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Effect of diazepam and chlordiazepoxide on the metabolism of other drugs

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Diazepam and chlordiazepoxide, even at high single doses, do not inhibit the metabolic transformation of *p*-nitroanisol, aniline and aminopyrine by the 9,000 *g* fraction of rat liver. Although diazepam and chlordiazepoxide increase the sleeping time induced by pentobarbitone, this effect is not explained by an increase in the brain level of pentobarbitone. Repeated administrations of the two benzodiazepines increase the metabolism of *p*-nitroanisol, aniline, amidopyrine and shorten the sleeping time induced by pentobarbitone in rats. There was a concomitant reduction of the concentration of brain pentobarbitone.

In recent years, increasing evidence has been obtained about the possibility that drugs may interfere with the activity of drug-metabolizing enzymes in liver microsomes. More than two hundred compounds have been found to stimulate drug metabolism and many of these also reduce drug metabolism in given experimental conditions. Chlordiazepoxide was included among the "inducers" (Conney, 1967). Hoogland, Miya & Bousquet (1966) suggested that tolerance to the skeletal muscle relaxant effects of chlordiazepoxide could be explained on the basis of a stimulation of liver microsomes responsible for chlordiazepoxide metabolism. The purpose of this investigation was to compare the activity of chlordiazepoxide and diazepam, *in vivo* and *in vitro*.

EXPERIMENTAL

Materials and methods

Female Sprague-Dawley rats, 150 ± 10 g maintained on food and water *ad libitum*, were used. Diazepam and chlordiazepoxide were dissolved in carboxymethylcellulose (0·5% v/v) and were given by oral intubation. Experiments were always made in the afternoon.

In vitro *experiment*. Pretreated animals were killed by decapitation, livers were quickly removed, placed in dry-ice, weighed and homogenized in 1·15% KCl solution. This homogenate was centrifuged at 4° at 9,000 *g* and the supernatant fraction used (Kato & Takanaka, 1967).

Substrates were: *p*-Nitrophenol 2 (μmol), aniline (5 μmol) and aminopyrine (5 μmol). At the end of the incubation period (30 min) aliquots of the incubation medium were removed and analysed for metabolites, *p*-nitrophenol, *p*-aminophenol and 4-aminoantipyrine respectively (Gilbert & Golberg, 1965).

In vivo *experiment*. Chlordiazepoxide- or diazepam- pretreated rats were given pentobarbitone (30 mg/kg i.p.). Sleeping time was evaluated as that time elapsing between loss and regaining of the righting reflex.

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Pentobarbitone was measured in the whole brain 90 min after the administration (Brodie, Burns & others, 1953). The weight of the liver and the liver proteins were not affected by the repeated treatments with diazepam.

RESULTS

Effect of acute pretreatment on microsomal enzyme activity in vitro. Oral pretreatment with diazepan (50 and 100 mg/kg) or chlordiazepoxide (100 mg/kg) in a single dose, does not inhibit the liver microsomal enzyme systems responsible for the metabolism of aniline, *p*-nitrophenol and aminopyrine (Table 1).

Table 1. *Effect of diazepam and chlordiazepoxide on liver metabolic activity of other compounds*

Treatment	mg/kg oral	*p*-Nitrophenol nmol ± s.e.	4-Aminoantipyrine nmol ± s.e.	*p*-Aminophenol nmol ± s.e.
Saline	—	(15) 440 ± 30	(4) 64 ± 10	(4) 520 ± 50
Diazepam ..	50	(15) 400 ± 30	(4) 66 ± 6	(4) 597 ± 70
Diazepam ..	100	(4) 300 ± 30	(4) 59 ± 10	(4) 590 ± 60
Chlordiazepoxide	100	(15) 440 ± 20	(4) 49 ± 4	(4) 530 ± 50

Chlordiazepoxide, diazepam or saline were given 2 h before the assay.
Enzyme activity is shown as nmol of metabolites formed per g liver/h.
Figures in brackets refer to the number of experiments.

Effect of acute pretreatment on pentobarbitone response. Pretreatment with diazepam (25 mg/kg, orally) or chlordiazepoxide (50 mg/kg, orally) results in a significant increase in pentobarbitone-induced sleeping time when intervals between benzodiazepines and barbiturate treatment were 1, 2 and 4 h. The results in Table 2 show also that the pretreatment caused non appreciable change in brain pentobarbitone levels measured 90 min after its injection.

Experiments made with higher doses of diazepam or chlordiazepoxide were not conclusive because of the increased toxicity of the combined treatments.

Table 2. *Effect of diazepam and chlordiazepoxide on pentobarbitone brain level and sleeping time in rats*

No. of rats	Treatment	mg/kg oral	Hours between treatment and pentobarbitone	Sleeping time min ± s.e.	Pentobarbitone concentration μg/g brain ± s.e.
15	Saline	—	—	42 ± 4	15·25 ± 1
6	Diazepam ..	25	1	65 ± 5	15·66 ± 2·8
8	Diazepam ..	25	2	68 ± 5*	13·19 ± 1·3
6	Diazepam ..	25	4	72 ± 10*	15·8 ± 1·3
5	Chlordiazepoxide	50	2	80 ± 3*	16·3 ± 1·5
5	Chlordiazepoxide	50	4	84 ± 1*	18·6 ± 0·9

* $P < 0.01$.

Pentobarbitone concentration in brain was measured 90 min after its administration (30 mg/kg i.p.).

Effect of repeated treatments on enzyme activity in vitro. The results in Table 3 indicate that when treatments with diazepam or chlordiazepoxide were repeated for at least 3 days, 24 h after the last treatment, the activity of liver enzymes present in the 9,000 *g* supernatant fraction is significantly increased.

Diazepam and chlordiazepoxide show a similar type of activity.

Table 3. *Effect of repeated treatments with diazepam or chlordiazepoxide on liver metabolic activity of other compounds*

Treatment	mg/kg oral	*p*-Nitrophenol nmol ± s.e.	4-Aminoantipyrine nmol ± s.e.	*p*-Aminophenol nmol ± s.e.
Saline	—	(13) 320 ± 30	(8) 40 ± 7	(8) 500 ± 30
Diazepam ..	50	(13) 370 ± 40	(8) 60 ± 7*	(8) 680 ± 70*
Diazepam ..	100	(9) 510 ± 30*	(9) 120 ± 10*	(9) 860 ± 80*
Diazepam ..	10 × 3†	(5) 410 ± 50	(5) 80 ± 10*	(5) 720 ± 100*
Chlordiazepoxide	100	(13) 630 ± 30*	(10) 100 ± 9*	(10) 890 ± 80*

Treatments were repeated on 3 consecutive days and determinations were made 24 h after the last administration.
Enzyme activity is indicated as nmol of metabolites formed per g liver per h.
Figures in brackets refer to the number of experiments.
* $P < 0.01$ in respect to saline.
† Diazepam was given three times a day at 8 a.m., 2 p.m. and 8 p.m.

Effect of repeated pretreatment on pentobarbitone response. The results in Table 4 indicate that repeated treatment with diazepam or chlordiazepoxide reduced the sleeping time to pentobarbitone when it was given intraperitoneally 24 h after the final dose of the benzodiazepines. The corresponding pentobarbitone concentrations in brain measured 90 min after its administration, were significantly lower in benzodiazepine-pretreated rats than in controls.

Table 4. *Effect of repeated treatments with diazepam or chlordiazepoxide on brain pentobarbitone level and sleeping time in rats*

No. of rats	Treatment	mg/kg oral	No. of days	Pentobarbitone effect: Sleeping rats (%)	Pentobarbitone effect: Sleeping time (min ± s.e.)	Pentobarbitone concentration μg/g brain ± s.e.
24	Saline	—	–	100	60 ± 2	15·2 ± 0·5
8	Diazepam ..	25	1	100	68 ± 5	14 ± 0·4
16	Diazepam ..	50	1	100	48 ± 3	15 ± 0·6
5	Diazepam ..	6·25	3	100	47 ± 4	16·9 ± 0·5
10	Diazepam ..	12·5	3	90	49 ± 4	12·9 ± 1·3
10	Diazepam ..	25	3	100	38 ± 5†	15 ± 1·3
15	Diazepam ..	50	3	94	43 ± 2*	12·2 ± 0·9†
14	Diazepam ..	100	3	72	31 ± 4*	12 ± 0·7†
10	Chlordiazepoxide	12·5	3	90	29 ± 2*	14·3 ± 1·5
10	Chlordiazepoxide	25	3	80	32 ± 4*	10·6 ± 1·2†
5	Chlordiazepoxide	50	3	80	29 ± 7†	8·4 ± 1·8*
16	Chlordiazepoxide	100	3	69	30 ± 3*	7·7 ± 1.0*

Pentobarbitone (30 mg/kg i.p.) was given 24 h after the last treatment and 90 min before the determination of brain levels.
P versus saline: * <0.01.
† < 0.05.

DISCUSSION

Measurements of *in vitro* liver enzymatic activity after acute pretreatment with diazepam or chlordiazepoxide show no significant difference from the controls. Similar results were obtained when the levels of brain pentobarbitone were measured *in vivo* although the sleeping time was increased. This evidence suggests that the two benzodiazepines should not be considered to be inhibitors of liver microsomal enzymes in rats, probably also because they are rapidly metabolized (Zbinden & Randall, 1967; Marcucci, Guaitani & others, 1968).

Increased activity of liver microsomal metabolizing enzymes was demonstrated after administration of diazepam and chlordiazepoxide in experiments *in vivo* and

in vitro. Our results show that diazepam's "inducing" activity is similar to that exerted by chlordiazepoxide. It should be remembered, however, that diazepam is more potent than chlordiazepoxide (Zbinden & Randall, 1967) and that the doses active in our experiments exceeded the minimal doses exerting specific pharmacodynamic responses (Zbinden & Randall, 1967; Marcucci & others, 1968).

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