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Metabolism of diazepam and its metabolites by guinea pig liver microsomes

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LIVER microsomal preparations of various animal species metabolize diazepam added *in vitro*. This drug is predominantly hydroxylated in C_3 position and only slightly *N*-demethylated by rat liver[1] while it is more *N*-demethylated than hydroxylated in mice.[1] Previous studies *in vivo* indicated that guinea pigs are also able to metabolize diazepam.[2] This note summarizes the findings obtained by adding diazepam and some of its known metabolites (see Fig. 1) to guinea pig liver microsomes.

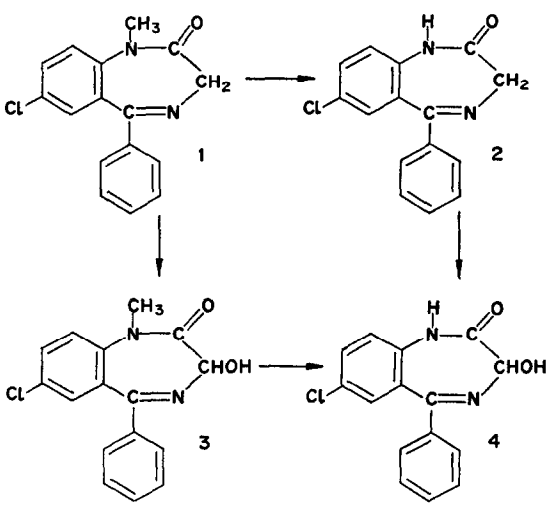

FIG. 1. (1) Diazepam. (2) *N*-demethyldiazepam. (3) *N*-methyloxazepam. (4) Oxazepam.

Materials and Methods

(1) *Animals.* Male Albino guinea pigs (body weight 300–350 g) fed with Alcom-Alal pellets, were used in all experiments.

(2) *Microsome preparation.* Animals were killed and the livers were immediately removed and homogenized in ice-cold 1·15% KCl solution (1:4 w/v) with a teflon glass homogenizer. The homogenate was centrifuged at 9000 *g* for 20 min and then the supernatant fraction was again centrifuged at 105,000 *g* for 1 hr (rotor 40′—Beckman Model L ultracentrifuge). The liver microsomes were suspended in 1·15% KCl solution.

(3) *Incubation* in vitro. The incubation mixture contained substrate, enzyme source, pyridine nucleotides and an NADPH-generating system according to that described by Kato *et al.*[3] The incubation volume of 5 ml consisted of 2·5 ml of microsomal suspension equivalent to 1 g of liver,

TABLE 1. *In vitro* METABOLISM OF DIAZEPAM AND DIAZEPAM METABOLITES BY GUINEA PIG LIVER MICROSOMES

Drug added (nmoles)	Drugs after incubation (nmoles ± S.E.)				
	Diazepam	*N*-Methyl-oxazepam	*N*-Demethyl-diazepam	Oxazepam	Recovery*
Diazepam 35	0·016	†	32 ± 0·45		91·4
70	0·016		65 ± 0·29		92·8
105	37 ± 0·1		69 ± 0·1		100·9
140	62 ± 0·7		80 ± 0·7		101·4
210	76 ± 0·7		130 ± 3·6		98·0
350	134 ± 0·3		187 ± 4·4		91·7
525	243 ± 0·7		254 ± 1		94·6
700	395 ± 0·3		284 ± 3·6		97·0
1050	754 ± 0·7		309 ± 3·6		101·2
1700	1471 ± 4·2		295 ± 4·4		100·9
N-methyl-oxazepam 33				7 ± 0·3	21·2
66				16 ± 1·0	24·2
99		1·6		20 ± 0·6	20·2
199		126 ± 3·9		35 ± 3·8	80·9
332		274 ± 4·9		24 ± 1·7	89·7
664		623 ± 10·2		17 ± 1·0	96·3
1661		1641 ± 16·6		10 ± 0·6	99·3
N-demethyl-diazepam 36			32 ± 1·1		88·8
73			73 ± 3·6		100·0
110			102 ± 2·9		92·7
220			215 ± 3·6		97·7
367			361 ± 4·0		98·3
735			735 ± 4·7		100·0
1830			1823 ± 5·5		99·6
Oxazepam 34				31 ± 0·3	91·0
69				65 ± 1·2	94·2
104				102 ± 1·8	98·0
209				207 ± 2·1	99·0
349				346 ± 3·6	99·1
699				601 ± 3·6	85·9
1748				1730 ± 4·2	98·9

* These figures represent the sum of diazepam and its metabolites found after the incubation. The values are expressed as per cent of the added diazepam or diazepam metabolite.

† When there is a blank space the compounds were not present. (The limit of sensitivity of the method was 0·05 μg.)

NADP (1·5 μmoles), glucose-6-phosphate (50 μmoles), glucose-6-phosphate dehydrogenase (0·5 units), magnesium chloride (25 μmoles), nicotinamide (50 μmoles), 1·4 ml of 0·2 M phosphate buffer pH 7·4, the substrate (diazepam; *N*-demethyldiazepam; *N*-methyloxazepam or oxazepam) dissolved in ethanol and added in amounts ranging from 10 to 500 μg and 0·45 ml of 1·15% KCl.

The incubation was carried out at 37° for 10 min under air in a Dubnoff metabolic shaker.

(4) *Chemical determinations.* After the incubation the entire content of each flask was extracted twice with 10 ml of fresh peroxide-free ethylether. The combined extracts were evaporated to dryness, redissolved in a suitable amount of acetonitrile and then gas-chromatographed according to the method previously reported.[4,5]

Results and discussion

Table 1 summarizes the findings obtained by incubating *in vitro* guinea pig liver microsomes with diazepam and three among its known metabolites. It appears that only *N*-demethylated products

accumulate in these experimental conditions. In fact *N*-demethyldiazepam and oxazepam are obtained after incubating diazepam and *N*-methyloxazepam respectively, while *N*-demethyldiazepam and oxazepam are not furtherly metabolized and they are almost completely recovered as such. The rate of *N*-demethylation of diazepam at saturating concentrations is calculated as 1854 nmoles/*g* of tissue/hr. In the case of low concentrations of *N*-methyloxazepam the recovered amount of the unmetabolized product added to the respective formed metabolite does not account for the added substrate. Since unpublished studies showed a recovery of *N*-methyloxazepam added to guinea pig liver microsomes of about 90 per cent (the presented data are corrected for the respective recovery factor), the noticed loss of this substrate may suggest a possible alternate metabolic pathway which leads to the formation of unknown compounds.

The findings obtained for diazepam and *N*-demethyldiazepam are consistent with the *in vivo* studies showing that no C_3-hydroxylated metabolites accumulate in blood brain and adipose tissue.[2] It is interesting to notice that diazepam is metabolized by guinea pigs both *in vitro* and *in vivo*[2] in a different manner than by rats or mice. As far as the *in vitro* metabolism is concerned, *N*-demethyldiazepam is slightly hydroxylated by rats and mice but not by guinea pigs while *N*-methyloxazepam is demethylated by mice and guinea pigs but not by rats. Oxazepam is not further metabolized *in vitro* by the liver microsomes of the three animal species here considered.[1]

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Nachweis der 16-Hydroxylierung bei Pregnanen in Ratten nach Barbiturat-Gabe durch Radiospirometrie

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DAS PRINZIP der Stoffwechsel-labilen Tritium-Markierung verwendeten wir zur *in-vivo* Messung der Hydroxylierung bereits Hydroxylgruppen tragender Pregnanderivate bei Ratten. Untersucht wurden die folgenden Steroide,* die alle in Position 16 stabil mit Tritium markiert waren[1]

3α, 17α, 20β-Trihydroxy-5β-pregnan (Trihydroxypregnan)
3α, 17α-Dihydroxy-20-oxo-5β-pregnan (Dihydroxypregnan)
3α-Hydroxy-20-oxo-5β-pregnan(Pregnanolon)

* Für die Überlassung der Steroide haben wir Herrn Dr. J. Livet, Paris, zu danken (vergl. 1).