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Good Correlations between Brain Levels of Benzodiazepines determined by Radioreceptorassay and Central Nervous System Activity

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A radioreceptorassay with ^{3}H-diazepam and benzodiazepine receptors was used to determine the levels of receptor reactive substances (diazepam activity) in various brain regions after *i.p.* injection of six benzodiazepines (2 mg/kg) into rats. All the compounds tested gave nearly the same regional distribution. The correlations between diazepam activities and pharmacological or clinical potencies were better than those between *in vitro* binding data and central nervous system activities. This result indicates that the determination of diazepam activity in the brain by the radioreceptorassay technique is useful for evaluating the pharmacological and clinical potencies of benzodiazepines and related compounds.

Keywords——benzodiazepine; benzodiazepine receptor; radioreceptorassay; diazepam activity; brain level of benzodiazepine; correlation with clinical doses

Benzodiazepines display their pharmacological activities through interaction with high affinity binding sites in the brain membrane designated as benzodiazepine receptors.[1,2] Important evidence for this concept is, as many investigators have reported,[2–4] that the affinities of a variety of benzodiazepines for the receptors are correlated closely with their pharmacological and clinical potencies. However, in a strict sense, the affinity determined *in vitro* would not always reflect the *in vivo* central nervous system (CNS) activity, because most benzodiazepines administered systemically are known to be metabolized in the body into a variety of pharmacologically active or inert metabolites,[5] and some of them may not pass through the blood-brain barrier.

By using a radioreceptorassay (RRA) for benzodiazepines, we previously demonstrated that peptido-aminobenzophenones, which have only low affinity for benzodiazepine receptors, are converted *in vivo* into active metabolites having high affinity for the receptors, and that the brain levels of active metabolites are high enough to explain the CNS activities of the parent compounds.[3,6] These results led us to consider that the brain levels of receptor reactive substance(s), which are hereafter designated as diazepam activity, may be a better measure than *in vitro* binding data of the pharmacological and clinical potencies of benzodiazepines and other compounds having similar activities.

In the present work, we administered to rats six benzodiazepines which are currently in clinical use, and the diazepam activities in seven brain regions were examined in relation to various pharmacological and clinical parameters.

Experimental

Materials——All benzodiazepines used in this study were obtained from Dr. K. Hirai of this laboratory. ^{3}H-Diazepam (47 Ci/mmol) was obtained from the Radiochemical Centre, Amersham.

Determination of Diazepam Activity in the Brain——Male Wistar rats (body weight 200—220 g) received *i.p.* injections of benzodiazepines at a dose of 2 mg/kg of body weight. Thirty minutes later, the rats were killed by exposing their heads for 1.5 s to a focused microwave beam (2450 MHz, 4.5 kW). The brains were carefully removed and dissected on an ice-cooled glass plate according to the methods of Glowinski and Iversen into seven regions: cortex, cerebellum, striatum, hippocampus, midbrain, hypothalamus, and medulla oblongata plus pons.[7] Each region was homogenized in ten volumes of ethanol and centrifuged at $10000 \times g$ for 20 min, and then a portion of the supernatant was used for measurement of the diazepam activity.

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Diazepam activity was determined by RRA as described previously.[3] Briefly, a P_2-crude synaptosomal fraction prepared from whole forebrains of male Wistar rats was suspended in five times the original tissue weight of 50 mM Tris-HCl buffer (pH 7.4), and portions were stored at −70°C. The frozen suspension was thawed and diluted 1: 10 in the same buffer prior to use for RRA. In the binding assay, 500 μl of this suspension was incubated with 25 μl of ^{3}H-diazepam (final concentration of 2.0 nM) and 10 μl of a standard solution of diazepam or brain extract in an ice bath for 30 min. After filtration through a Whatman GF/C glass fiber filter and subsequent washing, the filter was counted for tritium. Specific binding was calculated by subtracting the nonspecific binding obtained in the presence of 3 μM diazepam. Diazepam activity in the brain extract was calculated from the standard curve prepared for diazepam. When the extract resulted in binding inhibition equal to that caused by 1 μg of diazepam we defined this as 1 diazepam unit.

Results and Discussion

Preliminary observations with several benzodiazepines revealed that maximum or nearly maximum diazepam activity in the brain was obtained 30 min after *i.p.* injection of 2 mg/kg of these drugs. Thus, the diazepam activities in the seven brain regions were compared at 30 min after drug administration (Table I). Differences in the activity were marked for the different drugs. Moreover, it is noticeable that medazepam, which has only low affinity for the receptors,[3] gave activities comparable to that obtained with flurazepam. On the other hand, although marked regional variation exists in the density of benzodiazepine receptors in the brain,[4,8] the differences observed among diazepam activities in the seven brain regions for each drug were small.

Table I. Diazepam Activity in Extracts of Various Brain Regions of Rats treated with Benzodiazepines (2 mg/kg) and Its Correlation with Clinical Doses

Benzodiazepine (*N*)	Cerebral cortex	Cerebellum	Striatum	Hippo-campus	Midbrain	Hypo-thalamus	Medulla-pons
Diazepam (6)	0.27±0.04 (100)	0.29±0.04 (108±5)	0.27±0.04 (101±4)	0.27±0.03 (105±12)	0.28±0.04 (106±7)	0.28±0.04 (103±5)	0.37±0.05 (138±3)
Nitrazepam (6)	0.40±0.03 (100)	0.45±0.03 (112±8)	0.33±0.02 (82±3)	0.35±0.03 (89±10)	0.34±0.01 (86±5)	0.36±0.03 (91±7)	0.32±0.01 (81±5)
Flurazepam–HCl (5)	0.14±0.01 (100)	0.15±0.02 (106±17)	0.11±0.01 (80±8)	0.16±0.01 (119±15)	0.16±0.03 (94±9)	0.11±0.01 (78±6)	0.14±0.02 (98±15)
Estazolam (5)	0.77±0.02 (100)	0.87±0.06 (114±10)	0.71±0.02 (93±3)	0.72±0.03 (95±5)	0.75±0.03 (98±2)	0.69±0.03 (90±4)	0.78±0.03 (103±5)
Medazepam (5)	0.13±0.02 (100)	0.12±0.01 (101±10)	0.13±0.02 (101±3)	0.11±0.01 (96±10)	0.11±0.01 (94±9)	0.12±0.01 (98±11)	0.13±0.01 (110±10)
Lorazepam (5)	2.09±0.43 (100)	2.16±0.33 (123±26)	1.81±0.40 (82±7)	1.56±0.26 (82±9)	1.60±0.34 (79±8)	1.61±0.38 (82±12)	1.81±0.40 (93±13)
Correlation with average therapeutic dose	$r=0.978$ $p<0.001$	$r=0.984$ $p<0.001$	$r=0.980$ $p<0.001$	$r=0.984$ $p<0.001$	$r=0.985$ $p<0.001$	$r=0.987$ $p<0.001$	$r=0.940$ $p<0.01$

Each value is the mean±S.E. of 5 or 6 rats. The values represent diazepam units/g tissue. The values in parentheses represent percent of that in the cerebral cortex.

Next, we investigated the correlations of pharmacological and clinical potencies with diazepam activity and *in vitro* binding affinity. Figure 1 shows the results obtained with the cerebral cortex, where the highest density of benzodiazepine receptors is found.[4,8] Although therapeutic doses of benzodiazepines showed no significant correlation with their *in vitro* binding affinities, they showed a close correlation with the diazepam activities in the cortex. Moreover, the slope of the regression line obtained for the doses *versus* diazepam activities was closer to a 1: 1 relationship than that for doses *versus in vitro* binding data. As shown in Table I, similarly good correlations were also found for the other brain regions.

A similar tendency appeared in correlations with some pharmacological effects on experimental animals, *e.g.*, the intensifying effect on chlorprothixene hypnosis, taming of mice, and

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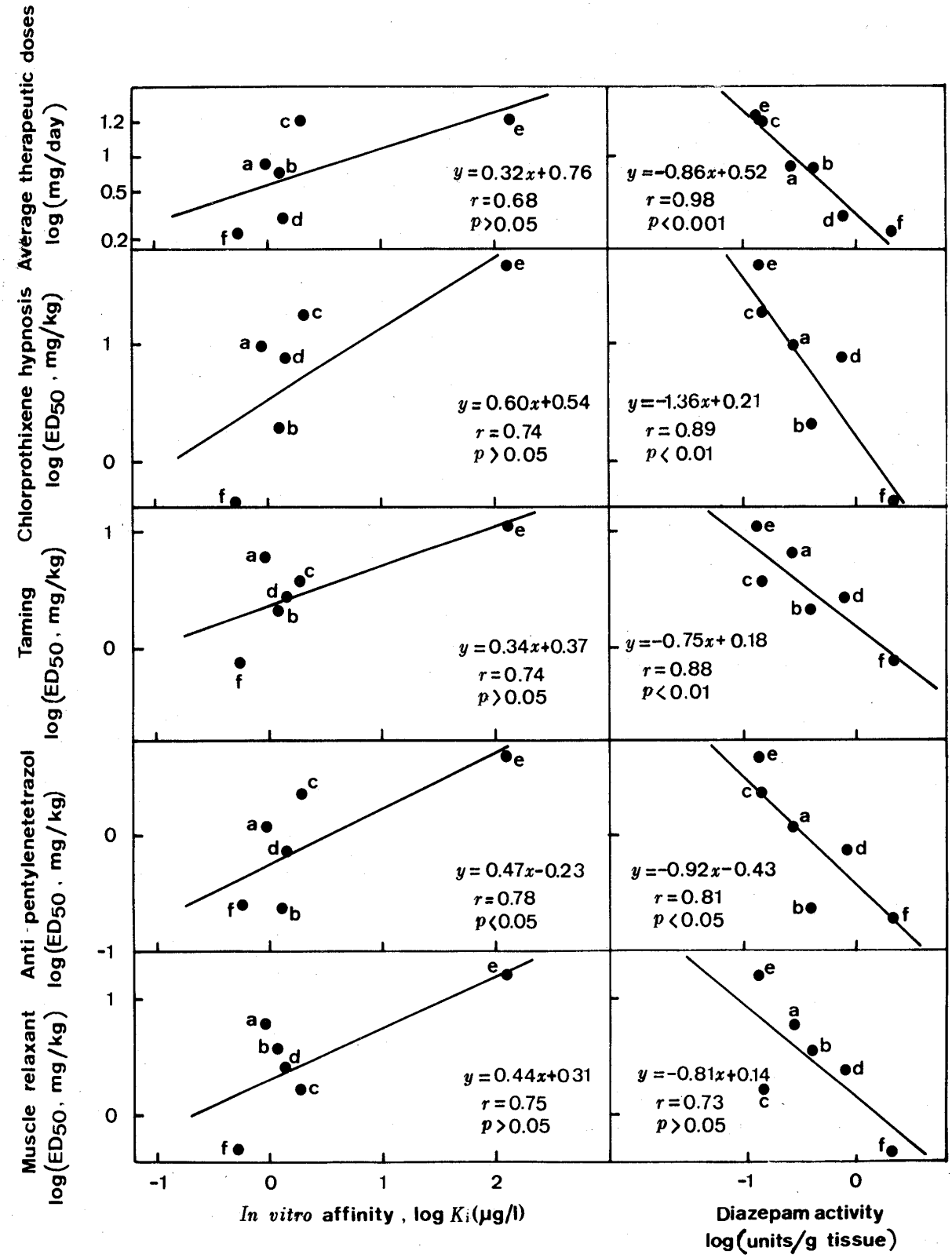

Fig. 1. Correlations of Clinical and Pharmcological Potencies of Benzodiazepines with *in Vitro* K_i Values in the Inhibition of ^{3}H-Diazepam Binding by Benzodiazepines (Left) and with Diazepam Activities in the Cerebral Cortex of Rats treated with Benzodiazepines (2 mg/kg) (Right)

Pharmacological data and *in vitro* K_i values are from reference 3. a, diazepam; b, nitrazepam; c, flurazepam; d, estazolam; e, medazepam; f, lorazepam.

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anti-pentylenetetrazol convulsion activity in mice. However, the cat muscle relaxant effect showed no correlation with the brain levels of diazepam activity or with the *in vitro* binding data.

Benzodiazepines which are currently in clinical use possess more or less varied pharmacological spectra.[9,10] Thus, nitrazepam, which is effective as a sleep inducer, is especially potent pharmacologically as an intensifier of chlorptothixene hypnosis.[10] An experiment, the results of which are presented in Table I, was performed to observe whether or not different regional distributions in the brain of diazepam activity might explain the different pharmacological spectra. As is clear from the data, this is not the case.

Besides *in vitro* receptor binding techniques, *ex vivo* and *in vivo* binding techniques have also been developed using major and minor tranquilizers.[11–14] These methods, which measure the receptor occupancy after systemic administration of drugs, are of course useful, but the procedures are fairly complicated and the results are sometimes affected by metabolism of the labeled drugs.[11,12] Furthermore, the possibilities that dissociation of bound drugs from the receptors and the rebinding of free drugs to the receptors occur during the experimental manipulation cannot be completely excluded.

The present method, in which a constant amount of benzodiazepines is administered systemically to rats and the brain levels of receptor reactive substances are compared, does not give information on receptor occupancy. However, the method is simple and yet can still give fairly precise information on the pharmacological and clinical potencies of the administered drugs (Fig. 1, Table I).

Recently, Campbell *et al.* investigated the relationship between a behavioral effect and the level of neuroleptic activity determined by RRA after administration of haloperidol.[15] They found that the activity determined 1 h after drug administration showed a very close correlation ($r=0.99$) with cataleptic activity, although this relationship did not hold for the entire experimental period (0 to 12 h).

In conclusion, determination of brain levels of receptor reactive substance(s) by RRA seems to be a useful means for evaluating the *in vivo* activities of benzodiazepines and related drugs, as well as neuroleptics.

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