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Species differences in clobazam metabolism and antileptazol effect

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The antileptazol effect of clobazam lasts longer in the mouse than in the rat. After intraperitoneal injection of clobazam (10 mg kg^{-1}) plasma and brain concentrations of the drug and its rate of disappearance were similar in both species, whereas the metabolite *N*-demethylclobazam was present at higher concentrations and for longer in the mouse than in the rat. Although the exact contribution of the *N*-desmethylclobazam to the anticonvulsant effect of clobazam cannot be assessed, the longer duration in mice than in rats seems to be associated with different brain accumulations of the metabolite.

The metabolic pathways of compounds of the 1,4-benzodiazepine series include N_1-dealkylation, which results in accumulation of pharmacologically active metabolites in blood and tissue (Schwartz 1973; Garattini et al 1973, 1977). Studies of the metabolism of clobazam, a newly developed 1,5-benzodiazepine with anticonvulsant activity (Barzaghi et al 1973; Chapman et al 1978), indicate that N_1-desmethylation is an important metabolic pathway in several animal species (Volz et al 1979; Caccia et al 1979).

The purpose of this work was to investigate the kinetics of clobazam and its *N*-desmethyl metabolite after administration of clobazam to mice and rats. The duration of the anticonvulsant activity (antileptazol effect) of the drug was compared in these species and the correlation between the pharmacological effect and brain concentrations of drug and metabolite was investigated.

MATERIALS AND METHODS

Animals

Male CD Sprague Dawley rats, 200–250 g and male CD_1 Albino Swiss mice, 20–25 g (Charles River, Italy) were used.

Analysis of clobazam and N-*desmethylclobazam*

Animals were injected intraperitoneally with clobazam (10 mg kg^{-1}) suspended in 0·5% carboxymethylcellulose and killed at various times after drug administration. Blood samples were collected in heparinized tubes, centrifuged and the plasma was stored at −20°C. Brains were immediately removed and stored at −20°C. Clobazam and metabolite were extracted from plasma and homogenized brain and analysed by g.l.c. (Caccia et al 1979).

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Kinetic calculations

Plasma and brain half-lives ($T\frac{1}{2}$) were calculated by linear regression of the terminal log-linear part of the plasma and brain concentrations—time curves. The areas under the curves (AUC) were calculated with the trapeizoidal rule and extrapolated to infinity.

Leptazol (metrazol) antagonism

Clobazam (10 mg kg^{-1} i.p.) was administered at various intervals before leptazol (120 mg kg^{-1}, i.p.) to groups of six animals at each interval. Prevention of the tonic phase of the convulsions within 30 min of leptazol injection was considered as protective effect.

RESULTS

Kinetics of clobazam and N-*desmethylclobazam*

Fig. 1 shows the plasma concentration-time curves of clobazam and *N*-desmethylclobazam after intraperitoneal injection of clobazam to rats. Clobazam was rapidly absorbed rising to a peak at 5 min

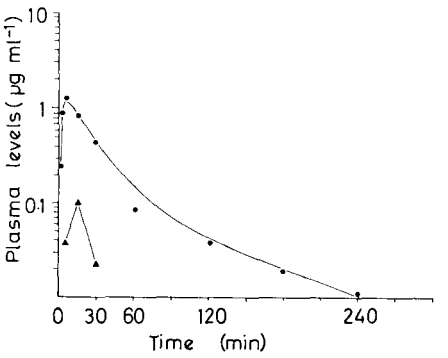

FIG. 1. Plasma concentrations-time curves of clobazam (●) and *N*-desmethylclobazam (▲) after intraperitoneal injection of clobazam (10 mg kg^{-1}) to rats. Each point is the mean of six animals.

(1·32 ± 0·06 μg ml^{-1}). Plasma concentrations showed a biphasic decline thereafter with an initial phase lasting 60 min followed by a second, slower phase. The half-life of the β-phase was 64 min. *N*-Desmethylclobazam was detectable within 5 to 30 min, reaching a peak of 0·1 ± 0·1 μg ml^{-1} at 15 min.

Fig. 2 shows the plasma concentration-time curves after intraperitoneal injection of clobazam to mice. The only substantial difference between the plasma concentrations in rats and mice was that the peak was significantly higher in the mouse (2·9 ± 0·01 μg ml^{-1}). However, plasma concentrations of *N*-desmethylclobazam were higher in mouse plasma and lasted at least 20 h declining at a slower rate (plasma $T_{\frac{1}{2}}$ 202 min) than the parent compound (plasma $T_{\frac{1}{2}}$ 66 min). The levels of the metabolite expressed as area under the curve (AUC) were about 20 times higher than those of clobazam (see Table 1).

Table 2 summarizes the brain concentrations of clobazam and *N*-desmethylclobazam in mice and rats. Peak brain concentrations of clobazam were similar in the two species. However, as in plasma, the desmethyl metabolite was present at higher concentrations and for longer in mouse than in rat brain. The brain to plasma ratio of clobazam and *N*-desmethylclobazam gradually rose to peak concentrations (close to 1·00) and then remained constant. Brain $T_{\frac{1}{2}}$ and AUC were similar to those for plasma (see Table 1).

Effect of clobazam on leptazol-induced convulsions

The dose of 120 mg kg^{-1}, i.p. of leptazol elicited tonic convulsions in 100% of animals species considered. The responses to leptazol given at various intervals after clobazam (10 mg kg^{-1} i.p.) show the duration of the protective effect differed markedly in the two species. In the rat, maximum protection was seen within 1 to 15 min of drug injection declining rapidly to zero by 60 min. In the mouse the protective effect lasted for about 4 h, after which the leptazol convulsions gradually increased as shown by the larger numbers of animals exhibiting tonic convulsions between 4 and 20 h.

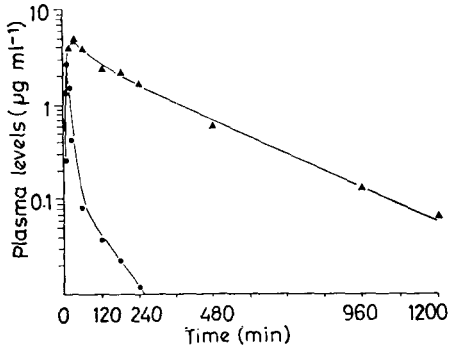

FIG. 2. Plasma concentrations-time curves of clobazam (●) and *N*-desmethylclobazam (▲) after intraperitoneal injection of clobazam (10 mg kg^{-1}) to mice. Each point is the mean of six animals.

Table 1. Half-life ($T_{\frac{1}{2}}$) and area under the curve (AUC) of clobazam (C) and *N*-desmethylclobazam (DC) after intraperitoneal injection of clobazam (10 mg kg^{-1}).

Species	$T_{\frac{1}{2}}$ (min)		AUC (μg ml^{-1} or μg g^{-1} × min)	
Rat	C	DC	C	DC
Plasma	64	n.d.*	39	1·6**
Brain	56	n.d.	37	1·6**
Mouse				
Plasma	66	202	54	1159
Brain	53	208	39	929

* n.d. = not detectable.
** Calculated only up to 30 min.

Table 2. Brain concentrations (μg g^{-1} ± s.e.) of clobazam (C) and *N*-desmethylclobazam (DC) after intraperitoneal injection of clobazam (10 mg kg^{-1}) to the rat and mouse.

Time after clobazam (min)	Rat		Mouse	
	C	DC	C	DC
1	0·13 ±0·02 (0·5)	<0·02	0·16 ±0·01 (0·3)	<0·02
5	0·75 ±0·02 (0·6)	0·03 ±0·004 (0·7)	1·07 ±0·16 (0·4)	0·36 ±0·06 (0·2)*
15	1·10 ±0·06 (1·4)	0·10 ±0·01 (1·0)	1·63 ±0·10 (1·1)	1·58 (±0·23 (0·4))*
30	0·49 ±0·02 (1·1)	0·03 ±0·003 (1·0)	0·50 ±0·07 (1·1)	2·94 ±0·13 (0·6)*
60	0·09 ±0·01 (1·1)	<0·02	0·09 ±0·01 (1·0)	3·40 ±0·25 (0·9)**
120	0·04 ±0·003 (1·0)	<0·02	0·04 ±0·01 (0·9)	2·09 ±0·18 (0·8)**
180	0·02 ±0·002 (1·0)	<0·02	0·02 ±0·001 (0·8)	1·71 ±0·14 (0·8)**
240	<0·01	<0·02	<0·01	1·47 ±0·06 (0·9)**
480			<0·01	0·60 ±0·06 (1·0)
960			<0·01	0·12 ±0·01 (0·9)
1200			<0·01	0·06 ±0·01 (0·9)

6 animals for each point.
Brain: plasma concentration ratio is shown in parenthesis.
Differences between rat and mouse:
* $P<0·01$ Mann-Whitney Rank Sum Test; ** $P<0·001$ *t*-test for differences between a sample mean and a constant (C = 0·02).

DISCUSSION

Species differences in the pharmacological activity of single benzodiazepines have often been ascribed to the different metabolic profile of the drug in various animal species (Marcucci & Mussini 1968; Marcucci et al 1968–71). Previous studies have shown that clobazam is extremely active in the mouse against leptazol-induced convulsions (Barzaghi et al 1973).

Table 3. Percent protection from leptazol-induced convulsions after injection of clobazam (10 mg kg^{-1} i.p.). Leptazol (120 mg kg^{-1}, i.p.) elicited tonic convulsions in 100% of rats and mice pretreated with vehicle.

Time between clobazam and leptazol injection (min)	Protection % Rat	P*	Mouse	P*
1	100	0·02	100	0·002
5	100	0·02	100	0·002
15	100	0·02	100	0·002
30	67	0·03	100	0·002
60	0		100	0·002
120			100	0·002
180			100	0·002
240			83	0·01
480			50	
960			17	
1200			0	

6 animals for each point.
* Fisher's exact test (one side tail). Compared versus control groups.

Our results indicate that this effect is weaker and shorter-lasting in the rat, although the brain concentrations of clobazam and its rate of disappearance were similar in the two species.

Conversely N_1-desmethylation of clobazam to *N*-desmethylclobazam occurs extensively in mice whereas only traces of the metabolite were found in rat brain and even these were only detectable for 30 min. Whether the low values of *N*-desmethylclobazam are due to slower formation or to faster elimination of the metabolite in the rat cannot be established on the basis of our experiments. However the differences in brain concentrations of *N*-desmethylclobazam seem associated with the different duration of clobazam's anticonvulsant effect in the two species. In the rat, the protective effect of clobazam against leptazol-induced convulsions correlates directly with brain disposition of the drug. Maximum protection lasts only 15 min, declining rapidly in parallel with disappearance of the drug from the brain. The rapid speed with which clobazam enters the brain and the about 4 min latency of the occurrence of tonic convulsions after leptazol injection can explain the complete protection observed at 1 min after clobazam administration in spite of the low brain drug concentrations found at this time.

In the mouse, protection is maximal for about 4 h. As in rat brain, after 3 h clobazam in mouse brain is <10 ng g^{-1} but the concentration of *N*-desmethylclobazam is still high. The slower rate of disappearance of the metabolite from the brain appears to be associated with the gradual fall-off in protective effect between 3 and 20 h.

Although the contribution of other metabolites of clobazam cannot be excluded, these findings suggest that *N*-desmethylclobazam might be the reason why the antileptazol effect of clobazam lasts longer in mice than in rats. Previous independent studies (Fielding & Hoffmann 1979) indicate that *N*-desmethylclobazam is active in the mouse against leptazolinduced convulsions, though at doses higher than the parent compound.

The studies reported underline an analogy between the effects of diazepam and clobazam. Both compounds are *N*-desmethylated, with greater, longer-lasting accumulation of the *N*-desmethyl metabolite in mice than in rats. Both drugs have a longer-lasting antileptazol effect in mice than in rats. It has been previously shown that the anticonvulsant effect of diazepam in mice involves *N*-desmethyldiazepam and its hydroxylated compound oxazepam (Marcucci et al 1969, 1970a; Garattini et al 1970). It is possible that a hydroxylated metabolite of clobazam contributes to the antileptazol effect in mice.

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