Biochemical Pharmacology, Vol. 34, No. 13, pp. 2377–2383, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

INHIBITORY EFFECT OF PROPYLTHIOURACIL ON THE DEVELOPMENT OF METABOLIC TOLERANCE TO ETHANOL

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(*Received* 1 *August* 1984; *accepted* 31 *December* 1984)

Abstract—Chronic ethanol administration (4–5 weeks) to female spontaneously hypertensive (SH) rats led to a marked increase in the rate of ethanol metabolism. This was accompanied by an increase in hepatic alcohol dehydrogenase (ADH) and by an increase in the rate of oxygen consumption in perfused livers of these animals. Treatment with the antithyroid drug 6-*n*-propyl-2-thiouracil (PTU) during the last 9 days (40 mg/kg/day) of the chronic administration of ethanol reduced hepatic oxygen consumption, resulting in a net diminution of the metabolic tolerance to ethanol, despite a further elevation in ADH activity. In these animals, microsomal ethanol-oxidizing system (MEOS) activity was not affected by chronic ethanol administration or by treatment with PTU. Data strongly suggest that in the female SH rat all the metabolic tolerance to ethanol proceeds via the ADH pathway, and that the increase in hepatic oxygen consumption is more important in the development of metabolic tolerance to ethanol than the increased ADH levels.

Chronic ethanol intake results in an increase in the rate of ethanol metabolism (metabolic tolerance to ethanol) of the order of 30–100% in experimental animals [1–9] and in humans [10–16].

Two main mechanisms have been proposed to be responsible for the development of metabolic tolerance to ethanol: (1) an increase in ethanol oxidation through the alcohol dehydrogenase (ADH) pathway [17–19], and (2) an increase in ethanol metabolism via the microsomal ethanol-oxidizing system (MEOS) pathway [2, 20]. There is a general agreement, by investigators in the field, that most of the increase in ethanol metabolism following chronic ethanol intake is primarily mediated by the ADH system [3, 9, 21–25].

Metabolic tolerance to ethanol via the ADH pathway may occur by: (1) an increase in the actual amount (level) of ADH (V_{max} determined under optimal conditions of substrate and cofactor availability), and/or (2) an increase in the rate of mitochondrial oxidation of NADH, which may be expressed as an increase in the rate of hepatic oxygen consumption.

Experiments conducted in our laboratory have shown that chronic ethanol administration to female SH rats leads to the development of a marked and highly reproducible metabolic tolerance to ethanol, which is accompanied by increases in both hepatic oxygen consumption and ADH activity [26]. The present studies were carried out in order to assess the relative importance of an increased hepatic oxygen consumption versus an increased ADH activity to the development of metabolic tolerance to ethanol in the female SH rat. This was done by chronically administering the antithyroid drug 6-*n*-propyl-2-thiouracil (PTU) which has been found to reduce the rate of hepatic oxygen consumption [27], but to increase hepatic ADH levels [28]. Thus, since PTU has opposing actions on the two main controlling factors of ethanol metabolism via the ADH pathway, the net effect of PTU administration on the rate of ethanol metabolism can give an indication as to which of the two factors plays a more prominent role in the development of metabolic tolerance following chronic alcohol consumption.

In these studies, we have investigated the effect of PTU administration on the rate of ethanol metabolism, liver ADH activity, hepatic oxygen consumption and MEOS activity in female SH rats chronically fed with ethanol and in their carbohydrate-fed controls. Data obtained suggest that the contribution of the increased rate of oxidation of NADH, expressed as an increased rate of oxygen consumption, is more important for the development of metabolic tolerance to ethanol than an increased ADH level.

MATERIALS AND METHODS

Chronic ethanol treatment and PTU administration. Female spontaneously hypertensive (SH) rats (Taconic Farms, Germantown, NY) 4 weeks of age (body weight: 51 ± 1 g) were housed individually in wiremesh cages. One group of animals received Lieber-type liquid diets containing 35% of the total calories as ethanol, 19% as proteins, 41% as fat and 5% as sucrose [29], for a period of 4–5 weeks. The control animals were pair-fed with

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equivalent diets in which ethanol was substituted isocalorically with sucrose.

PTU was administered to these animals only in the last 9 days of chronic ethanol treatment, by adding it to their ethanol and sucrose liquid diets at a final concentration of 100 mg/liter. For the administration of PTU, the animals were divided into groups of four, according to body weight. Each group consisted of: (a) ethanol-fed rat + PTU, (b) sucrose-fed rat + PTU, (c) ethanol-fed control rat and (d) sucrose-fed control rat. In each group, the caloric intake during PTU treatment was controlled by the animal with the lowest caloric intake per day. The average caloric intake per kg body weight throughout the chronic ethanol treatment was higher in the ethanol-fed rats than in the sucrose controls (464 ± 10 vs 397 ± 6 kcal/kg body wt/day, $P < 10^{-5}$, in the ethanol-fed and sucrose-fed rats respectively; and 478 ± 13 vs 411 ± 9 kcal/kg body wt/day, $P < 10^{-3}$, in the ethanol-fed + PTU and sucrose-fed + PTU rats respectively), due to a lower growth rate in the former animals.

Eighteen to twenty hours before the *in vivo* determination of the rates of ethanol metabolism or of the rates of oxygen consumption in the perfused livers, the rats receiving ethanol and ethanol + PTU diets were switched to sucrose and sucrose + PTU diets respectively. All diets were administered *ad lib.* for this period.

Determination of the rate of ethanol metabolism in vivo. Ethanol was determined in tail-vein blood samples taken 2, 3, 4, 5 and 6 hr after the intraperitoneal administration of 2.5 g/kg ethanol in saline, as a 12.5% (w/v) solution. The samples were deproteinized and ethanol was assayed enzymatically, as described by Hawkins *et al.* [1]. Blood ethanol concentrations were plotted as a function of time, and the intercept at the abscissa was taken as the time at which all the administered ethanol was metabolized. Ethanol metabolic rate in terms of mmoles ethanol/kg/hr was obtained by dividing the dose of ethanol administered (mmoles/kg body wt) by the period of time required for its complete elimination (hr). The value obtained was divided by the ratio of wet liver weight to body weight (g liver/kg body weight) and multiplied by 1.0×10^3, in order to express the rate of ethanol metabolism in terms of μmoles ethanol/g liver/hr.

Liver perfusion. A non-recirculating liver perfusion system was used. The animals were anesthetized with pentobarbital (60 mg/kg, i.p.) and heparinized (2000 U.S.P. units injected into the spleen), and the portal vein was cannulated following the procedure of Miller [30]. The perfusion medium was hemoglobin-free bicarbonate buffer [31], containing 5.5 mM glucose and saturated with 95% O_2:5% CO_2, with a pH of 7.4 and temperature of 37°. The portal vein was quickly cannulated and flow through the liver was started immediately, such that the interruption of tissue oxygenation did not exceed 15–30 sec. The inferior vena cava below the right kidney was immediately cut to allow outflow of the perfusate. A cannula was placed in the inferior vena cava above the liver, and the inferior vena cava above the right kidney was then tied off. The liver was removed and transferred to the perfusion apparatus while maintaining perfusion flow. The oxygen tension was measured in the perfusion fluid entering and leaving the liver using a Clark oxygen electrode (Yellow Springs Instruments, Yellow Springs, OH, U.S.A.). Perfusion flow was regulated to keep the outflow oxygen tension between 71 and 142 mm Hg, using a variable speed peristaltic pump. Total liver oxygen consumption was calculated by taking the product of the flow rate and the difference between inflow and outflow oxygen content of the buffer. This value was divided by the animal's body weight and by the animal's liver weight to give liver oxygen consumption per kg body weight and per g liver respectively.

After a 30-min control period, ethanol was added to the perfusion medium at a concentration of 1.5 mM. The rate of ethanol metabolism in the perfused livers was determined by measuring inflow and outflow ethanol concentrations at 10 and 15 min following the start of perfusion with ethanol. The differences between the inflow and outflow concentrations of ethanol across the liver were determined in triplicate. Minor losses of ethanol from the perfusion system were corrected for by measuring inflow and outflow ethanol concentrations without a liver present.

Alcohol dehydrogenase (ADH) activity. ADH activity in the liver was determined essentially as described by Crow *et al.* [32]. Liver samples were homogenized in 3 vol. of 0.05 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), pH 8.4, containing 0.33 mM dithiothreitol and centrifuged at 40,000 *g* for 45 min at 4°. Aliquots (0.02 ml) of the supernatant fractions obtained were assayed for ADH activity at 37° in 2 ml of a reaction mixture containing 0.5 M Tris buffer, pH 7.4, 2.8 mM NAD and 10 mM ethanol. The activity was calculated from the initial rate of NADH production and expressed per g of liver and per kg body weight.

Microsomal ethanol oxidizing system (MEOS) activity. MEOS activity was assayed in washed liver microsomes as described by Lieber *et al.* [33]. For each sample, duplicate incubations were performed for periods of 0, 5 and 10 min to verify the linearity of the reaction. After an overnight diffusion period at room temperature, the concentration of acetaldehyde bound to the semicarbazide was determined spectrophotometrically at 224 nm as described by Gupta and Robinson [34]. MEOS activity was calculated from the rate of the reaction (taken as the slope obtained from linear regression analysis of a plot of optical absorbance vs reaction times) and expressed per mg protein, per g liver and per kg body weight. Values per g liver and per kg body weight were further corrected for microsomal recovery.

Cytochrome P-450 content. Cytochrome P-450 content in the homogenate was measured by the method of Greim [35], as described by Estabrook *et al.* [36]. Homogenate cytochrome P-450 content was calculated from the difference in absorbance of reduced cytochrome P-450 bound to CO between 450 and 510 nm, using the extinction coefficient of 100 $mM^{-1}\,cm^{-1}$. Baseline difference in absorbance was subtracted from this value. Microsomal cytochrome P-450 content was measured by the method

Table 1. Body weight, liver weight, and thyroid weight in female SH rats given chronically ethanol or sucrose liquid diets with and without 9 days of PTU treatment*

Treatment group	Body wt (g)	Liver wt (g)	Liver wt/ Body wt (%)	Thyroid wt (mg)
Ethanol	114 ± 2 $P < 0.001$†	6.92 ± 0.18	6.08 ± 0.17 $P < 0.001$†	5.4 ± 0.2
Sucrose	129 ± 2	6.60 ± 0.15	5.16 ± 0.16	5.9 ± 0.4
Ethanol + PTU	123 ± 3	7.98 ± 0.22 $P < 10^{-5}$‡ $P < 0.02$§	6.51 ± 0.21 $P < 10^{-5}$‡ $P < 0.001$§	11.8 ± 0.8 $P < 10^{-7}$‖ $P < 10^{-7}$§
Sucrose + PTU	127 ± 3	6.48 ± 0.14	5.13 ± 0.12	13.1 ± 0.4

* Values represent means ± S.E.M. (N = 16 per group). Only statistically significant differences are indicated ($P < 0.05$).
†, ‡ Effect of ethanol: † Ethanol vs Sucrose; and ‡ Ethanol + PTU vs Sucrose + PTU.
§, ‖ Effect of PTU; §Ethanol + PTU vs Ethanol; and ‖Sucrose + PTU vs Sucrose.

of Omura and Sato [37]. Cytochrome P-450 content in the microsomes was calculated from the difference in absorbance of reduced cytochrome P-450 bound to CO between 450 and 490 nm using the extinction coefficient of 91 $mM^{-1}\,cm^{-1}$, minus the difference in absorbance for baseline.

Protein determination. Protein was determined by the method of Lowry *et al.* [38], using bovine serum albumin as a standard.

Statistical analysis. Results are presented as means ± S.E.M. Statistical comparisons were made using the two-tailed Student's *t*-test for unpaired data, unless otherwise indicated. Differences were considered to be significant when a probability value of less than 0.05 was obtained.

RESULTS

Body weight, liver weight and thyroid weight. Chronic ethanol administration to female SH rats resulted in significant increases in the liver to body weight ratios in the ethanol-fed rats (+18%) and PTU-treated ethanol-fed rats (+27%), when compared to their respective sucrose controls (Table 1). In the ethanol-fed rats, the increase in liver to body weight ratio was due primarily to a reduction in body weight relative to sucrose-fed controls; while in the PTU-treated ethanol-fed rats increased liver size relative to PTU-treated sucrose-fed controls can account for most of the increase in liver to body weight ratio. PTU administration resulted in small but significant increases in liver weight and liver to body weight ratio (+15% and 7% respectively) in the ethanol-fed rats, while having no effect in the sucrose-fed rats. The weight of the thyroid glands was markedly elevated in the ethanol-fed and sucrose-fed rats following the 9 days of treatment with PTU, indicating that the animals became hypothyroid.

In vivo *ethanol metabolism and* in vitro *ADH activity.* The effects of chronic ethanol treatment and of PTU administration on the rate of ethanol metabolism and on ADH activity are presented in Table 2. The rate of ethanol metabolism, expressed per kg body weight, was increased markedly in the chronically ethanol-fed rats relative to that found in the sucrose-fed controls (+49%). This increase in the rate of ethanol metabolism was accompanied with an increase in ADH activity (+31%). PTU administration for 9 days reduced the rate of ethanol metabolism by 31% in the ethanol-fed rats, while having only a small effect in the sucrose control rats (−12%). The reduction in ethanol metabolism by PTU in the ethanol-fed rats occurred despite a 32%

Table 2. Effect of 9 days of PTU treatment on the rate of ethanol metabolism and ADH activity in female SH rats chronically fed with ethanol and their sucrose controls*

	Ethanol metabolism		ADH activity	
Treatment group	(mmoles/kg body wt/hr)	(μmoles/g liver/hr)	(mmoles/kg body wt/hr)	(μmoles/g liver/hr)
Ethanol	15.35 ± 0.53 $P < 10^{-7}$†	270.0 ± 7.4 $P < 10^{-5}$†	13.03 ± 0.64 $P < 0.002$†	229.2 ± 1.0
Sucrose	10.27 ± 0.26	215.9 ± 3.3	9.96 ± 0.48	209.4 ± 8.3
Ethanol + PTU	10.59 ± 0.30 $P < 0.001$‡ $P < 10^{-6}$§	175.0 ± 4.8 $P < 10^{-8}$§	17.20 ± 0.35 $P < 10^{-5}$‡ $P < 10^{-4}$§	284.3 ± 7.4 $P < 0.05$‡ $P < 0.001$§
Sucrose + PTU	9.00 ± 0.16 $P < 0.001$‖	185.9 ± 5.2 $P < 0.001$‖	11.83 ± 0.74	245.0 ± 16.5

* Values represent means ± S.E.M. (N = 10 per group). Only statistically significant differences are indicated ($P < 0.05$).
†, ‡ Effect of ethanol: †Ethanol vs Sucrose; and ‡Ethanol + PTU vs Sucrose + PTU.
§, ‖ Effect of PTU: §Ethanol + PTU vs Ethanol; and ‖Sucrose + PTU vs Sucrose.

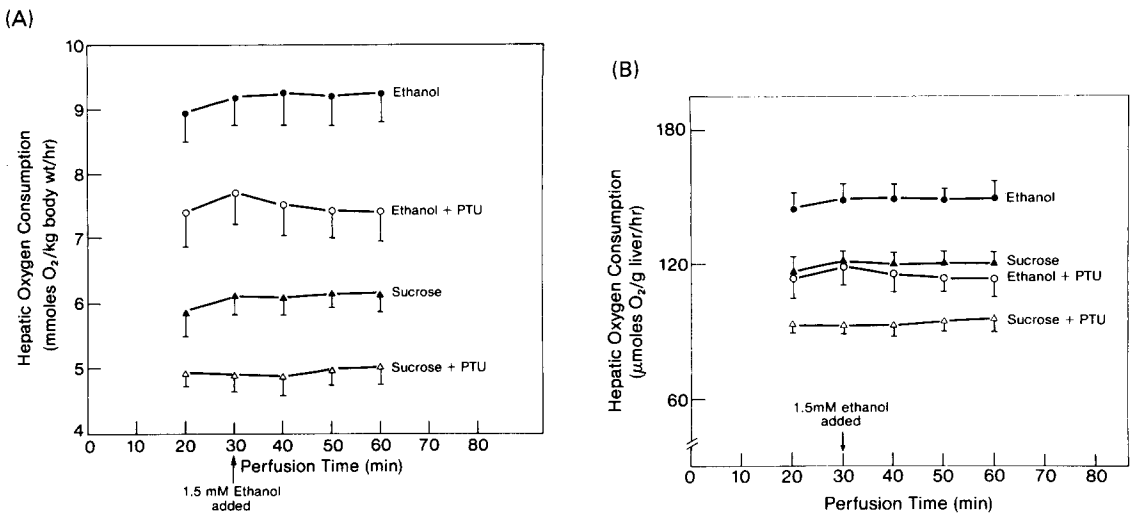

Fig. 1. Effect of 9 days of PTU treatment on oxygen consumption per kg body weight (A) and per g liver (B) in the perfused livers of chronically ethanol-fed female SH rats and their sucrose controls. Each point represents mean ± S.E.M. (N = 11–13 animals per treatment group). Statistical significance was assessed by analysis of variance, and only statistically significant differences are indicated. Overall effect of ethanol, $P < 10^{-3}$; overall effect of PTU, $P < 10^{-3}$; interaction between ethanol and PTU (per kg body weight), $P < 0.01$.

stimulation in ADH activity, relative to ethanol-fed controls. Alcohol dehydrogenase activity was not changed significantly by PTU in the sucrose-fed controls. The absolute metabolic tolerance to ethanol in the presence of PTU was reduced by 70% ($P < 10^4$), when compared to the absolute metabolic tolerance in the absence of PTU. This occurred despite a 75% increase ($P < 0.03$) in the absolute difference in ADH activity between ethanol and sucrose rats, when compared in the presence and absence of PTU treatment. A similar pattern emerges when ethanol metabolic rate and ADH activity are expressed per g liver (Table 2), except that the differences in these parameters between ethanol-fed with and without PTU treatment and their respective sucrose controls are smaller. This is due to higher liver to body weight ratios observed in the ethanol-fed rats relative to their controls (Table 1).

Oxygen consumption and ethanol metabolism in perfused livers. As shown in Fig. 1A, chronic ethanol treatment to female SH rats resulted in large significant increases in the rate of hepatic oxygen consumption, expressed per kg body weight, relative to sucrose controls (+49 to +53%). These increases occurred both in the presence and absence of ethanol in the perfusion medium. PTU treatment for 9 days significantly reduced the rate of oxygen consumption in the livers of both ethanol-fed and sucrose-fed rats. The net decrease in the rate of oxygen consumption by PTU was greater in the ethanol-fed rats ($P < 0.01$). When hepatic oxygen consumption is expressed per g liver, a similar pattern was also obtained (Fig. 1B).

The rates of ethanol metabolism in the perfused livers of chronically ethanol-fed rats were increased markedly relative to sucrose controls (+ 168% per kg body weight and + 134% per g liver, Table 3). The increase in oxygen consumption could stoi-

Table 3. Ethanol metabolism in the perfused livers of chronically ethanol-fed female SH rats treated with PTU and their respective controls*

	Ethanol metabolism	
Treatment group	(mmoles/kg body wt/hr)	(μmoles/g liver/hr)
Ethanol	7.49 ± 0.49	121.7 ± 8.7
	$P < 10^{-5}$†	$P < 10^{6}$†
Sucrose	2.80 ± 0.29	52.0 ± 4.8
Ethanol + PTU	4.96 ± 0.48	76.5 ± 8.0
	$P < 0.002$‡	$P < 0.05$‡
	$P < 0.002$§	$P < 0.001$§
Sucrose + PTU	2.87 ± 0.22	55.7 ± 5.2

* Values represent means ± S.E.M. (N = 11–12). Only statistically significant differences are indicated ($P < 0.05$).

†, ‡ Effect of ethanol: †Ethanol vs Sucrose; and ‡Ethanol + PTU vs Sucrose + PTU.

§ Effect of PTU: Ethanol + PTU vs Ethanol.

Table 4. Effect of 9 days of PTU treatment on microsomal ethanol-oxidizing system activity in female SH rats chronically fed with ethanol and their sucrose controls*

	MEOS activity		
	nmoles acetaldehyde	μmoles acetaldehyde	mmoles acetaldehyde
Treatment group	mg protein × min	g liver × hr	kg body wt × hr
Ethanol	5.69 ± 0.63	23.06 ± 2.96	1.53 ± 0.17
Sucrose	4.55 ± 0.50	19.32 ± 1.98	1.13 ± 0.13
Ethanol + PTU	4.38 ± 0.40	16.46 ± 2.07	1.18 ± 0.14
Sucrose + PTU	4.38 ± 0.46	20.22 ± 1.69	1.13 ± 0.09

* Values represent means ± S.E.M. (N = 6 per group). No statistically significant differences were observed in MEOS activity expressed per mg protein, per g liver and per kg body wt. Values expressed per g liver and per kg body weight are corrected for microsomal recovery. The recovery factors were calculated from the ratio of microsomal P-450 content to homogenate cytochrome P-450 content (see Table 5). The average recovery factors, which were not significantly different between the treatment groups, were: ethanol, 43.3 ± 3.9%; sucrose, 41.7 ± 1.3%; ethanol + PTU, 46.7 ± 2.7%; and sucrose + PTU, 43.1 ± 2.0%.

chiometrically account for 63% of the metabolic tolerance to ethanol in these livers. In line with the *in vivo* findings, the administration of PTU significantly reduced the rate of ethanol metabolism in the perfused livers of the ethanol-fed rats, while having no effect in the sucrose control rats. Thus, increases in the rate of ethanol metabolism in the PTU-treated ethanol-fed rats relative to PTU-treated sucrose-fed rats were much smaller than in ethanol-fed versus sucrose-fed control rats.

MEOS activity and cytochrome P-450 content. Chronic ethanol administration to female SH rats had no significant effect on MEOS activity, whether expressed per mg protein, per kg body weight or per g liver (Table 4). Similarly, PTU treatment had no significant effect on MEOS activity in the ethanol-fed rats nor in the sucrose-fed rats. Liver homogenate and microsomal cytochrome P-450 content were also not affected by chronic ethanol administration in the female SH rat (Table 5). In the sucrose-fed rats, PTU treatment resulted in significant increases in liver homogenate and microsomal cytochrome P-450 content, when expressed per mg protein, per g liver and per kg body weight. PTU administration to chronically ethanol-fed rats resulted in small statistically non-significant increases in cytochrome P-450 content, except when microsomal P-450 was expressed per mg protein (+29%, $P < 0.02$; Table 5).

To determine whether the female SH rats also show a relative insensitivity to another known microsomal inducer, other than ethanol, they were administered phenobarbital (50 mg/kg, i.p.) for 6 days. This treatment resulted in a marked increase in cytochrome P-450 in liver homogenates, when expressed as nmoles per g liver (31.3 ± 1.4 vs 83.6 ± 5.7; +167%, $P < 10^{-5}$), or as μ moles per kg body weight (1.49 ± 0.07 vs 4.56 ± 0.32; +206%, $P < 10^{-5}$). Thus, cytochrome P-450 can indeed be markedly induced in the female SH rat but this depends on the inducer substance used.

DISCUSSION

Chronic ethanol administration to female SH rats results in a large increase in the rate of ethanol metabolism per kg body weight (approximately 50%), which is accompanied by increases in both hepatic oxygen consumption and ADH levels. The administration of PTU, which reduced hepatic oxygen consumption but increased ADH levels, resulted in a significant reduction in the rate of ethanol metabolism in the chronically ethanol-fed rats. The latter strongly suggests that, in the female SH rat, the contribution of increased mitochondrial oxidation of NADH (expressed as an increase in hepatic oxygen consumption) plays a more important role in the

Table 5. Effect of 9 days of PTU treatment on liver homogenate and microsomal cytochrome P-450 content in female SH rats chronically fed with ethanol and their sucrose controls*

	Homogenate cytochrome P-450			Microsomal cytochrome P-450		
Treatment group	nmoles/mg protein	nmoles/g liver	μmoles/kg body wt	nmoles/mg protein	nmoles/g liver	μmoles/kg body wt
Ethanol	0.23 ± 0.01	55.99 ± 3.14	3.76 ± 0.19	0.84 ± 0.04	23.94 ± 2.05	1.61 ± 0.15
Sucrose	0.24 ± 0.01	54.25 ± 3.39	3.16 ± 0.22	0.76 ± 0.04	22.47 ± 1.13	1.31 ± 0.08
Ethanol + PTU	0.30 ± 0.03	68.03 ± 7.77	4.88 ± 0.51	1.08 ± 0.07 $P < 0.02$†	32.23 ± 4.95	2.30 ± 0.33
Sucrose + PTU	0.35 ± 0.02 $P < 0.002$‡	84.50 ± 7.00 $P < 0.02$‡	4.70 ± 0.36 $P < 0.005$‡	1.09 ± 0.08 $P < 0.01$‡	36.79 ± 3.98 $P < 0.01$‡	2.04 ± 0.21 $P < 0.01$‡

* Values represent means ± S.E.M. (N = 6 per group). Only statistically significant differences ($P < 0.05$) are indicated.
†, ‡ Effect of PTU: †Ethanol + PTU vs Ethanol; and ‡Sucrose + PTU vs Sucrose.

development of metabolic tolerance to ethanol than the increased ADH levels. PTU also depressed the rate of ethanol metabolism *in vivo* in the control female SH rats, suggesting that reoxidation of NADH is the main rate-determining factor in ethanol metabolism in these animals. The reductions in hepatic oxygen consumption and ethanol metabolism were greater in the ethanol-fed female SH rats than in the controls. In other studies in Wistar rats, oxygen consumption measured in liver slices [27] and the rate of ethanol metabolism measured *in vivo* [39] have also been observed to be more sensitive to PTU administration in ethanol-fed than in control animals. In addition, recent studies in perfused livers of Sprague–Dawley rats have shown that increases in oxygen consumption and in ethanol metabolism, induced by a single large dose of ethanol given before sacrifice, can both be abolished by pretreatment with PTU [40].

PTU is a potent antithyroid drug, which inhibits both thyroid synthesis and peripheral deiodination of thyroxine to triidothyronine [41, 42]. Previous studies in our laboratory suggest that thyroid hormones may play a permissive role in the production of the liver hypermetabolic state following chronic ethanol consumption [18]. This may explain the finding that the increased rates of hepatic oxygen consumption and of ethanol metabolism in the ethanol-fed rats are more responsive to the action of PTU than the basal rates. Another possibility is that the greater sensitivity of ethanol-fed rats to PTU is due to a modification of the pharmacokinetic properties of this drug by chronic ethanol consumption. However, since the dose of PTU administered to the animals was quite high, at least eighty times the acute dose necessary to completely inhibit thyroidal incorporation of iodide [43], changes in pharmacokinetic parameters are not likely to influence drug effect. Further, thyroid enlargement induced by PTU was not different in the ethanol-treated and control animals.

We have reported previously that in female Sprague–Dawley rats chronic ethanol administration does not increase ADH activity, and even slightly reduces it [44]. These strain differences in ADH inducibility by ethanol are in line with recent studies by Wang and Singh [45], who showed that in different inbred strains of mice liver ADH activity can be either "induced" or "repressed" by chronic alcohol feeding. These latter studies suggested a single gene difference for ADH inducibility.

While in the perfused liver preparations from female SH rats the rates of ethanol metabolism were lower than those *in vivo*, qualitatively the pattern of ethanol metabolism rates in perfused livers of the different treatment groups was similar to that observed *in vivo*. Quantitatively, the increase in the rate of ethanol metabolism in the chronically ethanol-fed rats relative to sucrose rats was greater *in vitro* then *in vivo* (+168% vs +49%), while the small reduction in the *in vivo* rate of ethanol metabolism in the PTU-treated sucrose-fed rats relative to sucrose controls was not observed in the perfused liver preparations of these animals. These differences might be due to different conditions *in vitro* from those *in vivo*, such as the type of perfusion medium and the flow rates that were employed, in these experiments, as compared with circulating blood in the whole animal, or to the low concentration of ethanol (1.5 mM) that was used in the liver perfusion experiments.

Oxygen consumption measured in the perfused livers of female SH rats showed a similar pattern to the rate of ethanol metabolism *in vivo*. Chronic ethanol consumption resulted in a large increase in hepatic oxygen consumption, while PTU treatment for 9 days significantly reduced the rate of oxygen consumption in the livers of the chronically ethanol-fed rats and their sucrose controls, the net reduction being larger in magnitude in the former animals. In addition to the increase in hepatic oxygen consumption induced by chronic ethanol treatment, the proportion of the total oxygen consumption that was utilized for ethanol metabolism in the perfused livers of the chronically ethanol-fed animals was greater than that observed in sucrose control livers (+81% vs 38%), which would further indicate the importance of the contribution of mitochondrial oxidation of NADH and oxygen consumption to the development of metabolic tolerance to ethanol in the female SH rat.

Studies in the literature, using Sprague–Dawley and Wistar rats, have shown that chronic ethanol intake leads to proliferation of the smooth endoplasmic reticulum, and to increases in cytochrome P-450 content and MEOS activity in the liver [46]. In the present study with female SH rats, chronic ethanol administration did not have an effect on hepatic cytochrome P-450 content or on MEOS activity. The latter suggests that increased MEOS activity is not obligatory for the development of metabolic tolerance to ethanol, at least in females of this rat strain. We have reported previously that, in male spontaneously hypertensive rats, MEOS is indeed induced by ethanol [47]. The lack of inducibility of cytochrome P-450 by ethanol in the female SH rat does not seem to be due to a general insensitivity to microsomal inducers, since we observed that phenobarbital administration to these animals resulted in marked increases in hepatic cytochrome P-450 content. As well, PTU treatment significantly increased the level of cytochrome P-450 in the livers of the sucrose-fed control female SH rats. Moreno *et al.* [48] observed that the administration of PTU (7 days, 50 mg/kg) increases MEOS activity in female Sprague–Dawley rats. In the female SH rats, we found that PTU (9 days, 40 mg/kg) had no effect on MEOS activity.

In conclusion, in the female SH rat, metabolic tolerance to ethanol appears to occur via the ADH pathway. The increase in the rate of ethanol metabolism is accompanied by increases in both liver oxygen consumption and in the amount of hepatic ADH. However, the contribution of the increased hepatic oxygen consumption appears to be more important to the development of metabolic tolerance to ethanol in the female SH rat than the increased ADH levels. The studies support the hypothesis that an increased reoxidation of NADH mediated by an increased oxygen consumption constitutes an important mechanism of metabolic tolerance induced by chronic alcohol intake.

Acknowledgements—The authors are grateful to Mr. B. Oporto for his valuable technical assistance during this work.

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