J. Pharm. Pharmacol. 1986, 38: 631–632
Communicated January 27, 1986

The effect of proadifen on the metabolism of adinazolam

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Binding affinities of adinazolam and its metabolite mono-*N*-demethyladinazolam, U-42352, to the brain tissue are not altered by the presence of proadifen (SKF-525A) in [^{3}H]flunitrazepam ([^{3}H]FNZ) binding assays in-vitro. Pretreatment of mice with proadifen significantly blocked the ability of intravenously administered adinazolam to inhibit [^{3}H]FNZ binding in the studies ex-vivo. The binding profile of [^{3}H]FNZ to the brain tissue was not significantly different when animals were treated with U-42352 or proadifen with U-42352. These results suggest that proadifen may block the conversion of adinazolam to its active metabolite U-42352.

Adinazolam has both antidepressant and anxiolytic activities in animals (Hester et al 1980; Lahti et al 1983). Also, in man this compound has been found to be an antidepressant with low sedative properties (Pyke et al 1983). It was reported previously that the mono-methyl aminomethyl analogue of adinazolam (U-42352) is about 25 times more potent than adinazolam in the in-vitro [^{3}H]flunitrazepam ([^{3}H]FNZ) binding assay. However, in the in-vivo tests such as protection against pentetrazol (leptazol, metrazol), or nicotine-induced seizures, and against hypoxic stress, adinazolam and U-42352 seem to be equipotent (Sethy et al 1984). The metabolic profile of adinazolam in rodents has been determined at The Upjohn Company. In mice, one of the metabolites of adinazolam is mono-*N*-demethyladinazolam (U-42352, E. G. Daniels, personal communication). Similarly, high plasma concentrations of U-42352 have been reported at various time intervals in humans after oral administration of adinazolam (Peng 1984). These observations suggest that adinazolam may lead to the formation of a more active compound in-vivo.

Proadifen (SKF-525A) inhibits the in-vivo metabolism of several drugs by inhibiting liver microsomal enzymes which catalyse reactions such as *N*-demethylation, side chain oxidation, deamination, and hydroxylation (Conney & Burns 1962). It is not known if it would block the in-vivo metabolism of adinazolam in mice. Furthermore, the effect of proadifen on binding affinities of adinazolam and its metabolite in the [^{3}H]FNZ binding assay has not been investigated. Therefore, we have undertaken a study to determine the effect of proadifen on adinazolam and U-42352 in in-vitro and ex-vivo [^{3}H]FNZ binding assays.

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Methods

[^{3}H]FNZ binding to brain membrane preparations in-vitro for the determination of the inhibition constant (K_i) of adinazolam and U-42352 was carried out by the method described previously (Sethy & Harris 1982). Proadifen at a concentration of 10^{-5} M was used for studies involving the presence of a metabolic inhibitor. Each concentration of adinazolam (10^{-5} to 10^{-9} M) and U-42352 (10^{-7} to 10^{-11} M) was investigated in triplicate during the binding assay. Each drug was studied in two independent experiments.

Male CF-1 mice (20–25 g) bred by The Upjohn Company were used for ex-vivo binding studies. Animals were kept under constant temperature conditions and diurnal lighting before use, and all were killed at aproximately the same time of day.

The methanesulphonate salt of both adinazolam and U-42352, or proadifen hydrochloride were dissolved in 0·9% sodium chloride. Proadifen at a dose of 100 μmol kg^{-1} was injected intraperitoneally. Control mice received an equal volume (10 ml kg^{-1}) of vehicle. Sixty minutes after the injection of proadifen, animals received either adinazolam (10 μmol kg^{-1}), U-42352 (10 μmol kg^{-1}), or vehicle intravenously. Animals were killed 1, 3, 10 and 30 min after the second injection. Whole brains minus cerebellum were dissected and placed on dry ice for subsequent measurement of ex-vivo binding of [^{3}H]FNZ by the method previously reported (Sethy et al 1983a).

Brains were thawed and then 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 6. [^{3}H]FNZ binding was measured by incubating 1·0 ml aliquots of the homogenate with 0·1 ml of [^{3}H]FNZ (sp. act. 82·8 Ci mmol^{-1}, NEN, Boston) to give a final concentration of 0·68 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 2·0 ml. The mixture was incubated for 30 min at 25 °C and then filtered under vacuum through a Whatman GF/B filter. The incubation tube was rinsed with 5·0 ml ice cold buffer and this rinse was applied to the filter. The filter was then washed three times with 5·0 ml aliquots of buffer. Finally, the filter was placed in a scintillation vial to which 15 ml of ACS (Amersham) cocktail was added. The vials were shaken for 30 min on a mechanical shaker (Eberbach). The radioactivity was counted by a liquid scintillation spectrometer.

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 [^{3}H]FNZ bound: fmol (mg tissue)$^{-1}$. It was assumed in this study that ex-vivo binding of [^{3}H]FNZ was inversely proportional to the concentration of parent compound and its active metabolite in the brain.

Results and discussion

The relative potency of adinazolam, U-42352, and diazepam for inhibiting [^{3}H]FNZ binding in-vitro is shown in Table 1. Adinazolam was about 19 times less potent than U-42352 in inhibiting [^{3}H]FNZ in-vitro. This finding is consistent with the observation reported previously by Sethy et al (1984). Binding affinities of adinazolam and U-42352 were not significantly altered by the presence of proadifen, suggesting that these compounds are not metabolized by brain membrane preparations when incubated in-vitro, and that proadifen does not interact directly with the [^{3}H]FNZ.

Table 1. Effect of proadifen (10 μM) on K_i of adinazolam and U-42352 in [^{3}H]FNZ binding assays.

	K_i (nM)	
Compound	Without proadifen	With proadifen
Adinazolam	203·5	207·7
U-42352	11·3	10·3
Diazepam	7·7	7·6

The peak effect of adinazolam (10 μmol kg^{-1}) was observed between 10 to 30 min after i.v. administration of drug. Similar results have been reported with adinazolam in the past (Sethy et al 1983b). The delayed achievement of peak effect after i.v. administration of adinazolam suggests that the drug may be metabolized into an active compound in-vivo. This suggestion is confirmed in studies involving administration of adinazolam 60 min after intraperitoneal injection of proadifen. Pretreatment with proadifen significantly ($P < 0.001$) increased the binding of [^{3}H]FNZ. These results indicate that proadifen may have blocked the conversion of adinazolam to its active metabolite, and thus decreased the concentration of this metabolite in brain tissue (Fig. 1).

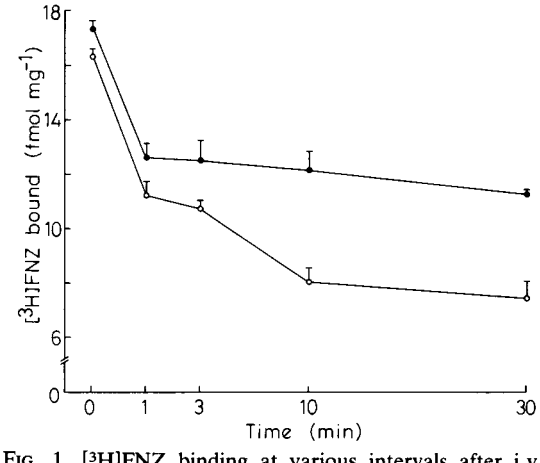

FIG. 1. [^{3}H]FNZ binding at various intervals after i.v. administration of adinazolam (10 μmol kg^{-1}) (○) or proadifen (100 μmol kg^{-1}) + adinazolam (●) to mice. Each point is the mean ± s.e. of 4 or 5 determinations.

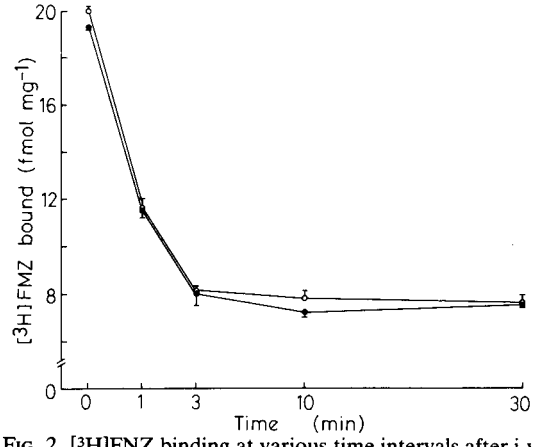

FIG. 2. [^{3}H]FNZ binding at various time intervals after i.v. administration of U-42352 (10 μmol kg^{-1}) (○) or proadifen (100 μmol kg^{-1}) + U-42352 (●) to mice. Each point is the mean ± s.e. of 4 or 5 determinations.

U-42352 is an active metabolite of adinazolam. Intravenous administration of 10 μmol kg^{-1} significantly inhibited ex-vivo binding of [^{3}H]FNZ to brain tissues. The peak effect was obtained at 3 min, and this effect persisted at 10 and 30 min. Pretreatment with proadifen did not alter the effect of U-42352 in ex-vivo binding of [^{3}H]FNZ. These results suggest that either proadifen does not inhibit the metabolism of U-42352, or U-42352 is not significantly metabolized during the 30 min period of investigation, or its metabolism leads to the formation of compounds which have potencies similar to U-42352.

Thus, U-42352 is the likely candidate for benzodiazepine-like activity of adinazolam, both in animals and man.

REFERENCES

Conney, A. H., Burns, J. J. (1962) Adv. in Pharmacol. 1: 31–58

Hester, J. B., Rudzik, A. D., VonVoigtlander, P. F. (1980) J. Med. Chem. 23: 392–402

Lahti, R. A., Sethy, V. H., Barsuhn, C., Hester, J. B. (1983) Neuropharmacology 22: 1277–1282

Peng, G. W. (1984) J. Pharm. Sci. 73: 1173–1175

Pyke, R. E., Cohn, J. B., Feighner, J. P., Smith, W. T. (1983) Psychopharm. Bull. 19: 96–98

Sethy, V. H., Harris, D. W. (1982) J. Pharm. Pharmacol. 34: 115–116

Sethy, V. H., Daenzer, C. L., Russell, R. R. (1983a) Ibid. 35: 194–195

Sethy, V. H., Russell, R. R., Daenzer, C. L. (1983b) Ibid. 35: 524–526

Sethy, V. H., Collins, R. J., Daniels, E. G. (1984) Ibid. 36: 546–548