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J. Pharm. Pharmacol. 1983, 35: 194–195
Communicated September 27, 1982

0022–3573/83/030194–02 $02.50/0

Interaction of *N*-alkylaminobenzophenones with benzodiazepine receptors

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Triazolobenzodiazepines like alprazolam and triazolam have been found to be potent anxiolytics and hypnotics in man (Fabre & McLendon 1979; Chatwin & John 1976). Correspondingly, both alprazolam and triazolam have been reported to be very potent in inhibiting the binding of [^{3}H]flunitrazepam ([^{3}H]FNZ) to rat crude rat brain membrane preparations. The result of in-vitro receptor binding assays indicates that these triazolobenzodiazepines may mediate their pharmacological activity through benzodiazepine receptors (Sethy & Harris 1982). *N*-Alkylaminobenzophenones have been reported to have anxiolytic activity in animals (Gall et al 1976). The activity of *N*-alkylaminobenzophenones on benzodiazepine receptors is not known. We have undertaken a study to determine the activity of these compounds on benzodiazepine receptors by using [^{3}H]FNZ binding to the mouse crude brain membrane preparation both in vitro and ex vivo preparations. The results of in-vitro [^{3}H]FNZ binding assays were compared with ex-vivo [^{3}H]FNZ binding assays and with in-vivo ED50's of these compounds required to protect the mice against leptazol (metrazol)- and nicotine-induced seizures.

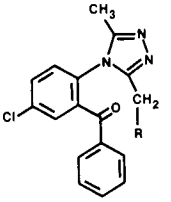
Methods

In-vitro [^{3}H]FNZ binding to crude mouse brain membrane preparation for the determination of inhibition constants (K_i) of alprazolam and *N*-alkylaminobenzophenones was carried out by the method previously described (Sethy & Harris 1982). Ex-vivo [^{3}H]FNZ binding assay was based on the guideline described by Nakajima et al (1981).

Male albino CF-1 mice bred at The Upjohn Company were used for ex-vivo [^{3}H]FNZ binding assays. Animals were kept under constant diurnal lighting and temperature conditions before use and were killed at approximately the same time of day.

Alprazolam was dissolved in 2% ethanol. Compounds I, II and III were dissolved in distilled water. All drugs were administered by the intravenous route. Control mice received equal volumes (1 ml/100 g) of the vehicle. Alprazolam was injected at a dose of 3 μmol kg^{-1}. Compounds I, II and III were administered at a dose of 10 μmol kg^{-1}. Animals were killed at 1, 3, 10, 30 and 60 min after administration of drug. Whole brain, minus cerebellum, was quickly removed and homogenized in 50 volumes of cold (4 °C) 50 mM Tris-HCl buffer, pH 7·4, using a Brinkman polytron PCU-2-110 homogenizer for 30 s at setting No. 6. [^{3}H]FNZ binding was measured by incubating 1·0 ml aliquots of the homogenate with 0·1 ml [^{3}H]FNZ (spec. act. 84·8 Ci mmol^{-1}, NEN, Boston, Massachusetts), to give a final concentration of 0·7 nM, 0·1 ml of distilled water or flurazepam (10 μm) and 0·8 ml of 50 mM Tris-HCl buffer, pH 7·4, to give a final volume of 2 ml. The mixture was incubated for 30 min at 25 °C and then filtered under vacuum through a Whatman GF/B filter.

Table 1. Effect of alprazolam and n-alkylaminobenzophenones on in-vitro [^{3}H]flunitrazepam binding to crude mouse brain membrane preparation and its correlation with in-vivo pharmacological tests.



Drug	R	Ki (nM)	ED50: μmol kg^{-1} i.p. Leptazol	Nicotine
Alprazolam		3·4	0·42	0·18
Compound I*		>100 000	3·2	0·35
Compound II*		>100 000	3·1	1·4
Compound III*		>100 000	1·3	0·5

* In-vivo test data were obtained from published report of Gall et al (1976) J. Med. Chem. 19: 1057.

* Correspondence.

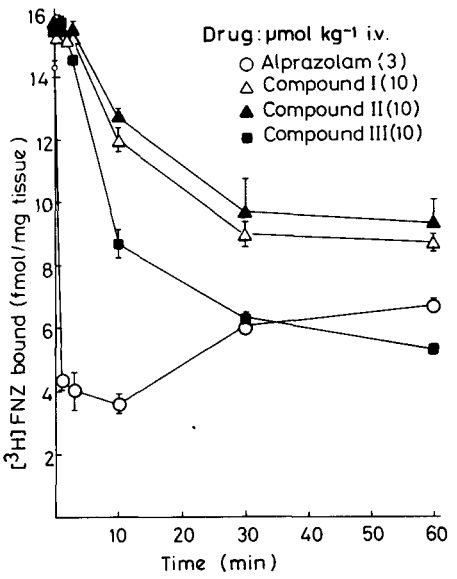


FIG. 1. [³H]FNZ binding at various time intervals after i.v. administration of alprazolam and *N*-alkylaminobenzophenones to mice. Each point is the mean ± s.e. of 4 determinations.

The incubation tube was rinsed with 5 ml of ice-cold buffer, and this rinse was applied to the filter. The filter was finally washed three times with 5 ml amounts of buffer. Finally the filter paper was placed in a scintillation vial containing 15 ml of Amersham Searle ACS cocktail and the radioactivity was counted by liquid scintillation spectrometry.

Specific binding was defined as total binding minus binding in the presence of 10 μM flurazepam. Specific binding represented over 95% of total binding. The results are expressed as: [³H]FNZ bound: fmol mg^{-1} tissue. It is assumed in this study that ex-vivo binding of [³H]FNZ is inversely proportional to the concentration of active drug in the brain.

Results

The relative potency of alprazolam and compounds I, II and III is shown in Table 1. the K_i of alprazolam was 3·4 nM. This value is identical to that reported previously (Sethy & Harris 1982). This compound was also found to be very potent in protecting mice against leptazol- and nicotine-induced seizures. Compounds I, II and III were practically inactive in inhibiting the binding of [³H]FNZ to the crude membrane preparation in-vitro. However, these compounds were found to be very potent in protecting mice against leptazol- and nicotine-induced seizures (Table 1). These results suggest that compounds I, II and III may be activated in-vivo. This has been confirmed by ex-vivo [³H]FNZ binding (Fig. 1).

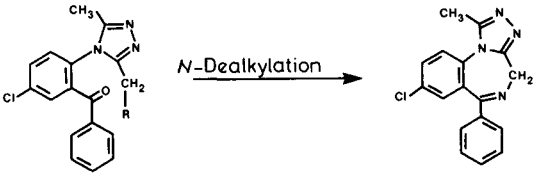


FIG. 2. *N*-Dealkylation of *N*-alkylaminobenzophenones and formation of triazolobenzodiazepines.

Alprazolam (3 μmol kg^{-1} i.v.) was found to be very potent in inhibiting the binding of [³H]FNZ. Peak action of alprazolam was achieved within 1 min after the i.v. injection. Fifty-three percent inhibition of [³H]FNZ binding was observed at 60 min, the last time investigated. The peak effect of compounds I, II and III was observed at 30 min after i.v. administration. At 60 min, compounds I, II and III inhibited [³H]FNZ binding by 43, 41 and 65% respectively (Fig. 1).

The high binding affinities of compounds I, II and III in ex-vivo [³H]FNZ binding assays indicate that *N*-alkylaminobenzophenones may be activated in-vivo by *N*-dealkylation into triazolobenzodiazepines as shown in Fig. 2 and reported by Lahti & Gall (1976). The use of in-vitro and ex-vivo binding assays together may serve as a quick method for determining the in-vivo activation of drugs.

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