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THE SWIFT INCREASE IN ALCOHOL METABOLISM

INHIBITION BY PROPYLTHIOURACIL*

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Abstract—Administration of a single large dose of ethanol (5 g/kg) to rats elevates the rates of ethanol metabolism and of oxygen consumption in perfused livers in 2–3 hr. Pretreatment with the antithyroid drug propylthiouracil (PTU) for 10 days abolished both of these effects. Under all treatment conditions studied (controls; PTU-pretreatment; acute ethanol treatment; PTU-pretreated + acute ethanol treatment), a significant correlation between ethanol metabolism and oxygen consumption was observed ($r = 0.86$). It is concluded that a normal thyroidal state is required to evoke the swift increase in alcohol metabolism (SIAM) and an elevation of oxygen consumption.

Numerous studies have documented the fact that chronic treatment with ethanol increases the rate of ethanol elimination in humans [1–3], experimental animals [4–7], and a variety of *in vitro* preparations of liver [6, 8]. Recently, Yuki and Thurman [9] showed that, after a large dose of ethanol, this adaptive increase was observed in a few hours, both *in vivo* in the rat [10] and in the perfused liver (i.e. the swift increase in alcohol metabolism, SIAM). Ethanol uptake by the perfused liver was nearly doubled 2.5 hr after ethanol pretreatment *in vivo* (5.0 g/kg). This phenomenon was associated with a marked elevation in hepatic oxygen uptake which could be accounted for, in part, by depressed rates of glycolytic ATP production [9].

Using liver slices, Israel *et al.* [11] showed that the increases in ethanol uptake and oxygen consumption following chronic treatment with ethanol could be blocked by treatment with the antithyroid drug, 6-*n*-propyl-2-thiouracil. Hypothyroidism and propylthiouracil treatment are known to increase glycogen reserves in the liver which might lead to an increase in the glycolytic rate [12, 13]. The purpose of these studies, therefore, was to examine the effect of propylthiouracil on the swift increase in alcohol metabolism, oxygen uptake, and rates of glycolysis in the perfused rat liver. The data indicate that propylthiouracil pretreatment indeed prevents the SIAM effect, although its action is not mediated by an effect on glycolytic flux.

MATERIALS AND METHODS

Animals. Female albino rats (Sprague–Dawley, 80–150 g) received laboratory chow and water *ad lib.* Animals in the ethanol-pretreated group received one single dose of 10% ethanol orally (5.0 g/kg) 2.5 hr prior to surgery. Controls were intubated with corn oil. Propylthioracil (50 mg/kg daily; in corn oil) was administered by gastric intubation for 10 days. A hypothyroid state was evidenced by marked enlargement in the thyroid glands.

Non-recirculating liver perfusion. The non-recirculating liver perfusion technique has been described previously [14]. Krebs–Henseleit bicarbonate buffer (pH 7.4) [15], saturated with oxygen (95%) and carbon dioxide (5%), was pumped into the liver via a cannula in the vena cava and flowed past an oxygen electrode before it was discarded. Ethanol was infused into the influent perfusate. The oxygen concentration in the effluent perfusate was determined with a Teflon-shielded platinum electrode. Metabolic rates were calculated from influent–effluent concentration differences and the constant flow rate and were expressed per gram wet weight of the liver.

Analytical. Samples of effluent perfusate were collected every 2 min and analyzed for glucose, lactate, pyruvate, and ethanol by standard enzymatic procedures [16].

RESULTS

Experiments of the type depicted in Fig. 1 were carried out on livers from four groups of animals: (a) controls, (b) ethanol (2.5 hr) pre-treated, (c) propylthiouracil-pretreated and (d) ethanol + propylthiouracil-pretreated rats. After 20 min of preperfusion, samples of effluent perfusate were collected every 2 min for the enzymatic determination of rates of glucose production and rates of glycolysis (lactate + pyruvate production). In the period from 20 to 40 min of perfusion, rates of glucose production and rates of glycolysis ranged from 70 to 90 and from 70 to 80 μmoles$\cdot$g$^{-1}\cdot$hr^{-1}, respectively. During the same period, oxygen uptake was of the order of 100 μmoles$\cdot$g$^{-1}\cdot$hr^{-1} (Fig. 1). At 40 min of perfu-

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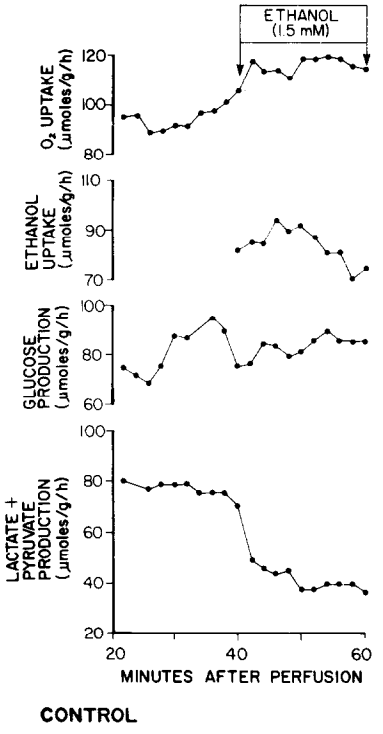

Fig. 1. Effect of ethanol infusion on oxygen and ethanol uptake, glucose production and rates of glycolysis. The liver from a control, well-fed Sprague–Dawley female rat was perfused as described in Materials and Methods. Oxygen uptake was monitored with a Clark type platinum electrode placed in the effluent perfusate. Glucose, lactate, pyruvate and ethanol were measured enzymatically in perfusate samples (Materials and Methods). Infusion of ethanol is depicted by horizontal bar and arrows. This was a typical representative experiment.

sion, ethanol (final concentration = 1.5 mM) was infused. Ethanol uptake by livers from normal rats was about 80 μmoles·g^{-1}·hr^{-1} during the period between 40 and 60 min of perfusion. Ethanol infusion under these conditions reduced the rate of glycolysis and caused a slight but detectable stimulation of oxygen uptake. Rates of glucose production were unaffected by the infusion of ethanol (Fig. 1).

Table 1. Ethanol uptake by the perfused rat liver*

Pretreatment *in vivo*	Ethanol uptake (μmoles·g^{-1}·hr^{-1})
None	83.9 ± 2.4
Propylthiouracil	61.1 ± 1.7†
Ethanol	132.4 ± 2.5†
Propylthiouracil + ethanol	54.6 ± 1.1†

* Rates of ethanol uptake were measured in perfused [17] rat livers during an infusion of ethanol (1.5 mM final concentration) for 20 min following stabilization of hepatic oxygen uptake. Samples of effluent perfusate were measured at 2-min intervals and were analyzed for ethanol enzymatically [16]. Female Sprague–Dawley rats were treated for 10 days with propylthiouracil (50 mg/kg). Ethanol (5 g/kg) was administered via gastric intubation 2.5 hr prior to perfusion. Normal rats were untreated. Results are means ± S.E.M. for all values measured in each treatment group (N = 4–6).
† $P < 0.001$.

A comparison of the rates of ethanol uptake by livers from the four groups of rats studied is shown in Table 1. Rates of ethanol uptake were diminished slightly by propylthiouracil pretreatment. *In vivo* treatment of rats with ethanol increased the rates of ethanol uptake by the perfused livers from 84 μmoles·g^{-1}·hr^{-1} to 132 μmoles·g^{-1}·hr^{-1} in livers from normal animals; however, in livers from propylthiouracil-pretreated rats, this increase was not observed.

Livers from propylthiouracil-pretreated rats consumed oxygen at rates that were about two-thirds those of the normal untreated animals (Table 2). Following *in vivo* pretreatment with ethanol, the oxygen uptake of the liver (in the absence of ethanol in the perfusate) was doubled (Table 2). In contrast, ethanol pretreatment had little if any effect on oxygen uptake in livers from propylthiouracil-pretreated rats (i.e. the stimulation of oxygen uptake by the *in vivo* ethanol treatment was blocked by propylthiouracil).

Table 2. Oxygen uptake by the perfused rat liver*

Pretreatment *in vivo*	Oxygen uptake (μmoles·g^{-1}·hr^{-1})	
	Basal 30–40 min	+ Ethanol (40–60 min)
None	98.2 ± 2.4	116.5 ± 0.9
Propylthiouracil	64.4 ± 0.7†	78.6 ± 1.4†
Ethanol	213.8 ± 0.6†	216.0 ± 0.9†
Propylthiouracil + ethanol	81.2 ± 1.1	90.8 ± 0.9

* Basal rates were measured for 10 min following stablization of hepatic oxygen uptake. Rates of oxygen uptake were then determined during a 20-min infusion of ethanol (1.5 mM final concentration). Oxygen uptake was monitored continuously with a Teflon-shielded platinum electrode placed in the effluent perfusate. The influent oxygen concentration was maintained by an oxygenator and calibrated from the arterio-venous concentration differences, the flow rate and the liver wet weight. Results are means ± S.E.M. for all values measured in each treatment group (N = 4–6).
† $P < 0.001$.

Lactate + pyruvate production is a good index of the rate of glycolysis, since pyruvate concentrations are below the K_m of pyruvate dehydrogenase under these conditions [17]. During the 20–40 min perfusion period, rates of glycolysis were between 70 and 80 μmoles$\cdot g^{-1}\cdot hr^{-1}$ in livers from the normal rats (Table 3). In livers from propylthiouracil-pretreated, ethanol-pretreated, and propylthiouracil + ethanol-pretreated rats, basal rates of glycolysis were much lower (*ca.* between 28 and 39 μmoles$\cdot g^{-1}\cdot hr^{-1}$). Following the addition of ethanol to the perfused liver, marked inhibition in glycolytic flux was observed in livers from normal and propylthiouracil-pretreated rats (Table 3); however, ethanol addition to the perfusate had no effect on the rates of glycolysis in livers from ethanol-pretreated or ethanol + propylthiouracil-pretreated rats.

Glucose production by the liver was between 70 and 90 μmoles$\cdot g^{-1}\cdot hr^{-1}$ in livers from normal and propylthiouracil-pretreated rats (Table 4). Ethanol pretreatment *in vivo* for 2.5 hr markedly diminished the rate of glucose production (*ca.* 30 μmoles$\cdot g^{-1}\cdot hr^{-1}$; Table 4). In contrast, ethanol pretreatment *in vivo* did not diminish the rate of glucose production in livers from propylthiouracil-pretreated (*in vivo*) rats. Addition of ethanol to the perfusion fluid during the 40–60 min perfusion period did not change the basal rate of glucose production.

Fructose stimulates oxygen uptake by increasing the supply of ADP to the mitochondrial respiratory chain subsequent to its phosphorylation by the highly active ketohexokinase [18]. It is by this mechanism that fructose stimulates hepatic ethanol uptake. Since propylthiouracil pretreatment decreased glycolysis (Table 3), an ADP consuming process, and, also, decreased oxygen uptake (Table 2), the effect of fructose was examined. As expected, fructose increased oxygen and ethanol uptake in livers from normal rats (Table 5); however, fructose had no effect on either variable in livers from propylthiouracil-pretreatment (*in vivo*) rats (Table 5).

DISCUSSION

Chronic ethanol administration has been reported to increase the rates of oxygen consumption and ethanol metabolism in the livers of experimental animals [4–8]. Recent studies [9] have shown that this effect can also be obtained in 2–3 hr following the administration of one large dose of ethanol (the "SIAM" effect). A hypermetabolic state induced by either of these techniques is dependent on adrenergic mechanisms since adrenergic blocking agents [9, 19] and adrenalectomy block this phenomenon [19–21]. A hypermetabolic state following chronic ethanol treatment has also been shown to be dependent on thyroidal status. Thyroidectomy and propylthiouracil pretreatment have been reported to markedly reduce or block the increase in oxygen uptake and ethanol metabolism produced by chronic ethanol treatment [11]. Present findings show that PTU treatment also can prevent the effect of ethanol on both oxygen consumption and ethanol metabolism in the hypermetabolic state induced by acute addition of ethanol (Tables 1 and 2). Therefore, thyroid hormones are necessary for ethanol to stimulate ethanol and oxygen uptake irrespective of whether it is administered acutely or chronically.

It is known that there is a direct relationship, under most circumstances, between the rate of oxygen consumption and the rate of ethanol metabolism [6, 8] because the respiratory chain is responsible for the oxidation of NADH produced by alcohol and acetaldehyde dehydrogenase reactions. It should be noted, however, that not all investigators have observed an increase in oxygen uptake following ethanol treatment [22]. This relationship between oxygen consumption and ethanol metabolism was also observed in the four experimental conditions employed in the present studies ($r = 0.86$). For example, propylthiouracil pretreatment prevented most of the elevation of oxygen uptake due to ethanol pretreatment *in vivo* in perfused livers both in the presence and absence of ethanol in the perfusate (Table 2). Thus, by preventing a more rapid oxidation of reducing equivalents at the mitochondrial level, PTU may also prevent an increase in the rate of ethanol metabolism. It is unlikely that propylthiouracil reduces the rate of ethanol metabolism by decreasing alcohol dehydrogenase activity; the activity of this enzyme is actually increased following PTU treatment ([23]; Y. Israel, R. Britton, G. Rachamin and A. MacDonald, unpublished observations).

By its well-documented antithyroid actions, PTU

Table 3. Glycolysis in the perfused rat liver*

Pretreatment *in vivo*	Lactate + pyruvate production (μmoles$\cdot g^{-1}\cdot hr^{-1}$)	
	Basal (30–40 min)	+ Ethanol (40–60 min)
Normal	75.4 ± 1.5	41.5 ± 1.3
Propylthiouracil	39.6 ± 1.3†	17.2 ± 0.2†
Ethanol	28.6 ± 1.0†	24.7 ± 0.4†
Propylthiouracil + ethanol	28.6 ± 2.6†	24.0 ± 0.5†

* Glycolysis was measured by summation of lactate and pyruvate production [16] both in the presence and absence of 1.5 mM (final concentration) ethanol infusion into the rat liver (see legend of Table 1). Results are means ± S.E.M. for all values measured in each treatment group (N = 4–6).

† $P < 0.001$.

Table 4. Glucose production by perfused rat liver*

Pretreatment *in vivo*	Glucose production (μmoles·g^{-1}·hr^{-1}) Basal (30–40 min)	+ Ethanol (40–60 min)
None	89.4 ± 1.7	84.2 ± 2.9
Propylthiouracil	71.4 ± 1.9	71.8 ± 1.2
Ethanol	29.2 ± 0.5†	25.1 ± 0.7†
Propylthiouracil + ethanol	73.0 ± 6.1	62.3 ± 1.5

* Glucose production was measured as described in Ref. 6.
† $P < 0.01$.

affects the rates of several intracellular ATP utilizing processes [24]. It is via this mechanism that PTU affects O_2 uptake by the liver. We have proposed previously that both the chronically induced hypermetabolic state and the SIAM effect increase ATP utilizing reactions, although the exact nature of the ATP-consuming reactions is still not established clearly [8, 20, 25, 26].

Effect of ethanol added in vitro. We have shown previously [17] that addition of ethanol to the perfused liver of fed rats leads to a small (5–10%) but reproducible increase in the rate of oxygen uptake (Fig. 1 and Table 2). This effect is due to inhibition of glycolysis at glyceraldehyde-3-phosphate dehydrogenase due to elevation in the NADH/NAD$^+$ redox state as a consequence of ethanol metabolism [27]. Since glycolysis is an ATP-producing reaction, this inhibition by ethanol leads to a greater rate of suppiy of ADP to the respiratory chain thus increasing oxygen uptake. This effect is only evidenced in the presence of adequate glycogen stores (e.g. in the fed state). In these studies, with the exception of the PTU-pretreated rat, a good correlation was observed between the reduction in lactate + pyruvate production and the increase in oxygen uptake following addition of ethanol to the perfusate (Tables 2 and 3).

Effect of SIAM and propylthiouracil on glycolysis. One possible mechanism for the increase in oxygen consumption in the SIAM state is a reduction in cellular glycogen stores, thus resulting in lower rates of glycolysis [9]. Since it is well known that after propylthiouracil treatment glycogen levels increase, we predicted that after PTU treatment the increase in oxygen uptake due to ethanol treatment would be diminished.

It is known that large doses of ethanol cause the release of epinephrine and norepinephrine [28]. These hormones elevate blood glucose and deplete intrahepatic glycogen stores [20]. Therefore, substrate depletion in all likelihood accounts for the lower rates of glycolysis observed in the SIAM animals (Table 3).

It was our expectation that, since hypothyroidism elevates glycogen stores, an increased oxygen uptake following ethanol treatment would be reversed by propylthiouracil by making substrate available for glycolysis. An increase in substrate availability occurred since rates of glucose production remained high in perfused livers of propylthiouracil- or propylthiouracil + ethanol-pretreated rats (Table 4), and propylthiouracil pretreatment prevented the inhibition of glucose production induced by acute treatment with ethanol. However, propylthiouracil abolished the increased rate of oxygen consumption following the acute ethanol treatment without preventing the reduction in glycolysis. This is most likely due to the fact that propylthiouracil treatment *per se* leads to a reduction in energy demands. PTU is known to reduce the phosphorylation potential [29] which in turn reduces glycolysis [30]. Thus, propylthiouracil does not prevent the elevation in oxygen consumption produced by chronic ethanol treatment by affecting glycolysis.

The question then arose, if rates of glycolysis are decreased by PTU treatment, then why isn't oxygen

Table 5. Effects of fructose on oxygen and ethanol uptake in perfused livers from normal and propylthiouracil pretreated (*in vivo*) rats*

Pretreatment *in vivo*	Addition	Uptake (% of control) Oxygen	Ethanol
None	None	100 ± 6	100 ± 12
None	Fructose, 4 mM	126 ± 3	164 ± 4†
Propylthiouracil	None	62 ± 1†	30 ± 4†
Propylthiouracil	Fructose, 4 mM	65 ± 1†	35 ± 2†

* Conditions were as in Tables 1 and 2. Ethanol (1.5 mM final concentration) infusion was initiated at 20 min of perfusion and represents the control condition for this experiment. At 40 min, fructose (4 mM) was infused. Data are represented as percent of control of data from values similar to those shown in Tables 1–4. N = 4.
† $P < 0.05$.

uptake stimulated? This is a crucial question, since Yuki and Thurman [9] have postulated a role of diminished glycolysis in the mechanism of SIAM. Some insight into this question came from experiments with fructose. Fructose, like decreased glycolysis, increased the supply of ADP subsequent to its phosphorylation via the highly active ketohexokinase [18]. As expected, fructose stimulated ethanol and oxygen uptake in livers from normal rats (Table 5). In contrast, fructose did not stimulate oxygen uptake in livers from PTU-pretreated rats (Table 5). If we assume that the phosphorylation of fructose occurs in livers from PTU-pretreated rats, then it can be concluded that PTU treatment has somehow prevented ADP stimulation of hepatic respiration. If this hypothesis is correct, then one would not expect decreased glycolysis (Table 3) to stimulate respiration in livers from PTU-pretreated rats.

Effect of acute ethanol administration and treatment with PTU on glucose production. The rates of glucose production did not increase (Table 4) with PTU alone. However, the inhibition of glucose production following chronic ethanol pretreatment was prevented by propylthiouracil pretreatment. Thus, propylthiouracil pretreatment probably prevented the effect of adrenergic hormones released by acute ethanol treatment on the glycogen phosphorylase system [31].

We have postulated previously that hypoxia due to a faster utilization of oxygen by the liver can result in zone 3 and lead to alcoholic hepatitis and liver necrosis [11, 32]. Recently, Ji *et al.* [33] have demonstrated that alcohol treatment increases the oxygen gradient between the periportal and pericentral regions of the liver lobule. Propylthiouracil administration to patients with alcoholic hepatitis has resulted in a faster improvement in their condition [32]. Further, propylthiouracil has been shown to minimize hepatic necrosis in animals subjected to low oxygen tensions after both acute and chronic alcohol administration [11, 34]. The protective effects of this drug on liver cell necrosis may be related to the effect of PTU on oxygen consumption observed in this study.

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