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Benzodiazepine Hypnotics: Time Course and Potency of Benzodiazepine Receptor Occupation After Oral Application

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MÜLLER, W. E. AND A. E. STILLBAUER. *Benzodiazepine hypnotics: Time course and potency of benzodiazepine receptor occupation after oral application.* PHARMACOL BIOCHEM BEHAV **18**(4) 545–549, 1983.—Ex vivo benzodiazepine receptor occupation by six benzodiazepine hypnotics in the mouse brain was investigated with respect to the time course after oral administration and with respect to the in vitro potency as benzodiazepine receptor ligands. All drugs were administered as solutions. Receptor occupation, as indicated by the inhibition of specific [^{3}H] flunitrazepam binding, occurs very rapidly after oral administration of all benzodiazepines tested with about 80% or even more of maximal inhibition already seen within 30 min after oral administration. However, the onset is somewhat faster with midazolam and temazepam than with the other four derivatives. The duration of benzodiazepine receptor occupation in the mouse brain differed markedly for the six benzodiazepines in agreement with their very different pharmacokinetics in this species. Except for midazolam, there was a good correlation between in vitro receptor affinity and ex vivo receptor occupancy, suggesting that all drugs except midazolam reach the receptor in the CNS similarly. Midazolam has a large first pass metabolism after oral administration which seems the reason for the lower ex vivo potency relative to the in vitro affinity. It is concluded that a fast onset of receptor occupation is a rather general property of benzodiazepines when administered orally as solutions.

Benzodiazepine hypnotics Benzodiazepine receptor Ex vivo binding Oral application

THERE is convincing evidence that most of the pharmacological and clinical effects of the benzodiazepines are mediated via benzodiazepine specific receptors present in the central nervous system of most vertebrate species including man. Furthermore, the affinity of the benzodiazepines for these receptor sites seems to be the major determinant for the pharmacological and clinical potency of these drugs [9, 13, 14]. On the other hand, a variety of data accumulated so far also indicates that differences of the pharmacokinetics of the benzodiazepines, e.g., onset and duration of activity, might also significantly contribute to variations of the in vivo potencies of the benzodiazepines [1,12] and might explain that receptor affinity in vitro may be not in any case strongly correlated with in vivo activity.

Benzodiazepine receptor binding, usually determined in brain homogenates in vitro, has also been demonstrated in vivo in several species [2, 3, 4, 7, 11, 15, 17, 18]. Obviously, the determination of benzodiazepine receptor occupation in vivo provides a very specific parameter of the CNS pharmacokinetics, taking into account differences of receptor affinity as well as differences of the pharmacokinetics. Unfortunately, only little comparable data are available about the relationship between in vitro and in vivo receptor affinity, especially after oral administration, the most usual way of administration for the benzodiazepines. Accordingly, we report in the present communication time course and potency of receptor occupation of six benzodiazepine derivatives after oral administration using a previously described ex vivo technique [4]. The aim was to obtain some further data about the most relevant parameter of the CNS pharmacokinetics of these drugs and about the relationship between in vitro and in vivo potency as benzodiazepine receptor ligands. Most of the derivatives are used as hypnotics in man, where a rapid onset of action is desirable to ensure a fast induction of sleep in insomniac patients. Oxazepam, which is considered to have a relative slow onset of action in man and therefore is less often used as hypnotic [12], has been included for comparison.

METHOD

In Vitro Receptor Binding

Male NMRI mice (18–20 g) were killed by decapitation. The brains were removed and homogenized in 200 volumes 100 μmol/l Tris-HCl buffer pH 7.4. 900 μl aliquots of this tissue suspension were incubated in triplicate at 4°C for 30 min together with 50 μl buffer containing [^{3}H] flunitrazepam (FNT) and 50 μl buffer containing the blank (diazepam) or

TABLE 1

AFFINITIES OF THE SIX BENZODIAZEPINE DERIVATIVES INVESTIGATED FOR THE MOUSE BRAIN BENZODIAZEPINE RECEPTOR IN VITRO

Drug	IC_{50} [^{3}H] flunitrazepam [nM] Mean ± S.E.M.	Relative Potency
Triazolam	2.9 ± 1.0	5.9
Midazolam	8.0 ± 1.0	2.1
Diazepam	17 ± 2	1.0
Nitrazepam	20 ± 3	0.85
Oxazepam	42 ± 8	0.4
Temazepam	50 ± 7	0.34

Affinity is indicated by the inhibitory concentrations 50% (IC_{50}) for specific [^{3}H] FNT binding. IC_{50} values were determined by log probit analysis using four to six concentrations of the displacers. Data are means of four to six individual determinations. Relative potencies are determined on the basis diazepam = 1.0.

various displacers. The final [^{3}H] FNT concentration was always 0.2 nmol/l. The incubation was terminated by rapid filtration through Whatman GF-B filters. The filters were washed three times with 3 ml ice-cold incubation buffer, placed in minivials, and dried for 30 min at 60°C. The radioactivity on the filters was determined by liquid scintillation spectrophotometry in 4 ml Quickszint 402 (Zinsser, Frankfurt, FRG). Unspecific binding was determined by parallel experiments performed in the presence of 10 μmol/l diazepam (blank) and accounted for less than 10% of total binding.

Ex Vivo Receptor Binding

Male NMRI mice (18–20 g) received orally by stomach tube (PO) 200 μl saline containing the benzodiazepines and 10–20 percent ethanol. The mice were killed by decapitation at different intervals after the PO administration and the brains were removed, cleaned, weighed and homogenized in 3 ml ice-cold 100 μmol/l Tris-HCl buffer pH 7.4 within 1 min after decapitation. Three hundred μl aliquots of the homogenate were incubated together with about 2.5 pmol [^{3}H] FNT (added in 50 μl buffer) and either 50 μl buffer alone or buffer containing diazepam as blank (final concentration 10 μmol/l) for 30 min at 4°C. The incubation was terminated by rapid filtration through Wharman GF-B filters which were washed three times with 3 ml ice-cold incubation buffer. For each mouse brain, triplicate determinations for specific and for unspecific binding were performed. The specific binding obtained in these experiments was corrected for brain weight and calculated as percentage of specific binding obtained from parallel experiments using mice treated with the solvent only. Previous studies [4] have demonstrated that for several benzodiazepine receptor ligands this ex vivo binding technique gives fairly similar results than other in vivo techniques for benzodiazepine receptor binding. Moreover, Pieri *et al.* [16] have recently reported some data about benzodiazepine receptor binding of diazepam and midazolam in the mouse brain in vivo, which are quite similar to our findings using the ex vivo technique. Accordingly, the ex vivo method described in this paper will not only measure all the receptor ligands present in the brain, but will also give information about the receptor occupation in vivo.

Materials

[^{3}H] Flunitrazepam ([^{3}H] FNT) specific activity 88 Ci/mmol) was obtained from New England Nuclear (Dreieich, FRG). The benzodiazepine derivatives were gifts from the German manufacturers (diazepam, midazolam, nitrazepam from Hoffmann-La Roche, Grenzach; oxazepam from Boehringer, Ingelheim; temazepam from Farmitalia Carlo Erba, Freiburg; triazolam from Upjohn, Heppenheim). All other chemicals were obtained from commercial suppliers.

RESULTS

The affinities of the 6 benzodiazepine derivatives investigated for the mouse brain benzodiazepine receptor in vitro (see the IC_{50} values in Table 1) are similar to previous findings in other species including man [7, 9, 14]. Compared with diazepam, triazolam and midazolam are more (about 6 or 2 times, respectively) potent, nitrazepam is similar, and oxazepam and temazepam are less than half as active at the receptor in vitro than diazepam (Table 1). Thus, the in vitro potencies of the 6 benzodiazepines vary by more than one order of magnitude.

When diazepam is given orally to mice as a solution at a dose of 7.5 μmol/kg which corresponds to its ED_{50} in various pharmacological tests [7,9], inhibition of benzodiazepine receptor binding in the mouse brain can be seen very rapidly (Fig. 1). Specific [^{3}H] FNT binding is reduced by about 30% already 5 min after oral administration and the maximal inhibition by this dose (about 50%) is reached already 10 min after administration (Fig. 1). The maximal effect remains relatively stable for the period of 10 min to 2 hr after administration and after that time inhibition decreases slowly with an apparent biological half-life of about 14 hr as determined from the linear phase in the half-logarithmic system (Fig. 1, right). When mice received the diazepam derivative temazepam (3-hydroxydiazepam) at an oral dose of 21.8 μmol/kg, which is similar to the diazepam dose relative to the receptor affinity in vitro (Table 1), some differences in respect to the inhibition of specific [^{3}H] FNT binding ex vivo were observed (Table 2). The onset of receptor occupation is faster for temazepam than for diazepam, with about 30% inhibition seen already 2 min after oral administration and about 80% of maximal inhibition present already 5 min after administration, at which time the diazepam effect accounts for only 50% of the maximal effect. Maximal inhibition by this dose of temazepam (70% inhibition of specific [^{3}H] FNT binding) is somewhat higher than that of the comparable (relative to the receptor affinity in vitro) dose of diazepam, giving a first indication for a slightly higher in vivo potency of temazepam compared to diazepam (Table 2). Similar to diazepam, the maximal effect of temazepam is relatively stable over the period of 10 min to 2 hr after oral administration, after which time the effect also decreases. Compared with diazepam, the time course is much slower with an apparent biological half-life of about 30 hr, which is in agreement with the faster elimination of diazepam compared to temazepam in the mouse [8]. This contrasts sharply to the conditions in man, where diazepam is eliminated much more slowly than temazepam [1]. The reason seems to be that, in contrast to man [5,6], temazepam is metabolized in the mouse to a considerable extent to oxazepam and that oxazepam has an exceptionally long elimination half-life in the mouse [8].

Similar experiments on the time course of benzodiazepine receptor binding ex vivo were performed for all 6 benzodiaz-

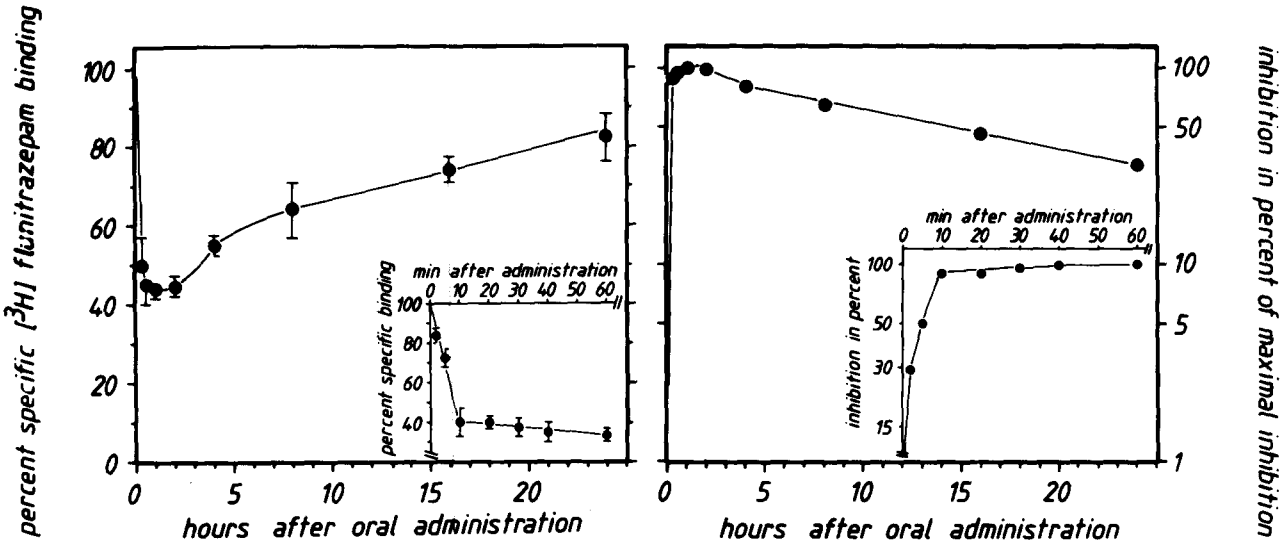

FIG. 1. Time course of benzodiazepine receptor occupation ex vivo after oral administration of diazepam (7.5 μmol/kg). Left: Inhibition of specific [^{3}H]FNT binding at various time intervals after oral administration of a diazepam solution (inset: same data for the time from 2–60 min after administration). Data are means±S.E.M. of four to six individual determinations. Right: Benzodiazepine receptor occupation by diazepam at various time intervals after oral administration expressed as percent of the maximal effect (inset: same data for the time from 2–60 min after administration).

epine derivatives. The data are summarized in Table 2. All benzodiazepines were given orally as solutions at doses similar to diazepam on a molar basis and relative to the receptor affinity in vitro (Table 1), except midazolam, whose dose was twice of the calculated dose in order to reach between 50 and 70% inhibition of specific [^{3}H] FNT binding in vivo [16]. The data were transformed as described for diazepam and temazepam (Fig. 1) and several parameters of the time course of benzodiazepine receptor occupation were calculated and are summarized in Table 3. All benzodiazepines produced maximal inhibition (between 55 to 75%) of specific [^{3}H] FNT binding within 20 to 60 min after oral administration. The time to reach 50% of the peak effect was less than 10 min for all 6 derivatives but was only about 3 min in the case of midazolam and temazepam (Table 3). Ninety percent of maximal inhibition was found after midazolam and temazepam within less than 10 min, was reached by diazepam, nitrazepam, and triazolam in about 20 min, but in

TABLE 2

TIME COURSE OF INHIBITION OF SPECIFIC [^{3}H] FNT BINDING IN THE MOUSE BRAIN EX VIVO AFTER ORAL ADMINISTRATION

	Specific [^{3}H] Flunitrazepam Binding in Percent of Controls											
	Time after PO Administration											
	(min)						(hr)					
Drug (μmol/kg)	2	5	10	20	30	40	1	2	4	8	16	24
Diazepam (7.5)	84 ± 4	72 ± 5	50 ± 7	50 ± 3	46 ± 5	44 ± 5	44 ± 3	45 ± 2	55 ± 2	64 ± 7	74 ± 3	82 ± 6
Midazolam (7.5)	74 ± 5	48 ± 4	49 ± 2	44 ± 4	57 ± 2	54 ± 3	54 ± 5	63 ± 4	67 ± 5	74 ± 5	97 ± 4	98 ± 1
Nitrazepam (9.0)	80 ± 9	65 ± 4	48 ± 1	52 ± 9	46 ± 4	42 ± 2	44 ± 4	44 ± 4	50 ± 5	62 ± 2	83 ± 5	93 ± 2
Oxazepam (18.8)	87 ± 5	69 ± 9	56 ± 5	49 ± 6	47 ± 4	32 ± 2	38 ± 3	35 ± 5	37 ± 6	45 ± 6	49 ± 4	60 ± 2
Temazepam (21.8)	73 ± 8	43 ± 7	37 ± 3	35 ± 5	31 ± 2	38 ± 3	30 ± 1	33 ± 2	34 ± 4	37 ± 2	46 ± 1	58 ± 2
Triazolam (1.5)	91 ± 1	76 ± 6	34 ± 8	36 ± 4	30 ± 2	27 ± 2	25 ± 2	35 ± 2	37 ± 2	63 ± 4	93 ± 5	85 ± 2

Data are means of four to six individual determinations.

TABLE 3

KINETIC PARAMETERS OF THE OCCUPATION OF BENZODIAZEPINE RECEPTORS IN THE MOUSE BRAIN

	Diazepam	Midazolam	Nitrazepam	Oxazepam	Temazepam	Triazolam
E_{max} (%)	56	56	58	68	70	75
t_{max} (min)	40	20	40	40	60	60
$t_{50\%}$ (min)	5	3	9	6	3	7
$t_{90\%}$ (min)	15	7	15	35	9	20
$t_{1/2}$ (hr)	14	5	7	>35	30	6
$E_{0.5\ hr}$ (%)	96	78	93	78	99	93
$E_{1.0\ hr}$ (%)	100	82	97	91	100	100
$E_{8\ hr}$ (%)	64	46	66	81	90	49
$E_{24\ hr}$ (%)	32	4	12	59	60	20

E_{max} and t_{max} are intensity (in percent inhibition of specific [^{3}H] FNT binding ex vivo) and time after oral administration of the maximal effect (see Table 2). $t_{50\%}$ and $t_{90\%}$ are the time after administration to reach 50 or 90% of maximal inhibition, respectively. $t_{1/2}$ is the biological half life of the inhibition of benzodiazepine receptor binding in vivo. $E_{0.5\ hr}$ to $E_{24\ hr}$ are inhibition of benzodiazepine receptor binding ex vivo in percent of the maximal effect at different time intervals after oral administration.

the case of oxazepam was not found before 30 min after oral administration (Table 3). Thus, distinct differences can be seen for the onset of benzodiazepine receptor occupation by the 6 benzodiazepines after oral administration. However, these differences might be not very relevant from a more practical point of view, since all 6 derivatives show between 80 and 100% of the maximal inhibition already 30 min after administration (Table 3).

On the other hand, more significant differences of the ex vivo receptor occupation are present 8 or 24 hr after oral administration (Table 3). Accordingly, the biological half-lives of benzodiazepine receptor occupation ex vivo of the 6 benzodiazepines vary considerably, with values between 5 and 7 hr for midazolam nitrazepam, and triazolam, of about 15 hr for diazepam, but of more than 30 hr for oxazepam and temazepam. Thus, there might be large differences in the elimination of the 6 benzodiazepines in the mouse, which however, do not parallel the conditions in man [1,12].

As mentioned above, all 6 derivatives exhibit 80% or more of their maximal effect at 30 min after oral administration (Table 3). Therefore, we determined the potency of these drugs as inhibitors of specific [^{3}H] FNT binding ex vivo at 30 min after oral administration. The doses of the benzodiazepines required to produce 50% inhibition of specific [^{3}H] FNT binding under these conditions are summarized in Table. 4. Similar to the conditions in vitro (Table 1) the ex vivo potencies range over more than one order of magnitude (Table 4) with triazolam being considerably more potent than diazepam and all other compounds being similar or slightly less potent than diazepam. But more important, the ratios between the relative potencies in vitro and ex vivo vary only about twofold for diazepam, midazolam, oxazepam, nitrazepam, and temazepam (Table 4). Thus, for the derivatives investigated, in vitro affinity for the receptor seems to be the most important determinant for ex vivo potency, even after oral administration. Although temazepam seems to be somewhat more active ex vivo compared to the other derivatives, the only important exception is midazolam which is about four times more potent in vitro than ex vivo (Table 4). At present, we interpret the relative low ex vivo activity by assuming that the fraction of the administered drug which reaches the brain is smaller than that of the other five benzodiazepines.

DISCUSSION

Ex vivo receptor occupancy occurs very rapidly after oral administration of benzodiazepine solutions. Although there are significant differences of the time courses of receptor occupation for the 6 benzodiazepines investigated, e.g., the extremely fast onset in the case of midazolam and temazepam and the considerably slower onset in the case of oxazepam, these differences might be less important from a therapeutical point of view, since all six derivatives reach 80% or even more of maximal inhibition within 30 min after oral administration.

It is quite clear that the duration of receptor occupancy in the mouse will differ fundamentally from that in man due to the large differences of the metabolism of these drugs in the two species [1, 8, 12]. On the other hand, the mechanisms of absorption in the gastrointestinal tract and the penetration into the brain might differ less between man and mouse. Thus, one feels entitled to speculate that the time courses of receptor occupation in man might also be not very different when all six drugs are given orally as solutions.

Unfortunately, we do not know about comparable data on the time course of absorption in man for the benzodiazepines given as solutions. However, significant differences of the time course of absorption can be seen in man when the drugs are taken orally as tablets [12]. This has been studied in detail for the drug temazepam [5,6] which is now sold as a soft gelatin capsule containing the drug in a dissolved form. Absorption of temazepam in man from the soft gelatin capsule is nearly as fast as absorption from a temazepam solution with peak plasma levels already seen 30 min after administration [5,6] which is remarkable similar to our observations in the mouse. When given as tablets or hard gelatin capsules, time course of absorption of temazepam was considerably slower, obviously due to the slow dissolution of the compound [5,6]. Thus, our present studies support the con-

TABLE 4

EX VIVO POTENCIES OF THE BENZODIAZEPINES AS BENZODIAZEPIN RECEPTOR LIGANDS

Drug	ED_{50} [^{3}H] Flunitrazepam (μmol/kg) Means ± S.E.M. (mg/kg)	Relative Potency
Triazolam	0.7 ± 0.18 (0.24)	9.0 (0.7)*
Diazepam	6.3 ± 0.9 (1.8)	1.0 (1.0)
Nitrazepam	7.4 ± 1.8 (2.1)	0.85 (1.0)
Temazepam	8.8 ± 1.3 (2.6)	0.72 (0.5)
Midazolam	10.6 ± 1.5 (3.5)	0.59 (3.6)
Oxazepam	16.8 ± 3.1 (4.8)	0.38 (1.0)

Given are the oral doses needed to reach 50% inhibition of benzodiazepine receptor binding ex vivo 30 min after oral administration (ED_{50}). ED_{50} values were determined by log probit analysis using three different doses of the benzodiazepines. The data are means of four individual determinations. Relative potencies were determined on the basis diazepam = 1.0.
*Relative potency in vitro/relative potency ex vivo.

cept to give benzodiazepines in a dissolved form [5] to ensure fast onset of action in man. Moreover, the data indicate that fast absorption is a rather general property of the benzodiazepines when given orally as solutions and seems to be present even in the case of oxazepam which is considered to be relatively slowly absorbed if given as tablets in man [12].

The relationship between in vitro and ex vivo potency does not differ very much for the benzodiazepines investigated except for midazolam, indicating similar biovailabilities and suggesting that ex vivo receptor affinity is closely related to in vitro affinity. Midazolam is a considerably less potent inhibitor of specific [^{3}H] FNT binding ex vivo than in vitro, possibly because the fraction of the oral dose which reaches the brain is smaller that that of the other five benzodiazepines. This goes parallel with the conditions in man, where a large first pass metabolism has been found [10]. The metabolites, although of similar potency at the receptor in vitro, are much less active in vivo than midazolam, presumably due to their reduced ability to penetrate the blood-brain barrier [16]. Similar factors may account for the relatively weak in vivo potency of midazolam in the mouse.

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