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# Interaction of Peptido-aminobenzophenones with Benzodiazepine Receptors

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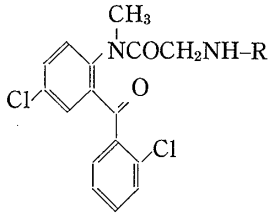
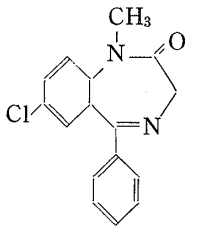
Peptido-aminobenzophenones exhibit potent central nervous system activities similar to those of benzodiazepines in experimental animals, despite their lack of the diazepine skeleton. These compounds have low affinity for benzodiazepine receptors, but are converted into compound(s) having high affinity for the receptors by incubation with crude synaptosomes from rat brains or rat liver homogenates. This conversion seems to involve an enzymatic process, because it is temperature-dependent and stereospecific. The results suggest that peptido-aminobenzophenones show their pharmacological activities through conversion into benzodiazepine(s) *in vivo*.

**Keywords**——peptido-aminobenzophenone; benzodiazepine; diazepine ring; benzodiazepine receptor; $^3$H-diazepam binding

Several peptido-aminobenzophenones were synthesized as ring-opened derivatives of 1,4-benzodiazepines (Fig. 1).[2] These compounds exhibited potent central nervous system (CNS) activities similar to that of diazepam in mice on oral administration, despite earlier indications that the specific CNS effect of the 1,4-benzodiazepines is a function of the diazepine ring.[3]




diazepam

**1a** : R=Gly
**1b** : R=L-Phe
**1c** : R=D-Phe
**1d** : R=$COCH(CH_3)_2$
**1e** : R=$COCH_2CH_2CH_3$
**1f** : R=$COCH_2NHCH_3$

Fig. 1. Chemical Structure of Peptido-aminobenzophenones and Diazepam

Recently, high-affinity specific binding sites for benzodiazepines, so-called "benzodiazepine receptors," have been identified in mammalian brains,[4,5] and substantial evidence exists that these sites represent the pharmacological sites of action of the drugs.[6–8] In this study, we investigated the interaction of peptido-aminobenzophenones with benzodiazepine receptors. Although peptido-aminobenzophenones themselves had low affinity for the receptors, their incubation with crude synaptosomes from rat brains or rat liver homogenates resulted in the formation of compound(s) having high affinity for the receptors.

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2) K. Hirai, T. Ishiba, H. Sugimoto, K. Sasakura, T. Fujishita, Y. Tsukinoki, and K. Hirose, *Chem. Pharm. Bull.*, **26**, 1947 (1978).
3) H. Oelschläger, W.Z. Behrendt, and H. Hoffmann, *Arzneim. Forsch.*, **23**, 802 (1973).

## Experimental

**Preparation of Crude Synaptosomal Fraction and Procedure for $^3$H-Diazepam Binding**——Specific $^3$H-diazepam binding and displacement by drugs were determined according to the method of Braestrup and Squires[7] with minor modifications. Whole forebrains of male Wistar rats (180—200 g) were homogenized in 20 volumes of 0.32 M sucrose, centrifuged at $1000\times g$ for 10 min and recentrifuged at $30000\times g$ for 10 min to give a crude $P_2$-synaptosomal fraction. The $P_2$ pellets were resuspended in 50 times the original tissue weight of 50 mM Tris-HCl (pH 7.4) and used directly.

In the binding experiments, 500 μl of this $P_2$-suspension was incubated with 25 μl of $^3$H-diazepam (to give a final concentration of 2.0 nM) and 10 μl of nonradioactive drug at various concentrations in an ice bath for 30 min. The incubation was terminated by addition of 8 ml of ice-cold buffer and immediate filtering through Whatman GF/C glass fiber filters. The filters were washed twice with 4 ml each of the buffer, placed in a counting vial, shaken thoroughly with 1 ml of $H_2O$ and 10 ml of scintillation cocktail (1 vol. of Triton X-100, 2 vol. of toluene, 0.5 weight % PPO and 0.05 weight % bis-MSB), and counted for tritium. Specific binding was calculated by subtracting the nonspecific binding obtained in the presence of 3 μM diazepam. Routinely, the assay was performed in duplicate or triplicate.

**Preincubation of Peptido-aminobenzophenones with Crude Synaptosomes**——Preincubation was performed by incubating the drugs in the complete assay mixture at 37° for the indicated times, and after changing the incubation temperature to 0°, the incubation was continued for an additional 30 min to determine the binding. This procedure was simple and gave essentially the same results as experiments where the drug was incubated first with crude synaptosomes at 37° then $^3$H-diazepam was added at the time of the temperature shift.

**Activation of Peptido-aminobenzophenones by Rat Liver Homogenates**——Livers of male Wistar rats were excised and homogenized in four volumes of iced 0.25 M sucrose in 50 mM Tris-HCl (pH 7.4) and the homogenate was diluted with an equal volume of Tris buffer. Next, 0.6 ml of the liver homogenates was incubated with $1.78\times10^{-6}$ M peptido-aminobenzophenone at 37° for various times, and the incubation was terminated by adding 1.4 ml of iced ethanol. Samples were centrifuged for 10 min at $10000\times g$ at 0°, then 10 μl of the supernatant (to give a final concentration of $10^{-8}$ M of the original compound) was assayed.

**Materials**——$^3$H-Diazepam (42—47 Ci/mmol) was obtained from The Radiochemical Centre, Amersham. All peptido-aminobenzophenones and benzodiazepines used in this study were prepared in this laboratory.

## Results and Discussion

When determined at 0°, specific binding of $^3$H-diazepam to crude synaptosomes could be saturated with increasing concentrations of the ligand. From Scatchard analysis, $K_d$ and $B_{max}$ were calculated as $3.0\pm0.2$ nM and $0.44\pm0.07$ pmol/mg protein, respectively. These values roughly coincide with those described previously.[4-7]

Table I compares the $K_i$ values of peptido-aminobenzophenones and diazepam for benzodiazepine receptors and the $ED_{50}$ values in pharmacological tests. As indicated in the following paper,[9] various benzodiazepines, most of which are currently in clinical use, showed high affinity for the receptors, and their $K_i$ values correlated well with $ED_{50}$ values in pharmacological tests. In sharp contrast, peptide-aminobenzophenones showed affinities two to three orders of magnitude lower than that of diazepam, although some of them showed CNS activities comparable to or higher than that of diazepam. These results raise two possibilities: one is that peptido-aminobenzophenones act through a different mechanism from that of benzodiazepines and the other is that they are converted into active benzodiazepines *in vivo*.

To study these possibilities, we performed preincubation experiments at 37°. Fig. 2 shows that the incubation of assay mixtures without added drug caused about a 20% increase in $^3$H-diazepam binding. On the other hand, the presence of peptido-aminobenzophenones

4) R.F. Squires and C. Braestrup, *Nature*, (London) **266**, 732 (1977).
5) H. Möhler and T. Okada, *Science*, **198**, 849 (1977).
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Table I. Affinity for "BenzodiazepineReceptors" and Pharmacological Activities of Peptido-aminobenzophenones and Diazepam

| Compound | $K_i$, nM[a] | | $ED_{50}$, mg/kg[c] | |
|---|---|---|---|---|
| | Without preincubation | After preincubation[b] | Anti-pentylene-tetrazole | Rotated Performance test |
| **1a** | 925 | 3.07 | 0.58 | 15.4 |
| **1b** | 128 | 1.09 | 0.92 | — |
| **1c** | 718 | 104 | 4.8 | 78.0 |
| **1d** | 394 | 497 | 6.0 | >100 |
| **1e** | 227 | 227 | 0.98 | 50—100 |
| **1f** | 1075 | 844 | 1.55 | — |
| Diazepam | 3.23 | — | 1.19 | 13.4 |

*a*) The $K_i$ values were calculated using the following equation:
$K_i = IC_{50}/(1 - C/K_d)$
where $IC_{50}$=the concentration causing 50% inhibition of specific $^3$H-diazepam binding, $C$=$^3$H-diazepam concentration (2 nM) and $K_d$=dissociation constant (3.2 nM).
*b*) The complete assay mixtures were preincubated for 15 min at 37°, after which $^3$H-diazepam binding was determined.
*c*) The compounds were administrated orally to mice for pharmacological tests according to previously described procedures.[10-12]

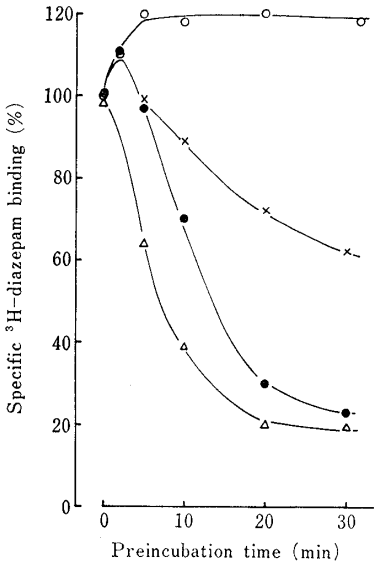


Fig. 2. Effect of Preincubation on the Binding of Peptidoaminobenzophenones to "Benzodiazepine Receptors"

Complete assay mixtures containing 530 μg, as protein, of brain crude synaptosomes per tube were incubated at 37° for the indicated times in the absence or presence of $1 \times 10^{-8}$ M peptido-aminobenzophenones. The tubes were transferred to an ice bath and incubated for a further 30 min, then the specifically bound radioactivity was determined as described in the Experimental section.
—○—, no addition; —●—, **1a**; —△—, **1b**; —×—, **1c**.

resulted in a decrease of specific $^3$H-diazepam binding in a time-dependent manner. This indicates that during the incubation, peptido-aminobenzophenones are converted into compound(s) having higher affinity for the receptors. In another experiment, we confirmed that this activation hardly occurred at 0° (results not shown); thus the process seems to be temperature-dependent.

Table I also shows $K_i$ values of peptido-aminobenzophenones determined after preincubation with crude synaptosomes at 37° for 15 min. The value for **1a** (Fig. 1), in which R is glycine, decreased from 925 to 3.07 nM. This activated $K_i$ value is comparable

Table II. Activation of Peptido-aminobenzophenones by Incubation with Liver Homogenates

| Compound | $IT_{50}$, min[a] |
|---|---|
| **1a** | 1.8 |
| **1b** | <0.5 |
| **1c** | >84.0 |
| **1d** | 22.0 |
| **1e** | 7.0 |
| **1f** | 42.0 |

*a*) $IT_{50}$=incubation time required to yield metabolite(s) able to inhibit specific$^3$H-diazepam binding by 50%.

10) L.O. Randall, W. Schallek, L.H. Sternbach, and R.Y. Ning in "Psychopharmacological Agents," Vol. III, ed. by M. Gordon, Academic Press, New York, N.Y., 1974, p. 175.
11) G. Garattini, E. Mussin, and L.O. Randal (ed.), "The Benzodiazepines," Raven Press, New York, N.Y., 1973.
12) M. Ogata, H. Matsumoto, and K. Hirose, *J. Med. Chem.*, **20**, 776 (1977).

to that of diazepam and is consistent with pharmacological data. Note the difference between the susceptibility to activation of **1b**, in which R is L-Phe, and of **1c**, where R is D-Phe.

Three compounds in which R was not an amino acid (**1d**, **1e**, and **1f**) were not activated by incubation with synaptosomes, although they had some CNS activities. However, they were activated by incubation with rat liver homogenates. Table II, in which the susceptibility of compounds to activation is expressed as the time required for activation sufficient to result in a 50% decrease in $^{3}$H-diazepam binding, indicates that here again **1a** and **1b** are the most susceptible to activation, and that **1c** is more resistant than **1b**. However, the order of susceptibility of peptido-aminobenzophenones to activation differs slightly from that obtained with synaptosomes.

The results presented in the present work imply that the activation is due to the formation of benzodiazepine(s), and indicate that an enzymatic process is involved in the activation process. In a subsequent study,[9] we found that such activation does occur *in vivo*, although the overall sequence of the activation reaction is more complicated than was anticipated on the basis of the present experiments.