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Benzodiazepines: Relationship Between Pharmacological Activity in the Rat and in vivo Receptor Binding

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MENNINI, T., S. COTECCHIA, S. CACCIA AND S. GARATTINI. *Benzodiazepines: Relationship between pharmacological activity in the rat and in vivo receptor binding.* PHARMAC. BIOCHEM. BEHAV. 16(4) 529–532, 1982.—When given at ED_{50} antileptazol several benzodiazepines displace ^{3}H-Diazepam bound in vivo to rat brain by about 50%, in spite of the differences between pharmacological potencies and metabolism. No displacement is found in cerebellum, even when the concentrations of displacing drugs are comparable. The possibility of the presence of multiple benzodiazepine receptors in brain and cerebellum is discussed in relation with their pharmacological activity.

Benzodiazepine receptors Diazepam Clobazam Camazepam Temazepam Oxazepam
Antileptazol activity Anxiolytic effect In vivo receptor binding

THE high-affinity, stereospecific binding sites for tritiated benzodiazepines found in brain tissues of many animal species including man [1, 2, 10, 15, 16, 20] and the relation between their pharmacological potencies and their ability to displace ^{3}H-benzodiazepines binding in vitro and in vivo [3, 4, 10, 15, 20] suggest that specific receptors for benzodiazepines in the brain might represent a neuronal substrate which would explain their mechanism of action. Correlations have been found between the inhibitory effect of benzodiazepine pretreatment on ^{3}H-diazepam binding in vitro and these drugs' anticonvulsant [17,18] and anticonflict [14] activity.

However these studies only partially contribute to the knowledge of the mechanism of action of benzodiazepines because from ex vivo experiments it is difficult to compare pharmacological activity and receptor binding of drugs in the same experimental conditions. There is also the possibility of active metabolites forming after drug administration, and these may interact with benzodiazepine receptors independently from the parent compound [11,12].

In the present study we describe the relationship between the pharmacological effects of some benzodiazepines and their ability to displace ^{3}H-diazepam binding in vivo, considering the actual brain concentrations of drugs and of their known metabolites.

METHOD

Female CD-COBS rats (Charles River, Italy) average weight 250 g, were injected in lateral tail vein with 50 μCi/rat of ^{3}H-diazepam (S.A. 94 Ci/mmol, Radiochemical Centre), dissolved in 0.4 ml of saline, and decapitated one minute after injection. ^{3}H-diazepam binding in vivo was determined according to the method of Williamson *et al.* [21]. Tissues were immediately removed, hemisected and half was homogenized in 25 volumes of ice-cold Tris HCl buffer (0.05 M pH 7.4) using an Ultra Turrax TP 18.10 (20 sec, full speed).

The other half of the brain was homogenized in Tris buffer containing 3 μM of diazepam and incubated at 0°C for at least 30 min to determine non-specific binding. 0.5 ml aliquots of the tissue homogenate were filtered through Whatman GF/B filters. The filters were immediately washed twice with 5 ml of Tris buffer and counted in 10 ml of dioxane scintillator. Radioactivity was measured with a Packard Tri-Carb liquid scintillation spectrometer model 2450; counting efficiency averaged 40%.

The percentage of specific binding was defined as the amount of radioactivity specifically retained on the filter divided by the total amount of radioactivity present in the tissue sample multiplied by 100. Drugs were given to rats according to a pre-set schedule i.e. time of pre-treatment, route of administration and dosage were selected to provide 50% protection against leptazol-induced convulsions and the minimal dose effective in the conflict test [5, 7, 8, 9].

Brain concentrations of drugs were determined by gas-chromatography according to methods described elsewhere [5,6].

The following drugs (and sources) were utilized: camazepam (7 chloro-3, N,N dimethylcarbamoyloxi-s-phenyl-l-methyl, 1-3, dihydro-2H-1,4 benzodiazepine-2-one) (Simes); Clobazam (Hoechst); CP 1414S (7-nitro-2 amino 5 phenyl-3H-1,5 benzodiazepine) (Continental Pharma); Diazepam (Ravizza); Oxazepam (Ravizza); Temazepam (N-methyl-oxazepam) (Ravizza).

RESULTS

Table 1 shows that equiactive doses of different benzodi-

TABLE 1

PERCENT ^{3}H-DIAZEPAM BOND IN VIVO

Treatment	Brain	Cerebellum
Vehicle (5)	36 ± 4	14 ± 3
Camazepam (4) 147.8 μmol/kg PO 30 min	28 ± 6*	19 ± 5
Temazepam (7) 13.7 μmol/kg PO 30 min	19 ± 4†	13 ± 2
Oxazepam (5) 24.0 μmol/kg PO 30 min	22 ± 3†	14 ± 3
CP 1414 S (4) 29.1 μmol/kg IP 15 min	22 ± 7†	14 ± 3

Data are mean ± S.D. Number of replications is given in parenthesis.

*$p<0.05$. Statistically different from vehicle.

†$p<0.01$. Dunnett's test on arc sin transformation of data.

azepines (ED_{50} antileptazol) cause about the same degree of displacement of ^{3}H-diazepam bound in vivo to the brain, but not to cerebellum. The actual levels of CP 1414 S (taken as an example of this group) in the brain and cerebellum were respectively 8.4±0.9 and 9.8±1.5 nmoles/g,15 min after IP injection of 29.1 μmol/kg, suggesting that the lack of effect in cerebellum was not due to pharmacokinetic factors.

We further investigated the effect of benzodiazepine on ^{3}H-diazepam displacement in various brain areas. Table 2 shows that the highest percentage of bound ^{3}H-diazepam was in the forebrain, followed by hippocampus, brainstem, striatum and cerebellum, confirming other authors' data [20].

The displacing effect of the ED_{50} antileptazol of camazepam, clobazam and diazepam seemed to be higher in forebrain than in other cerebral areas, and no significant effect was found in the cerebellum, confirming the finding presented in Table 1. The levels of diazepam found in the brain areas considered 15 minutes after IP injection of 4.6 μmol/kg were homogeneous (cortex 0.52±0.14; hippocampus 0.56±0.07; brainstem 0.66±0.21; striatum 0.56±0.03; cerebellum 0.63±0.21) indicating that, as for CP 1414 S, the differences in displacement of in vivo bound ^{3}H-diazepam were not due to pharmacokinetic factors. Table 3 summarizes the findings in the brain, setting out the actual brain levels of drugs and metabolites found after injection of the ED_{50} antileptazol [12].

DISCUSSION

When given at equiactive doses (ED_{50} anti-leptazol) several benzodiazepines displace ^{3}H-diazepam bound to the rat brain in vivo to the same extent (about 50%), independently of the doses administered (Table 3). This finding agrees with a report by Chang and Snyder [10] indicating a fairly good correlation between ED_{50} anti-leptazol of several benzodiazepines and ED_{50} for displacement of ^{3}H-flunitrazepam bound in vivo to mice brain. In our conditions, the amount of bound ^{3}H-diazepam displaced in cerebellum was lower than in other areas (Tables 1 and 2). Since cerebellum levels of diazepam and CP 1414 S were comparable to that found in other brain regions, the limited displacement of ^{3}H-diazepam bound to cerebellum is not explained in terms of lower drug concentrations, suggesting either that binding sites in the cerebellum are not involved in the benzodiazepines' anticonvulsant activity or that there are multiple benzodiazepines receptors, as indicated by other authors [13,19]. In addition the difference is in agreement with other data in the literature [19] showing that the benzodiazepine receptors in the cerebellum have different sensitivity from other parts of the brain. An alternative explanation might be that the distribution of the "endogenous ligand" for benzodiazepine receptors is different in various parts of the brain.

Table 3 attempts to correlate pharmacological effects, brain levels and high affinity binding sites of several benzodiazepines. All the values are expressed in μmoles or pmoles for easy comparison. In the case of clobazam it is clear that the anticonvulsant and anticonflict activities in rats are probably related much more to the presence of brain clobazam than N-desmethylclobazam, because the latter is less effective than the former in displacing ^{3}H-diazepam in vitro. In the case of diazepam it is more difficult to decide whether the parent compound or its metabolite is of importance for the anticonvulsant and anticonflict activities. For camazepam it can probably be excluded that the parent compound is effective, the metabolites temazepam and oxazepam probably being responsible for its anticonvulsant effect.

In conclusion the present report indicates that, in spite of the differences between pharmacological potencies and metabolism of benzodiazepines [11,12], a common interaction

TABLE 2

PERCENT ^{3}H-DIAZEPAM BOUND IN VIVO

Treatment	Cortex	Hippocampus	Brainstem	Striatum	Cerebellum
Vehicle	37.1 ± 1.5	24.8 ± 2.2	25.0 ± 6.0	21.0 ± 0.8	16.2 ± 4.8
Camazepam 148 μmol/kg PO 30 min	21.4 ± 1.4†	15.3 ± 2.1†	14.0 ± 4.0*	11.7 ± 4.5†	12.0 ± 3.6
Clobazam 29.0 μmol/kg IP 15 min	18.3 ± 4.1†	9.2 ± 3.0†	16.0 ± 5.0*	8.0 ± 1.6†	12.2 ± 8.7
Diazepam 4.6 μmol/kg IP 15 min	20.0 ± 12.8†	17.0 ± 6.9*	17.0 ± 6.0*	12.7 ± 6.1*	9.7 ± 4.5

Data are mean ± S.D. of 4 replications.

*$p<0.05$. Statistically different from vehicle. Dunnett's test on arc sin transformation of data.

†$p<0.01$.

TABLE 3

CORRELATION BETWEEN PHARMACOLOGICAL ACTIVITIES AND BRAIN HIGH AFFINITY BINDING FOR SEVERAL BENZODIAZEPINES IN RATS

				^{3}H-Diazepam	
Benzodiazepine	Test	ED_{50} μmol/kg (mg/kg)	Brain level* pmol/g	% displacement in vivo*	IC_{50} in vitro (pmol/ml)
Diazepam	AL-CT	4.6	D 596	45.7	6.4
		(1.31)	DD 185		5.7
Clobazam	AL-CT	29.0	C 4266	50.2	260.0
		(8.70)	DC 210		580.0
Camazepam	AL	147.8	CZ 699		950.0
		(55.00)	T 598	46.4	24.0
			OX 385		43.0
Temazepam	AL	13.7	T 498	59.0	24.0
		(4.10)	OX 385		43.0
Oxazepam	AL	24.0	OX 910	50.2	43.0
		(6.70)			
CP 1414 S	AL-CT	29.1	CP 7857	39.4	490.0
		(11.40)			

Antileptazol test (AL); Conflict test (CT); Diazepam (D); N-desmethyldiazepam (DD); Clobazam (C); N-desmethylclobazam (DC); Camazepam (CZ); Temazepam (T); Oxazepam (OX); CP 1414 S (CP).

*Time and route of drugs' administration were the same as in Table 1 and 2.

with brain binding sites in vivo is associated with the presence of the antileptazol activity in the rat. This results in about 50% displacement of ^{3}H-diazepam bound in vivo to brain regions, but not to cerebellum, which is less sensitive to displacement even when the concentrations of displacing drugs are comparable. The possibility that benzodiazepines may bind different receptors, thus explaining different pharmacological effects in the brain and cerebellum, merits further investigation.

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