Effect of Propylthiouracil on the Ethanol-induced Increase in Liver Oxygen Consumption in Awake Rats

FREDERICK J. CARMICHAEL,[1, 2, 3, 4] HECTOR ORREGO,[2, 4] VICTOR SALDIVIA[2] AND YEDY ISRAEL[2, 4]

Departments of [1]Anesthesia and [2]Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8; [3]Department of Anesthesia, Toronto Western Hospital, Toronto, Ontario M5T 2S8; and [4]the Addiction Research Foundation of Ontario, Toronto, Ontario M5S 2S1, Canada

It has been postulated that the beneficial effects of the antithyroid drug propylthiouracil in the treatment of alcoholic liver disease depend primarily on the action of propylthiouracil in suppressing the increase in hepatic oxygen consumption induced by ethanol. The evidence for this effect of propylthiouracil is derived from studies in which liver oxygen consumption has been determined in *in vitro* preparations. In our study the effects of ethanol and propylthiouracil on liver oxygen consumption were assessed *in vivo* in an unrestrained and unanesthetized rat model, where liver blood flow and hepatic vein and portal vein oxygen content can be measured. Data show that the liver oxygen consumption increased in rats treated with ethanol-containing liquid diets for 4 to 6 wk, both on withdrawal of alcohol (30%, $p < 0.01$), and after readministration of ethanol (50%, $p < 0.01$). Single-dose ethanol administration increased portal tributary blood flow without affecting hepatic arterial blood flow in both controls and rats withdrawn from long-term ethanol treatment. Long-term ethanol administration *per se* had no effect on portal tributary blood flow; however, hepatic arterial blood flow was increased by 38% ($p < 0.01$). Treatment with propylthiouracil for 5 days resulted in complete suppression of the increase in liver oxygen consumption induced by long-term ethanol administration. Propylthiouracil treatment also attenuated the increase in portal tributary blood flow after the administration of a single dose of ethanol. These determinations were made 24 hr after the last dose of propylthiouracil. In conclusion, use of a new *in vivo* model with unrestrained and unanesthetized rats has confirmed that long-term administration of ethanol increases liver oxygen consumption. For the first time, it has been shown *in vivo* that propylthiouracil abolishes the ethanol-induced increase in liver oxygen consumption that follows long-term ethanol administration. (HEPATOLOGY 1993; 18:415-421.)

The antithyroid drug propylthiouracil (PTU) has been shown to markedly reduce the risk of mortality in patients with alcoholic liver disease (ALD) (1). The use of PTU in the treatment of ALD is based on the assumption that the drug reduces the hepatic oxygen demand that is elevated after the administration of ethanol (2-5). The hypoxic theory of ethanol-induced liver necrosis postulates that when increases in oxygen consumption after ethanol intake are not accompanied by increases in the delivery of oxygen, necrosis in zone 3 of the liver acinus ensues (2, 5, 6). PTU has been shown in *in vitro* studies to prevent the ethanol-induced increase in liver oxygen consumption (2, 4, 7, 8). This drug has also been shown to increase portal vein blood flow in *in vivo* studies (9), which would further increase the oxygen supply/demand ratio. However, the effect of PTU on the hepatic oxygen supply/demand ratio after ethanol administration has not been studied *in vivo*.

For this study we developed an *in vivo* model that allows the simultaneous determination of splanchnic hemodynamics and liver oxygenation in awake and unrestrained rats. In this model arterial, portal vein and hepatic vein oxygen tensions were determined, along with hepatic blood flow through both the portal and arterial tributaries with radiolabeled microspheres. Application of this model has allowed the demonstration of a hypermetabolic state *in vivo* induced by long-term alcohol consumption and its complete suppression by the antithyroid drug PTU.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 225 to 300 gm were used with the approval of the University of Toronto Animal Care Committee. The animals were housed in a temperature- and humidity-controlled environment with a 12-hr light/dark cycle. The rats were fasted for 16 hr with water *ad libitum* before the study.

Long-term Ethanol Administration. Rats were pair-fed liquid diets containing ethanol (long-term ethanol group) as 36% of calories for 4 to 6 wk with an automated system as described by Israel, Oporto and MacDonald (10). In the control diet ethanol calories were replaced by maltose-dextrin. The

Received December 4, 1992; accepted March 25, 1993.

This work was supported by the Medical Research Council of Canada and the National Institutes of Alcohol Abuse and Alcoholism (United States).

Address reprint requests to: F.J. Carmichael, M.D., Department of Pharmacology, Medical Sciences Building, Rm 4352, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

0270-9139/93 $1.00 + .10 **31/1/47591**

general composition of the diet was ethanol plus dextrose as 46% of calories, protein as 21.4% and fat as 42.4%. The rats were randomly assigned to each group.

Cannulation of Left Ventricle and Femoral Artery. Under ether or halothane anesthesia, a 1-cm incision was made in the skin of the right neck, and the common carotid artery was exposed. A ligature was passed around the vessel, and a temporary clip was placed on the artery. A polyethylene cannula (PE-50; Becton Dickinson, Parsippany, NJ) was inserted into the vessel through a small incision and was advanced toward the heart after the clip was released. A pressure waveform (Narco Biosystems, Houston, TX) was used for monitoring as the catheter was advanced into the left ventricle. The diastolic waveform returning to zero indicated proper localization of the catheter in the left ventricle.

A 1.5-cm skin incision was made over the right medial thigh, the femoral artery was isolated and temporarily clipped and a ligature was passed around the vessel. The cannula (PE-50) was then passed up the artery through a small incision in the vessel to a position in the aorta 0.5 to 1 cm above the femoral bifurcation. The time for insertion of these two lines was 7 to 9 min.

Cannulation of Hepatic Vein. A laparotomy was performed through a 3-cm midline incision, and the median and left lateral lobes of the liver were exposed and separated to expose the hepatic vein. The hepatic vein catheter tip (PE-50) was advanced into the hepatic vein by direct puncture to a depth of approximately 1.5 mm. When free blood flow, assessed by withdrawal and infusion with a syringe, was adequate, the cannula was secured in place with glue (Histoacryl blau; B. Braun Melsungen AG, Melsungen, Germany). The placement of the catheter in the hepatic vein was confirmed in each rat after death. In addition, if a sample showed oxygen tensions in the inferior vena cava range, the sample was repeated.

Cannulation of Ileocolic Vein. Through the laparotomy a distal branch of the ileocolic vein was isolated by elevating the large intestine. The ileocolic catheter tip (PE-50) was advanced into the vein by direct puncture to a distance of about 0.5 to 1 cm from the liver porta hepatis. Free flow of blood was assessed with a syringe. This cannula sampled portal blood from the portal tributaries. The cannula was secured in place with glue, as above.

The four cannulas were then tunneled subcutaneously and were brought out onto the skin surface on the midback. The incisions were infiltrated with local anesthetic, sutured, and the rats were allowed to wake up and recover for 3 to 4 hr in standard 22-cm by 11-cm cages. The total preparation for each of the animals required 20 to 25 min.

Cardiac Output and Organ Blood Flow Determination. Cardiac output and organ blood flows were determined with radiolabeled microspheres as we have previously described (11, 12). In brief, in awake and unrestrained rats approximately 50,000 microspheres, labeled with either Co-57 or Sc-46 were infused into the left ventricle through the left ventricular cannula over a period of 20 sec with an infusion pump. Ten seconds before microsphere infusion, a reference sample was withdrawn from the femoral artery catheter at a rate of 0.6 ml/min, for a period of 1 min. After this withdrawal period virtually no radioactivity was found in the circulation. Body temperature was then measured, and the rats were killed with a bolus infusion of KCl into the left ventricle. The following organs were removed for counting: heart, lungs, kidneys, liver, spleen, stomach, small and large intestine and brain. The pancreas and omentum were isolated with the stomach and intestine samples.

Cardiac output *(Qt)* (ml/min/kg body wt) was calculated as follows: $Qt = (Ci \times R)/(Cr \times w)$, where *Ci* is the net counts injected, *R* is the reference sample withdrawal rate, *Cr* is the net counts in the reference sample and *w* is the body weight (kg).

Organ blood flows *(Qo)* (ml/min/kg) were calculated as follows: $Qo = (Qt \times Co)/Ci$, where *Co* is the net counts in the organ.

Oxygen Delivery, Uptake and Excretion Determinations. Blood samples for hemoglobin concentration and oxygen saturation were withdrawn from the femoral artery, hepatic vein and ileocolic vein catheters with a 1-ml syringe. The blood samples were analyzed with an oximeter to measure hemoglobin concentration and the percentage of saturation (Radiometer OSM 2; Radiometer, Copenhagen, Denmark).

Oxygen delivery to the liver *($DhaO_2$)* (ml O_2/min/kg body wt) was calculated for the hepatic artery as follows: $DhaO_2 = HABF \times CaO_2$, where *HABF* is the hepatic arterial blood flow (ml/min/kg body wt) and CaO_2 is the content of oxygen in arterial blood calculated as the percentage of saturation multiplied by 1.34 multiplied by the hemoglobin concentration (ml O_2/ml). Oxygen delivery for the portal vein *($DpvO_2$)* was calculated as follows: $DpvO_2 = PBF \times CpvO_2$, where *PBF* is the portal tributary blood flow and *$CpvO_2$* is the content of oxygen in portal venous blood, calculated as the percentage of saturation multiplied by 1.34 multiplied by the hemoglobin concentration.

Total oxygen delivery to the liver *($DtotO_2$)* was taken as the sum of the following: $DtotO_2 = DhaO_2 + DpvO_2$ and was expressed as milliliters of O_2 per minute per kilogram of body weight.

Hepatic vein oxygen flowing out of the liver *($DhvO_2$)* (ml O_2/min/kg body wt) was calculated as follows: $DhvO_2 = TLBF \times ChvO_2$, where *TLBF* is the total liver blood flow and *$ChvO_2$* is the oxygen content of hepatic vein blood.

Oxygen consumption by the liver *(VLO_2)* (ml O_2/min/kg body wt) was taken as the difference between total oxygen delivery to the liver and hepatic vein oxygen flowing out of the liver, and it was calculated as follows: $VLO_2 = DhaO_2 + DpvO_2 - DhvO_2$.

Hepatic oxygen extraction, expressed as a percentage, was calculated as follows: $Extraction = VLO_2/(DhaO_2 + DpvO_2) \times 100$.

In previous publications it was shown that after catheter insertion and anesthesia, the animals show no obvious evidence of being under undue stress and that the prior anesthetic does not interfere with systemic or splanchnic hemodynamics in awake rats at the time of study (11-14). We have also shown that catheter insertion and ethanol administration do not alter the hematocrit; thus no evidence exists of changes in circulating blood volumes. Just before hemodynamic studies the cannulas were connected for pressure monitoring and for the infusion and withdrawal of blood samples for hemoglobin and oxygen analysis. This procedure did not appear to upset the animals. All the hemodynamic and blood flow measurements were carried out while the rats were awake and freely mobile. During the experiments heating lamps were used to warm the rats. Rectal temperatures taken at the end of the studies were 37.2° ± 0.3° C.

Experimental Design. All rats were pair-fed liquid diets containing 36% of calories as ethanol (ethanol diet) or diets in which dextromaltose replaced ethanol (control diets) for 28 to 42 days. On the evening before the study began, the diets were withdrawn, and the rats were allowed free access to water.

In PTU-treated rats, PTU 50 mg/kg was given by gavage daily for the last 5 days of feeding the control or ethanol liquid

TABLE 1. Effect of long-term and of single-dose ethanol administration on splanchnic hemodynamics in rats with or without PTU treatment

Treatment	Hepatic arterial blood flow[a]	Portal tributary blood flow[a]	Spleen[a]	Stomach[a]	Small intestine[a]	Large intestine[a]
Rats without PTU						
Control diet						
+ Water (n = 12)	13.4 ± 1.5	34.7 ± 3.0	4.0 ± 0.8	2.5 ± 0.4	19.8 ± 1.6	7.6 ± 1.0
+ Ethanol (n = 11)	11.7 ± 2.1	50.4 ± 3.9[b]	5.2 ± 0.6	4.1 ± 0.8	31.2 ± 2.5[b]	9.8 ± 1.0
Ethanol diet						
+ Water (n = 12)	18.6 ± 2.3	36.2 ± 3.1	3.5 ± 0.5	3.0 ± 0.4	23.2 ± 1.9	6.5 ± 0.6
+ Ethanol (n = 13)	18.3 ± 3.7	47.1 ± 3.1[c]	3.8 ± 0.4	3.7 ± 0.4	30.7 ± 2.2[d]	8.7 ± 0.7
Rats with PTU						
Control diet						
+ Water (n = 9)	12.3 ± 1.3	32.9 ± 2.8	3.3 ± 0.5	2.1 ± 0.3	21.0 ± 1.8	6.5 ± 0.8
+ Ethanol (n = 7)	9.8 ± 2.2	39.4 ± 5.3[e]	4.5 ± 0.7	2.7 ± 0.5	24.0 ± 3.0[e]	8.2 ± 1.3
Ethanol diet						
+ Water (n = 7)	11.2 ± 1.0[e]	37.6 ± 1.7	3.2 ± 0.2	2.3 ± 0.2	25.1 ± 1.4	6.9 ± 0.3
+ Ethanol (n = 7)	13.0 ± 2.4	37.1 ± 4.3	3.5 ± 0.6	2.8 ± 0.2	23.6 ± 3.3[e]	7.1 ± 0.6

All data expressed as mean ± S.E.M.
[a]Blood flows in ml/min/kg.
[b]$p < 0.01$ vs. control-plus-water group.
[c]$p < 0.05$ vs. control-plus-water or long-term ethanol-plus-water groups.
[d]$p < 0.025$ vs. control-plus-water or long-term ethanol-plus-water groups.
[e]$p < 0.05$ vs. no PTU treatment.

diets. Because the short-term administration of PTU is known to increase portal blood flow after both oral and intraarterial administration of the drug (9), no PTU was given on the day of study.

On the study day, after the insertion of the four cannulas, the rats were allowed to wake up and recover for 3 to 4 hr before measurement of cardiac output, blood flows and oxygen content. The long-term ethanol-treated rats were withdrawn from ethanol for 20 hr, and their blood alcohol levels were zero at the time of study. The rats were then randomly assigned to receive water or 2 gm/kg ethanol by gavage. After 1 hr blood flow and liver oxygen delivery and consumption were determined in the eight groups of animals.

Data are presented as mean ± S.E.M. Data were analyzed with a three-way ANOVA with SAS programming (SAS Institute Inc., Cary, NC). Intergroup differences were assessed with the least significant difference method (15).

RESULTS

Effect of Diets on Liver and Body Weight

No statistical differences were found in body weight between the various groups of pair-fed animals. The rats fed ethanol-containing diets for 4 to 6 wk had significantly larger livers per kilogram body weight than the rats pair-fed dextromaltose diets (control diet, 2.89% ± 0.10%; ethanol diet, 3.90% ± 0.15%; $p < 0.01$).

Blood Flow Studies

Effect of Administration of a Single Dose of Ethanol on Splanchnic Hemodynamics. An important aspect of this study was the demonstration that this new model for the study of splanchnic hemodynamics produced blood flow values that were similar to those observed in previous work without the portal venous and hepatic venous catheters (11-14) and also that the four cannulas did not alter the increase in portal tributary blood flow that normally follows the administration of a single dose of ethanol ($F = 13.04$; $p < 0.0006$) (Table 1). The administration of a single dose of ethanol resulted in a 49% increase in portal tributary blood flow in naive rats (control diet plus ethanol). This increase in blood flow was mainly the result of an increase in small intestinal blood flow ($F = 13.54$; $p < 0.003$) (Table 1). Blood flow to the spleen, stomach and large intestine, although slightly increased, was not significantly different from that of the respective controls receiving water. Consistent with our previous work, a single dose of ethanol did not affect hepatic arterial blood flow.

Effect of Long-term Ethanol Administration on Splanchnic Hemodynamics. Portal tributary blood flow in rats that had been withdrawn from ethanol for 20 hr (ethanol diet plus water) was not different from that of the pair-fed controls (Table 1). The increase in portal tributary blood flow after single-dose ethanol administration also occurred in rats receiving long-term ethanol administration (ethanol diet plus ethanol) (Table 1).

Long-term ethanol treatment (ethanol diet) significantly increased hepatic arterial blood flow ($F = 7.07$; $p < 0.01$) compared with control diets (Table 1).

Effect of PTU Treatment on Portal Tributary and Hepatic Arterial Blood Flow. Short-term administration of PTU did not affect portal blood flow in these studies conducted 24 hr after the last dose of PTU. The

TABLE 2. Effect of long-term and of single-dose ethanol administration on systemic hemodynamics in rats with or without PTU treatment

Treatment	Cardiac output (ml/min/kg body wt)	Mean arterial pressure (mm Hg)	Systemic vascular resistance (mm Hg/[ml/min/kg])	Coronary flow (ml/min/kg)	Renal flow (ml/min/kg)
Rats without PTU					
Control diet					
+ Water (n = 12)	264 ± 22	110 ± 6	0.45 ± 0.04	22.5 ± 8.3	47.6 ± 3.2
+ Ethanol (n = 11)	292 ± 12	108 ± 4	0.40 ± 0.02	31.9 ± 6.2	52.5 ± 3.4
Ethanol diet					
+ Water (= 12)	272 ± 20	120 ± 6	0.54 ± 0.05	21.3 ± 3.9	52.2 ± 7.7
+ Ethanol (n = 13)	289 ± 26	110 ± 7	0.46 ± 0.04	25.8 ± 5.3	59.0 ± 6.3
Rats with PTU					
Control diet					
+ Water (n = 9)	237 ± 20	119 ± 4	0.53 ± 0.05	15.9 ± 3.0	34.4 ± 2.8
+ Ethanol (n = 7)	208 ± 22[a]	109 ± 6	0.56 ± 0.06[a]	12.2 ± 1.7[a]	37.5 ± 2.6
Ethanol diet					
+ Water (n = 7)	220 ± 15	121 ± 4	0.57 ± 0.03	13.5 ± 1.9	29.4 ± 1.1[a]
+ Ethanol (n = 7)	190 ± 19[a]	105 ± 7	0.57 ± 0.05	10.9 ± 1.6	32.7 ± 3.8[a]

All data are expressed as mean ± S.E.M.
[a] $p < 0.05$ vs. no PTU treatment.

PTU treatment resulted in a reduction in portal tributary blood flow ($F = 4.62$; $p < 0.04$) (Table 1). PTU also suppressed the ethanol-induced increase in portal tributary blood flow. PTU treatment *per se* did not affect small intestinal blood flow. Contrary to what had been observed in animals after long-term administration of ethanol diets for 4 to 6 wk, in the animals treated with PTU hepatic arterial blood flow was not increased (Table 1).

Systemic Hemodynamic Effects of Single-dose and Long-term Administration of Ethanol and PTU

Cardiac outputs were not affected by long-term ethanol diets or by single-dose administration of ethanol (Table 2). Treatment with PTU for 5 days resulted in a small reduction in cardiac output ($F = 17.71$; $p < 0.0001$). Mean arterial pressures did not differ between any of the eight groups (Table 2). Systemic vascular resistance, likewise, did not differ between the various groups; however, treatment with PTU significantly increased this variable ($F = 17.13$; $p < 0.0001$) (Table 2).

Blood flow to the lungs did not differ between the different groups, indicating that the treatments used did not affect shunting in the animals (data not shown). Renal blood flow was not affected by long-term ethanol administration but was increased by single-dose ethanol administration ($F = 5.05$; $p < 0.03$) (Table 2). Renal blood flow was reduced in the animals treated with PTU ($F = 39.46$; $p < 0.0001$). Coronary blood flow was not affected by long-term or single-dose ethanol administration; however, coronary blood flow was reduced by PTU treatment ($F = 21.50$; $p < 0.0001$) (Table 2).

Splanchnic Oxygen Consumption

Effect of Single-dose and Long-term Ethanol Administration on Liver Oxygen Supply and Demand. Another major finding in our study was the demonstration that in this *in vivo* model with unanesthetized and unrestrained rats, the long-term administration of ethanol resulted in a significant increase in liver oxygen consumption ($F = 20.45$; $p < 0.0001$) (Table 3). In contrast, the single-dose administration of ethanol did not affect liver oxygen consumption (Table 3).

The delivery of oxygen through the hepatic artery was increased in rats treated with long-term ethanol diets ($F = 6.12$; $p < 0.02$), whereas oxygen delivery through the portal vein was unchanged with long-term ethanol treatment (Table 3). This small increase in total oxygen delivery in rats withdrawn from ethanol was insufficient to compensate for the increase in liver oxygen consumption and resulting in a significant decrease in hepatic vein oxygen saturation ($F = 17.14$; $p < 0.0001$) (Table 3).

The single-dose administration of ethanol increased delivery of oxygen through the portal vein ($F = 11.78$; $p < 0.001$) (Table 3). Hepatic arterial oxygen delivery did not change after single-dose ethanol administration (Table 3). This increase in delivery of oxygen in the presence of ethanol resulted in an increase in hepatic vein oxygen saturation in both groups resulting in full compensation for the increase in liver oxygen consumption after the long-term administration of ethanol ($F = 9.61$; $p < 0.003$) (Table 3).

Effect of PTU Treatment on Ethanol-induced Changes in Splanchnic Oxygen Balance. PTU treatment for 5 days resulted in a reduction in liver oxygen consumption in all groups ($F = 36.86$; $p < 0.0001$)

TABLE 3. Effect of long-term and single-dose ethanol administration on splanchnic oxygen delivery and consumption with and without PTU treatment

Treatment	O_2 delivery		Liver oxygen consumption (ml O_2/min/kg)	Percentage of extraction	Hepatic vein oxygen saturation (%)
	Hepatic artery (ml O_2/min/kg)	Portal vein (ml O_2/min/kg)			
Rats without PTU					
Control diet					
+ Water	2.37 ± 0.27	4.20 ± 0.43	4.28 ± 0.23	60.9 ± 1.0	30.1 ± 2.0
+ Ethanol	2.28 ± 0.41	6.75 ± 0.67[a]	4.20 ± 0.26	49.8 ± 4.4	41.3 ± 4.8
Ethanol diet					
+ Water	3.64 ± 0.44	4.24 ± 0.60	5.58 ± 0.43[b]	68.6 ± 4.7	20.8 ± 7.4
+ Ethanol	3.31 ± 0.65	5.71 ± 0.49[c]	6.27 ± 0.42[a]	67.3 ± 2.5	30.5 ± 4.8
Rats with PTU					
Control diet					
+ Water	1.83 ± 0.20	3.70 ± 0.40	3.01 ± 0.22[d]	60.0 ± 3.7	37.1 ± 2.3
+ Ethanol	1.73 ± 0.40	5.34 ± 0.93	2.78 ± 0.26[d]	41.1 ± 4.4	49.1 ± 3.2
Ethanol diet					
+ Water	1.90 ± 0.13[e]	4.14 ± 0.32	3.91 ± 0.29[f]	65.1 ± 4.2	28.7 ± 3.0
+ Ethanol	2.01 ± 0.42	3.85 ± 0.49	3.25 ± 0.30[f]	58.9 ± 3.8	35.7 ± 3.8

All data expressed as mean ± S.E.M.
[a] $p < 0.001$ vs. water, control diet.
[b] $p < 0.01$ vs. water, control diet.
[c] $p < 0.05$ vs. water, control diet.
[d] $p < 0.01$ vs. controls without PTU.
[e] $p < 0.05$ vs. controls without PTU.
[f] $p < 0.001$ vs. controls without PTU.

(Table 3). In rats receiving ethanol (single-dose or long-term), PTU resulted in a suppression of the ethanol-induced increase in oxygen consumption. Despite the fact that PTU treatment reduced the delivery of oxygen through the portal vein ($F = 9.81$; $p < 0.003$) and the hepatic artery ($F = 10.78$; $p < 0.002$), the saturation of oxygen in the hepatic vein increased in each group after PTU treatment ($F = 8.61$; $p < 0.004$).

DISCUSSION

The use of PTU in the treatment of ALD is based on the finding that the increase in liver oxygen consumption after long-term ethanol administration is dependent on normal thyroid function and can be suppressed by thyroidectomy or the administration of methimazole or PTU (2, 4, 7, 8). The hypoxic theory for alcohol-induced liver necrosis postulates that hepatocellular necrosis in ALD is caused by hypoxic damage that results from the combination of the ethanol-induced increase in liver oxygen consumption plus a decrease in oxygen delivery to the liver (5, 6). A reduction in oxygen delivery can occur in a variety of physiological conditions including anemia, sleep apnea and respiratory dysfunction (3, 5, 6). It is logical then to postulate that interventions resulting in either or both a suppression of the ethanol-induced increase in liver oxygen consumption and an increase in oxygen delivery to the liver could potentially prevent ethanol-induced liver necrosis. Moreover, it would appear that PTU does not act on the liver oxygen supply/demand ratio only by reducing oxygen consumption but that it also increases oxygen supply through an increase in portal blood flow (9). This effect of PTU has been confirmed by a recent study (16), which demonstrated an increase in sinusoidal oxygen concentration after the single-dose administration of PTU.

The above hypothesis appears to be supported by the fact that in rats treated for a long time with ethanol, the administration of PTU was able to prevent hepatocellular necrosis resulting from the combination of long-term ethanol administration plus exposure to a hypoxic environment (3). More recently, in a long-term clinical trial, it was found that treatment with PTU markedly increases survival in patients with ALD (1).

On the other hand, it should be considered that the evidence supporting the effects of PTU on liver oxygen balance may not be representative of *in vivo* conditions in animals treated with ethanol because it was derived entirely from *in vitro* studies with either perfused livers or liver slices (2, 4, 8). Moreover, these animals had been withdrawn from alcohol, a situation that may not reflect conditions existing in animals actively metabolizing alcohol.

This study was designed to allow, in a new *in vivo* model in unanesthetized and unrestrained rats, the determination of the effect of ethanol and of PTU on oxygen balance in the liver. Prior studies that had demonstrated an increase in liver oxygen consumption after long-term ethanol administration *in vivo* in rats had the possible confounding factor that the animals were restrained, anesthetized or both (17, 18). Our study shows that the effects of ethanol are also present in awake and unrestrained rats. The data obtained also confirm reports from other authors showing that

long-term ethanol administration increases liver oxygen consumption *in vivo* in rats (17, 18).

On the other hand, in contrast to previous studies, in the present model an effect of single-dose administration of ethanol on liver oxygen consumption was not found (4, 19). Part of this difference could be caused by the time of measurement—which in the present experiments was only 1 hr after ethanol administration, whereas previous workers had waited 3 hr—and the fact that we used a smaller alcohol dose (4). The possibility also exists of species differences in this type of response.

Our study further demonstrates that in the rat the increase in oxygen consumption induced by long-term ethanol administration persists after the withdrawal of alcohol, a finding that is in agreement with Kessler et al. (20) and Hadengue et al. (21) in human beings. On the other hand, our results appear at odds with the report of Bredfeldt et al. (17), who found that in rats the increase in liver oxygen consumption was not different from control levels after the discontinuation of alcohol. The reasons for this discrepancy are not clear from the present data. This point is of importance because a persistent increase in oxygen consumption after ethanol withdrawal, at a time when the ethanol-induced increase in splanchnic blood flow (11) would not be present, creates a situation in which the liver is at an increased risk of becoming hypoxic.

Long-term ethanol treatment did not alter portal tributary blood flow in conditions where no ethanol was present, consistent with our previous findings (11). Likewise, long-term ethanol treatment did not alter the portal blood flow response to the administration of a single-dose of alcohol, in agreement with previous authors (11, 17, 18). The increase in hepatic arterial blood flow seen after long-term ethanol treatment is in contrast to that reported by previous authors (11, 17). However, it is in keeping with the findings of Tsukamoto and Xi (18), who found a nearly significant 20% increase in hepatic arterial blood flow in their long-term ethanol-treated rats.

An interesting finding in our study was that the animals receiving PTU for 5 days did not demonstrate the increase in portal blood flow that normally follows ethanol administration. The reason for this effect of PTU is not known but might be related to the induction of mild hypothyroidism by PTU (9). We have previously shown that thyroidectomy results in a marked decrease in both portal and hepatic arterial blood flow (22). This observation should not be construed as evidence of the suppression of the hypermetabolic state of the liver as being the only mechanism by which PTU prevents liver necrosis. Indeed, the single-dose administration of PTU is followed by a 43% increase in portal blood flow even in the presence of mild hypothyroidism (9).

Our study shows for the first time in an *in vivo* model with unanesthetized and unrestrained animals that PTU treatment blocks the increase in oxygen consumption induced by the long-term administration of ethanol. The fact that the suppression of the long-term ethanol-induced increase in liver oxygen consumption was observed 24 hr after the last dose of PTU is in line with the postulate that this effect is a result of a PTU-induced hypothyroid state (7). Moreover, the observation that 24-hr after the last dose of PTU the increase in liver oxygen consumption after the readministration of ethanol is also suppressed further supports this idea.

In clinical studies Hadengue et al. (21) have postulated that during withdrawal the increase in liver oxygen consumption is the result of the elevation in circulating catecholamines that accompanies the discontinuation of alcohol. In support of this hypothesis, the authors present data showing a correlation between the increase in circulating catecholamines and oxygen consumption by the liver. Our data do not rule out a contribution by catecholamines in the increase in liver oxygen consumption during withdrawal. It is clear, however, that in the rat the withdrawal state cannot be the only cause of the ethanol-induced hypermetabolic state because the increase in oxygen consumption was also demonstrated in experiments in which rats were actively metabolizing alcohol (see also 17, 18). Our study shows that the state of withdrawal is not a necessary condition for the increase in liver oxygen consumption in animals treated for a long time with ethanol. If the increase in liver oxygen consumption were caused exclusively by the effects of withdrawal, a reduction in oxygen consumption would be expected when ethanol is readministered. This was not seen in our experiments, where liver oxygen consumption remained elevated after the readministration of ethanol to rats treated long-term with ethanol.

In summary, a new methodology that allows for the simultaneous measurement of liver blood flow and oxygen consumption in awake and unrestrained rats is presented. This study confirms that in rats long-term ethanol administration induces an increase in oxygen consumption by the liver of rats. Further, the administration of PTU results in a complete suppression of this hepatic hypermetabolic state that follows the long-term administration of ethanol. Moreover, it has been recently postulated that hepatic oxygen delivery may be the rate-limiting factor for certain hepatic functions in cirrhosis (23). Therefore the improvement in oxygenation induced by PTU might have important consequences in terms of improving and maintaining liver function. The metabolism of alcohol can use as much as 80% of the available liver oxygen (5); therefore a reduction in this consumption would make more oxygen available for different hepatic functions along the sinusoidal length. Single-dose PTU administration also increases oxygen delivery to the liver by an increase in portal blood flow (9), which results in an increase in sinusoidal oxygenation (16). Although these data do not exclude the possibility of PTU also acting on alcoholic liver damage caused by another coexisting mechanism or mechanisms, it does provide the first published evidence that PTU blocks the ethanol-induced increase in liver oxygen consumption *in vivo* in unanesthetized and unrestrained rats.

REFERENCES

1. Orrego H, Blake JE, Blendis LM, Compton K, Israel Y. Long-term treatment of alcoholic liver disease with propylthiouracil. N Engl J Med 1987;317:1421-1427.
2. Israel Y, Kalant H, Orrego H, Khanna JM, Videla L, Phillips MJ. Experimental alcohol-induced hepatic necrosis: suppression by propylthiouracil. Proc Natl Acad Sci USA 1975;72:1137-1141.
3. Israel Y, Orrego H. Hepatocyte demand and substrate supply as factors in the susceptibility to alcoholic liver injury: pathogenesis and prevention. Clinics in Gastroenterology 1981;10:355-373.
4. Yuki T, Israel Y, Thurman RG. The swift increase in alcohol metabolism: inhibition by propylthiouracil. Biochem Pharmacol 1982;31:2403-2407.
5. Israel Y, Orrego H. Hypermetabolic state, hepatocellular expansion, and liver blood flow: an interaction triad in alcoholic liver injury. Ann NY Acad Sci 1987;492:303-323.
6. Orrego H, Carmichael FJ. Effects of alcohol on liver hemodynamics in the presence and absence of liver disease. J Gastroenterol Hepatol 1991;6:101-121.
7. Israel Y, Videla L, Bernstein J. Hypermetabolic state after chronic ethanol consumption: hormonal interrelations and pathogenic implications. Fed Proc 1975;34:2052-2059.
8. Rachamin G, Okuno F, Israel Y. Inhibitory effect of propylthiouracil on the development of metabolic tolerance to ethanol. Biochem Pharmacol 1985;34:2377-2383.
9. Kawasaki T, Carmichael FJ, Giles HG, Saldivia V, Israel Y, Orrego H. Effects of propylthiouracil and methimazole on splanchnic hemodynamics in awake and unrestrained rats. HEPATOLOGY 1989;10:273-278.
10. Israel Y, Oporto B, MacDonald AD. Simultaneous pair-feeding system for the administration of alcohol-containing liquid diets. Alcohol Clin Exp Res 1984;8:505-508.
11. McKaigney JP, Carmichael FJ, Saldivia V, Giles HG, Sandrin S, Israel Y. Role of ethanol metabolism in the ethanol induced increase in splanchnic circulation. Am J Physiol 1986;250:G518-G523.
12. Verma-Ansil B, Carmichael FJ, Saldivia V, Varghese G, Orrego H. Effect of ethanol on splanchnic hemodynamics in awake and unrestrained rats with portal hypertension. HEPATOLOGY 1989;10:946-952.
13. Carmichael FJ, Saldivia V, Varghese GA, Israel Y, Orrego H. Ethanol-induced increase in portal blood flow: role of acetate and A1 and A2 adenosine receptors. Am J Physiol 1988;255:G417-G423.
14. Orrego H, Carmichael FJ, Saldivia V, Giles HG, Sandrin S, Israel Y. Ethanol-induced increase in portal blood flow: role of adenosine. Am J Physiol 1988;254:G495-G501.
15. Snedecor GW, Cochran WG. Statistical methods. Ames, Iowa: Iowa State University Press, 1967.
16. Matsunaga T, Kawano S, Okumura S, Yoshihara H, Takei Y, Goto M, Fukui H, et al. Effect of propylthiouracil on sinusoidal hemodynamics and oxygenation: a study by intravital microscopy [Abstract]. HEPATOLOGY 1991;14:160A.
17. Bredfeldt JE, Riley EM, Groszmann RJ. Compensatory mechanisms in response to an elevated hepatic oxygen consumption in chronically ethanol fed rats. Am J Physiol 1985;248:507-511.
18. Tsukomoto H, Xi XP. Incomplete compensation of enhanced hepatic oxygen consumption in rats with alcoholic centrilobular liver necrosis. HEPATOLOGY 1989;9:302-306.
19. Villeneuve JP, Pomier G, Huet PM. Effect of ethanol on hepatic blood flow in unanesthetized dogs with chronic portal and hepatic vein catheterization. Can J Physiol Pharmacol 1981;59:598-603.
20. Kessler BJ, Lieber JB, Bronfin GJ, Saso M. The hepatic blood flow and splanchnic oxygen consumption in alcohol fatty liver. J Clin Invest 1954;33:338-345.
21. Hadengue A, Moreau R, Lee SS, Gaudin C, Rueff B, Lebrec D. Liver hypermetabolism during alcohol withdrawal in humans: role of sympathetic overactivity. Gastroenterology 1988;94:1047-1052.
22. Kawasaki T, Carmichael FJ, Saldivia V, Roldan L, Orrego H. Relationship between portal venous and hepatic arterial blood flows: spectrum of response. Am J Physiol 1990;259:G1010-G1018.
23. Morgan DJ, McLean AJ. Therapeutic implications of impaired hepatic oxygen diffusion in chronic liver disease. HEPATOLOGY 1991;14:1280-1282.