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LIGANDS FOR BENZODIAZEPINE RECEPTORS WITH POSITIVE AND NEGATIVE EFFICACY

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Receptors for benzodiazepines were characterized in 1977 by radio ligand binding studies using ^{3}H-diazepam [1, 2]. It appeared that the benzodiazepine group of minor tranquillizers exerted their anticonvulsant and anxiolytic effects by combining with these receptors in the central nervous system.

Benzodiazepine receptors are localized exclusively in the brain. Multiplicity seems to exist among these brain specific receptors located on the plasma membrane of neurons. At least two classes of proteins have been identified [3] which seems to represent benzodiazepine receptors with slightly different recognition properties (BZ_1 and BZ_2 receptors). Some pharmacological agents, for example CL 218.872 and β-CCE, express a 5–10 fold higher affinity for BZ_1 than for BZ_2 receptors [4–6]. When these selective agents were discovered it was anticipated that more selective compounds would appear and that the pharmacological significance of BZ_1 and BZ_2 receptors would be identified. The hope was that among all the effects common to benzodiazepines, i.e. anxiolytic, anticonvulsant, sedative, and muscle relaxant, some would depend on BZ_1 and others would depend on BZ_2 or on as yet unknown subtypes. This hope has not been fulfilled. Specific compounds for BZ_1 or BZ_2 receptors have not been presented.

In addition to the brain BZ-receptors, binding sites for ^{3}H-diazepam exist in peripheral tissue [7]. These binding sites are not related to any known pharmacological effects of benzodiazepines, It should be noted, however, that "peripheral" benzodiazepine binding site in addition are located in some structures in the CNS; glial cells in particular accomodate "peripheral" BZ-binding sites [8].

The benzodiazepine receptor ligands to be described below exhibit a spectrum of pharmacological effects. This spectrum is probably not directly related to BZ-receptor subtype selectivity, but seem to reflect the existence of various types of ligands. This presentation will summarize findings‡, which can explain how the diverse pharmacological effects, in principle, can be ascribed to the presence of a single class of receptors, which respond to receptor occupation in different ways depending on the nature of the ligand.

‡ This summary is not fully referenced. Further references can be found at the end of the paper.

NEW TYPES OF BZ RECEPTOR LIGANDS

It was initially thought that only pharmacologically active benzodiazepines, and a few benzodiazepine-like agents, interacted with high affinity with the benzodiazepine receptors. However, the β-carboline ethyl β-carboline-3-carboxylate (β-CCE) which was discovered in a search for endogenous ligands for BZ-receptors [9] has high affinity for benzodiazepine receptors but lack the anticonvulsant and anticonflict effects of benzodiazepines [10, 11]. β-CCE and also the more recently discovered agents Ro 15-1788 and CGS 8216 can block or reverse benzodiazepines from eliciting their usual effects, indicating that they all act as benzodiazepine antagonists.

The discovery of benzodiazepine antagonists was not unexpected, antagonists exist for many types of receptors. It has been common practice in pharmacology to name antagonists according to the agents they antagonize; β-CCE and Ro 15-1788 accordingly have been called benzodiazepine antagonists by some authors. This nomenclature, however, seems inappropiate. It was discovered that a third class of ligands for benzodiazepine receptors existed which produced pharmacological effects which were exactly opposite to those of benzodiazepines [5, 12] (see below). These compounds were also antagonized by β-CCE and Ro 15-1788. On this background, we proposed, that at least three groups of ligands existed for BZ receptors, which were characterized as having either positive efficacy (benzodiazepine like agents), nil efficacy (receptor antagonists) or negative efficacy (convulsant/anxiogenic ligands) [12, 13]. We will suggest to specify antagonists according to the receptor with which they interact in order to accomodate the finding that different types of ligands can be antagonised by the same antagonists. β-CCE and Ro 15-1788 will then be BZ *receptor* antagonists; they will antagonize benzodiazepines and all other agents which interact with the benzodiazepine receptor recognition site. Other types of compounds like coffein, pentylenetetrazol, bicuculline and picrotoxin do inhibit the effects of benzodiazepines, but the inhibition is produced by mechanisms different from simple competition at BZ receptors.

Recently, a new β-carboline, ZK 93426, has been discovered which is an almost silent benzodiazepine receptor antagonist (Jensen *et al.*, in preparation). This compound possesses almost nil efficacy; in

contrast to β-CCE and Ro 15-1788, which have weak negative and positive efficacies, respectively [21].

The existence of agonists and antagonists at benzodiazepine receptors would indicate that partial agonists exist also. Partial agonists would be agents with less efficacy than benzodiazepines. They would be capable of producing those benzodiazepine like effects that require low receptor stimulus but not those where high stimulus is required. Two such agents have recently been disclosed, ZK 91296 and CGS 9896. Both agents are potent anticonvulsants in rodents [14; Petersen *et al.*, in preparation), in addition ZK 91296 is a potent anticonvulsant in the photoepileptic baboon [15]. At the same time both agents lack the characteristic ataxia producing potential of benzodiazepines. The anticonvulsant effect of ZK 91296 is apparently mediated via benzodiazepine receptors because benzodiazepine receptor antagonists extinguish the anticonvulsant effect.

The partial agonist character of ZK 91296 will be clear from Table 1 which shows that some full agonists produce an antipentylenetetrazol effect at 20–50% receptor occupancy. ZK 91296 is active at 80% occupancy (= low efficacy) while the receptor antagonists were not active even at > 96% occupancy.

While the existence of benzodiazepine receptor antagonists and partial agonists would be expected with reference to classical pharmacology it was quite unexpected that some very potent ligands for benzodiazepine receptors produced pharmacological effects which were exactly opposite to those of benzodiazepines (ligands with negative efficacy, inverse agonists). DMCM, for example, produces clonic–tonic convulsions in mice and rats after doses of 5–10 mg/kg i.p. These convulsions are very similar to convulsions produced by pentylenetetrazol. Several lines of evidence indicate that DMCM produce convulsions by interaction with benzodiazepine receptors and not due to some unrelated mechanisms. One of the crucial pieces of evidence is the finding that benzodiazepine receptor antagonists, which are not general anticonvulsants, antagonize convulsions produced by DMCM [12].

DMCM seems to have high efficacy as a BZ receptor inverse agonist. It cannot be stated whether ligands with higher negative efficacy will be discovered and consequently it cannot be stated with certainty whether DMCM is a full inverse agonist. However, assuming that DMCM is a full inverse agonist the next question would be whether partial inverse agonists exist. This again seems to be the case. FG 7142 is a ligand for benzodiazepine receptors which was developed for administration to man due to a weak, general stimulant effect in rodents which suggested effect on vigilance; FG 7142 is not convulsant. Surprisingly, FG 7142 produced attacks of severe anxiety in human volunteers [16]. These findings suggest a partial inverse agonistic effect on benzodiazepine receptors (anxiety without convulsions). Later studies have used the knowledge of selective anxiogenic properties of FG 7142 to develop animal models which predict anxiogenic properties [17–19]. It has also been shown that FG 7142 under certain conditions has proconvulsant properties [20, 21].

Before accepting that the benzodiazepine receptor represents a unique system in pharmacology where a receptor can respond in two opposite directions depending on the nature of the ligand, alternative

Table 1. Receptor occupancy of BZ receptor ligands at doses giving 50% protection against pentylenetetrazol convulsions

Compound	i.p. Administration to mice 30 min: ^{3}H-FNM binding *in vivo* ID_{50} (mg/kg)	Antagonism of pentylenetetrazol convulsions ED_{50} (mg/kg)	(%) Receptor occupancy at the $ED^{*}_{50\ (pentylenetetrazol)}$
Diazepam†	1.5	0.7	32
Flunitrazepam†	0.2	0.06	23
Zopiclone†	9.6	2.0	17
ZK 93423§	1.0	0.6	38
CL 218.872§	18.0	18.0	50
CGS 9896§	1.4	5.0	78
ZK 91296‡	4.0	15	79
Ro 15-1788§	4.0	> 100	> 96
ZK 93426‡	1.0	> 100	> 99

* (%) Occupancy was calculated as $\frac{100\%}{1 + \frac{ID_{50(^3H\text{-}FNM)}}{ED_{50\ (pentylenetetrazol)}}}$, assuming that the binding isotherm follows the law of mass action.

† Values from Braestrup *et al.* [12].

‡ Values from Petersen *et al.* in preparation; Jensen *et al.* in preparation.

§ Unpublished.

ZK 93423: Ethyl 6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate
CGS 9896: 2-(4-Chlorophenyl)-2, 5-dihydropyrazolo[4,3-c]quinoline-3(3H)-one
ZK 91296: Ethyl 5-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate
ZK 93426: Ethyl 5-isopropyloxy-4-methyl-β-carboline-3-carboxylate.

Table 2. Efficacies and pharmacological effects of selected BZ receptor ligands

Type	Efficacy	Examples	Effects
Agonist	++	Benzodiazepines CL 218.872 Zopiclone ZK 93423	Anticonvulsant Anticonflict Ataxic Sedative
Partial agonist	+	ZK 91296 CGS 9896	Anticonvulsant
Receptor antagonist	0	Ro 15-1788 ZK 93426	Antagonize all classical effects of benzodiazepines and of inverse agonists
Partial inverse agonist	−	FG 7142	Anxiogenic Proconvulsant
Inverse agonist	−−	DMCM β-CCM	Convulsant

explanations must be considered. One alternative to the existence of ligands with opposite efficacy at one single receptor would be the existence of a tonically active endogenous ligand with partial agonist activity. The "receptor antagonists" would then have similar efficacy as the endogenous ligand and would not exert effects by themselves. Inverse agonists, following this alternative possibility, would correspond to antagonists of the partial agonistic endogenous ligand. This alternative explanation is unlikely [22].

Table 2 summarizes pharmacological properties of these various types of BZ receptor ligands.

Efforts have been made to correlate the pharmacological efficacy observed in animal pharmacology with biochemical parameters (Table 3). These studies have been quite fruitful even though it has not yet been possible to identify one single biochemical variable which predicts the pharmacological characteristics of BZ receptor ligands.

The GABA ratio reflects the ability of GABA to enhance (GABA ratio > 1) or decrease (GABA ratio < 1) the affinity of ligands for benzodiazepine receptors [12].

The photoshift reflects the ability of partial photoinactivation of BZ receptors to affect allosterically the affinity of the remaining sites for various BZ ligands [23].

The barbiturate shift reflects the effect of allosteric modulation of the BZ receptor via the chloride ionophor system. Barbiturate shifts above 1 indicates that stimulation of the barbiturate site with pentobarbital will enhance the affinity of benzodiazepine receptors for that ligand, barbiturate shifts below unity indicate that the affinity is reduced (Honoré *et al.* submitted).

Table 3. Various biochemical parameters related to BZ receptor ligand efficacy

Compound	^{3}H-flunitrazepam* binding IC_{50} (nM) 0°‖	30°‖	GABA† ratio 0°¶	Photo‡ shift 0°**	Barbiturate* shift 30°‖	TBPS§ shift 22°**
Diazepam	25	240	2.30	0.02	3.14	1.27
Flunitrazepam	3.9	27	2.45	0.03	1.96	1.46
Zopiclone		400	1.53	0.29	2.03	1.24
ZK 93423		1.0	2.2	1.3	1.22	1.54
CL 218.872	320	580	1.98	0.37	1.03	0.97
ZK 91296	0.6	1.6	1.4	0.6	1.13	1.16
Ro 15-1788	1.9	4.5	1.22	0.85	1.09	1.01
ZK 93426	0.4	1.2	1.37	0.66	0.81	0.88
FG 7142	300	680	0.87	1.0	0.51	0.79
β-CCM	2.4	6.9	0.61	1.4	0.50	0.77
DMCM	9.0	4.7	0.46	2.6	0.56	0.57

Methodological details see
* Honoré *et al.* (submitted).
† Braestrup *et al.* [12].
‡ Karobath and Supavilai [23].
§ Supavilai and Karobath [24], with modifications (unpublished).
Values from
‖ Honoré *et al.* (submitted).
¶ Braestrup *et al.* [12],
** Unpublished.

The TBPS shift relates to the ability of BZ receptor ligands to regulate the specific binding of ^{35}S-TBPS. ^{35}S-TBPS is a cage convulsant which binds to the chloride ionophore part of the GABA/BZ receptor chloride channel complex. Benzodiazepines enhance binding of ^{35}S-TBPS (shift > 1) while inverse agonists reduce ^{35}S-TBPS binding (shifts < 1) [24].

The results depicted in Table 3 suggest that various biochemical parameters to some extent predict pharmacological efficacy. Investigation of additional BZ receptor ligands of various chemical classes seems warranted before it can be clarified how reliably the parameters predicts pharmacological efficacy and if any of the biochemical parameters are predictive for any particular pharmacologic action.

The considerations above indicate how our studies with β-carbolines have revealed new insight in the nature of benzodiazepine receptors. The studies have also brought us to the discovery of a new concept in pharmacology, the concept of receptors which can respond in two opposite directions depending on whether ligands with positive or negative efficacy occupy the receptor.

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