Brain concentrations of clobazam and *N*-desmethylclobazam and antileptazol activity

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Clobazam, a 1,5-benzodiazepine (Rossi et al 1969), is extremely active in the mouse against leptazol (metrazol) induced convulsions (Barzaghi et al 1973).

In a previous communication we reported that this pharmacological effect was weaker and short-lasting in the rat than in the mouse although brain concentrations of clobazam and its brain half-life were similar in the two species (Caccia et al 1980). However, *N*-desmethylclobazam, the main metabolite of clobazam in several animal species (Voltz et al 1979), was present in mouse brain at higher concentrations while only traces were found in rat brain (Caccia et al 1980). *N*-Desmethylclobazam has antileptazol activity in the mouse, though at higher doses than clobazam (Fielding & Hoffman 1979); in addition, its half-life is longer than the parent compound (Rupp et al 1979) suggesting that the antileptazol effect may last longer in mice than in rats because of the different brain accumulation of this metabolite among other possibilities.

This supposition was investigated in the present experiments by comparing brain concentrations of *N*-desmethylclobazam after administration of clobazam or *N*-desmethylclobazam at doses effective against leptazol-convulsions.

Male CD_1 albino mice, about 25 g, and male CD-COBS rats about 250 g, (Charles River, Italy) were used. Clobazam and *N*-desmethylclobazam were suspended in 0·5% carboxymethylcellulose and were injected intraperitoneally at doses corresponding to their antileptazol ED50, i.e. the dose (mg kg^{-1} i.p.) protecting 50% of the animals, measured at various times, from the tonic convulsions elicited by leptazol (120 mg kg^{-1}, i.p.). Each ED50 was calculated, in separated experiments, on at least 5 dose levels with 10 animals for each dose.

Brain concentrations of the two compounds were measured gas-chromatographically according to Caccia et al (1979) in different groups of animals treated with an ED50 of clobazam or *N*-desmethylclobazam.

Table 1 shows that in the rat the active brain concentrations of clobazam are much higher than those required to produce the same effect in mice. Clobazam brain concentrations of 0·3–0·4 $\mu g\ g^{-1}$ were necessary in the rat to ensure significant anticonvulsant protection, whereas in the mouse brain lower concentrations were present at 30 min and the drug was undetectable ($<0{\cdot}01$ $\mu g\ g^{-1}$) 90 and 180 min after administration. However, at the same intervals *N*-desmethylclobazam was found at high concentrations in the mouse brain though it was practically undetectable in rat brain.

Table 1. Brain concentrations of clobazam and *N*-desmethylclobazam in mice and rats after administration of clobazam at the ED50 of leptazol.

Species	Time*	ED50 (95% confidence limits) mg kg^{-1} i.p.	Brain concentrations $\mu g\ g^{-1}$ (s.d.)** Clobazam	*N*-Desmethylclobazam
Mouse	30	1·37 (1·59–1·19)	0·11 ± 0·05	0·49 (0·11)
	90	2·00 (2·32–1·68)	<0·01	0·78 (0·15)
	180	4·93 (5·60–4·33)	<0·01	1·18 (0·29)
Rat	30	10·28 (12·30–8·59)	0·44 ± 0·09	0·04 (0·01)
	90	32·73 (38·18–28·06)	0·36 ± 0·09	<0·02
	180	>100	—	—

* Minutes between clobazam pretreatment and leptazol (120 mg kg^{-1} i.p.).
** Each value is the mean of 8 animals.

Table 2. Brain concentrations of *N*-desmethylclobazam in mice after administration at the ED50 of leptazol.

Time*	ED50 (95% confidence limits mg kg^{-1} i.p.)	Brain concentrations** $\mu g\ g^{-1}$ (s.d.)
30	12·20 (15·40–9·70)	0·64 (0·13)
90	13·00 (17·38–11·37)	0·70 (0·14)
180	14·69 (18·75–11·51)	0·97 (0·30)

* Minutes between *N*-desmethylclobazam pretreatment and leptazol (120 mg kg^{-1} i.p.).
** Each value is the mean of 8 mice.

This suggests that in mice the *N*-desmethyl-metabolite might be responsible for the antileptazol effect at these intervals after clobazam administration. Table 2 confirms this, showing that when *N*-desmethylclobazam is injected intraperitoneally in mice at doses effective against leptazol convulsions it reaches brain concentrations comparable to those found at the same times after clobazam injection.

These results indicate that the antileptazol effect in mice is mediated by both clobazam and *N*-desmethylclobazam. Since the metabolite has a longer half-life than the parent compound, this probably explains why the antileptazol effect is longer in animal species in which *N*-desmethylclobazam is accumulated in significant amounts.

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MIF (Pro-Leu-Gly-NH_2): failure to affect oxotremorine effects in mice and rats as well as fluphenazine catalepsy or amphetamine hyperactivity in rats

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Oxotremorine antagonism by the tripeptide prolylleucylglycineamide, MIF, was first reported by Plotnikoff et al (1972). All evaluated symptoms of oxotremorine in mice, viz tremor, head twitch, ataxia, lachrymation, salivation and diarrhoea, were attenuated or abolished by MIF, 1–16 mg kg^{-1}. The peptide was active after s.c., i.p., i.v. or oral administration (Plotnikoff & Kastin 1974). These results were partially confirmed in studies where only the tremor-inhibiting effect was evaluated by means of electronic tremor measurement (Castensson et al 1974; Björkman & Sievertsson 1977). In contrast, Kruse (1977) reported a failure of MIF to antagonize oxotremorine tremor in mice. In view of the conflicting results reported for this effect of MIF and also for its influence on opiate tolerance and dependence (Mucha & Kalant 1979) we wish to report an observed lack of effect of MIF in oxotremorine-treated mice and rats as well as in fluphenazine-treated (cf. Voith 1977) or amphetamine-treated rats.

Oxotremorine-treated mice. Male NMRI mice (22–26 g) were given 0·9% NaCl (saline), 3, 10 or 30 mg kg^{-1} MIF s.c. and, 1 h later, oxotremorine sesquioxalate 0·4 mg kg^{-1} (as free base) *s.c.* (6 mice in each group). Their body temperature was recorded every 20 min by means of a thermistor probe and the symptoms enumerated by Plotnikoff et al 1974 were estimated by an experienced observer who was unaware of drug treatment. The mice were monitored for 4 h. The effects of oxotremorine were as earlier reported with the exception that no head twitches were observed. MIF, 10 mg kg^{-1}, seemed to cause a transient reduction of tremor. However, statistical significance was not achieved (Fig. 1). MIF had no appreciable effect on the other symptoms caused by oxotremorine, i.e. hypokinesia, rigidity, hypothermia, lachrymation, salivation and diarrhoea. MIF itself, 10 mg kg^{-1} s.c., had no effect on body temperature.

Oxotremorine-treated rats. Male Sprague-Dawley rats (230–250 g) in an analogous experiment were treated with saline, 0·3, 1, 3, 10 or 30 mg kg^{-1} MIF s.c. followed by oxotremorine (sesquioxalate) 0·4 mg kg^{-1} s.c. (3 rats in each group). The intense whole-body tremor caused by oxotremorine in mice was not observed in the rats, but the tremor was visible in the forepaws and the muscles of the jaws at 20 min, fading to palpable tremor of the jaws at 40–60 min with no response at 100 min. Strong head twitches were also observed. MIF had no effect either on these symptoms or on the oxotremorine-induced hypothermia, rigidity, lachrymation, salivation and diarrhoea.

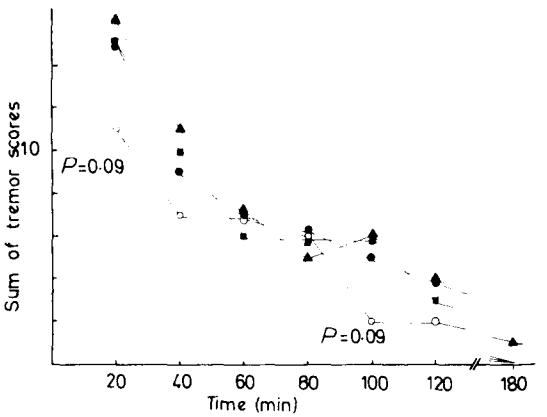

FIG. 1. Oxotremorine tremor, expressed as sums of the individual tremor scores in each group of 6 mice, at various doses of MIF and times after oxotremorine injection. The tremor was scored as: 3 = continuous tremor, 2 = intermittent tremor and 1 = tremor elicited by touch (Cho & Jenden 1964). The *P* values refer to MIF, 10 mg kg^{-1}, vs saline (Mann-Whitney U test; Siegel 1956).

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