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In Vitro Correlates of Benzodiazepine Cerebrospinal Fluid Uptake, Pharmacodynamic Action and Peripheral Distribution[1]

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ABSTRACT

Factors influencing the rate and extent of benzodiazepine uptake into cerebrospinal fluid (CSF), peripheral tissue distribution and electroencephalographic (EEG) effects were evaluated in a model utilizing anesthetized male cats. A single (0.25–10 mg/kg) dose of the following eight benzodiazepines was administered i.v.: diazepam, desmethyldiazepam, midazolam, lorazepam, alprazolam, triazolam, flunitrazepam and clobazam. Multiple samples were simultaneously drawn from arterial blood and cisternal CSF over the next 4 hr and the EEG was continuously monitored. Concentrations of benzodiazepines in plasma and CSF samples were measured by electron-capture gas-liquid chromatography and plasma protein binding determined by equilibrium dialysis. Physicochemical properties of lipophilicity of each benzodiazepine were determined by measurement of the octanol/buffer partition ratio at physiologic pH and by the high-pressure liquid chromatographic (HPLC) retention on a reverse-phase C_{18} column at neutral pH. Disappearance of all benzodiazepines from plasma was consistent with a linear sum of two or three exponential terms. After correction for individual differences in protein binding, volume of distribution (V_d) of unbound drug was highly correlated with HPLC retention ($r = 0.91$), but not significantly related to octanol/buffer partition coefficient. Diazepam and midazolam, having the longest HPLC retention also had the largest unbound V_d. All benzodiazepines rapidly entered CSF, with peak concentrations usually attained within 15 min of dosage. More lipophilic drugs tended to enter CSF most rapidly, but associations of entry rate and *in vitro* lipophilicity were not significant. After distribution equilibrium was attained, disappearance of benzodiazepines from both plasma and CSF occurred in parallel. Equilibrium CSF/total plasma concentration ratios of all drugs were much less than unity. This was explained by plasma binding because equilibrium ratios were highly correlated with free fraction ($r = 0.93$, regression line slope = 0.98). All benzodiazepines produced diffuse low-frequency (1–5 Hz) EEG activity of very rapid onset after the i.v. dose. Onset rate was not correlated with *in vivo* lipophilicity. However, the duration of slow-wave activity differed markedly among drugs, having a high negative correlation with log of HPLC retention ($r = 0.77$) and with log of *in vivo* unbound V_d ($r = 0.87$). Thus, benzodiazepine distribution *in vivo* is determined largely by lipid solubility, which in turn is reflected best by retention on a reverse-phase HPLC system. The rate of CSF entry, as well as the rate of onset of EEG slow-wave effects, were rapid and not significantly related to lipophilicity, but duration of EEG effects were longest for the least lipophilic drugs having the smallest V_d.

Benzodiazepine derivatives are a class of sedative and tranquilizing agents extensively used in clinical practice (Greenblatt and Shader, 1974a,b; Usdin *et al.*, 1982). Despite the structural similarity among these compounds, they differ widely in their physicochemical properties, their profile of distribution and clearance *in vivo* and in the time course and intensity of clinical sedative and tranquilizing effects after single doses (Greenblatt *et al.*, 1982a, 1983; Usdin *et al.*, 1982; Klotz *et al.*, 1980; Biagi *et al.*, 1980; Borea and Bonora, 1983). Clinical differences among benzodiazepines have so far been documented mainly in descriptive terms, without a systematic framework relating such differences to physicochemical properties.

Classic studies performed more than two decades ago first indicated that lipophilicity of drugs can be an important determinant of the rate at which they traverse biologic membranes such as the blood-brain barrier (Rall *et al.*, 1959, 1961; Mayer *et al.*, 1959; Brodie *et al.*, 1960). This in turn might influence the onset and intensity of pharmacodynamic action of centrally acting compounds. The most widely used approach to evaluating lipophilicity *in vitro* involves partition ratios and variants

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ABBREVIATIONS: CSF, cerebrospinal fluid; EEG, electroencephalogram; HPLC, high-pressure liquid chromatographic; V_d, total volume of distribution using the area method.

thereof, whereby relative drug concentrations or amounts at equilibrium between "aqueous" and "organic" phases are taken to reflect relative solubility in the two phases (Leo *et al.*, 1971; Ritschel and Hammer, 1980). More recently, reverse-phase liquid chromatography has been evaluated for a similar purpose. The partitioning of a drug between mobile and stationary phases also appears to reflect lipophilicity, with less polar compounds retained longer (Wells *et al.*, 1981; Hulshoff and Perrin, 1976; Mirrlees *et al.*, 1976; Jandera *et al.*, 1982; Baker *et al.*, 1979). The present study was undertaken to evaluate two lipophilicity indexes for a series of benzodiazepines (fig. 1) in relation to their pharmacokinetic properties of distribution, clearance and uptake into CSF, as well as the time course and intensity of their effects on the EEG.

Methods

***In vitro* studies.** The partitioning of eight benzodiazepine derivatives (fig. 1; table 1) between an aqueous phosphate buffer (pH 7.4) and *n*-octanol was determined *in vitro*. Aqueous solutions of benzodiazepines labeled with ^{14}C or ^{3}H were shaken with equal volumes of *n*-octanol. The mixtures were centrifuged and separated. Radioactivity in both phases was determined using a liquid scintillation counter. Octanol/buffer partition ratios were calculated as the ratio of radioactivity in the octanol phase divided by that in the aqueous phase.

Retention time on a HPLC system was determined using a reverse-phase C_{18}-microBondapack column (Waters Associates, Milford, MA). The mobile phase was methanol-acetonitrile-0.001 M sodium acetate buffer (25:25:50), having a final pH of 7.0. The mobile phase flow rate was 1.5 ml/min. Methanolic solutions of the pure benzodiazepines were sequentially injected, with column effluent quantitated by ultraviolet detection at 254 nm. All analyses were performed at room temperature.

Animal studies. Adult male mongrel cats, weighing between 3 and 5 kg, were anesthetized in a chamber with 4% halothane in 2:1 nitrous oxide-oxygen (deJong *et al.*, 1981, 1982; deJong and Davis, 1981). Anesthesia was subsequently maintained with 0.5 to 1% halothane in 3:1 nitrous oxide-oxygen. Central (esophageal) temperature was maintained at 38 ± 0.5°C using heating pads. Lactated Ringer's solution was infused at the rate of 15 ml/kg/hr, based on continuous monitoring of urine output and hematocrit, to maintain adequate intravascular volume. Muscle relaxation was maintained using gallamine, which was administered as needed at an overall rate of 10 to 20 mg/hr. Aortic blood pressure was continuously monitored *via* a femoral arterial cannula. The femoral vein was cannulated as well. The EEG was continuously monitored using needle electrodes inserted through the skull to be in contact with the dura. Left and right frontotemporo-occipital bipolar arrays were used. The electrocardiogram was monitored using extremity leads.

The animals were mechanically ventilated. Arterial oxygen tension was maintained above 100 mm Hg and arterial CO_2 tension at 31 ± 2 mm Hg. Arterial pH was maintained at 7.4 ± .02. The alveolar (end-expiratory) carbon dioxide percentage was 4.4 ± 0.2%.

Table 1 shows benzodiazepine doses administered, chosen to be

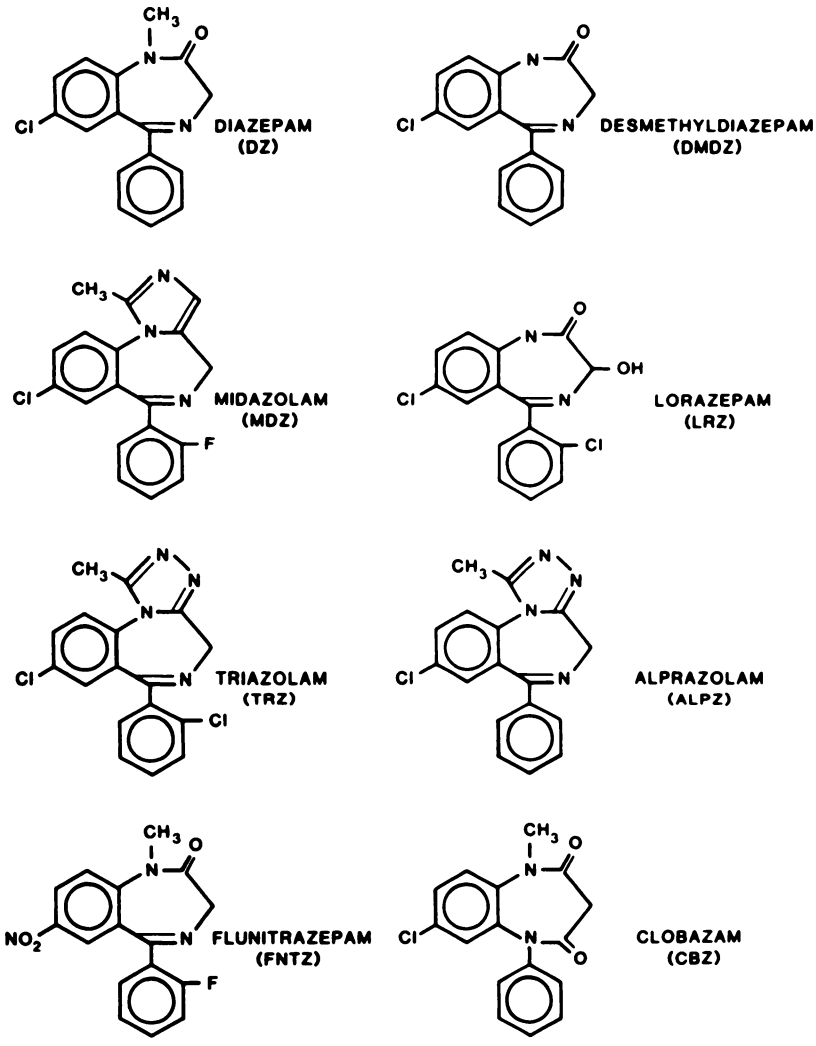

Fig. 1. Structures of benzodiazepines evaluated in the present study.

TABLE 1
Doses and analytic methods for benzodiazepine studies

Drug	Abbreviations	Dose	Analytic Method Reference
		mg/kg	
Diazepam	DZ	2	Greenblatt *et al.*, 1980c
Desmethyldiazepam	DMDZ	2.2–2.5	Greenblatt *et al.*, 1980c
Midazolam	MDZ	10	Greenblatt *et al.*, 1981b
Lorazepam	LRZ	0.5	Greenblatt *et al.*, 1978
Triazolam	TRZ	0.25	Greenblatt *et al.*, 1981a
Alprazolam	ALPZ	0.5	Greenblatt *et al.*, 1981a
Flunitrazepam	FNTZ	0.25	Greenblatt *et al.*, 1982b
Clobazam	CBZ	10	Greenblatt, 1980a

approximately comparable clinically. Each drug was given to three animals and each animal received only one drug. Medications were administered either as the commercially available parenteral preparation or as a propylene glycol-ethanol (50:50) solution. Total volumes injected were always less than 2 ml. Drugs were administered by rapid i.v. injection into the femoral vein. Femoral arterial blood samples (2–4 ml) each were drawn into heparinized syringes at the following times after each dose: 1, 5, 10, 15, 30 and 45 min, 1, 1.5, 2.0, 2.5, 3, 3.5 and 4 hr. CSF (0.2–1.0 ml each) samples were also obtained *via* a catheter inserted into the cisterna magna at 1, 5, 10, 15 and 30 min, 1, 2, 3 and 4 hr. Heparinized blood samples were centrifuged and the plasma separated. Plasma and CSF samples were frozen at −20°C until the time of assay. Total drug concentrations and free fraction of drug in plasma were not influenced by storage under these conditions. Analyses were always performed within 1 week of sample collection.

Concentrations of benzodiazepines and their pertinent metabolites in all plasma and CSF samples were determined by electron-capture gas-liquid chromatography using methods referenced in table 1. After measurement of total (free plus bound) benzodiazepine concentrations in each plasma aliquot for each animal, samples containing the highest drug concentration (*i.e.*, 5 and 10 min after dosage) were pooled, as were those containing lower concentrations (*i.e.*, 2, 3 and 4 hr after dosage). Three-milliliter plasma samples were spiked to contain 2 to 10 nCi of the ^{14}C or ^{3}H analogs of the benzodiazepine derivatives. The samples were subjected to equilibrium dialysis at 37°C for 12 to 18 hr. After quantitation of radioactivity by liquid scintillation counting, free fraction was determined as the ratio of radioactivity in the protein-free dialysate divided by that inside the dialysis membrane (Woo and Greenblatt, 1979; Divoll and Greenblatt, 1982; Moschitto and Greenblatt, 1983).

Evaluation of the EEG. EEG tracings were read by a neurologist (B.L.E.) who had no knowledge of the *in vitro* or pharmacokinetic results. The reader estimated qualitative changes in the tracing as well as the time course of such changes.

Pharmacokinetic methods. Plasma benzodiazepine concentrations after i.v. injection were analyzed by weighted iterative nonlinear least-squares regression techniques (Greenblatt *et al.*, 1979b). Squared residual errors were weighted by a factor equal to the reciprocal of the concentration. Data points were fitted to the following two functions:

$$C = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

$$C = Ae^{-\alpha t} + Pe^{-\pi t} + Be^{-\beta t} \quad (2)$$

where C is the plasma concentration at time t after dosage. A, P and B are hybrid coefficients with units of concentration; α, π and β are hybrid exponents having units of reciprocal time. The choice between equations 1 and 2 as functions of best fit was made by comparison of weighted residual errors and by the scatter of actual data points about the fitted function. Using standard pharmacokinetic methods, coefficients and exponents were used to calculate the following kinetic variables: initial distribution $T_{1/2}$, $T_{1/2}$ of elimination during the terminal or β phase, volume of the central compartment, V_d, total volume of distribution using the steady-state method and total clearance. For each animal, V_d and clearance for total (free plus bound) drug were divided by mean value of free fraction, yielding corresponding values of unbound V_d and unbound clearance. For several benzodiazepines (diazepam, clobazam, midazolam), clearance of the parent compound was mirrored by formation of metabolic products known to possess at least some pharmacologic activity (Greenblatt *et al.*, 1983). However, the duration of sampling was not sufficient for formal analysis of metabolite kinetics. Furthermore, parent drug levels generally were far higher than metabolite levels.

Concentrations of benzodiazepines in CSF were similarly analyzed by weighted nonlinear least-squares regression techniques (Greenblatt *et al.*, 1979a, 1980b). CSF concentrations were fitted to the following two functions:

$$C = B\,(e^{-\beta t} - e^{-k_a t}) \quad (3)$$

$$C = -(A + B)e^{-k_a t} + Ae^{-\alpha t} + Be^{-\beta t} \quad (4)$$

where C is the CSF concentration at time t after parenteral dosage, A and B are hybrid coefficients (not the same as those in equations 1 and 2) and α and β are hybrid exponents. K_a represents the apparent first-order rate constant for drug entry into CSF, which was used to calculate a value of CSF entry half-life.

Statistical methods included linear regression analysis and Student's *t* test.

Results

Table 2 shows values of octanol/buffer partition ratio and HPLC retention time for each of the benzodiazepines. The two indexes of lipid solubility were poorly correlated with each other ($r = 0.25$). Diazepam was by far the most lipophilic benzodiazepine based on the partition ratio, whereas midazolam had the longest retention time in the HPLC system.

Figure 2 shows plasma and CSF drug concentrations as a function of time in two representative animals. V_d of total (free plus bound) drug for the various benzodiazepines (table 2) was unrelated to partition ratio ($r = 0.42$) or HPLC retention ($r = 0.44$). However, these relationships were importantly confounded by differences in protein binding. After correction for these differences, unbound V_d was highest for midazolam and diazepam and lowest for clobazam and alprazolam. Unbound V_d was highly correlated with HPLC retention ($r = 0.91$, $P < .005$; fig. 3), but was not significantly associated with octanol/buffer partition ratio ($r = 0.23$). These relationships were the same whether the area method or the steady-state method was used to calculate volume of distribution. V_d and total V_d using the steady-state method were highly intercorrelated ($r = 0.98$), with a mean ratio of 0.89 (range: 0.75–0.97).

Clobazam, diazepam and desmethyldiazepam had the longest values of plasma elimination $T_{1/2}$ whereas midazolam and triazolam have the shortest values. However, midazolam had by far the highest value of unbound drug clearance, whereas other benzodiazepines had considerably smaller values of clearance.

All benzodiazepine derivatives rapidly entered CSF. With one exception, peak CSF concentrations were reached within 15 min of the dose (figs. 2 and 4). The rapid rate of CSF entry caused computer-determined values of k_a (equations 3 and 4) to have large S.D.s. Values for $T_{1/2}$ of drug entry into CSF nonetheless were calculated from K_a and in no case exceeded 8 min. Although more lipophilic benzodiazepines tended to enter CSF the most rapidly, neither CSF peak time nor CSF entry $T_{1/2}$ were significantly correlated with either octanol/buffer partition ratio or HPLC retention (fig. 4). Disappearance of benzodiazepines from CSF always occurred in parallel with disappearance from plasma. Half-lives of elimination from the two compartments were highly correlated ($r = 0.85$, $P < .01$; fig. 5).

TABLE 2

***In vitro*, pharmacokinetic and pharmacodynamic properties of eight benzodiazepines**

	Diazepam	Desmethyl-diazepam	Midazolam	Lorazepam	Triazolam	Alprazolam	Flunitrazepam	Clobazam
In Vitro Characteristics								
Octanol-buffer partition ratio	309	54	34	73	43	19	48	9
HPLC retention (min)	31.4	25.8	49.3	16.5	19.1	18.9	13.6	14.9
Plasma kinetics[a]								
$T_{1/2}\alpha$ (min)[b]	13.7(±3.5)	8.7(±2.0)	1.2(±0.7)	0.9(±0.5)	0.8(±0.3)	3.2(±2.2)	2.8(±1.1)	6.2(±3.8)
$T_{1/2}\beta$ (hours)[c]	5.24(±0.35)	5.50(±0.71)	1.29(±0.41)	4.06(±0.73)	1.47(±0.12)	3.44(±0.40)	3.64(±1.17)	6.11(±1.80)
V_1 (liters/kg)[d]	0.77(±0.09)	0.64(±0.05)	0.59(±0.01)	0.19(±0.09)	0.11(±0.05)	0.53(±0.22)	0.52(±0.21)	0.44(±0.19)
V_d (liters/kg)	3.01(±0.4)	1.37(±0.19)	2.99(±0.46)	1.44(±0.21)	1.84(±0.21)	2.00(±0.31)	3.26(±0.67)	1.27(±0.09)
$V_{d_{ss}}$ (liters/kg)[e]	2.66(±0.37)	1.33(±0.18)	2.38(±0.38)	1.39(±0.20)	1.37(±0.13)	1.92(±0.29)	2.72(±0.54)	1.21(±0.10)
Unbound V_d (liters/kg)	17.3(±0.3)	10.1(±0.8)	33.9(±4.8)	11.5(±1.4)	5.5(±0.5)	4.7(±0.7)	11.0(±3.5)	3.85(±0.5)
Clearance (ml/min/kg)	6.6(±0.7)	2.9(±0.2)	30.2(±6.4)	4.2(±0.2)	15.0(±2.9)	6.6(±0.3)	11.2(±1.4)	3.0(±1.0)
Unbound clearance (ml/min/kg)	38.3(±2.0)	21.4(±1.1)	330.0(±56.0)	33.7(±2.7)	44.7(±7.3)	15.7(±1.0)	35.1(±0.3)	9.2(±3.3)
Protein binding and CSF entry[a]								
Free fraction in plasma	0.17(±0.02)	0.14(±0.01)	0.09(±0.01)	0.12(±0.01)	0.33(±0.01)	0.43(±0.02)	0.32(±0.04)	0.34(±0.04)
Equilibrium CSF/plasma concentration ratio	0.12(±0.02)	0.11(±0.02)	0.14(±0.03)	0.15(±0.01)	0.37(±0.04)	0.41(±0.02)	0.34(±0.04)	0.34(±0.04)
Time of peak CSF concentration (min after dose)	3.7(±1.3)	11.7(±1.7)	3.7(±1.3)	7.0(±4.2)	6.7(±1.7)	28.3(±15.9)	8.3(±1.7)	10.0(±2.9)
CSF entry $T_{1/2}$ (min)	0.44(±0.22)	1.35(±0.41)	0.28(±0.28)	1.60(±1.15)	2.94(±1.24)	3.86(±1.85)	1.14(±0.21)	0.72(±0.23)
Pharmacodynamic EEG effects[a]								
Onset of slow-wave activity (min after dose)	0.89(±0.31)	0.44(±0.29)	0.29(±0.04)	3.8(±3.1)	2.03(±1.49)	0.54(±0.10)	0.39(±0.31)	0.44(±0.16)
Duration of slow-wave activity (min)	7.5(±1.4)	23.3(±18.3)	6.3(±1.9)	28.3(±10.1)	60.0(±31.2)	35.0(±12.6)	38.3(±10.9)	62.7(±13.9)

[a] Each entry is the mean (±S.E.) for three animals.
[b] Initial distribution $t_{1/2}$.
[c] Elimination $t_{1/2}$ during the β-phase.
[d] Volume of the central compartment.
[e] V_d using the steady-state method.

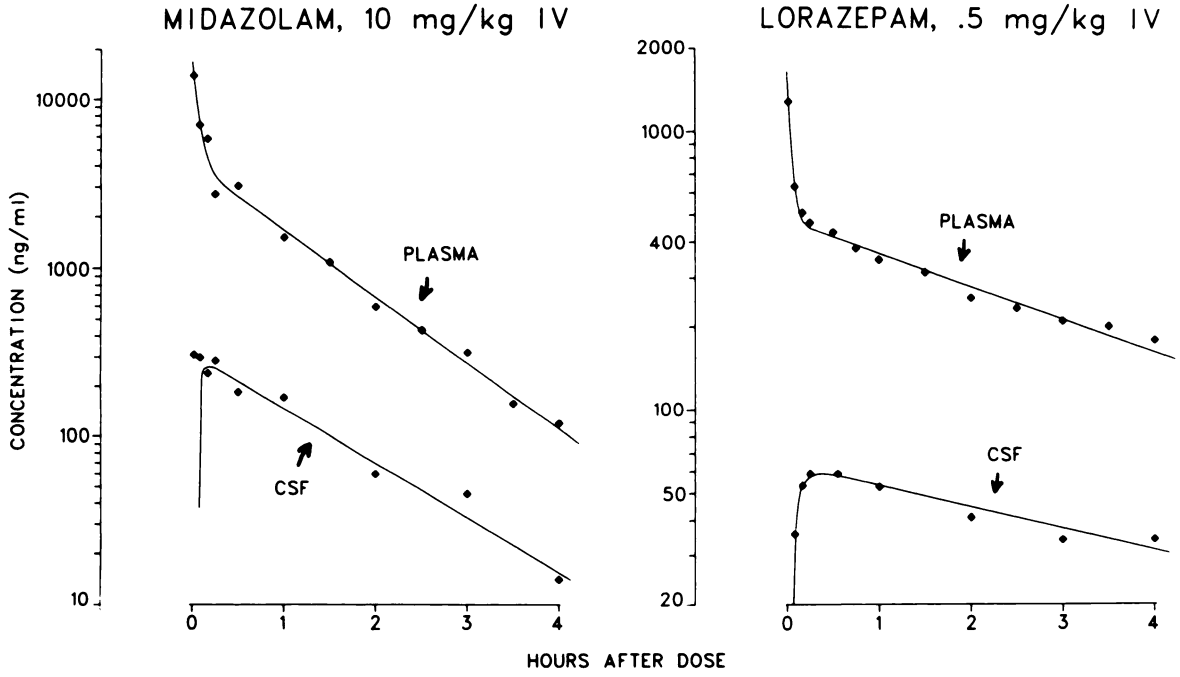

Fig. 2. Plasma and CSF concentrations of midazolam and lorazepam after a single i.v. dose to representative animals. Solid lines represent pharmacokinetic functions.

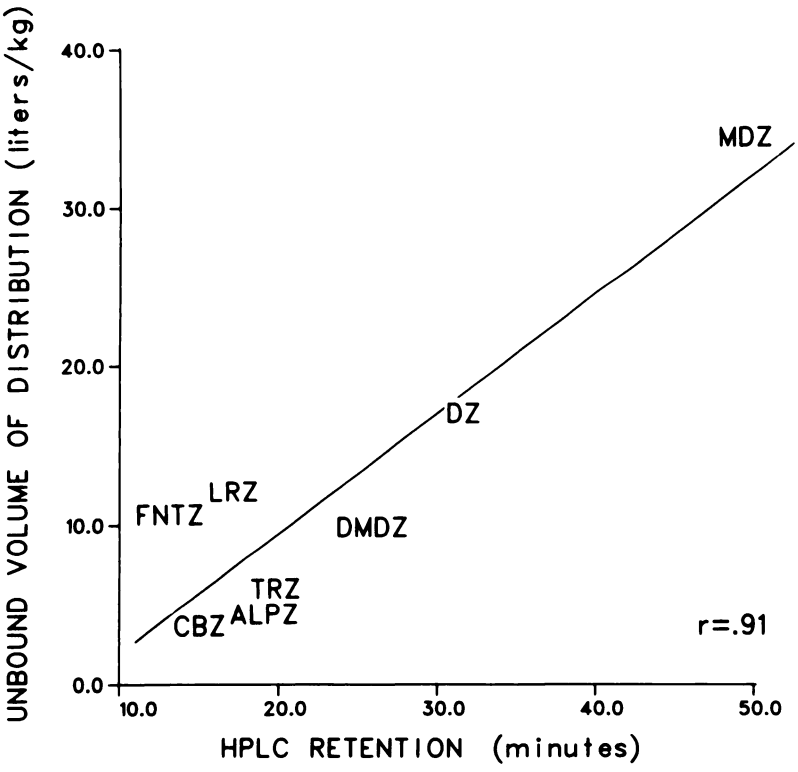

Fig. 3. Relation of HPLC retention to unbound volume of distribution for the eight benzodiazepines. Each value is the mean for three animals that received the same drug. Solid line was determined by least-squares regression analysis (r = 0.91). See table 1 and figure 1 for explanation of abbreviations.

The CSF/total plasma concentration ratios for all benzodiazepines were less than unity and ranged from 0.08 to 0.46 (table 2). The equilibrium CSF/total plasma ratio was highly correlated with free fraction of drug in plasma (fig. 6). The correlation coefficient was 0.93 and the slope of the regression line was 0.98. Consistent with a previous study of benzodiazepine binding in human plasma (Moschitto and Greenblatt, 1983) plasma protein binding of benzodiazepines in the present study was independent of total plasma concentration.

All benzodiazepines produced a principal EEG change characterized as medium to high voltage, low frequency (1–5 Hz) activity (fig. 7). The onset of these effects was in all cases rapid, occurring within 4 min of the i.v. injection. The time of onset of EEG slow-wave activity was unrelated to octanol/buffer partition ratio (r = 0.07) or to HPLC retention (r = 0.31). The duration of these EEG effects, estimated as the time for 50 to 75% disappearance, varied widely among drugs (table 2). Duration was highly correlated with logarithm of HPLC retention

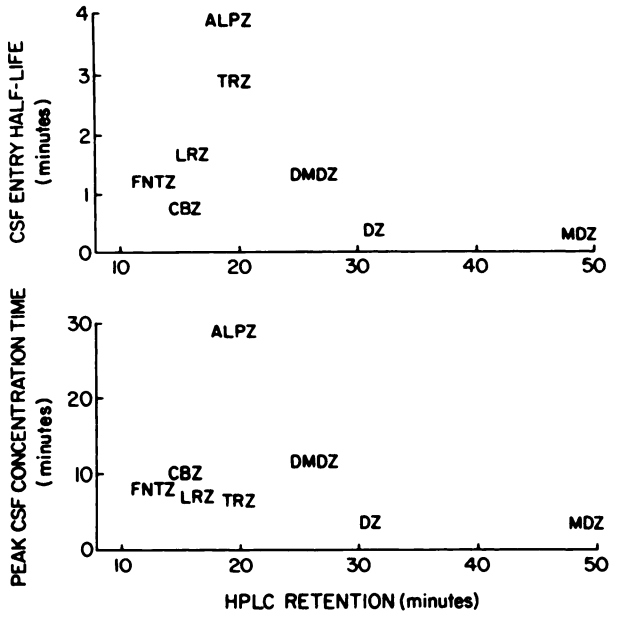

Fig. 4. Relation of HPLC retention to CSF entry $T_{1/2}$ (above) and time of peak CSF concentration (below). Each value is the mean for three animals that received the same drug. Associations did not reach significance. See table 1 and figure 1 for explanation of abbreviations.

(r = −0.77, P = .03) and with logarithm of unbound V_d (r = −0.87, P < .005; fig. 8). However, EEG duration was unrelated to plasma elimination $T_{1/2}$ (r = 0.06) or CSF disappearance $T_{1/2}$ (r = 0.01; fig. 9). Resolution of EEG slow-waves was coincident with appearance of low to medium voltage higher frequency (15–25 Hz) "rebound"-like activity which persisted for 1 to 3 hr after dosage. This was most evident for the most lipophilic benzodiazepines (midazolam, diazepam) for which slow-waves disappeared most rapidly (fig. 7).

Discussion

The variability in pharmacokinetic profile among eight benzodiazepines in this animal model is similar to the variability observed in humans (Greenblatt *et al.*, 1982a, 1983; Klotz *et al.*, 1980; Usdin *et al.*, 1982). The eight compounds differed greatly in elimination $T_{1/2}$, V_d and total metabolic clearance. After correction for individual differences in protein binding, *in vivo* V_d was highly correlated with HPLC retention on a reverse-phase system. This is consistent with the postulated mechanism of reverse-phase liquid chromatography which partitions and retains compounds depending on their polarity. The most lipophilic benzodiazepines, diazepam and midazolam, had the longest HPLC retention and also the largest *in vivo* V_d. Other less lipophilic benzodiazepines had shorter retention and smaller *in vivo* V_d. This association was not evident if total rather than unbound V_d was used to estimate distribution because unbound V_d correctly describes the extent of distribution of the unbound drug which is actually available for distribution (Greenblatt *et al.*, 1982c). Furthermore, V_d was not associated with octanol/buffer partition ratio, suggesting that this traditional measure of lipophilicity describes different physicochemical properties than does HPLC retention.

Previous studies have suggested that the rate of entry of drugs into CSF is related to their lipophilic properties, provided that CSF uptake is determined by passive diffusion (Mayer *et al.*, 1959; Brodie *et al.*, 1960; Rall *et al.*, 1959, 1961; Oldendorf, 1974a,b; Lorenzo and Spector, 1976). However, most studies have evaluated a series of compounds ranging from very lipophilic to very hydrophilic. The benzodiazepines evaluated in the present study differed among themselves in their characteristics of lipid solubility, but all were relatively lipid soluble compounds, having octanol/buffer partition ratios considerably in excess of unity. Thus, all benzodiazepines rapidly entered

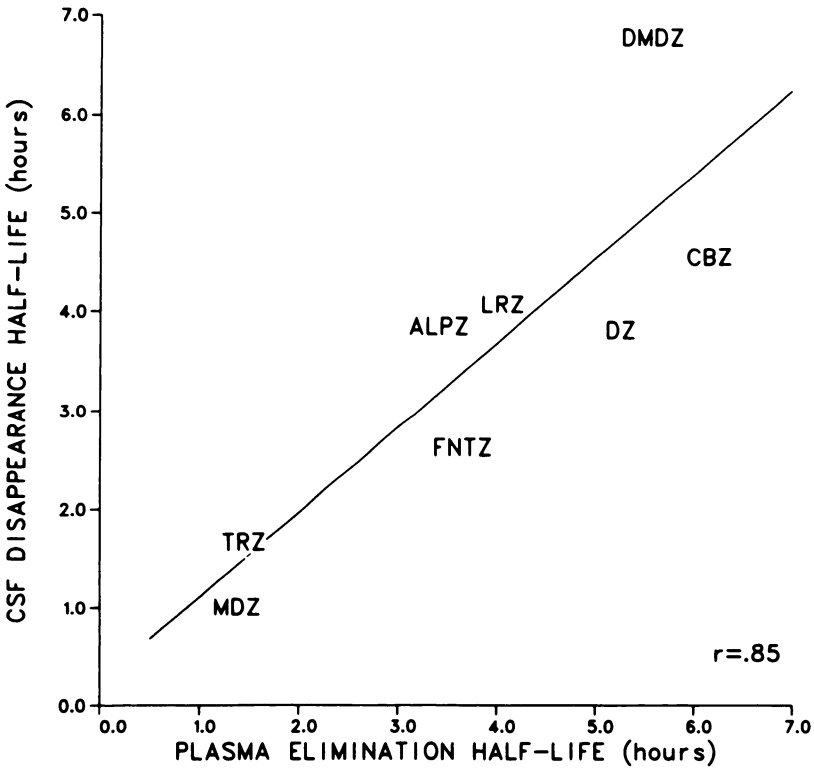

Fig. 5. Relation of benzodiazepine elimination $T_{1/2}$ from plasma *vs.* the $T_{1/2}$ of disappearance from CSF. Each value is the mean for three animals that received the same drug. Solid line was determined by least-squares regression analysis (r = 0.85). See table 1 and figure 1 for explanation of abbreviations.

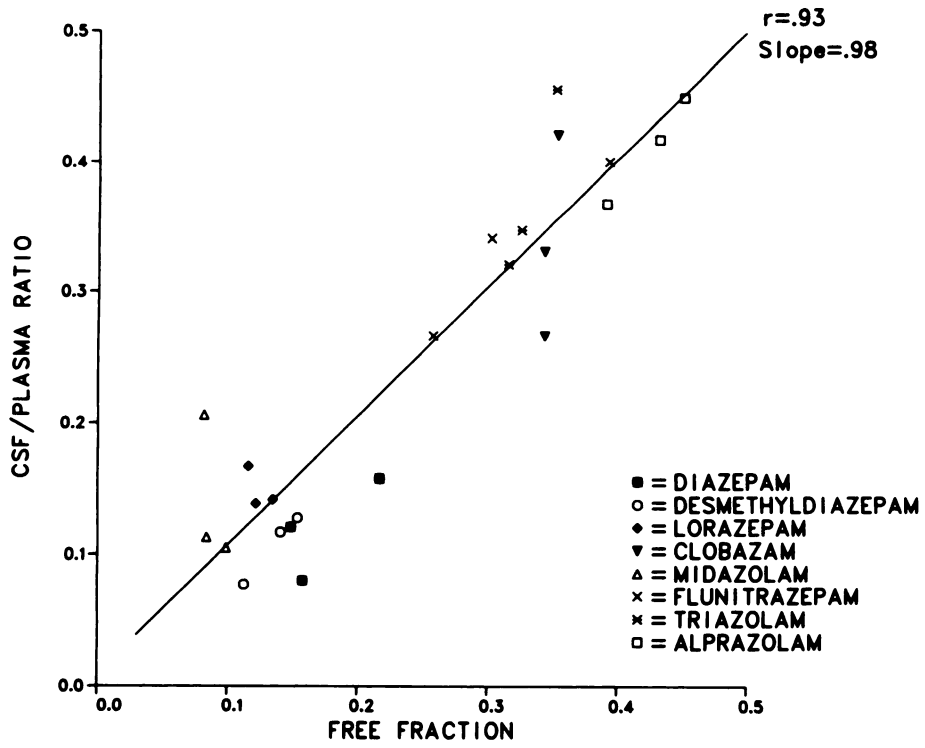

Fig. 6. Relation of free fraction of benzodiazepines in plasma to CSF/total plasma concentration ratio at equilibrium. Solid line was determined by least-squares regression analysis (r = 0.93).

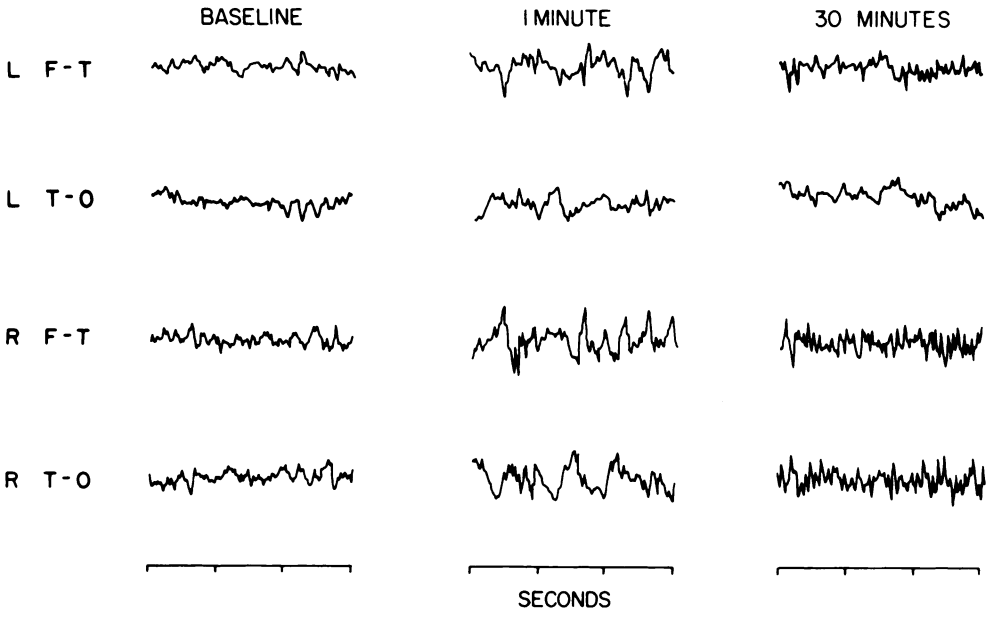

Fig. 7. Three-second segments of the EEG tracing from a representative animal taken before and after i.v. injection of midazolam. L, left; R, right; F-T, frontotemporal; T-O, temporo-occipital.

and equilibrated between CSF and plasma. After equilibrium was attained, disappearance from CSF always occurred in parallel with disappearance from plasma. This is consistent with pharmacokinetic theory indicating that disappearance of drug from all compartments occurs in parallel once distribution equilibrium is attained (Gibaldi and Perrier, 1975).

Despite the rapid rate of benzodiazepine entry into CSF, equilibrium CSF/total plasma concentration ratios were always less than unity. This phenomenon was essentially completely explained by plasma protein binding. The correlation between free fraction in plasma and CSF/total plasma concentration ratio was high, with a regression line slope of essentially unity. Thus, partitioning of benzodiazepines into a protein-free body compartment such as CSF appears to occur by passive diffusion, with the extent of entry limited by protein binding in plasma.

Intravenous injection of all benzodiazepines produced characteristic medium to high voltage low-frequency (1–5 Hz) EEG activity (Nagy and Desci, 1973). As in the case of CSF entry, the onset of these EEG changes was rapid and was unrelated to *in vitro* parameters of lipophilicity. However, the rate of disappearance of EEG effects was variable among drugs and had a clear inverse relation to *in vivo* unbound V_d and to *in vitro* HPLC retention. Thus, increases in lipophilicity and *in vivo* distribution are associated with reduced duration of pharmacodynamic action of benzodiazepines after single i.v. doses. Half-life of drug elimination, on the other hand, is unrelated to single-dose pharmacodynamic action.

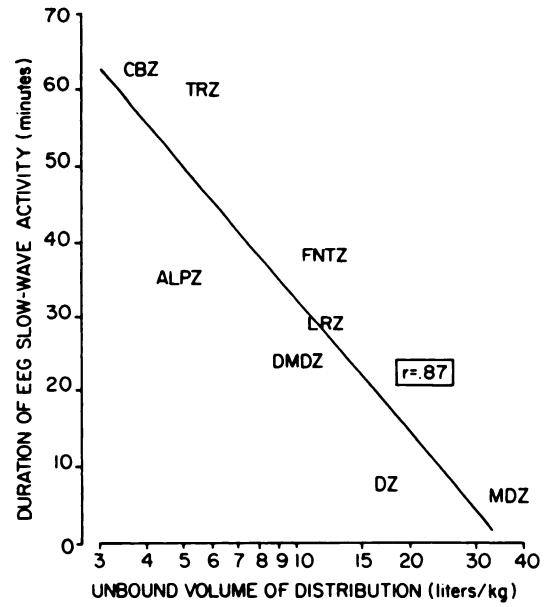

Fig. 8. Relation of unbound volume of distribution (plotted on a logarithmic axis) to duration of EEG slow-wave activity. Each value is the mean for three animals that received the same drug. Solid line was determined by least-squares regression analysis (r = −0.87). See table 1 and figure 1 for explanation of abbreviations.

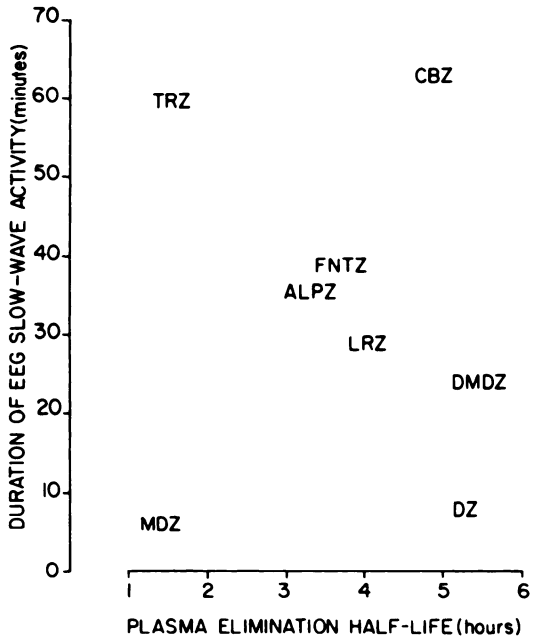

Fig. 9. Relation of plasma elimination $T_{1/2}$ to duration of EEG slow-wave activity. Each value is the mean for three animals that received the same drug. The association did not approach significance. See table 1 and figure 1 for explanation of abbreviations.

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