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EFFECT OF 6-*n*-PROPYL-2-THIOURACIL ON THE RATE OF ETHANOL METABOLISM IN RATS TREATED CHRONICALLY WITH ETHANOL

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Abstract—Chronic ethanol administration results in an increase in the ethanol metabolic rate (EMR), and this may be related to the production of alcoholic liver disease. Treatment with the antithyroid drug 6-*n*-propyl-2-thiouracil (PTU) for a 10-day period (20 mg·kg^{-1}·day^{-1}) reduced the EMR of chronically ethanol-treated rats, but had no effect on the EMR of control rats. This preferential inhibitory effect of PTU was observed either when PTU treatment was started after 20 days of ethanol consumption, or when ethanol and PTU administration were started at the same time. A single dose of PTU (20 mg/kg), given 1 hr before the experiment, had no effect on the EMR of rats treated chronically with ethanol, or on controls. Ten days of PTU treatment did not alter the hepatomegaly which had resulted from chronic ethanol treatment. These results are consistent with the hypothesis that thyroid hormones play a direct or permissive role in producing the increase in EMR seen after chronic ethanol treatment and are in agreement with an increased reoxidation of reducing equivalents in the liver of chronically ethanol-fed animals.

Chronic ethanol consumption results in an increased ethanol metabolic rate (EMR) in both laboratory animals [1–8] and in man [9–14]. The elevated EMR may be associated with an enhanced rate of hepatic oxygen consumption [7, 15, 16], and may be related to the production of alcoholic liver damage [16–18]. Treatment with the antithyroid drug 6-*n*-propyl-2-thiouracil (PTU) improves the rate of recovery of patients with active alcoholic liver disease [19]. It has also been observed that PTU administration depresses the respiratory rate of liver slices from chronically ethanol-treated rats, before significantly affecting the respiratory rate of liver slices from control animals [20, 21]. It was of interest, therefore, to determine if the EMR of rats treated chronically with ethanol would be preferentially decreased by treatment with PTU.

METHODS

Male Wistar rats with starting weights of 150–195 g were used. Ethanol was given to the rats in a nutritionally adequate, high fat liquid diet, which provided 35% of the total energy (calories) as ethanol, 19% as protein, 41% as fat and 5% as sucrose. In the control (sucrose) diet, ethanol was replaced isocalorically with sucrose. These liquid diets [22] are modelled after those originally described by Lieber *et al.* [23]. Three experiments were performed.

In experiment A, the effect of one dose of PTU on the EMR of ethanol-treated and control rats was examined. Rats were given the ethanol liquid diet for 12 days; control animals were pair-fed with the sucrose liquid diet. Twenty hours before the determination of the EMR, the rats that had received the ethanol diet were switched to the sucrose diet. Both ethanol and control rats received 70 ml of the sucrose diet for the 20-hr period before the measurement of EMR. One hour before the EMR determination, one half of the ethanol-treated animals and their controls were given PTU at a dose of 20 mg/kg body weight, p.o. (dissolved in 0.9% NaCl, to give a volume of 2 ml/100 g). The other half of the animals received an equivalent volume of the vehicle alone.

In experiments B and C, the effects of 10 days of PTU treatment on the EMR of rats treated chronically with ethanol and on controls were studied. In these experiments, the rats were placed in groups of four on the basis of weight (within 5 g). Within every group, each of the following treatments was represented: ethanol, sucrose, ethanol plus PTU, and sucrose plus PTU. PTU was administered by adding it to the diet to give a concentration of 100 mg/liter. This resulted in a daily PTU consumption of approximately 20 mg/kg body weight. In experiment B, rats were treated with ethanol for 30 days, with PTU given for the last 10 days. Each control rat was pair-fed with an ethanol rat during the period before PTU treatment began. In experiment C, the rats were given ethanol diet for 10 days and PTU was administered concurrently. It was observed that rats receiving the ethanol diet plus PTU consumed slightly less diet than rats receiving the ethanol diet alone. For this reason, the amount of diet consumed by a rat receiving the ethanol diet plus PTU on one day was given to the other three rats in the group on the following day. Eighteen to twenty hours before the experimental determination of the EMR, the rats receiving ethanol and ethanol plus PTU diets were switched to sucrose and sucrose plus PTU diets, respectively. All rats were given 70 ml of diet for the

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20-hr period before the experiment. Thus, all rats were fed until the determination of the EMR, but the ethanol rats were withdrawn from ethanol.

The rate of ethanol metabolism was determined after an intraperitoneal injection of a 2.5 g/kg dose of ethanol, given as a 12.5% (w/v) solution in 0.9% NaCl. Samples (100 μl) of capillary blood were taken from the cut tails of the animals, using glass microsampling pipettes at 2, 3, 4, 5 and 6 hr after the administration of the test dose. Ethanol was determined enzymatically after deproteinization of the samples as described previously [1]. The animals were decapitated, and the wet weight of each liver was measured. In experiment A, hepatic DNA content was measured using the method of Martin *et al.* [24]. To determine the EMR, a graph of blood ethanol concentration vs time was plotted for each rat. The linear portion of the curve was extrapolated to the abscissa, to yield the theoretical time when the blood concentration was zero. This time value (in hours) was divided into the dose of ethanol administered (in mg/kg) to give mg ethanol metabolized per kg body weight per hr. To calculate the rate of ethanol metabolism in mg ethanol per g liver per hr, the term in $mg \cdot kg^{-1} \cdot hr^{-1}$ was divided by the ratio of liver weight to body weight (in g/kg). This value, in turn, was converted to mg ethanol per total liver per hr by multiplying it by the liver weight in g. The data were either subjected to analysis of variance with differences between mean values assessed by the Newman–Keuls modification of the Studentized Range Test [25], or were analyzed using Student's *t*-test [25].

RESULTS

Ethanol treatment of rats for 12 days resulted in a 21 per cent increase in the liver/body weight ratio, without changing the body weight or the total amount of DNA in the liver (Table 1). This increase in liver weight does not appear to be due to a proliferation of hepatocytes since the total liver content of DNA does not increase, but rather it is caused by elevated amounts of lipid, protein and water in the hepatocytes [26, 27]. In ethanol-treated rats with hepatomegaly, therefore, each gram of liver contains fewer hepatocytes (less DNA) than a gram of liver from control rats. In this situation, the EMR calculated per mg hepatic DNA gives a better indication of the EMR per hepatocyte than the EMR expressed per gram of liver. Alternatively, since total liver DNA was virtually identical in ethanol-treated and control animals (Table 1), the data may be equivalently expressed as metabolic rate per total liver mass.

The results from experiment A are shown in Table 2, with the averages of the EMR expressed per mg hepatic DNA, per total liver, per kg body weight and per g liver. Twelve days of ethanol treatment resulted in an increase in the EMR, relative to controls, when the values were expressed per mg hepatic DNA, per total liver, or per kg body weight, but not when expressed per g liver. The increase in the average EMR per mg hepatic DNA and per total liver in the ethanol-treated rats, without an increase per g liver, is a reflection of the hepatomegaly induced by ethanol. A single dose of PTU (20 mg/kg), given 1 hr before measuring the EMR had no significant effect on the EMR averages of ethanol-treated rats or controls.

The results from experiment B are presented in Fig. 1, with the EMR averages expressed as $mg \cdot (kg\ body\ weight)^{-1} \cdot hr^{-1}$, $mg \cdot (total\ liver)^{-1} \cdot hr^{-1}$ and $mg \cdot (g\ liver)^{-1} \cdot hr^{-1}$. Regardless of units used, 10 days of treatment with PTU reduced the average EMR of the ethanol-treated rats without significantly affecting the average EMR of the control rats. The decrease was 19 per cent when the EMR was expressed per total liver. As in experiment A, chronic consumption of ethanol resulted in an increase in the average EMR relative to the sucrose group, when EMR was calculated per kg body weight (+17 per cent) or per total liver (+16 per cent), but not when expressed per g liver.

Figure 2 presents the EMR averages for experiment C. The results show a pattern very similar to that of experiment B. Ten days of concurrent treatment with PTU depressed the average EMR in the ethanol-treated rats, without significantly affecting the average EMR of the control rats. The magnitude of the reduction in the ethanol-treated rats was 24 per cent, when the EMR was expressed per total liver. Rats consuming the ethanol diet for the 10-day period had an elevated average EMR, compared to those on sucrose diet, when the EMR was expressed per kg body weight or per total liver, as in the above experiments. PTU treatment for 10 days had no significant effect on liver/body weight ratios or liver

Table 1. Effect of chronic ethanol treatment on liver weight, hepatic DNA content, and body weight*

	Sucrose	Ethanol	P
Number of animals	16	16	
Starting weight (g)	169 ± 3	169 ± 3	NS
Final weight (g)	220 ± 3	223 ± 2	NS
Liver weight (g)	8.16 ± 0.14	10.01 ± 0.17	< 0.001 (+23%)
Liver/body weight (g/100 g)	3.71 ± 0.04	4.50 ± 0.07	< 0.001 (+21%)
DNA (mg/g liver)	3.72 ± 0.07	3.09 ± 0.06	< 0.001 (−17%)
DNA (mg/total liver)	30.4 ± 0.9	30.9 ± 0.7	NS

* Rats were fed ethanol or sucrose liquid diets for 12 days (experiment A). Statistical significance was assessed using Student's *t*-test; NS means not significant. The percentage change in the average values for the ethanol-treated animals compared to the sucrose controls is given in parentheses.

Table 2. Effect of acute PTU administration on the rate of ethanol metabolism in rats treated for 12 days with ethanol and controls*

Treatment	Rate of ethanol metabolism [mg·(mg hepatic DNA)$^{-1}$·hr^{-1}]	[mg·(total liver)$^{-1}$·hr^{-1}]	[mg·(kg body wt)$^{-1}$·hr^{-1}]	[mg·(g liver)$^{-1}$·hr^{-1}]
Ethanol	3.19 ± 0.12	104 ± 2	458 ± 11	10.1 ± 0.1
	$P < 0.01$	$P < 0.01$	$P < 0.01$	NS
Sucrose	2.54 ± 0.12	79 ± 2	355 ± 11	9.6 ± 0.2
Ethanol + PTU	3.43 ± 0.12	99 ± 2	454 ± 10	10.2 ± 0.3
	$P < 0.01$	$P < 0.01$	$P < 0.01$	NS
Sucrose + PTU	2.67 ± 0.10	78 ± 2	359 ± 7	9.6 ± 0.2

* Rats were fed ethanol or sucrose liquid diets for 12 days; half of the rats received PTU (20 mg/kg, p.o.) 1 hr before the EMR was determined *in vivo*. Acute PTU administration had no significant effect on the EMR in ethanol-treated or sucrose control rats. There were eight animals per group. NS means not significant.

weights of ethanol-treated or control rats in experiment B or C [for experiment B, liver/body weight ratios (g/100 g) were: ethanol, 4.71 ± 0.10; ethanol + PTU, 4.74 ± 0.09; sucrose, 3.89 ± 0.10; sucrose + PTU, 4.03 ± 0.10; mean ± S.E.M.].

DISCUSSION

PTU preferentially decreased the EMR of chronically ethanol-treated rats when given for a 10-day period, whether started concurrently with ethanol (experiment C) or started after 20 days of ethanol treatment (experiment B). This finding is in agreement with the concept of a thyroid hormone-dependent increase in reoxidation of reducing equivalents playing an important role in metabolic tolerance to ethanol [16, 28]. The rate of hepatic oxygen consumption in chronically ethanol-treated rats is also depressed more quickly by PTU. Israel *et al.* [20] reported that 3, 6 and 10 days of PTU administration (50 mg·kg^{-1}·day^{-1}, p.o.) reduced the respiratory rate of liver slices from rats treated chronically with ethanol, whereas the respiratory rate of liver slices from control animals was only significantly decreased after 12 days of PTU treatment.

PTU is a potent antithyroid drug that inhibits both thyroid hormone synthesis and the peripheral deiodination of thyroxine to triiodothyronine [29, 30]. Repeated administration of PTU is necessary to reduce the thyroidal state of the animal. A single dose of PTU did not reduce the EMR, suggesting that the effect of chronic PTU treatment on ethanol metabolism is most likely related to its anti-thyroid actions. The data in the present study suggest that the EMR of chronically ethanol-treated animals is more sensitive to a reduction in the thyroidal state than the EMR of controls. It is possible that thyroid hormones play a direct or permissive role in producing the increase in EMR seen after chronic ethanol consumption, making the increase in ethanol metabolism more responsive than the basal EMR to the action of PTU. Recent data indicate that the blood levels of thyroid hormones remain unchanged or are slightly decreased after chronic ethanol treatment in rats [31, 32,†]. Thus, an effect of thyroid hormones is more likely to be related to other factors that modify hormonal expression such as the tissue level [28], the number and affinity of thyroid hormone receptors, or the coupling of receptor binding to the effector system. Catecholamines have also been implicated in metabolic tolerance to ethanol [16], and it is known that thyroid hormones play a permissive role in many of the effects of the catecholamines [33]. Thus, the relationship between thyroid hormone expression and catecholamine function is also likely to be of importance in determining the effects of chronic ethanol treatment. It is unlikely that the preferential sensitivity of ethanol-treated rats to PTU is due to a modification of the pharmacokinetic properties of this drug after ethanol administration, since the dose of PTU administered was at least forty times the acute dose necessary to completely inhibit the thyroidal incorporation of iodide [34]. It is also unlikely that PTU reduces the

† Orrego *et al.*, personal communication.

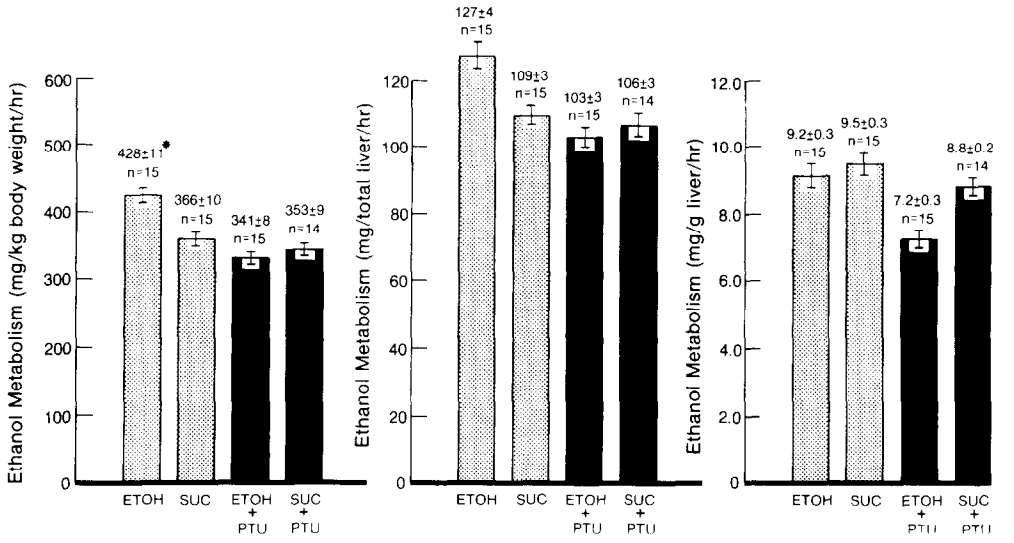

Fig. 1. Effect of 10 days of PTU administration on the rate of ethanol metabolism in rats treated for 30 days with ethanol, and controls. Rats were fed ethanol (ETOH) or sucrose (SUC) liquid diets for 30 days: for the last 10 days, some rats were given PTU in the diet at a concentration of 100 mg/l. Ethanol metabolic rate was determined *in vivo* and expressed per kg body weight, per total liver, and per g liver. When expressed per kg body weight or per total liver: ETOH vs SUC, ETOH vs ETOH + PTU, ETOH vs SUC + PTU, $P < 0.01$; SUC vs ETOH + PTU, SUC vs SUC + PTU, ETOH + PTU vs SUC + PTU, not significant ($P > 0.05$). When expressed per g liver: ETOH vs ETOH + PTU, SUC vs ETOH + PTU, SUC + PTU vs ETOH + PTU, $P < 0.01$; ETOH vs SUC, ETOH vs SUC + PTU, SUC vs SUC + PTU, not significant ($P > 0.05$). The asterisk (*) indicates mean ± S.E.M.; n = number of animals.

EMR of ethanol-treated rats either by decreasing the level of alcohol dehydrogenase or by reducing microsomal metabolism of ethanol. Treatment with PTU has been reported to increase both alcohol dehydrogenase activity [35] and the activity of the microsomal ethanol oxidizing system [36].

Chronic ethanol treatment resulted in an increase in liver weight without changing the total amount of DNA in the liver. Previous studies have also reported that ethanol given with a high fat diet causes an increase in liver weight [26, 27, 37, 38]. Since the total amount of DNA in the liver does not change, this increase in weight does not appear to be due to the proliferation of hepatocytes but rather to elevated amounts of water, lipid and protein in the hepatocytes [26, 27]. As a result of this hepatomegaly, each gram of liver from an ethanol-treated rat contains fewer hepatocytes (less DNA) than a gram of liver from a control rat. Therefore, valid comparisons cannot be made of results expressed per g

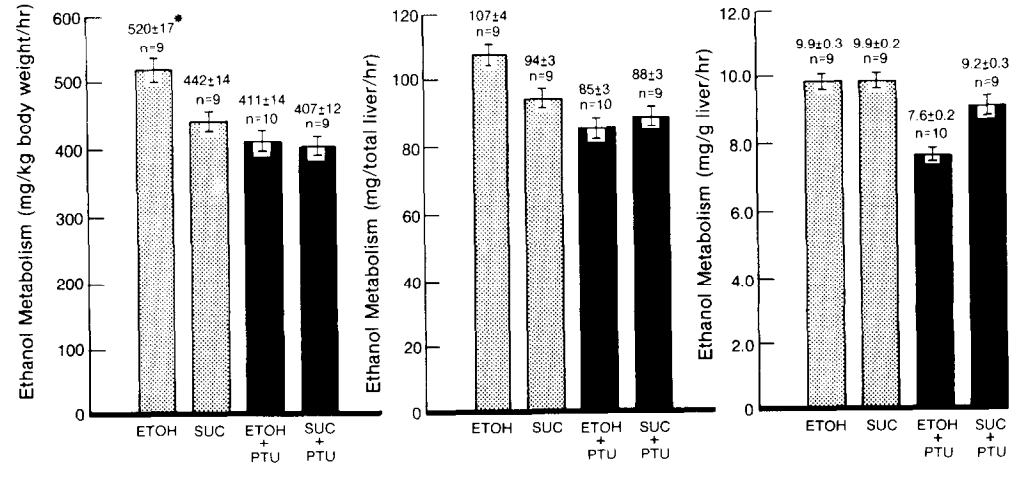

Fig. 2. Effect of concurrent administration of PTU on the rate of ethanol metabolism in rats treated for 10 days with ethanol, and controls. Rats were fed ethanol (ETOH) or sucrose (SUC) liquid diets for 10 days: some rats were given PTU in the diet at a concentration of 100 mg/l. Ethanol metabolic rate was determined *in vivo* and expressed per kg body weight, per total liver, and per g liver. The statistical significance of each comparison between groups was the same as in Fig. 1. The asterisk (*) indicates mean ± S.E.M.; n = number of animals.

liver in this siutation. The best indication of the metabolic rate per hepatocyte is given by expressing the results per unit of hepatic DNA. Alternatively, in cases where the total amount of hepatic DNA is not changed by treatment, results expressed per total liver mass can be compared.

In the present experiments, chronic ethanol treatment for 10, 12 or 30 days resulted in an increase in the EMR per hepatocyte. This increase in the EMR may be accompanied by an enhanced rate of total hepatic oxygen consumption [7, 15, 16], and may be related to alcoholic liver damage [16–18]. The action of PTU in preferentially reducing the EMR of ethanol-treated rats is consistent with the concept that thyroid hormones may play a direct or permissive role in producing the increase in EMR and attendant processes which may lead to liver damage.

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