to show regional differences in their affinity for BZRs and even specific involvement of receptor subtypes in the various pharmacological effects have been, prematurely, proposed. In contrast to the situation in vertebrates, GABA-receptors in invertebrates lack the BZR; it will be highly interesting to learn the reason for this difference.

The information most eagerly awaited from future work on the BZR, in particular by medicinal chemists, is the three-dimensional structure of the BZ binding area. Except for the presumed presence of a histidine residue critical for binding of BZs[14] virtually nothing is known about this area to explain the structure-activity relationship for ligands.

5. The Three Categories of Ligands of the BZR

The BZR is an allosteric modulatory site of the $GABA_A$-receptor complex by which the channel gating function of the $GABA_A$-receptor can be altered. On the basis of fundamental pharmacological concepts it could be expected – and this idea was expressed well before the biochemical identi-

fication of BZRs and the discovery of the three categories of ligands – that compounds might be found that acted as antagonists of BZ tranquilizers by blocking their receptors. Such antagonists indeed exist, however, the unique situation with the BZR is the existence of three prototypes of ligands with qualitatively different effects on the $GABA_A$-receptor function.

5.1. BZR Agonists

The classical BZ tranquilizers enhance GABAergic transmission and the effect of exogenous GABA produced via $GABA_A$-receptors. When single neurons, e.g. in cell culture, are recorded from intracellularly, these BZs produce a shift to the left of the concentration-response curve for the chloride conductance inducing action of GABA (see[15]). The shift depends on the concentration of the agonist and the maximal shift is about 2 to 3 fold, hence rather modest. The maximum GABA effect is not affected by BZR agonists. Recording from single $GABA_A$-receptor-operated chloride channels using the patch clamp technique revealed that the effect of a small dose of GABA in the presence of a BZR agonist is identical with the channel response to a larger dose of GABA in the absence of a BZ. BZR agonists can be said to increase the apparent affinity of the $GABA_A$-receptor for GABA. This allosteric modulatory action of BZR agonists on the channel gating efficiency of GABA has its counterpart in binding experiments. Agonists of the BZR enhance the binding of GABA or muscimol. while binding of BZR agonists is enhanced in the presence of GABA (it is still not clear whether this reciprocal allosteric interaction affects K_D or B_{max} for the respective ligand).

The positive modulatory action of BZR agonists on the $GABA_A$-receptor function nicely explains the effects of these agents on the neuronal activities studied in most areas of the CNS. Enhanced efficiency of GABAergic synaptic transmission also poses no conceptual difficulties for understanding the various pharmacological effects of BZR agonists on the intact CNS, although much remains to be done in order to identify in detail the neuronal networks mediating anxiolytic, anticonvulsant, sedative, and muscle relaxant effects.

5.2. Inverse Agonists at the BZR

It came as a great surprise when compounds were found in the early eighties that had a high affinity for BZRs but produced effects which were the perfect mirror image of the well known effects of agonists (anxiogenic, proconvulsant and convulsant, vigilance and muscle tone increasing effect). The first compounds discovered which act in this way were esters of β-carboline-3-carboxylate acid, but similar properties were subsequently found in other classes of chemicals, including BZs[2]. These ligands reduce the binding of GABA and, reciprocally, GABA reduces their binding. They depress the channel gating function of the $GABA_A$-receptor and, hence, GABAergic synaptic transmission.

Since no receptor has been shown so far to mediate diametrically opposite effects, a new terminology had to be created. Because these ligands induce a change in the receptor conformation leading to a pharmacological effect, they fulfill the definition of agonists. However, because they are negative modulators of the $GABA_A$-receptor function, we proposed the now widely accepted term «inverse agonists»[16].

5.3. Antagonists

Shortly before the advent of the first inverse agonists, BZ derivatives were synthesized in our laboratories which bound with high affinity to the BZR without producing relevant pharmacological effects by themselves, however, which highly specifically blocked those of agonists and, as realized soon afterwards, also those of inverse agonists. Antagonists at the BZR have no or only a minimal modulatory effect on the $GABA_A$-receptor function and, hence, do not affect GABAergic synaptic transmission. There is also no reciprocal allosteric interaction in the binding of GABA and BZR antagonists. BZR antagonists occur in several chemical classes, e.g. β-carbolines and pyrazolopyridazines[2]. Flumazenil[17], an imidazobenzodiazepine, is the first BZR antagonist available in therapy to treat overdosage with BZR agonists and to shorten the duration of sedative action of BZR agonists used in anesthesia.

How can we explain the existence of three categories of BZR ligands with such

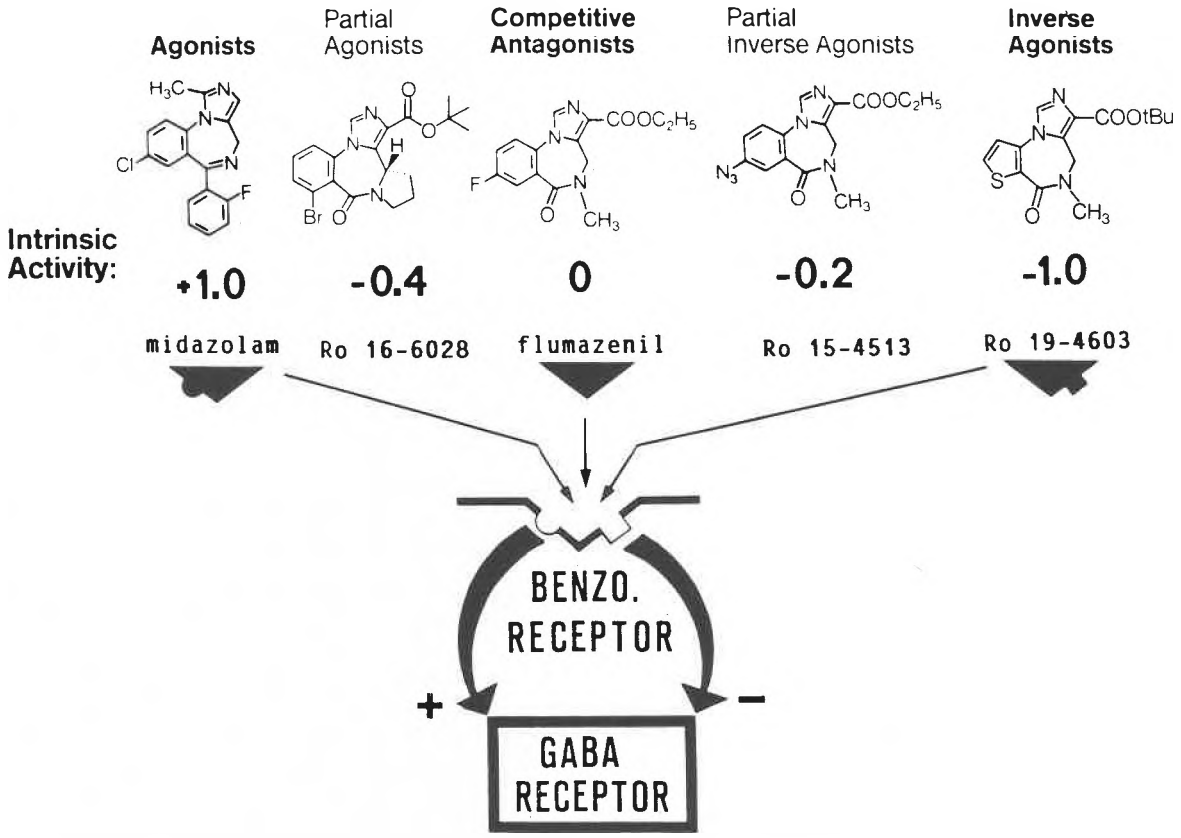

Fig. 7. Schematic presentation of the various classes of ligands of the benzodiazepine receptor (BZR).

different activities and their mode of interaction with the BZR? First of all, it is imperative to realize that the BZR is not the receptor on the $GABA_A$-receptor complex whose function is to operate the channel, i.e. to trigger the open conformation in the normally closed channel; this function is taken care of by the $GABA_A$-receptor, which is the primary receptor in the complex. The BZR is only a secondary receptor in this complex, mediating the fine-tuning or the gain of the $GABA_A$-receptor gating function (Fig. 6). BZR ligands differ by their intrinsic efficacy, the inherent property of drugs (and physiological signal substances) to induce a conformational change or isomerization of the receptor molecule (or domain). Prior to the discovery of the BZR ligands, receptor ligands were classified as agonists, characterized by an intrinsic efficacy (of differing degree) and as antagonists which ideally lacked any intrinsic efficacy. The novel situation with the BZR necessitates the notion of agonists (or positive allosteric modulators) with positive intrinsic efficacy, of inverse agonists (or negative allosteric modulators) with negative intrinsic efficacy, and of antagonists (blockers of allosteric modulators) with no intrinsic efficacy (Fig. 7).

Two hypothetical situations may explain these receptor-ligand interactions (Fig. 8). The «three-state model»[16] assumes that in the absence of a BZR ligand, the BZR is in a conformation that does not affect the $GABA_A$-receptor gating function. Agonists would induce a novel conformation that has a positive allosteric modulatory influence on the $GABA_A$-receptor, inverse agonists another conformation with a negative allosteric modulatory influence. Antagonists would bind to the BZR without changing its «resting», «neutral» or «ineffective» conformation, however, by their presence at the receptor would antagonize both agonists and inverse agonists. The «two-state model»[18] assumes that the BZR in the absence of a ligand oscillates spontaneously between two interconvertible corformational states, one corresponding to the «positive», the other to the «negative» modulatory state. The equilibrium between the two states would define the «resting» gain of the $GABA_A$-receptor function. Agonists would be agents binding preferably to the «positive» conformer, stabilize it and, therefore, shift the equilibrium in favor of the positive modulatory conformation leading to an increase in the gain of the $GABA_A$-receptor function. Inverse agonists would prefer binding to the «negative» conformer and thereby reduce the gain. Antagonists would bind equally well to both conformers and, therefore, not change the equilibrium and the gain. The nice aspect of this hypothesis is that it would allow intrinsic efficacy to be precisely defined as the ratio of affinities for the «positive» and for the «negative» conformation. There appears to be no basis at present to accept one and reject the other model.

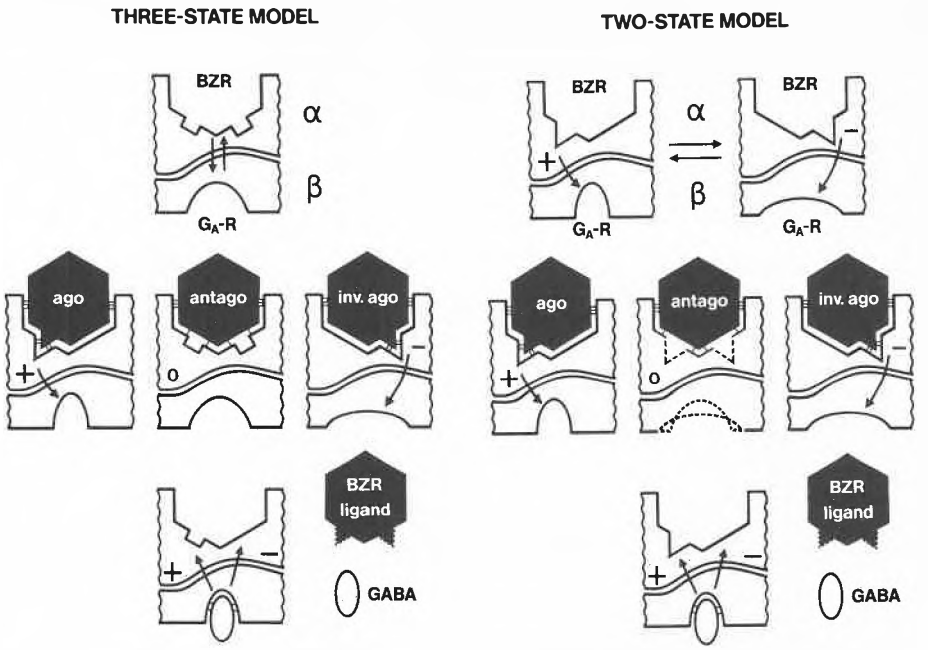

Fig. 8. Schematic presentation of the interaction of agonists, antagonists, and inverse agonists with the benzodiazepine receptor according to the three-state and the two-state model. Shown are small parts of the α- and the β-subunit with the benzodiazepine receptor (BZR) and $GABA_A$-receptor (G_A-R) and the interphase between the two.

6. Partial Agonists and Partial Inverse Agonists of the BZR

The thousands of known ligands of the BZR do not all fall into one of the three categories described above. Rather, the ligands cover a continuum of intrinsic efficacies between the two extremes, the full agonists on the one side and the full inverse agonists on the other side (Fig. 7). If we ascribe an intrinsic efficacy of +1 to those agonists that produce the most marked enhancement of the GABA effect, intrinsic efficacy zero to antagonists producing no GABA enhancement, and intrinsic efficacy −1 to inverse agonists with a maximum depression, then partial agonists are ligands with intrinsic efficacy between +1 and zero, and partial inverse agonists those with values between zero and −1.

Intrinsic efficacy is a property of ligands not coupled with affinity for the receptor, as demonstrated by antagonists which may have a very high affinity but zero intrinsic efficacy. As discussed above, intrinsic efficacy is the ability to induce an isomerization of the receptor after formation of a complex with it, or the selective affinity for one of the two «active» conformations. For a given intensity of allosteric modulation of the $GABA_A$-receptor function partial agonists and partial inverse agonists need to form more complexes with the receptors than full agonists or full inverse agonists, which is compatible with both the «induction» and the «selection» model presented in Section 5.3.

The relationship between fractional receptor occupancy (now directly measurable by the radioligand technique) and intensity of pharmacological effect is illustrated in Fig. 9. The individual neurons in the various regions of the CNS differ in their density of $GABA_A$-BZRs. Let us assume that neuron a) with a high density of receptors is part of the neuronal network responsible for the anxiolytic and anticonvulsant effects of BZR agonists. It is likely that a maximal conductance increase for chloride ions in response to GABA is achieved at a low fractional occupancy of $GABA_A$-receptors. GABA would act as indicated by the compound A, i.e. there would be a large receptor reserve. GABA might still produce a maximal increase of chloride conductance in neurons b) and c) involved in sedative-muscle relaxant effect of BZR agonists, however only at high fractional receptor occupancy. A full BZR agonist may act like compound B to potentiate GABA effects, i.e. to shift the GABA dose-response curve to the left. A maximal (e.g. threefold) shift would still be obtained with this full agonist in neuron b) at complete fractional receptor occupancy, however in neuron c) only a twofold shift would be possible even at receptor saturation. The situation just described explains why a full BZR agonist can produce anxiolytic and anticonvulsant effects at doses inducing no or little sedative side effects. Partial BZR agonists, such as indicated by compounds C and D may still produce a therapeutically relevant GABA potentiation in neurons a) and perhaps b), but none in a neuron of type c). In fact, partial agonists would act as antagonists of a full agonist on the latter cell. Partial agonism at the BZR, therefore, drastically reduces the pharmacological effectiveness for actions often appearing as undesired side effects.

Ro 16-6028 is a congener of the BZR

antagonist flumazenil (Fig. 1), whose pharmacological profile suggests that it might act much like compound C in Fig. 9. The compound has a very high affinity for the BZR. It is very potent in animal tests for anxiolytic and anticonvulsant activities. In various tests for reduction of vigilance, psychomotor activity, and muscle tone Ro 16-6028 is very weak or even ineffective, however, it blocks the effects of the full agonist diazepam. An illustration of the complex properties of Ro 16-6028 is given in Fig. 10. BZR agonists, by decreasing the activity of cerebellar neurons, reduce the content of these cells in cGMP. As can be seen, the full agonist diazepam reduces cGMP levels to about 20% of control. Ro 16-6028 is more potent than diazepam, however, its effect on cGMP levels off with increasing doses, resulting in about half the intrinsic activity of diazepam. When increasing doses of Ro 16-6028 are given together with a fully active dose of diazepam, the effect of the latter is reduced, but only to the point where the intrinsic cGMP reducing activity of Ro 16-6028 is reached (Fig. 10). As expected, the compound has a drastically reduced ability to enhance the central depressant effects of ethanol when compared to diazepam. A further advantage of Ro 16-6028 is the greatly reduced physical dependence liability found in animals. The compound is in clinical trials and initial results have already confirmed some of the predictions made in preclinical pharmacology.

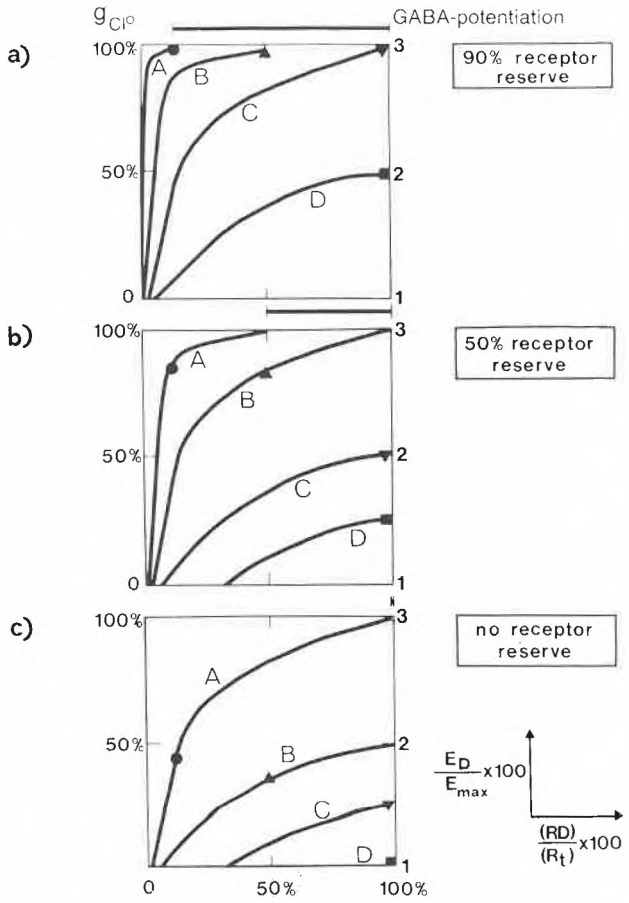

Fig. 9. Relationship between fractional receptor occupancy (on the abscissa) and intensity of effect (ordinate). The scale on the left indicates the intensity of chloride conductance (in percent of the maximum), the scale to the right the intensity of GABA potentiation, 1 being no, 2 a twofold, and 3 a threefold increase of the GABA-induced conductance change. The three squares illustrate the situation with four ligands (A, B, C, D) in three hypothetical neurons with differing densities of $GABA_A$-BZ-receptors (and, hence, receptor reserves).

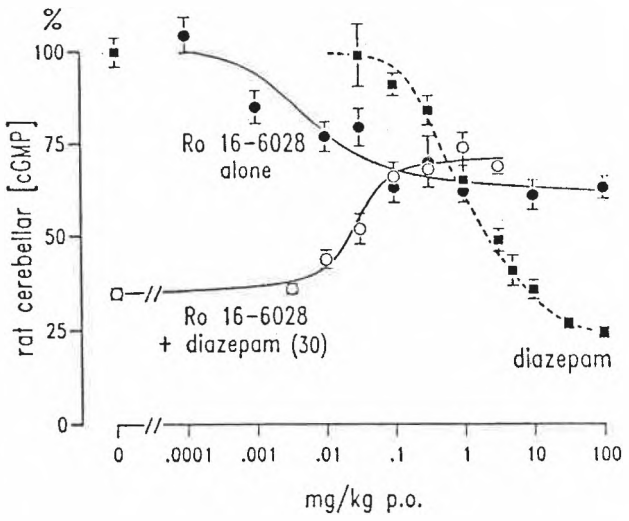

Fig. 10. The effect of a full agonist (diazepam) and of a partial agonist (Ro 16-6028) as well as of their interaction on the level of cylic guanosine-5'-monophosphate (cGMP) in rat cerebellum (in percent of controls on the ordinate). Doses are given in mg/kg on the abscissa.

Partial agonists, such as Ro 16-6028, would seem to offer the greatest therapeutic benefit emerging from the exploration of the fundamental events in the function of the BZR. They also resolve an old dilemma, namely why ligands interacting with a seemingly uniform receptor in the CNS can differ in their pharmacological profiles. As an example, the «classic» BZ clonazepam, in therapeutic use as an antiepileptic for years, has recently been shown to be a partial agonist, although with higher intrinsic efficacy than Ro 16-6028.

Partial inverse agonists are BZR ligands with less negative intrinsic efficacy than the overt convulsive, full inverse agonists. Compounds with minimal negative intrinsic efficacy increase vigilance and improve cognitive function in animals and may turn out to find clinical use in humans.

The existence of a *continuum of intrinsic efficacies* and varying receptor reserves for different CNS functions also explain why BZR antagonists are more potent in antagonizing sedative-muscle relaxant effects of agonists than their anxiolytic and anticonvulsant effects (which require smaller fractional receptor occupancy).

7. Endogenous Ligands of the Benzodiazepine Receptor

The discovery of an allosteric modulatory receptor on the $GABA_A$-receptor, through which drugs can alter the $GABA_A$-receptor function, immediately raised the question whether a natural compound in the CNS exists that acts as an endogenous modulator. This endogenous ligand could be an agonistic ligand, acting as a natural anxiolytic, anticonvulsant, sedative, and

myorelaxant factor, or an inverse agonistic ligand, acting as a natural anxiogenic factor. Although there is no a priori reason to postulate such an endogenous ligand[19], the possible existence of one or more of such compounds cannot be ruled out. Screening of tissue extracts for their ability to inhibit the in vitro binding of BZR ligands to its receptor has led to the identification of several active low molecular weight compounds and peptides[19, 20]. It is still not established whether any of them actually occurs in sufficient concentration at the right place to become active at BZRs.

8. Natural Benzodiazepines

The search for endogenous ligands for the BZR surprisingly led to the identification of pharmacologically active BZs (e.g. diazepam and *N*-methyldiazepam) in the brain of humans and animals as well as in plants used as food[21–23]. It remains to be shown whether microbial organisms and/or plants are able to synthesize these compounds believed until recently to be the exclusive products of the medicinal chemist.

9. Other Allosteric Sites of the $GABA_A$-Receptor

The BZR is the most intensively investigated allosteric site of the $GABA_A$-receptor and, in fact, of any other receptor for endogenous signal substances. The $GABA_A$-receptor, however, also carries allosteric sites different from the BZR through which the receptor-channel function can be modulated. Of particular interest is a site through which barbiturates, the old therapeutic forerunners of BZs, induce part of their effects[24]. Anesthetic and anticonvulsant barbiturates as well as steroid anesthetics[25] also enhance the effect of GABA, however, not by increasing the sensitivity of the channel gating function as do BZs, but by prolonging the opentime of the chloride channel and by opening the channel even in the absence of GABA[15]. The more dramatic potentiating of GABA effects by barbiturates as compared to BZs and the smaller therapeutic ratio are probably due to the fact that barbiturates markedly increase the maximal ability of GABA to increase chloride conductance. In addition, barbiturates affect a number of other processes that control neuron activity, thus being much less specific for the $GABA_A$-receptor than BZs.

An allosteric modulatory site possibly identical with the barbiturate receptor or, at least very closely coupled to it, is the site by which various convulsant agents, among them the well-known pentetrazol and picrotoxin, reduce the gating function of the $GABA_A$-receptor[24].

10. Conclusions and Outlook

Investigations aimed at identifying the mechanisms of action of BZ tranquilizers has led to the understanding of how one of the most important classes of neuropsychotropic drugs affect biological events in the CNS to produce a variety of therapeutic actions. The finding that the BZR is an allosteric modulatory site located on one of the most important neurotransmitter receptors in the CNS has revealed a fundamental mode of action for drugs that was virtually unknown before. The possibility of specific interaction with a distinct neurotransmitter system by allosteric fine-tuning of its receptor offers a more «physiological» means to affect normal or abnormal functions of the CNS than the direct interaction with transmitter binding sites. This basic mechanism will undoubtedly become a more frequently attempted approach for CNS drugs in the future. The most surprising and novel aspect of the BZR is the ability to mediate two opposite effects, a property which implies the allosteric modulatory function of this receptor. This does not mean that all modulatory sites to be found in the future need to have this bidirectional influence on a primary receptor.

The primary structures of the two subunits of the $GABA_A$-receptor-chloride channel complex have recently been elucidated. What we next need to know are the three-dimensional structures of the GABA and the BZ binding areas and the nature of their mutual interaction.

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