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Effects of ethanol and phenobarbital on the metabolism of propranolol by 9000 *g* rat liver supernatant

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Results from previous studies have suggested that propranolol might be of value in the treatment of alcoholism [1,2]. In addition, propranolol is widely used in the treatment of cardiac disorders and it might be expected that many patients consume significant amounts of ethanol while on propranolol. It is possible that ethanol might alter propranolol metabolism. Studies were undertaken to determine the effects of ethanol and phenobarbital administration on propranolol metabolism.

Male Sprague–Dawley rats (175–300 g) were used in all studies except where indicated. All rats were decapitated, the livers quickly removed and a 25% w/w homogenate was prepared. The 9000 *g* supernatant was prepared by centrifugation of the homogenate at 4° for 25 min. This preparation was selected as the source of propranolol-metabolizing enzymes since previous studies have shown that the 9000 *g* supernatant is as active as the microsomal fraction [3]. Aliquots of the 9000 *g* supernatant (1.5 ml) derived from 480 mg wet weight of rat liver were incubated in air at 37° with 500 nmoles propranolol and the following cofactors: NADP+ (0.5 μmole), $MgCl_2$ (150 μmoles), nicotinamide (100 μmoles), glucose 6-phosphate (50 μmoles) and 0.2 M phosphate buffer (pH 7.4) in a total volume of 4 ml as described by Shand and Oates [3]. After 15 min of incubation, the reaction was terminated by the addition of 1 ml of 2N NaOH. The unchanged drug was extracted into 1.5% isoamyl alcohol in heptane (12 ml). Propranolol was extracted from the organic layer (10 ml) with 0.1 N HCl (3 ml), and the fluorescent intensity of the aqueous layer was measured as described by Shand *et al.* [4]. The initial amount of propranolol added was determined from control samples prepared in the same manner except that 1 ml of 2 N NaOH was added before incubation. The amount of propranolol metabolized was the difference between the amount of propranolol present at zero time and that following incubation. Initial experiments established that the concentration of propranolol used was sufficient to saturate the enzyme system.

Aniline hydroxylase activity of the 9000 *g* supernatant fraction was measured as described by Imai and Sato [5] since previous studies have shown this enzyme activity to be induced by phenobarbital [6].

After phenobarbital pretreatment (75 mg/kg, i.p., once daily for 3 days), the rate of propranolol metabolism was increased to 142 per cent of control values (Table 1). Therefore, phenobarbital pretreatment enhances hepatic microsomal propranolol metabolism. In addition, phenobarbital pretreatment increased hepatic microsomal aniline hydroxylase activity by 279 per cent of control values (Table 1).

In order to study the effects of short-term administration of ethanol on the metabolism of propranolol, ethanol (2 g/kg, i.p., twice daily) was given to groups of rats for 3 and 7 days. Weight losses averaging 3–5 g/day were observed during the period of ethanol administration. The rats were sacrificed 24 hr after the last dose of ethanol. No significant decrease in the rate of propranolol metabolism was observed (Table 1). In contrast, a significant reduction in the activity of aniline hydroxylase was noted. This observation differs from that reported by Tobon and Mezey [7]. These workers found a significant increase in microsomal aniline hydroxylase activity after daily administration of ethanol (4 g/kg) either by gastric intubation (3 days) or in the diet (7 days) as an ethanol solution. An explanation for this descrepancy is not readily apparent but could be related to differences in route of administration of ethanol, in dietary intake, and in the source of aniline hydroxylase (9000 *g* supernatant versus washed microsomes).

To study the effect of chronic ethanol administration on propranolol metabolism, female Sprague–Dawley rats (initially weighing 60 *g*) were made chronically alcoholic by employing an adaptation of the Ratcliff method [8]. Drinking water was replaced by ethanol in 10% sucrose in tap water. The initial concentration of ethanol was 2.5%, gradually increasing to 25% during week 7 (the final week of the study). Only 10% sucrose in tap water was given to the control group. There was no significant difference between the weight of control and ethanol-treated rats at the end of the study period. Chronic ethanol administration enhanced the rate of propranolol metabolism to 206 per cent above control values. In contrast, no significant change in the activity of aniline hydroxylase activity was observed, suggesting that propranolol metabolism and aniline metabolism by the hepatic microsomes of the rat proceed via different mechanisms. The absence of change in aniline hydroxylase activity in these chronically treated animal is in agreement with the results of Tobon and Mezey [7]. These workers found an initial rise in aniline hydroxylase activity up to 7 days when ethanol was added

Table 1. Metabolism of propranolol and aniline by rat hepatic microsomes before and after treatment with phenobarbital and ethanol.

	N	Rate of propranolol metabolism (nmoles/g wet liver/hr) (mean ± S. E. M.)	Rate of propranolol metabolism Per cent of control	Rate of aniline hydroxylase metabolism (nmoles/g wet liver/hr) (mean ± S. E. M.)	Rate of aniline hydroxylase metabolism Per cent of control
Control	5	1077 ± 109	100	298 ± 34	100
Phenobarbital (3 day)	6	1529 ± 43†	142	833 ± 46†	279
Ethanol (3 day)	3	1032 ± 88	96	178 ± 30†	60
Ethanol (7 day)	6	917 ± 101	85	146 ± 28†	49
Control*	5	642 ± 169	100	494 ± 14	100
Ethanol* (7 weeks)	5	1324 ± 126†	206	525 ± 84	106

* Controls received rat chow *ad lib.* and 10% sucrose as a source of water. Ethanol-treated rats received the same diet except that increasing amounts of ethanol were administered in the 10% sucrose drinking solution. In these chronic studies, female Sprague–Dawley rats instead of males were used.

† $P < 0.01$.

to the diet. However, by 14 days, the aniline hydroxylase activity had returned to control levels even though the rats continued to receive ethanol. In contrast Dobbins *et al.* [9] found an increase in aniline hydroxylase activity after the addition of ethanol in the diet for 5 weeks. It is of interest to note that our ethanol-treated animals continued to receive the same amount of sucrose as the control group, whereas the ethanol-treated rats in the above study did not receive additional carbohydrate in the form of sucrose as they report for their control rats. The presence of sucrose might have suppressed the increased activity of aniline hydroxylase produced by ethanol. Carbohydrate repression of mammalian enzyme induction has been previously described [10–12].

In summary, phenobarbital and chronic ethanol administration to rats significantly enhanced the hepatic microsomal metabolism of propranolol. It is conceivable that phenobarbital and ethanol might cause enhanced propranolol metabolism in man. However, further studies are required to answer this question.

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