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Metabolism of propranolol by rat liver microsomes and its inhibition by phenothiazine and tricyclic antidepressant drugs*

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PROPRANOLOL appears to be extensively metabolized in animals and man, only small amounts of the drug being excreted unchanged.[1,2] In the present study the metabolism *in vitro* of propranolol by rat liver microsomes has been investigated and the inhibitory effects of certain phenothiazine and tricyclic antidepressant drugs tested.

Male Sprague–Dawley rats (150–200 g) were decapitated and the livers removed and homogenized in 2 vol. of ice-cold isotonic KCl in a motor-driven, Teflon-glass homogenizer. The 9000 *g* supernatant, the 100,000 *g* supernatant (soluble fraction) and the microsomal pellet were obtained by differential centrifugation. Various fractions of the liver preparation (in 1·5 ml) were incubated in a medium containing $MgCl_2$ (150 μmoles), glucose-6-phosphate (50 μmoles), nicotinamide (100 μmoles), and 1·8 ml of 0·2 M potassium phosphate buffer (pH 7·4) and brought to a final volume of 4 ml with water containing any drugs used. NADPH (12 μmoles) was added to the microsomal pellet or, in the case of the 9000 *g* and 100,000 *g* supernatant, generated from NADP (0·5 μmoles) and the glucose 6-phosphate in the presence of the glucose 6-phosphate dehydrogenase present in the soluble fraction. Incubations were carried out in a Dubnoff shaker and the reaction terminated by the addition of 1 ml of 2·5 N NaOH.

Propranolol metabolism was determined by measuring the disappearance of the drug using a method previously described.[2] Briefly, the alkalinized reaction mixture was extracted with 30 ml of heptane containing 1·5% isoamyl alcohol, a 10-ml aliquot of which was extracted into 3 ml of 0·1 N HCl. Their resulting fluorescence was read in an Aminco–Bowman spectrophotoflurometer (excitation at 295 mμ; emission at 360 mμ-uncorrected). The assay was linear over the range of substrate concentration used and 80–85 per cent recovery of the drug was obtained after correcting for the proportion of the organic phase transferred. Also, none of the drugs used interfered with the assay.

Propranolol was metabolized by the 9000 *g* supernatant fraction in the presence of NADPH-generating system, but not by the soluble fraction or microsomes alone (Table 1). However, the microsomes were active when NADH or especially NADPH was added. There was no metabolism in an atmosphere of nitrogen. These findings indicate that propranolol is metabolized by a microsomal enzyme system requiring NADPH and oxygen.

Pretreatment of rats with chlorpromazine or desmethylimipramine (DMI) markedly inhibited the metabolism of propranolol by the 9000 *g* supernatant fraction (Table 2). These drugs have also been shown to inhibit the metabolism of amphetamine[3,4] and guanethidine.[5] DMI can inhibit the metabolism of pentobarbital, tremorine and oxotremorine.[6,7]

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TABLE 1. METABOLISM OF PROPRANOLOL BY VARIOUS FRACTIONS OF RAT LIVER HOMOGENATE*

Fraction	Propranolol metabolized (μmole/g liver/hr)
Whole homogenate + NADP	1·38
9000 *g* supernatant	1·26
Soluble fraction + NADP	0·00
Microsomal pellet	0·00
Microsomes + NAD	0·00
Microsomes + NADP	0·00
Microsomes + NADH	0·34
Microsomes + NADPH	1·08
9000 *g* supernatant + nitrogen	0·00

* Various fractions of the liver homogenate, equivalent to 50 mg of liver, were incubated for 20 min with 50 nmoles of propranolol. NAD (0·5 μmole), NADP (0·5 μmole), NADH (12 μmoles) and NADPH (12 μmoles) were added where indicated.

The inhibitory effect of some other phenothiazines and tricyclic antidepressant drugs on the metabolism of propranolol was also investigated *in vitro*. The 9000 *g* supernatant fraction was preincubated with the potential inhibitor for 10 min prior to the addition of propranolol (10 μM). Three concentrations of inhibitor drug were used and log dose/response curves constructed to calculate the concentration producing 50 per cent inhibition of propranolol metabolism (ID_{50}), as shown in Table 3. It can be seen that the inhibitory properties of chlorpromazine and DMI are shared by other phenothiazines and tricyclic antidepressant drugs. However, a wide range in potency was found and no obvious structure–activity relationship was apparent.

TABLE 2. EFFECT OF PRETREATMENT WITH DESMETHYLIMIPRAMINE (DMI) AND CHLORPROMAZINE ON PROPRANOLOL METABOLISM*

Treatment	Propranolol metabolism (μmole/g liver/hr)	S.E.
Saline	1·29	0·24
Chlorpromazine	0·30	0·08
DMI	0·39	0·17

* Groups of six animals were given chlorpromazine or DMI (5 mg/kg, i.p.) 1 hr prior to sacrifice. Control animals were given saline. The metabolism of propranolol by the 9000 *g* supernatant equivalent to 100 mg of liver was measured and expressed as μmole per gram liver per hour. The metabolism following pretreatment was significantly different from control ($P < 0·01$ by Student's *t*-test).

The kinetics of the inhibition were determined for chlorpromazine and DMI using the 9000 *g* supernatant fraction from 50 mg of liver. A 10-min preincubation with the inhibitor drug was carried out before the addition of propranolol and the reaction terminated after a further 20 min. Under these conditions, the rate of disappearance of propranolol was linear with respect to time and enzyme concentration at all 5 substrate concentrations. Double-reciprocal plots were constructed (Fig. 1) showing the effect of DMI (10 and 15 μM) and of chlorpromazine (10 μM). The apparent K_m for propranolol was $2·5 \times 10^{-5}$M under these conditions and inhibition was competitive in nature.

The metabolism of propranolol by incubation mixtures may involve more than one pathway, since several metabolites have been identified, including 4-hydroxypropranolol and β-(1-napthoxy)-lactic acid.[1] Although the predominant pathway for the metabolism of propranolol in rat liver microsomes is unknown, the present study shows that its inhibition by chlorpromazine and DMI

can be overcome by increasing concentrations of substrate. It is, of course, impossible to extrapolate these findings to man, but the possibility that a similar interaction could occur in patients merits further investigation. In this regard, the nature of the kinetics involved is of some importance, for if inhibition is competitive, it should be reversible and dose-dependent.

TABLE 3. ID_{50} OF DRUGS INHIBITING PROPRANOLOL METABOLISM

Phenothiazines		Tricyclic antidepressant drugs	
Drug	ID_{50}, μM ± S.D.	Drug	ID_{50}, μM ± S.D.
Trifluprornazine (6)*	10·0 ± 1·4	Desmethylimipramine (11)*	21·5 ± 1·1
Chlorpromazine (11)	15·0 ± 0·7	Amitriptyline (9)	44·0 ± 4·5
Prochlorperazine (6)	34·6 ± 2·8	Imipramine (6)	50·5 ± 3·7
Promazine (8)	61·1 ± 5·9	Nortriptyline (6)	54·5 ± 5·4
Promethazine (8)	98·5 ± 7·8		
Trifluperazine (8)	125·0 ± 16·5		
Perphenazine (6)	350·0 ± 42·8		

* Numbers in parentheses indicate the number of experiments performed.

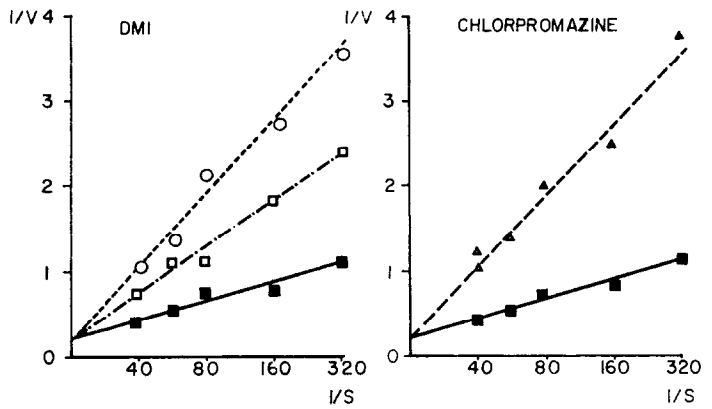

FIG. 1. Kinetics of desmethylimipramine (DMI) and chlorpromazine inhibition of propranolol metabolism. Lineweaver–Burk plots have been constructed for propranolol metabolism alone (■), and in the presence of 10 μM DMI (□), 15 μM DMI (○) and 10 μM chlorpromazine (Δ). The control points represent the mean of 7 experiments and those in the presence of inhibitor at least 3 determinations. Control data have been reproduced in the 2 diagrams for clarity of illustration. V = apparent velocity of reaction, μmole per gram liver per hour. S = substrate concentration, mM.

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Role of steroid hormones in hepatic microsomal enzyme induction*

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THE HYDROXYLATING enzymes associated with hepatic microsomes are active against a wide variety of substrates, including steroids, polycyclic hydrocarbons and many drugs.[1] They are adaptive enzymes; pretreatment of rats and other species with many substrates leads to the induction of increased levels of enzyme activity.[1] These are associated with an increase in the concentration of hepatic microsomal cytochrome P-450,[2] the terminal oxidase of the hydroxylating enzymes.

It seems likely that the principal endogenous substrates of the enzymes are steroids[3–5] and from observations on the effect of castration, adrenalectomy and steroid hormones on enzyme activity,[6] it has been suggested that the levels of microsomal enzyme activity are controlled by the concentration of circulating steroid hormones.[1,7,8] *In vitro* studies have shown that competition occurs when a drug substrate and a steroid hormone are incubated together with liver microsomes.[5] Such competition could occur also *in vivo*, and it has been postulated that the induction of the enzymes by foreign substances, for example, phenobarbitone, is mediated through changes taking place in an endogenous, steroid hormone controlled, regulatory system as a result of interference with endogenous steroid metabolism.[7,8]

There is some controversy as to the role of the steroid hormones in microsomal enzyme induction.[7–9] The experiments reported here represent an attempt to clarify the position, and the results suggest that enzyme induction is independent of these hormones.

A preliminary report of this work has already appeared.[10]

For these experiments castrated, adrenalectomized, castrated and adrenalectomized, sham-operated and intact male rats of the Carworth CFE strain (Sprague–Dawley derived) initially weighing about 140 g were purchased from Carworth Europe (Alconbury, Herts.). The animals were housed in mesh-floored cages in a conventional animal house. Their sole source of drinking water was 1% NaCl solution, available *ad lib.* Although 1% saline was given to all the rats in these experiments, subsequent investigation showed that giving saline to non-adrenalectomized rats had no effect on the enzyme activities measured. Food (modified diet 41B, Oxoid Ltd., Southwark Bridge Road, London, S.E.1) was available *ad lib.* also. All animals grew at a mean rate of between 3 and 6 g/day throughout the period of the experiments. Rats given phenobarbitone received daily i.p. injections of Na phenobarbitone in 0·15 M saline at a dose of 100 mg/kg. Controls were injected with an equivalent volume of saline. Regimes of drug administration were begun on the second day following operation and continued for 7 days, the rats being killed 24 hr after the last injection.

The efficacy of the adrenalectomies was checked by taking a group of four adrenalectomized rats at random from the same batch as those used in the experiments, and giving them water instead of saline. These rats all became moribund within 24 hr. On histological examination of tissue taken post mortem from the vicinity of the adrenals, no adrenal tissue was seen in any of the adrenalectomized animals. Neither was any ectopic adrenal tissue apparent on gross examination. The control rats used in the experiments described were not sham-operated, but in a separate series of experiments no significant differences were seen in any of the quantitites measured between groups of sham-operated and non-operated rats.

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