Ethanol Stimulates Glycogenolysis in Livers from Fed Rats (43488)

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Abstract. **To determine the reason for the lack of a hypoglycemic effect of ethanol in the fed state, the effect of ethanol on glucose turnover, liver glycogenolysis, and glucose metabolites was determined. Chronically catheterized awake and freely moving fed rats received either ethanol (blood ethanol, 37 ± 10 mmol/liter, $n = 11$) or saline ($n = 13$) intravenously for 4 hr. Glucose turnover was determined using a primed continuous infusion of [3-^{3}H]glucose. The liver was freeze clamped at 4 hr for glycogen and metabolite measurements. Plasma glucose (5.8 ± 0.3 mmol/liter vs 6.3 ± 0.2 mmol/liter at 4 hr, ethanol versus saline) and the rate of glucose turnover (61 ± 9 vs 58 ± 8 moles/kg · min) were similar during the ethanol and saline infusions. Plasma lactate was significantly higher in the ethanol (1.32 ± 0.05 mmol/liter) than in the saline (0.86 ± 0.06 mmol/liter, $P < 0.001$) study. Concentrations of gluconeogenic intermediates in the liver (glucose 6-phosphate, fructose 6-phosphate, glucose 1-phosphate, and pyruvate) were all significantly and ~30% lower in ethanol-infused than in saline-infused rats. The liver citrate content was similar in ethanol-infused than in saline-infused rats. The liver citrate content was similar in ethanol (0.38 ± 0.03 mmol/liter) and saline (0.37 ± 0.04 mmol/liter) studies. Liver glycogen was 75% lower in the ethanol-infused (61 ± 9 mmol/kg dry wt) than the saline (242 ± 27 mmol/kg dry wt, $P < 0.001$)-infused rats. These data demonstrate that in fed rats given ethanol, glucose turnover is maintained constant by accelerated glycogenolysis. Thus, inhibition of gluconeogenesis by ethanol does not lower hepatic glucose production unless compensatory glycogenolysis can be prevented.** [P.S.E.B.M. 1992, Vol 201]

It is well established that ethanol induces hypoglycemia in 48-hr fasted rats (1, 2) and humans (3–5). Hypoglycemia results from a decrease in the cytoplasmic free [NAD^+] to [NADH] ratio which is due to ethanol oxidation by alcohol dehydrogenase (EC 1.1.1.1.) and aldehyde dehydrogenase (EC 1.2.1.3) (1). This change in the redox state decreases the concentration of pyruvate and of other gluconeogenic intermediates (1, 6, 7), and inhibits gluconeogenesis from substrates such as lactate (1, 3, 8), glycerol (1, 8), and gluconeogenic amino acids (1).

In fed animals (2, 9) or in humans studied after an overnight fast (3, 5), ethanol does not produce hypoglycemia. Data regarding the mechanism by which hypoglycemia is prevented are controversial. Forsander *et al.* (2) observed that addition of ethanol to perfusion media of livers from fed rats had no effect on the perfusate lactate concentration, although the pyruvate concentration was 87% decreased by ethanol. Glucose output from ethanol-treated livers was higher than in those perfused with saline (2). In the study by Krebs *et al.* (1), ethanol abolished lactate uptake in perfused fed rat livers, but rates of gluconeogenesis or the glycogenolysis were not determined. *In vivo* ethanol inhibits glucose utilization during insulin stimulation in dogs (10) and humans (11–13), and according to some (12, 13), but not all (3, 8), studies, glucose utilization is also inhibited in the basal state by ethanol. These data raise three possibilities to explain the lack of a hypoglycemic effect of ethanol in the fed state. First, a decrease in hepatic glucose output could be counterbalanced by a decrease in peripheral glucose utilization. Second, inhibition of gluconeogenesis could be compensated by an increase in glycogenolysis resulting in unchanged overall hepatic glucose output. Third, ethanol might not inhibit gluconeogenesis in the fed state.

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The present study was designed to determine whether changes in glucose turnover, liver glycogen, or glucose metabolite concentrations occur in fed animals infused with ethanol *in vivo*.

Methods

Experimental Protocol. Two groups of rats were studied in the fed state. Three days before the experiments, catheters had been surgically inserted for blood sampling and infusions (see below). On the experimental day, the rats received 4-hr infusions of [3-^{3}H]glucose for measurement of glucose turnover, and either ethanol (n = 11) or saline (n = 13). Arterial blood samples were taken for measurement of plasma glucose (−5, 60, 120, 180, and 240 min), glucose specific activity (120, 180, and 240 min), serum insulin (−5 and 240 min), plasma lactate (120, 180, and 240 min), and plasma ethanol (240 min). At 4 hr, the rats were anesthetized with an intra-arterial injection of pentobarbital. Their livers were freeze clamped with aluminium tongs precooled in liquid nitrogen for measurement of the liver glycogen content and the concentration of ATP and glucose metabolites (glucose 6-phosphate, fructose 6-phosphate, glucose 1-phosphate, pyruvate, and citrate).

The study protocol was approved by the Ethical Committee for Animal Studies of Helsinki University.

Animals. Male Wistar rats (ethanol group, 245 ± 6 g, n = 11; saline group, 241 ± 7 g, n = 13) were used for the studies. The animals were housed in a controlled environment, exposed to a 12:12-hr light:dark cycle, and allowed standard rat chow (Hankkija, Helsinki, Finland) and water *ad libitum* for at least 2 weeks before the study.

Three days before the each experiment, rats were anesthetized with an intraperitoneal injection of pentobarbital (Mebumal; Orion, Espoo, Finland; 50 mg/100 g), and PE-50 (Meadox Surgimed, Stenlose, Denmark) catheters were surgically inserted into the left carotid artery and the right jugular vein for blood sampling and infusions, respectively. The catheters were exteriorized through an incision in the neck, filled with heparin (100 IU/ml), fixed to the skin, and sealed. The animals were thereafter allowed to recover from surgery for 3 days. During this time, they were allowed free access to the standard rat chow and water.

On the experimental day, the rats were placed in individual open cages 1 hr before start of the infusions and their jugular venous catheters were connected to infusion pumps (Braun perfusor; Medical Braun, Helsinki, Finland). During this time, the rats had access to water but not food. The arterial catheter was connected to a sampling syringe and a syringe containing heparin (10 IU/ml) for flushing the sampling line.

Ethanol/Saline Infusions. Ethanol was administered as a bolus at 0 min (100 μl of 20% ethanol or saline per 100 g of rat weight) and as a continuous infusion (20% ethanol or saline) over 4 hr at a rate of 10 μl/min (2 mg/g of rat weight).

Measurement of Glucose Turnover. At 0 min, a primed constant (1.5 μCi/hr) infusion of [3-^{3}H]glucose was started and continued until 240 min. The infusion rate was 10 μl/min. For measurement of glucose specific activity, 500 μl of blood were collected into tubes containing the glycolytic inhibitors sodium fluoride and potassium oxalate (Becton Dickinson, Rutherford, NJ). Glucose radioactivity was determined after deproteinization of plasma with an equal volume of chilled perchloric acid and evaporation of tritiated water (14). The ^{3}H from position 3 of glucose can only be lost as water (15). The dried glucose extract was reconstituted with 1 ml of water. An aliquot (900 μl) was taken for determination of tritium radioactivity by liquid scintillation counting, as described previously (8), and the remaining extract was used to determine plasma glucose (16). The glucose turnover rate was calculated by dividing the isotope infusion rate (dpm/kg·min) by glucose specific activity (dpm/μmol) between 180 and 240 min (14).

Measurement of Liver Glycogen, ATP, and Glucose Metabolites. The freeze-clamped livers were stored at −80°C until freeze drying (Edwards EF4 Modulyo; Edwards High Vacuum, Crawlew, West Sussex, England). The freeze-dried liver was dissected free of visible blood and connective tissue and divided into two parts for measurement of liver glycogen (1–3 mg) and metabolites (5–10 mg). Liver glycogen was measured using alkaline extraction and enzymatic hydrolysis using amyloglycosidase (17). For measurement of liver ATP, glucose 6-phosphate, fructose 6-phosphate, glucose 1-phosphate, pyruvate, and citrate concentrations, freeze-dried liver was extracted with perchloric acid and neutralized with $KHCO_3$. The metabolite concentrations were then measured using enzymatic fluorometric assays as described by Lowry and Passonneau (18), and the results were expressed as mmol/kg of dry liver. The wet to dry weight ratio was 3.47 ± 0.02 for the ethanol-infused rats and 3.47 ± 0.08 for the saline-infused rats. Fluorescence was measured using a Transcon 102FN analyzer (Orion Analytica, Espoo, Finland).

Analytical Methods. Plasma glucose was measured immediately after blood sampling with the glucose oxidase method using the Beckman glucose analyzer II (16). Serum insulin was determined using the Phadeseph radioimmunoassay kit (Pharmacia, Uppsala, Sweden) (19). Plasma ethanol was measured enzymatically using alcohol dehydrogenase (kit no. 332-UV, Sigma Chemical Co., St. Louis, MO). Blood for plasma lactate measurements was collected into chilled tubes containing inhibitors of glycolysis (Becton Dickinson). Plasma lactate was determined using an enzymatic fluorometric method (18).

Comparison of means between the ethanol and saline groups was performed using the unpaired t test, and, for repeated measures (glucose and lactate), using analysis of variance for repeated measures followed by the Bonferroni test.

Results

Plasma Ethanol, Glucose, Lactate and Insulin Concentrations, Glucose Turnover. Plasma ethanol averaged 37 ± 10 mmol/liter in the ethanol-infused rats and was undetectable in the saline group. Plasma glucose concentrations were similar throughout the study in the ethanol and saline groups, and averaged 5.8 ± 0.3 and 6.3 ± 0.2 mmol/liter at 240 min, respectively (Fig. 1). Plasma lactate was ~50% higher ($P <$ 0.001 for all time points) in the ethanol (1.32 ± 0.05 mmol/liter [mean ± SE], 120–240 min)-infused than in the saline (0.86 ± 0.04 mmol/liter)-infused rats between 120 and 240 min. Serum insulin averaged 3 ± 1 in the ethanol and 4 ± 1 mU/l in the saline infusion studies (NS, 240 min). The glucose turnover rate averaged 61 ± 9 and 58 ± 8 μmol/kg·min in the ethanol and saline groups (Fig. 2).

Liver Glycogen, ATP, and Glucose Metabolite Concentrations. The liver glycogen content was 75% lower at 240 min in the ethanol-infused (61 ± 21 mmol/kg of dry wt) than in the saline-infused rats (242 ± 13 mmol/kg of dry wt) (Fig. 3).

The concentration of ATP was similar in the ethanol (5.7 ± 0.7 mmol/kg of dry wt) and saline (6.1 ± 0.6 mmol/kg of dry wt) groups. The concentrations of liver pyruvate, glucose 6-phosphate, fructose 6-phosphate, and glucose 1-phosphate were all significantly and 24–37% lower in the rats receiving ethanol as compared with those receiving saline (Table I). The concentration of liver citrate was similar in the ethanol (0.38 ± 0.03 mmol/kg of dry wt) and the saline (0.37 ± 0.04 mmol/kg of dry wt) groups.

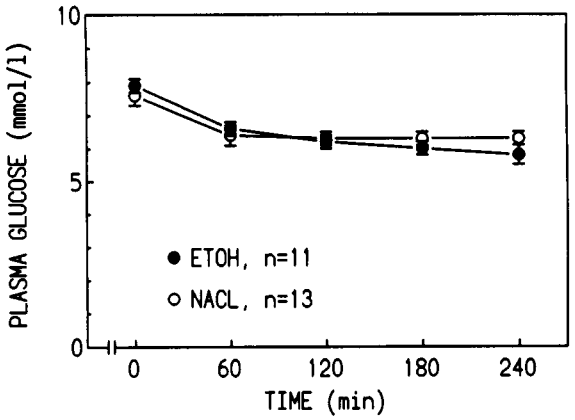

Figure 1. Plasma glucose concentrations in the ethanol (ETOH)- and saline (NACL)-infused rats.

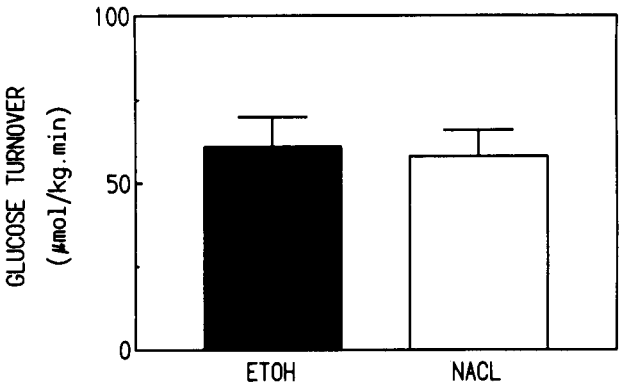

Figure 2. Rates of glucose turnover measured with a primed constant infusion of [3-^{3}H]glucose in the ethanol (ETOH)- and saline (NACL)-infused rats.

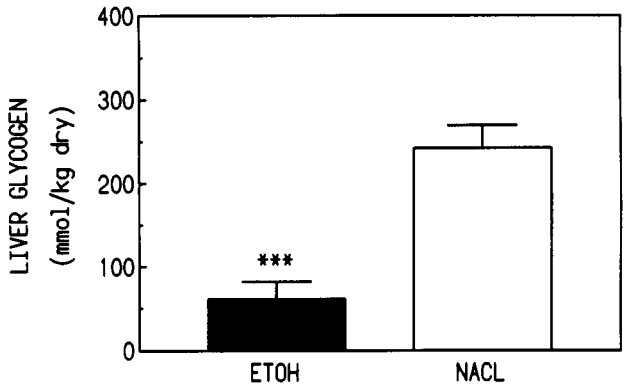

Figure 3. Liver glycogen in ethanol (ETOH)- and saline (NACL)-infused rats. ***$P < 0.001$ ethanol versus saline.

Discussion

In the present study, plasma glucose and hepatic glucose production remained unchanged in fed rats during ethanol infusion, but liver glycogen fell by 75%. These data demonstrate that the lack of a hypoglycemic effect of ethanol in the fed state can be attributed to increased glycogenolysis.

Quantitatively, the difference in liver glycogen content in ethanol-infused versus saline-infused rats was large enough to explain the lack of hypoglycemia in the rats. The liver was weighed from a few 300-g rats and averaged 8 g. The difference in liver glycogen was 181 μmol/g of dry or 628 μmol/g of wet liver wt (see Methods), which gives an average rate of glucose production of 21 μmol/min for 240 min. This rate of glycogenolysis is comparable to the measured rate of hepatic glucose production. These data indicate that hepatic glycogenolysis was the only source of glucose, that gluconeogenesis was completely inhibited, and that there would have been enough glycogen left in the rats to maintain normoglycemia via hepatic glycogenolysis for another ~1.5 hr (61 μmol/g of dry liver glycogen left at 240 min).

The rate of gluconeogenesis was not directly deter-

Table I. Liver Content of ATP and Glucose Metabolites in Ethanol- and Saline-Infused Rats[a]

	Ethanol study (n = 11)	Saline study (n = 13)	Significance (P)
ATP	5.7 ± 0.7	6.1 ± 0.6	NS
Glucose 6-phosphate	0.65 ± 0.08	1.03 ± 0.10	<0.02
Fructose 6-phosphate	0.31 ± 0.03	0.45 ± 0.04	<0.02
Glucose 1-phosphate	0.32 ± 0.02	0.41 ± 0.02	<0.05
Pyruvate	0.11 ± 0.01	0.15 ± 0.01	<0.005
Citrate	0.38 ± 0.03	0.37 ± 0.04	NS

[a] All data are expressed as mmol/kg dry liver, mean ± SE. For conversion of dry to wet weight values, multiply by 3.5 (see Methods).

mined in the present study, but the decrease in liver glycogen combined with the unchanged glucose production rate indicates that gluconeogenesis was inhibited by ethanol. In keeping with a reduced gluconeogenic flux, the levels of key gluconeogenic intermediates were ~30% reduced in the ethanol studies. Zakim (7) measured gluconeogenic intermediates in fasted ethanol-treated rats and found a ~50% reduction of fructose 6-phosphate and glucose 6-phosphate concentrations in the liver, but no change in liver pyruvate levels. Guynn and Pieklik (6) also gave ethanol to fasted rats and found, similar to the present data, no change in liver ATP, a 27% decrease in liver pyruvate, and no change in liver citrate concentrations. In perfused livers from fed rats, Krebs *et al.* (1) found an almost complete inhibition of lactate uptake by ethanol. The inhibition of gluconeogenesis has been shown to be a consequence of the increase in the NADH to NAD^+ ratio which results from ethanol oxidation by alcohol dehydrogenase. Liver slices from fasting rats metabolize alcohol about one half as rapidly as do slices from fed livers (20). This difference is largely explained by a higher NADH to NAD^+ ratio in fasted as compared with fed livers before alcohol administration, and a small capacity of the fasted liver to reoxidize NADH. These data suggest that ethanol inhibits gluconeogenesis not only in fasted, but also in fed rats.

The mechanism by which glycogenolysis was stimulated by ethanol is unclear, but it is not likely that it represents a direct effect of ethanol. In the perfused liver from starved rats, inhibition of gluconeogenesis or a decrease in hepatic glucose output is not observed if alcohol dehydrogenase is selectively inhibited by pyrazole (1). Stimulation of glycogenolysis via ethanol-induced counterregulatory hormone secretion is also unlikely under normoglycemic conditions. In rats (21) or humans (11, 12), ethanol concentrations comparable to those achieved in the present study do not stimulate secretion of growth hormone, cortisol, glucagon, or catecholamines.

Glucose 6-phosphate is a well-known inhibitor of glycogen phosphorylase, and an allosteric activator of glycogen synthase (22, 23). Therefore, the decrease in glucose 6-phosphate could provide one mechanism by which glycogenolysis was stimulated. Changes in other known regulators of glycogen synthase or phosphorylase, such as UDP-glucose, AMP, inorganic phosphate, and cAMP, might also be involved.

In patients with noninsulin-dependent diabetes mellitus, increased gluconeogenesis accounts for all of fasting hyperglycemia and increased hepatic glucose production (24). Consequently, inhibitors of gluconeogenesis might be useful hypoglycemic agents. Recently, we tested whether ethanol inhibited gluconeogenesis in these patients and, if so, whether this had any effect on overall glucose production. Ethanol decreased gluconeogenesis from lactate by ~70% and from glycerol by ~65%, but failed to alter hepatic glucose production (8). These data indirectly suggested that the inhibition of gluconeogenesis lead to a compensatory increase in glycogenolysis which allowed hepatic glucose production to remain constant. The present data provide direct evidence for the existence of such an intrahepatic regulatory mechanism. It should be emphasized that although ethanol-induced inhibition of gluconeogenesis leads to compensatory glycogenolysis, this may not be true for other inhibitors of gluconeogenesis, such as free fatty acid oxidation inhibitors (25). In concomitant studies by Jenssen *et al.* (26), the opposite phenomenon was described in normal subjects infused with lactate. Gluconeogenesis from lactate increased 3-fold but did not increase overall hepatic glucose production, which suggests that diminished glycogenolysis compensated for the increase in gluconeogenesis. These data indicate that in the fed state, the liver can both up- and down-regulate its glycogenolytic rate in response to diminished and increased gluconeogenesis, respectively. Although this type of regulation may complicate pharmacological inhibition of hepatic glucose production, it makes physiological sense in that it helps to maintain the blood glucose concentration constant, despite alterations in gluconeogenic substrate availability.

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