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Species differences in diazepam metabolism—I. Metabolism of diazepam metabolites*

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THE DISTRIBUTION and metabolism of *N*-demethyldiazepam and *N*-methyloxazepam have been studied in rats and in mice, after i.v. injection of these drugs (5 mg/kg). The levels of the compounds administered and their disappearance are similar in blood, brain and adipose tissue of rats and mice.

The accumulation of oxazepam coming from metabolization of both the parent compounds is observed in mice but not in rats.

Evidence is presented for the more rapid disappearance of oxazepam from rat than from mouse tissues.

Previous studies have shown that diazepam exerts a longer lasting anticonvulsant activity in mice than in rats.[1] In order to explain this species difference, the brain levels of diazepam and its metabolites were investigated.[2] These *in vivo* studies have shown that the level of diazepam is similar in both species, while there is an accumulation of *N*-demethyl metabolites in mice, but not in rats.

Since the *N*-demethyl metabolites of diazepam, namely *N*-demethyldiazepam and oxazepam, exert an anticonvulsant activity comparable to that of diazepam[1–3] these biochemical findings may explain why in mice the anticonvulsant activity of diazepam is longer lasting than in rats. Further studies were carried out *in vitro* considering the diazepam is *N*-demethylated and hydroxylated in the reticulum endoplasmic system of the liver[4, 5] in order to observe possible differences between rats and mice. By using liver microsomal preparations it was found that the mouse liver microsomes metabolize

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diazepam mostly with the formation of *N*-demethyldiazepam, while rat liver microsomes form mostly *N*-methyloxazepam. *N*-methyloxazepam is then demethylated by mouse but not rat liver microsomes, while *N*-demethyldiazepam is hydroxylated to form oxazepam by both species but only at a very low rate, at least *in vitro*.[6] Although these results could explain the observed difference *in vivo*, it was of interest to investigate the distribution and the metabolism of the considered diazepam metabolites, *N*-methyloxazepam, *N*-demethyldiazepam and oxazepam, in both rats and mice.

Methods

1. *Animals.* Male Sprague–Dawley rats (body wt. 200–250 g) and Male Albino Swiss mice (body wt. 20–25 g) were used in all experiments.

2. *Drug administration.* *N*-demethyldiazepam, *N*-methyloxazepam and oxazepam were administered by intravenous injection at a dose of 5 mg/kg, dissolved in a solvent containing propyl glycol, glycofurol, benzyl alcohol and water (30:30:2:48).

3. *Chemical determinations.* The preparation of blood, brain and adipose tissue extracts were made according to the method previously described.[7]

Gas chromatographic analysis were carried out using a gas chromatograph Model G 1 (Carlo Erba, Milan) equipped with a Ni 63 electron capture detector (voltage: 42 v). The stationary phase was OV_1 3% on Gas Chrom Q (60–80 mesh) packed into a 4m glass column (int. diam. 2 mm, ext. diam. 4 mm). The flow rate of carrier gas (nitrogen) was 33 ml/min and the column temperature was 245°.

The electron capture detector sensitivity for the various benzodiazepines was the following: oxazepam 50 ng, *N*-demethyldiazepam 5 ng and *N*-methyloxazepam 90 ng per 1 ml of blood or 1 g of tissue.

Results

1. *Levels of* N-*demethyldiazepam and its metabolite in blood, brain and adipose tissue of mice and rats.* In Table 1 are reported the levels of *N*-demethyldiazepam in blood, brain and adipose tissue of mice at different times after drug administration. The peak of *N*-demethyldiazepam for blood and brain was

TABLE 1. LEVELS (± S.E.) OF *N*-DEMETHYLDIAZEPAM (N.D.D.) AND ITS METABOLITE OXAZEPAM (OX) IN BLOOD, BRAIN AND ADIPOSE TISSUE OF MICE AFTER ADMINISTRATION OF *N*-DEMETHYLDIAZEPAM (5 mg/kg, i.v.)

Time after *N*-demethyl diazepam	Blood		Brain		Adipose tissue	
	NDD (μg/ml)	OX (μg/ml)	NDD (μg/g)	OX (μg/g)	NDD (μg/g)	OX (μg/g)
1 min	3·98 ± 0·12	—	8·16 ± 0·21	—	4·17 ± 0·08	—
5 min	1·76 ± 0·09	n.d.†	6·52 ± 0·12	n.d.	5·51 ± 0·07	n.d.
30 min	1·31 ± 0·03	0·10 ± 0	5·55 ± 0·05	1·53 ± 0·02	12·50 ± 0·62	0·64 ± 0·04
60 min	0·47 ± 0·03	0·13 ± 0·002	4·52 ± 0·18	2·37 ± 0·02	11·21 ± 0·22	1·02 ± 0·02
3 hr	0·12 ± 0·01	0·29 ± 0·01	0·62 ± 0·01	3·93 ± 0·07	1·30 ± 0·04	1·00 ± 0·03
5 hr	n.d.*	0·21 ± 0·0002	n.d.	0·75 ± 0·01	0·23 ± 0·01	0·69 ± 0·02
10 hr	n.d.	0·13 ± 0·007	n.d.	0·75 ± 0·02	n.d.	0·42 ± 0·01

* n.d. traces (less than 5 ng/g).
† n.d. traces (less than 50 ng/g).

reached immediately after its injection and then the levels tend to decline progressively so that after about 5 hr only traces are measurable. In the adipose tissue there is instead a progressive increase up to 30 min after injection followed by a gradual decrease with the time.

Oxazepam, the product formed by hydroxylation of *N*-demethyldiazepam, was found in considerable concentrations beginning 30 min after *N*-demethyldiazepam injection.

The peak of oxazepam was reached 3 hr after intravenous injection of *N*-demethyldiazepam, and even after 10 hr, when the drug administered is no longer detectable, oxazepam levels are still measurable in blood, brain and adipose tissue. Results of distribution of *N*-demethyldiazepam in rats (Table 2) were qualitatively similar to those obtained in mice, although in rats the rate of disappearance from blood, brain and adipose tissue was faster than in mice.

TABLE 2. LEVELS (± S.E.) OF *N*-DEMETHYLDIAZEPAM IN BLOOD, BRAIN AND ADIPOSE TISSUE OF RATS AFTER ADMINISTRATION OF *N*-DEMETHYLDIAZEPAM (5 mg/kg, i.v.)

Time after *N*-demethyl diazepam	Blood (μg/ml)	Brain (μg/g)	Adipose tissue (μg/g)
1 min	5·12 ± 0·18	7·82 ± 0·37	1·04 ± 0·05
5 min	3·29 ± 0·04	6·35 ± 0·09	3·88 ± 0·35
30 min	1·58 ± 0·14	3·01 ± 0·07	17·38 ± 0·76
60 min	0·098 ± 0·003	0·59 ± 0·007	2·45 ± 0·08
3 hr	0·007 ± 0·0005	0·021 ± 0·001	0·253 ± 0·020
5 hr	0·005 ± 0·0005	0·015 ± 0·0005	0·167 ± 0·008
10 hr	n.d.*	n.d.	0·100 ± 0·003

* n.d. traces (less than 5 ng/g).

It is of interest to note that in rats no oxazepam was present in the tissues examined, clearly showing that no accumulation of oxazepam occurs in rats.

2. *Levels of* N-*methyloxazepam and its metabolite in blood, brain and adipose tissue of mice and rats.* In Table 3 are shown the levels of *N*-methyloxazepam in blood, brain and adipose tissue of mice at different times after drug administration. The pattern of distribution of *N*-methyloxazepam is similar to that previously obtained using *N*-demethyldiazepam; the peak for blood and brain was reached immediately after its injection, while for adipose tissue the peak was reached only after 30 min. After 10 hr only traces of the drug administered are measurable.

Oxazepam, the metabolite coming from *N*-demethylation of the injected compound is present already 5 min after *N*-methyloxazepam injection and it is still measurable after 10 hr when *N*-methyl-

TABLE 3. LEVELS OF *N*-METHYLOXAZEPAM (N.M.O.) AND ITS METABOLITE OXAZEPAM (OX) IN BLOOD, BRAIN AND ADIPOSE TISSUE OF MICE AFTER ADMINISTRATION OF *N*-METHYL OXAZEPAM (5 mg/kg, i.v.)

Time after *N*-methyl oxazepam	Blood		Brain		Adipose tissue	
	N.M.O. (μg/ml)	OX (μg/ml)	N.M.O. (μg/g)	OX (μg/g)	N.M.O. (μg/g)	OX (μg/g)
1 min	3·54 ± 0·23	0·17 ± 0·01	6·98 ± 0·54	—	1·64 ± 0·22	—
5 min	1·39 ± 0·25	0·40 ± 0·02	5·25 ± 0·63	0·56 ± 0·01	6·20 ± 0·41	0·40 ± 0·03
30 min	0·84 ± 0·04	0·56 ± 0·03	2·63 ± 0·20	4·12 ± 0·14	8·60 ± 0·32	0·42 ± 0·02
60 min	0·15 ± 0·02	0·72 ± 0·05	0·44 ± 0·01	5·06 ± 0·18	3·27 ± 0·14	1·52 ± 0·03
3 hr	n.d.*	0·14 ± 0·003	0·21 ± 0·02	0·84 ± 0·02	0·28 ± 0·005	0·54 ± 0·01
5 min	n.d.	0·13 ± 0·01	n.d.	0·56 ± 0·01	0·21 ± 0·01	0·53 ± 0·01
10 hr	—	0·13 ± 0·001	n.d.	0·53 ± 0·01	n.d.	0·45 ± 0·02

* n.d. = not detectable (less than 90 ng/g).

TABLE 4. LEVELS (± S.E.) OF *N*-METHYLOXAZEPAM (N.M.O.) AND ITS METABOLITE OXAZEPAM (OX) IN BLOOD, BRAIN AND ADIPOSE TISSUE OF RATS AFTER ADMINISTRATION OF *N*-METHYLOXAZEPAM (5 mg/kg i.v.)

Time after *N*-methyl oxazepam	Blood		Brain		Adipose tissue	
	N.M.O. (μg/ml)	OX (μg/ml)	N.M.O. (μg/g)	OX (μg/g)	N.M.O. (μg/g)	OX (μg/g)
1 min	5·70 ± 0·04	—	9·40 ± 0·26	—	5·93 ± 0·08	—
5 min	1·74 ± 0·02	n.d.*	8·04 ± 0·34	1·78 ± 0·01	5·73 ± 0·08	1·28 ± 0·04
30 min	1·28 ± 0·05	0·21 ± 0·01	1·20 ± 0·19	0·46 ± 0·005	3·57 ± 0·17	0·23 ± 0·01
60 min	0·24 ± 0·03	0·09 ± 0·01	0·52 ± 0·02	0·32 ± 0·01	1·37 ± 0·04	0·19 ± 0·01
3 hr	n.d.†	—	n.d.	—	n.d.	—

* n.d. traces (less than 90 ng/g).
† n.d. traces (less than 50 ng/g).

oxazepam has disappeared. The rate of accumulation of oxazepam *in vivo* from *N*-methyloxazepam is faster than from *N*-demethyldiazepam.

The same experiment, carried out in rats, is shown in Table 4.

Blood and brain levels of *N*-methyloxazepam are similar to those seen in mice but in the adipose tissue the peak of *N*-methyloxazepam was reached immediately (from 1 to 5 min after injection).

Oxazepam is present in detectable amounts only between 5 to 60 min after *N*-methyloxazepam injection.

3. *Levels of oxazepam in blood, brain and adipose tissue of mice and rats.* After intravenous injection of oxazepam into mice, the highest blood and brain concentrations are observed between 1 and 5 min, while in adipose tissue the peak is reached at 30 min. Thereafter a gradual decline in level occurs up to 10 hr after administration, in all tissues examined. The disappearance of oxazepam from blood, brain and adipose tissue of rats (Table 6) was relatively rapid since only traces were present 5 hr after oxazepam injection.

TABLE 5. LEVELS (± S.E.) OF OXAZEPAM (OX) IN BLOOD, BRAIN AND ADIPOSE TISSUE OF MICE AFTER ADMINISTRATION OF OXAZEPAM (5 mg/kg i.v.)

Time after oxazepam	Blood	Brain	Adipose tissue
	OX (μg/ml)	OX (μg/g)	OX (μg/g)
1 min	3·16 ± 0·05	3·59 ± 0·05	3·16 ± 0·09
5 min	2·08 ± 0·05	14·30 ± 0·17	3·50 ± 0·04
30 min	1·51 ± 0·05	6·84 ± 0·12	7·35 ± 0·12
60 min	1·10 ± 0·02	5·21 ± 0·03	4·21 ± 0·18
3 hr	0·71 ± 0·05	3·12 ± 0·01	1·47 ± 0·01
5 hr	0·47 ± 0·02	2·78 ± 0·02	1·38 ± 0·01
10 hr	0·20 ± 0·01	2·25 ± 0·02	1·14 ± 0·01

TABLE 6. LEVELS (± S.E.) OF OXAZEPAM (OX) IN BLOOD, BRAIN AND ADIPOSE TISSUE OF RATS AFTER ADMINISTRATION OF OXAZEPAM (5 mg/kg i.v.)

Time after oxazepam	Blood	Brain	Adipose tissue
	OX (μg/ml)	OX (μg/g)	OX (μg/g)
1 min	1·63 ± 0·09	3·06 ± 0·05	0·88 ± 0·03
5 min	1·50 ± 0·04	4·50 ± 0·03	3·30 ± 0·05
30 min	0·55 ± 0·02	2·90 ± 0·02	2·57 ± 0·04
60 min	0·23 ± 0·01	1·43 ± 0·05	2·09 ± 0·05
3 hr	0·06 ± 0·002	0·20 ± 0·0005	0·14 ± 0·005
5 hr	n.d.*	n.d.	n.d.

* n.d. traces (less than 50 ng/g).

Discussion

N-Demethyldiazepam, one of the principal metabolites of diazepam in mice but not in rats, disappears from the blood stream and accumulates in the brain and adipose tissue similarly in the two animal species. Although the levels of *N*-demethyldiazepam are at given times (for example 3 hr after *N*-demethyldiazepam administration) several fold higher in mouse than in rat tissue, the very low levels of *N*-demethyldiazepam in rats *in vivo* after administration of diazepam are not likely to be due to a rapid disposition of *N*-demethyldiazepam.

These findings confirm therefore that the observed low rate of formation of *N*-demethyldiazepam when diazepam is incubated with rat liver microsomal enzymes[6] is the limiting factor for the lack of accumulation of *N*-demethyldiazepam in rats.

N-Methyloxazepam disappears also from the blood stream and accumulates in the brain of rats and

mice at about the same rate. However, the pattern of accumulation in the adipose tissue is different in the two animal species because *N*-methyloxazepam enters more slowly in the mouse than in the rat adipose tissue.

Both *N*-demethyldiazepam and *N*-methyloxazepam may be respectively hydroxylated or *N*-demethylated to form the common metabolite oxazepam.[3, 4] In rats there is no accumulation of oxazepam from *N*-demethyldiazepam while only limited concentrations are present in blood, brain or adipose tissue when *N*-methyloxazepam is given. On the contrary in mice high concentration of oxazepam are formed and accumulated for several hours both from *N*-demethyldiazepam and *N*-methyloxazepam. The reason for accumulation of oxazepam in mice and not in rats may be related to the different rate of disappearance of this metabolite from plasma and tissues in these two species. In fact when oxazepam was injected into rats it disappeared in about 3 hr while in mice with the same dose of oxazepam, it was still present in blood and tissues even after 10 hr.

It is possible therefore to conclude that in mice oxazepam is retained for longer time than in rats. This may explain why the administration of diazepam results in the presence of oxazepam only in mice and not in rats.[8] These metabolic findings also help to explain the longer duration of anticonvulsant action exerted by diazepam in mice than in rats.[1]

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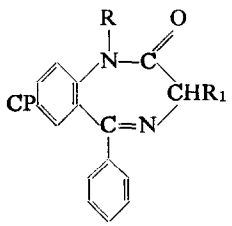

R	R_1	
CH_3	H	Diazepam
H	H	*N*-Demethyldiazepam
CH_3	OH	*N*-Methyloxazepam
H	OH	Oxazepam

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