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EFFECTS OF ACUTE ALCOHOL INTOXICATION ON GLUCONEOGENESIS AND ITS HORMONAL RESPONSIVENESS IN ISOLATED, PERFUSED RAT LIVER

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Abstract—Rats were acutely administered ethanol as a primed constant infusion in order to produce sustained blood ethanol levels of 8–12 or 55–65 mM. At the end of ethanol infusion the livers were either freeze-clamped *in vivo* or isolated and perfused for metabolic studies. The rate of gluconeogenesis and its responsiveness to phenylephrine (10 μM), prostaglandin $F_{2\alpha}$ (5 μM) and glucagon (10 nM), as well as the redox state of the cytosolic NAD^+–NADH system were assessed in livers isolated from acutely ethanol-treated rats, and subsequently perfused without ethanol. For liver clamped *in vivo*, high- but not low-ethanol treatment decreased the ATP content by 31% and slightly increased ADP and AMP content, resulting in a decreased energy charge (11%). Glutamate and aspartate content was also increased in high-dose ethanol-infused rats with no changes in malate and 2-oxoglutarate content. Gluconeogenesis with saturating concentrations of lactate (4 mM) + pyruvate (0.4 mM) was delayed in reaching a plateau in the livers of high-dose ethanol-treated rats and its response to all three stimulators was impaired. Low-dose ethanol treatment only decreased the liver response to phenylephrine. While the perfused livers of low-dose ethanol-treated rats displayed no changes in adenine nucleotide content, the livers of high-dose ethanol-treated rats had a decreased ATP (35%) and an increased AMP (77%) content, paralleled by a fall in the total adenine nucleotides (14%) and energy charge (14%). No differences were observed between the saline- and ethanol-treated rats with respect to malate–aspartate shuttle intermediate concentration in perfused livers. Also, the livers of high-, but not low-dose ethanol-treated rats had a more negative value of NAD^+–NADH redox state as compared to the livers of control rats. The data suggest that acute ethanol intoxication produces changes in liver metabolism and its responsiveness to hormones/agonists that are demonstrable for at least 2 hr after isolation and perfusion of the liver.

The effects of ethanol on the metabolism of various tissues and organs are mediated by two major mechanisms: biochemical processes associated with ethanol metabolism and interaction of ethanol with cellular membranes. It has been demonstrated that during ethanol oxidation to acetaldehyde, it generates bulk amounts of reducing power (NADH) in the alcohol dehydrogenase reaction [1], mainly, but not exclusively, in the liver [2], and in the aldehyde dehydrogenase reaction [3], in various tissues, including the liver. The conversion of acetaldehyde to acetate, a reaction that takes place in the liver and other tissues [3], further contributes to the generation of reducing power. This translates into a dramatic shift of the NAD^+–NADH towards a more reduced state [4], followed by a number of metabolic disturbances. One of them is the impairment of gluconeogenesis from reduced glucose precursors, such as lactate [2, 5], which is quantitatively most important under *in vivo* conditions. Another disturbance involves the generation of bulk amounts of acetate whose activation leads to the formation of AMP facilitating, thus, the decrease in the adenylate pool [6].

The existence of the second group of mechanisms, i.e. the interference of ethanol with cellular membranes, has been recognized relatively recently. Such an interference is attributed to ethanol *per se*, due to its ability to embede within the structure of various biological membranes [7–10], and by acetaldehyde, whose high chemical reactivity leads to the generation of adducts with both proteins [11, 12] and phospholipids [13]. In turn, this interaction may alter the structure and function of various cellular membranes. A direct effect of ethanol on biological membranes is seen, in general, when high concentrations of the drug are present in the internal milieu, or after a chronic treatment of animals with the drug [7–10].

An abundant literature exists regarding induction by chronic alcohol consumption of structural and functional alterations that persist after cessation of alcohol consumption. However, there are no studies that have examined whether the effects of acute alcohol administration are "imprinted" in cells or organs isolated subsequently to, and shortly after, the acute alcohol administration and assessed in the absence of the drug. Previous studies on the effects of acute alcohol administration on liver metabolism have dealt primarily with the changes produced by ethanol while the drug was still present in the body [14], or when administered to either perfused liver [5, 15] or isolated hepatocytes [16, 17].

The purpose of the present study was to determine the rate of gluconeogenesis and its hormonal

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responsiveness in the liver isolated from rats, treated acutely with ethanol, and subsequently perfused in the absence of ethanol, to assess to what extent and for how long the metabolic changes known to occur in liver *in vivo* are retained by the organ after its isolation. Thus, we have measured the rate of gluconeogenesis from saturating concentrations of lactate (4 mM) + pyruvate (0.4 mM) as well as its responsiveness to a hormone (glucagon), an α_1-adrenergic agonist (phenylephrine), and to prostaglandin (PG) $F_{2\alpha}$. We have chosen these agents because they are modulators of gluconeogenesis, using different transmembrane signalling pathways, i.e. cyclic-AMP (glucagon) and Ca^{2+} (the other two agents)-dependent, to stimulate gluconeogenesis. We also have selected two doses of ethanol leading to low (8–12 mM) and high (55–65 mM) blood concentrations, for 24 hr, to enable us to distinguish between the two groups of mechanisms of ethanol action mentioned above. Our data show that both the rate of gluconeogenesis and its response to stimulators were impaired in the livers isolated from acutely, high-dose ethanol-treated rats, perfused in the absence of ethanol.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats, weighing 300–380 g, were used in all experiments. Animals were anesthetized with Ketamine and Rompun (9 mg/100 g body wt and 0.9 mg/100 g body wt, respectively, intramuscularly), and catheters (PE50) were placed in the right jugular vein and the left carotid artery using sterile surgical procedures. The indwelling catheters were exteriorized on the dorsal surface and protected against chewing by passing them through a thick plastic sleeve (70 × 5 mm). The catheters were then affixed to the cage lid so as to allow the animal to move freely within the cage without exerting tension on the catheters.

Ethanol administration

Three to four hours after completion of surgery, at a time when all animals had recovered from the anesthesia, an intravenous (i.v.) infusion of ethanol was started. The animals received an i.v. injection of ethanol at a dose of 46 mg/100 g body wt (low-dose treatment) or 275 mg/100 g body wt (high-dose treatment) in sterile saline, followed by a constant infusion of the drug at a dose of 25 mg/100 g body wt/hr for both groups. This infusion was continued for the next 24 hr. Control animals received an equal volume of sterile saline. During ethanol or saline infusion the animals were unrestrained, deprived of food, with free access to water. We have used this method previously to maintain constant blood ethanol concentrations for up to 3 days [18]. The advantage of this procedure is that it rapidly increases circulating ethanol levels and maintains them relatively constant for the duration of the infusion.

Freeze-clamping liver in vivo

At the end of ethanol or saline infusion, coincident with approximately 36 hr of food deprivation, the rats were anesthetized with Nembutal® (5 mg/100 g body wt, i.p.), the abdominal cavity was opened and the large, left lobe of the liver was freeze-clamped with aluminium tongs, precooled in liquid nitrogen. The operation was carried out while the animals were breathing spontaneously.

Liver perfusion

In a separate group of rats, livers were perfused *in situ* at the end of the ethanol or saline infusion as detailed elsewhere [19]. The perfusion medium was a hemoglobin-free, Krebs–Ringer bicarbonate buffer, continuously gassed with O_2:CO_2 (19:1), at 36.5°. The perfusate was pumped into the portal vein at a rate of 37–39 mL/min, in a non-recirculating system. The period of time elapsed between portal vein cannulation and start of sample collection for glucose measurement (shown as zero time on figures) was 15 min. During this preperfusion period the liver was washed with at least 550 mL of oxygen-saturated Krebs–Ringer bicarbonate buffer. Therefore, the measurement of glucose output and the infusion of substrates and hormone/agonist were started at the same time for all livers. The substrates and the hormone or agonist were administered to the liver through an infusion chamber, at a rate of 1.5 mL/min, with the aid of computerized infusion pumps (model 22, Harvard Apparatus, South Natick, MA) for the period of time indicated in the figures, to achieve the final concentration in the perfusion medium. No ethanol was added to the perfusate in any study. Effluent samples were collected at 2-min intervals with the aid of a peristaltic pump (Minipulse 2, Gilson, Middleton, WI) and a fraction collector (Foxy, Isco, Lincoln, NE), and used for metabolite assays.

A separate series of experiments was performed to determine the content of adenine nucleotides and malate–aspartate shuttle intermediates in freeze-clamped, perfused livers. In these experiments, the large left lobe of the liver was freeze-clamped with aluminium tongs precooled in liquid nitrogen, while the liver was being perfused exactly as for glucose output measurements (including precursor infusion with or without phenylephrine—the only agonist examined in this series of experiments).

Metabolite assay

Ethanol in blood and liver perchloric acid extracts [20], and glucose [21], lactate [22], and pyruvate [23] in the perfusate were determined enzymatically according to standard enzymatic procedures. Adenine nucleotides [24, 25], glutamate [26], aspartate [27], malate [28] and 2-oxoglutarate [29] were assayed in the neutralized perchloric acid extracts of the powder obtained from the freeze-clamped liver, using enzymatic procedures. No correction was introduced for the blood present in the liver during its freeze-clamping *in vivo*.

Statistics

The data were analyzed using a one-way ANOVA procedure. $P \leq 0.05$ was considered statistically significant.

RESULTS

Ethanol concentration in blood

Ethanol concentration in blood measured at 8, 12

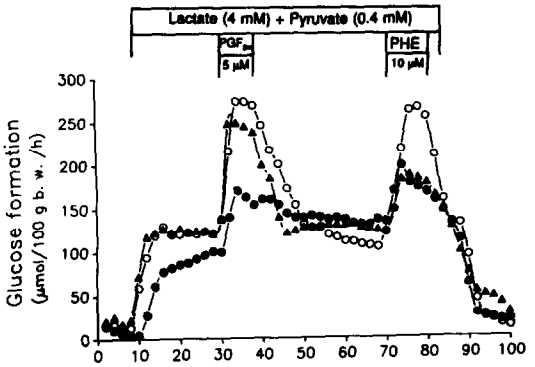

Fig. 1. Effect of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and phenylephrine (PHE) on the rate of gluconeogenesis with lactate + pyruvate in the perfused livers isolated from control (○), low-dose (▲), and high-dose (●) ethanol-treated rats. Ethanol was infused *in vivo*, but after liver isolation the drug was not included in the perfusate. In this and subsequent figures the horizontal columns show the time for which a compound (indicated inside the column) was infused into the perfusate to achieve the final concentration indicated in the columns. The plotted points are means of 4–5 individuals in each group. The SEM (which were between 7 and 13% of the means) were omitted for clarity.

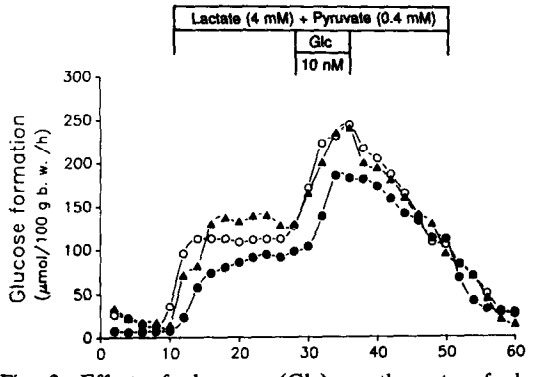

Fig. 2. Effect of glucagon (Glc) on the rate of gluconeogenesis in the perfused livers of control (○), low-dose (▲) and high-dose (●) ethanol-treated rats. Other details, as in Fig. 1.

and 22 hr after the bolus injection followed by continuous infusion varied between 8 and 12 (10.1 ± 1.8) mM for the low-dose and between 55 and 65 (59.6 ± 3.2) mM for the high-dose treated groups.

Gluconeogenesis and hormonal responsiveness

As shown in Figs. 1 and 2, the rate of gluconeogenesis with saturating concentrations of lactate (4 mM) + pyruvate (0.4 mM), at a physiological ratio of 10, reached a plateau approximately 12–14 min after the start of precursor infusion. A delay in reaching the plateau was seen only for the livers of high-dose ethanol-treated rats and it was evident for the perfusion interval between 10 and 26 min. Immediately prior to hormone/agonist or $PGF_{2\alpha}$ infusion, the difference in glucose output by the livers of high-dose ethanol-treated and control groups was not statistically significant. The stimulatory effect of specific agents was also examined by comparing the rate of gluconeogenesis attained immediately before hormone/agonist infusion with the rate observed at the peak of stimulation. Upon $PGF_{2\alpha}$ infusion, the control livers increased their glucose output by 126%. The glucose output from the livers of low-dose ethanol-treated rats increased by 102% ($P > 0.05$ vs control), while for the high-dose ethanol-treated rats glucose production increased only by 58% ($P < 0.05$ vs control). It should also be noted that, for $PGF_{2\alpha}$ infusion, the rate of gluconeogenesis in the livers of high-dose ethanol-treated rats did not return to the level observed before the start of its infusion and, therefore, the rate of glucose output before phenylephrine infusion actually represents a slightly stimulated rate of gluconeogenesis. Control livers showed an increase of 151%. A diminished responsiveness was also observed with phenylephrine. In this case, however, both low- and high-dose ethanol-treated groups displayed a suppressed responsiveness (49 and 48%, respectively, $P < 0.05$ vs control). Finally, as shown in Fig. 2, the liver response to glucagon was diminished only in high-dose ethanol-treated rats, where it reached 81% as compared to 93% in the control ($P < 0.05$ between the groups).

Redox state of the NAD^+–NADH system in liver cytosol

Since the redox state of the NAD^+–NADH system in the cytosolic compartment of the hepatocyte is known to be profoundly affected by ethanol [5, 15–17], we assessed the redox state of this couple by measuring the lactate-to-pyruvate ratio in the effluent upon infusion of a mixture of lactate + pyruvate at a physiological concentration (1 and 0.12 mM, respectively) and ratio (8.3). The lactate and pyruvate concentration in the infused stock mixture was both calculated and measured by assaying metabolites in the samples collected from the infusion chamber. The results presented in Fig. 3 indicate the maintenance of a lactate-to-pyruvate ratio of approximately 8.3 by the perfused livers of saline- and low-dose ethanol-treated rats during the whole perfusion period. This also demonstrates an oxygenation state of the liver similar to the *in vivo* conditions. In contrast, the lactate-to-pyruvate ratio in the effluent from high-dose ethanol-treated animals was constantly greater than the ratio from livers of control animals with a tendency to increase during the second half of the perfusion period.

Metabolite content in the liver

Adenine nucleotides. As shown in Table 1, high-dose ethanol treatment produced a significant decrease (31%) of ATP content, and a slight increase in ADP (21%) and AMP (22%) content *in vivo*. A fall in total adenine nucleotide content (10%) and in the energy charge of the adenylate pool (11%) was also observed. None of these parameters were

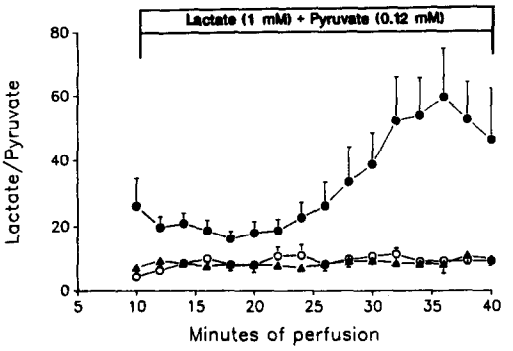

Fig. 3. Lactate-to-pyruvate ratio in the effluent of perfused livers, isolated from control (○), low-dose (▲), and high-dose (●) ethanol-treated rats. All points for livers from high- but not low-dose ethanol-treated rats were significantly greater ($P < 0.05$) than the matched control values. The SEM are represented by half vertical bars. Other details, as in Fig. 1.

affected by the low-dose ethanol treatment. The data presented in Table 1 also indicate that, when compared to control (i.e. no hormone addition) livers of saline-treated rats, the perfused livers from the high-dose ethanol-treated rats had a significantly lower ATP content (35%), a higher AMP content (77%) and a lower energy charge (14%). Although the total adenine nucleotide content tended to be reduced, this change was not statistically significant. The low-dose ethanol treatment of rats affected only the AMP content (increased by 66%, $P < 0.05$) and the energy charge (decreased by 6%, $P < 0.05$). The infusion of phenylephrine into livers of control or low-dose ethanol-treated animals did not affect these parameters significantly. However, phenylephrine did increase the ATP, and the total adenine nucleotide content and the energy charge, and decreased the AMP content in livers from high-dose ethanol-treated rats. No significant differences were seen between the livers of saline-treated rats either freeze-clamped *in vivo* or during the perfusion with respect to their concentration of adenine nucleotides and energy charge.

Malate–aspartate shuttle intermediates. High-dose ethanol treatment of rats tended to increase the hepatic content of glutamate (41%) and aspartate (18%) in *in vivo* freeze-clamped livers, although these changes were not statistically significant (Table 2). The low-dose ethanol treatment produced a low (12%), nonsignificant increase in glutamate content. When a comparison was made between the *in vivo* freeze-clamped livers and the livers perfused *in situ* with respect to their content in malate–aspartate shuttle intermediates, it could be seen that the livers *in vivo*, regardless of the treatment, had a higher content of glutamate, aspartate and malate, but not 2-oxoglutarate, than the perfused livers corresponding to the same treatment group. As shown in Table 2, there was no difference in the glutamate, aspartate, and malate content between the livers of saline- and ethanol-treated rats when perfused with only gluconeogenic precursors. The addition of phenylephrine significantly decreased the content of glutamate and increased the content of aspartate in all groups, without affecting the content of malate. These phenylephrine-induced changes were not different between control and ethanol-treated rats.

DISCUSSION

The present study shows that acute alcohol treatment of animals, by a 24-hr continuous drug

Table 1. Adenine nucleotide (AdN) content and energy charge (EC) of livers *in vivo* and during perfusion with glucose precursors with or without phenylephrine (PHE)

Experimental conditions	ATP	ADP	AMP	Total AdN	EC
	(μmol/g wet weight)				
In vivo					
Saline (4)	2.45 ± 0.04	1.38 ± 0.08	0.45 ± 0.09	4.29 ± 0.13	0.731 ± 0.010
Low ethanol (5)	2.60 ± 0.32	1.40 ± 0.21	0.45 ± 0.06	4.55 ± 0.46	0.725 ± 0.012
High ethanol (5)	1.69 ± 0.18*	1.68 ± 0.09*	0.55 ± 0.08	3.88 ± 0.21	0.652 ± 0.010*
Perfused livers					
Saline					
Control (4)	2.29 ± 0.13	1.35 ± 0.15	0.27 ± 0.02	3.90 ± 0.25	0.760 ± 0.011
PHE (5)	2.37 ± 0.13	1.52 ± 0.09	0.38 ± 0.07	4.27 ± 0.14	0.733 ± 0.008
Low ethanol					
Control (4)	2.35 ± 0.30	1.55 ± 0.18	0.45 ± 0.02†	4.35 ± 0.46	0.718 ± 0.012*‡
PHE (6)	2.38 ± 0.22	1.34 ± 0.11	0.46 ± 0.06	4.18 ± 0.27	0.729 ± 0.039‡
High ethanol					
Control (7)	1.49 ± 0.21†	1.37 ± 0.06	0.48 ± 0.04†	3.34 ± 0.21	0.651 ± 0.008†
PHE (3)	2.37 ± 0.16‡	1.43 ± 0.11	0.30 ± 0.06‡	4.10 ± 0.21‡	0.752 ± 0.008‡

Values are means ± SEM for the number of rats indicated in parentheses.
* $P < 0.05$ vs saline-treated rats.
† $P < 0.05$ vs control group of saline-treated rats.
‡ $P < 0.05$ vs control group of high-dose ethanol-treated rats.

Table 2. Glutamate, aspartate, malate, and 2-oxoglutarate content of livers *in vivo* and during perfusion with glucose precursors with or without phenylephrine (PHE)

Experimental conditions	Glutamate	Aspartate	Malate	2-Oxoglutarate
		(μmol/g tissue, wet wt)		
In vivo				
Saline (5)	3.39 ± 0.41	2.05 ± 0.18	1.01 ± 0.13	0.56 ± 0.06
Low ethanol (5)	3.81 ± 0.56	1.92 ± 0.18	0.72 ± 0.07	0.42 ± 0.02
High ethanol (4)	4.78 ± 0.65	2.43 ± 0.24	0.94 ± 0.04	0.57 ± 0.04
Perfused livers				
Saline				
Control (4)	2.00 ± 0.17	0.74 ± 0.07	0.41 ± 0.06	0.70 ± 0.06
PHE (5)	1.19 ± 0.05*	1.01 ± 0.06*	0.45 ± 0.03	0.67 ± 0.08
Low ethanol				
Control (7)	1.88 ± 0.24	0.52 ± 0.04	0.52 ± 0.05	0.40 ± 0.04
PHE (6)	1.24 ± 0.09*	0.62 ± 0.04*	0.54 ± 0.05	0.42 ± 0.02
High ethanol				
Control (7)	1.71 ± 0.15	0.68 ± 0.04	0.47 ± 0.02	0.54 ± 0.03†
PHE (3)	0.97 ± 0.06*	0.85 ± 0.05*	0.44 ± 0.05	0.60 ± 0.01

Values are means ± SEM for the number of rats indicated in parentheses.
* $P < 0.05$ vs control groups (i.e. livers perfused without PHE).
† $P < 0.05$ vs saline-treated rats, control group.

infusion, leading to blood concentrations of 55–65 mM, produced a lag in reaching a plateau by hepatic gluconeogenesis and, to a greater extent, suppressed its responsiveness to several modulators, when measured in the organ isolated shortly after cessation of ethanol infusion and perfused in the absence of ethanol. Treatment of animals with ethanol, leading to blood concentrations in the range of 8–12 mM, did not affect the rate of gluconeogenesis or its response to glucagon and $PGF_{2\alpha}$; however, the liver gluconeogenic response to phenylephrine was depressed to the same extent as for the high-dose ethanol-treated rats. These two doses were selected in order to allow us to distinguish between the effects of ethanol that can be ascribed to its metabolism or to its interference with cellular membranes. Previous experimental data have demonstrated that ethanol interference with biological membranes is likely to take place only in the presence of high ethanol concentrations (e.g. in the vicinity of 50 mM and higher [7–10]). Since such concentrations also cover the saturating levels of ethanol metabolism, the effects of ethanol can be ascribed both to ethanol metabolism and its interference with membranes. On the other hand, at the lower blood concentrations used in this study, i.e. 8–12 mM, it is likely that ethanol will affect liver metabolism predominantly by virtue of its conversion into acetaldehyde and acetate.

The following factors will be taken into consideration to explain the observed metabolic alterations: (a) the redox state of the NAD^+–NADH couple, (b) the energy state of the tissue, and (c) the potential perturbation of the hepatic plasma membrane by ethanol. These will be discussed below.

(a) *Redox state of the NAD^+–NADH couple.* Hepatic gluconeogenesis is known to be inhibited by ethanol through a mechanism involving primarily a decrease in the cytosolic and mitochondrial NAD^+/NADH ratio [2, 5]. Further support for such a mechanism was provided by experiments showing that gluconeogenesis from precursors that are more oxidized than glucose, i.e. pyruvate [2] and propionate [30], is either not affected or even stimulated by ethanol.

The procedure we used to assess the redox state of the cytosolic NAD^+–NADH couple was experimentally validated for the hemoglobin-free perfused liver [31]. It has also been demonstrated that ethanol induces a shift in the mitochondrial NAD^+–NADH, although it is less pronounced than in the cytosol [17].

Our results indicate that livers removed from high-dose ethanol-treated rats display a more negative redox potential in the cytosolic compartment, as reflected by the lactate-to-pyruvate ratio in the effluent. Because ethanol was not detected in the perchloric acid extracts of the liver (data not shown), it seems unlikely that the increased lactate-to-pyruvate ratio in the effluent is the result of continued ethanol oxidation. On the other hand, it is known that the shift of the redox state of the NAD^+–NADH couple in the perfused liver in the presence of ethanol is quickly reversed upon addition of inhibitors of ethanol oxidation [2]. A potential explanation for our results would be an alteration of shuttle pathways transporting the reducing equivalents from cytosol to mitochondria. Under several circumstances the concentration of malate–aspartate shuttle intermediates can become rate-limiting for ethanol oxidation [32]. However, our data show no difference between the perfused livers of control and ethanol-treated rats with respect to the concentration of four intermediates of the malate–aspartate shuttle. In spite of the existence of a more negative state of the NAD^+–NADH couple in the livers of high-dose ethanol-treated rats, the

concentration of glutamate and malate was not increased. Under the *in vivo* conditions, glutamate and aspartate were increased in high-dose ethanol-treated rats. This is in agreement with earlier data reported by Farbiszewski *et al.* [33] showing that ethanol induces accumulation of glutamate (+ glutamine) and aspartate (+ asparagine) in rat liver. On the basis of these data we suggest that, at least in the case of high-dose ethanol treatment, anion transporters, located within the inner mitochondrial membrane, may have been altered during the presence of ethanol. Alteration of mitochondrial processes carried out by specific proteins has been a common finding of studies dealing with the effect of alcohol on mitochondria [34–37]. We do not believe that the shift in the cytosolic redox state observed in our experiments was the cause of the suppressed hormonal responsiveness of gluconeogenesis. The livers were supplied with 0.4 mM pyruvate (+4 mM lactate), and an increase in the lactate/pyruvate ratio to 40 (Fig. 3) would still leave a pyruvate concentration of 0.1 mM, at which gluconeogenesis can proceed at high rates.

The decrease in glutamate and increase in aspartate concentration, observed upon phenylephrine infusion in both control and ethanol-treated rats, can be explained by an augmentation of oxaloacetate formation in the pyruvate carboxylase reaction [38, 39] associated with a higher transamination rate between glutamate and oxaloacetate. Additionally, an intensified glutamate oxidation as a consequence of citric acid cycle activation by phenylephrine and, in general, by α_1-adrenergic agonists, can also account for a decreased glutamate level.

(b) *Energy state of the tissue.* Our results show that the ATP content of perfused livers, isolated from high-, but not low-dose ethanol-treated rats was decreased to 65% of control values during the perfusion with gluconeogenic substrates. Although no significant changes occurred in the adenine nucleotide content of the perfused livers from low-dose ethanol-treated rats, a small but significant decrease of the energy charge was evident. Two aspects have to be considered here: the cause of these changes, and whether such changes can account for the inhibition of gluconeogenesis and for its suppressed hormonal responsiveness in the livers of ethanol-treated rats.

A number of studies have been published on the effects of acute ethanol administration *in vivo* or its infusion to the perfused organ on adenine nucleotide content in the liver [14, 40–42]. Among them one [14] is comparable to our study in terms of ethanol concentration, but not the duration of its presence in the blood. The authors measured adenine nucleotide content in *in vivo* freeze-clamped livers of starved rats after intraperitoneal administration of 46 mg ethanol/100 body wt (generating 8–10 mM ethanol in the liver) and observed no changes in ATP and ADP levels in the livers of animals killed within 30 min after ethanol administration; however, the AMP levels was increased as early as 5 min after ethanol administration and lasted up to 30 min, the last time-point of determinations. In our model of low-dose ethanol treatment no changes were seen in the amount of AMP either *in vivo* or in the perfused liver.

In the present study, the decreased ATP content in the livers of high-dose ethanol-treated rats, both under the *in vivo* conditions and during hemoglobin-free perfusion may be the result of: (a) alterations induced by ethanol and/or its metabolite, acetaldehyde, in mitochondrial ATP synthesis and/or associated processes (e.g. adenine nucleotide translocation across the inner mitochondrial membrane) [34–37], or (b) a drainage of the adenylate pool through a 5′-nucleotidase reaction, as a consequence of elevated acetate concentration and its activation to acetyl-CoA. It has been demonstrated that such alterations are most likely the result of an impairment in ATP synthesis [43, 44]. A correlation between the decreased ATP content of the livers of high-dose ethanol-treated rats and the rate of gluconeogenesis is likely since the rate of gluconeogenesis from lactate in the perfused rat liver is known to be highly dependent on intracellular concentration of ATP [45]. Thus, the difference in the hepatic ATP content between the two groups may account for the slower rate of gluconeogenesis by livers from high-dose ethanol-treated rats.

During phenylephrine infusion the ATP content in livers of both control and high-dose ethanol-treated rats was the same, yet the gluconeogenic response was decreased in the livers of the latter group. It is possible that phenylephrine increased the ATP production only in the livers of ethanol-treated rats. However, alternate explanations should also be considered. Phenylephrine, an α_1-adrenergic agonist, mobilizes intracellular Ca^{2+} which in turn activates a number of enzymes, including several mitochondrial dehydrogenases [46], in parallel with a stimulation of gluconeogenesis. As a consequence of a stimulated gluconeogeneis, ATP consumption and ADP production increase, leading to elevation of oxidative phosphorylation. More ATP becomes available to the hepatocyte, but more ATP is also consumed because of the increase in gluconeogenesis. Therefore, if a new, higher steady-state level between consumption and production is maintained, what appears to take place in the livers of control rats, no increase in ATP content should be seen. This was the situation in the livers from control rats. We postulate that in the livers of high-dose ethanol-treated rats, the increased ATP generation upon stimulation with phenylephrine is not proportional to its consumption, due to an already existing defect in the gluconeogenic pathway and/or its activation by the agonist (see the discussion under c). Thus, what might have prevented the livers of high-dose ethanol-treated rats from responding appropriately to the agonist was not the ability of the hepatocyte to generate ATP, but its failure to use it for gluconeogenesis.

(c) *Ethanol and cell membrane interaction.* It is becoming increasingly evident that ethanol may affect various tissues through mechanisms independent of biochemical events associated with its oxidation. Such mechanisms are primarily mediated by the interaction of ethanol with various cell membranes followed by alterations in receptor–ligand coupling involving the participation of G proteins [47–49],

transmembrane signalling [50], and ion pumps [51, 52].

The stimulatory agents used in our study act through different, highly specific receptors and use different transmembrane signalling pathways. Thus, phenylephrine, an α_1-adrenergic agonist, and $PGF_{2\alpha}$ recruit the Ca^{2+}-dependent pathway, while glucagon activates the cyclic-AMP dependent pathway. In spite of this diversity, all three agents failed to fully stimulate gluconeogenesis in the livers of high-dose ethanol-treated rats. It is also obvious that the liver response to these agents was not uniformly affected by the high-dose of ethanol treatment. Thus, the response to glucagon was much less affected than that to phenylephrine or $PGF_{2\alpha}$. In the liver of low-dose ethanol-treated animals only the response to phenylephrine was impaired. Such a pattern may result from the interaction with the plasma membrane, leading to alterations in the state of receptors, or in the enzymatic equipment of gluconeogenesis (or a metabolic pathway supporting gluconeogenesis, e.g. fatty acid oxidation). The fact that at the plateau phase, before the infusion of the stimulatory agent, the rate of gluconeogenesis was similar in the two groups tends to suggest that biochemical lesions in the pathway were not the cause of diminished gluconeogenic responsiveness. We are inclined to speculate that ethanol interference with membrane phenomena could account for such alterations. Our speculation is in agreement with previous studies demonstrating altered hormonal responsiveness induced by ethanol in a number of cell or tissue preparations [49].

We have made an attempt to discern between the effects of ethanol ascribable to its metabolism, or to its interaction with plasma membrane using rats with two different blood concentrations of the drug. It seems, however, that a clear-cut delineation between these two groups of effects cannot be made solely on the basis of ethanol concentration in the internal milieu. This assumption is supported by our data showing that at low blood ethanol concentrations—at which one would expect to see only the consequences of ethanol metabolism—an impaired response to phenylephrine could also be seen. Since neither the content of adenine nucleotide nor the gluconeogenic response to glucagon and $PGF_{2\alpha}$ was altered by the low-dose ethanol treatment, we conclude that the impaired response to phenylephrine could also be due to alterations in the hepatocyte plasma membrane, leading to disturbances of α_1-adrenergic receptors. In fact, several studies have demonstrated a high degree of sensitivity of α_1-adrenergic receptors to alcohol [51, 53]. This imposes a reconsideration of whether the membrane-mediated effects of ethanol can only be seen in the presence of high concentration of the drug. The nature of the membrane phenomena under examination should also be considered as a factor contributing to the interrelationship between ethanol and membrane structure and function.

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