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Influence of Ethanol Oxidation Rate on the Lactate/Pyruvate Ratio and Phosphorylation State of the Liver in Fed Rats

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The effect of the ethanol oxidation rate on the interaction between the phosphorylation state (the [ATP]/[ADP] × $[HPO_4]^{2-}$ ratio) and the redox state (the free [NAD+]/[NADH] ratio) of the liver cytosol was studied in intact fed rats. The rate of ethanol oxidation was inhibited to different degrees with pyrazole. The ethanol oxidation rate had no influence on the liver lactate level but correlated significantly with the pyruvate level. Accordingly, a significant correlation was also found between the ethanol oxidation rate and the lactate/pyruvate ratio. The rate of ethanol oxidation correlated significantly with the liver 3-phosphoglycerate level. No change in the glyceraldehyde-3-phosphate level was found. No correlation was found between the ethanol oxidation rate and the glyceraldehyde-3-phosphate/3-phosphoglycerate redox couple. Ethanol administration slightly increased the liver ATP level, but the simultaneous administration of pyrazole eliminated this effect. Other adenine nucleotides and HPO_4^{2-} were not changed. The changes in the rate of ethanol oxidation had no effect on the phosphorylation state in the fed liver. It is assumed that in the fed liver the phosphorylation state is so well stabilized that the redox level has no influence.

Interaction occurs between the ATP-ADP and NAD+-NADH couples in the glycolytic chain.[1] Hohorst *et al.*[2] have shown that this also holds for intact livers with various metabolic conditions. The redox state of the free NAD+—NADH couple in the liver cytosol is very stable during normal conditions, and the redox potential is controlled by the interaction between the phosphorylation state (*i.e.* the ratio [ATP]/[ADP][HPO_4]$^{2-}$) and the redox state of free NAD+-NADH couple.[3,4] Both the phosphorylation state and the level of the redox system of the cytosol can be changed by feeding different types of diets or by giving rats specific substances. It was suggested that these changes were mediated by the glyceraldehyde-3-phosphate—3-phosphoglycerate couple.[5] From this work it could not be stated whether the phosphorylation state influenced the redox level or the redox level influenced the phosphorylation state.

In the liver of the starved rat, ethanol influences the redox level as well as phosphorylation state of the cytosol.[5] In the liver of the fed rat, ethanol has a strong effect on the redox state, but the influence on the phosphorylation state is not so clear.[6] It has been claimed that the rate of ethanol oxidation is influenced by the redox state of the cytosol.[7,8] In two studies the rate of alcohol oxidation from differently treated rats has been compared to the lactate/pyruvate ratios.[7,9] Hillbom showed that the more reduced the redox level was, the lower was the rate of ethanol elimination.[7] Such a relationship was also observed by Smith and Newman [9] in their study on fed and fasted rats. In the present study we wanted to determine if the cytosolic redox changes induced by ethanol are reflected in the cytosolic phosphorylation state, in fed rats where the reaction catalyzed by alcohol dehydrogenase is modified with a specific inhibitor, pyrazole.[10] We also wanted to compare the ethanol oxidation rate and the cytosolic redox state.

EXPERIMENTAL

Animals. Female Sprague-Dawley rats, aged 2—3 months and weighing 200—250 g fed

with the ASTRA-EWOS (Södertälje, Sweden) laboratory diet and tap water *ad libitum* were used in all experiments.

Ethanol elimination rate. The rats were divided into 5 groups, 10 animals in each. The first served as the control group and received saline intraperitoneally (1 ml/kg body weight). The other four groups received pyrazole in saline, 0.1, 0.5, 1.0, or 2.0 mmol per kg body weight. 15 min after saline and pyrazole injections 1.5 g of ethanol in saline per kg body weight was given to all animals. Blood samples were taken from the tip of the tail 1.0, 1.5, 2.0, 2.5, and 3.0 h later and the concentration of ethanol in the blood was determined. The rate of ethanol oxidation was calculated from the slopes of the elimination curves according to Rawat and Kuriyama.[11] The liver was assumed to be 2.79 % of the total body weight.[12]

Determination of metabolites. The groups of rats and doses of pyrazole were the same as for the estimation of the ethanol elimination rate. 15 min after pyrazole administration each group of 12 animals was divided into two groups, one of which received 1.5 g of ethanol per kg body weight intraperitoneally and the other the same volume of saline. The liver samples were taken 1 h after ethanol or saline injections. The animals were anaesthetized with pentobarbital (Nembutal®, 1 % solution in saline, 40 mg/kg body weight) and the livers were quickly frozen *in situ* with aluminium tongs precooled in liquid nitrogen.[13] The powdered frozen liver tissue was suspended in ice-cold perchlorid acid (0.6 M). The protein precipitate was centrifuged down and the metabolites were determined from the neutralized supernatant within a few hours. Lactate and pyruvate were determined according to Hohorst *et al.*,[14] 3-phosphoglycerate according to Czok,[15] dihydroxyacetone phosphate according to Bücher and Hohorst,[16] ATP according to Adam,[17] ADP and AMP according to Adam,[18] and inorganic phosphate according to Bartlett.[19] The glyceraldehyde-3-phosphate concentration was calculated from the dihydroxyacetone phosphate concentration, according to Veech *et al.*[4] 60 % of the inorganic phosphate measured was assumed to be in the form of HPO_4.[2-5]

Ethanol was determined gas chromatographically (Perkin Elmer F 40) by the head space technique. *t*-Butyl alcohol was used as an internal standard.[20]

Statistical analysis. Statistical correlations between the concentrations of metabolites and the ethanol oxidation rate were calculated by linear regression analysis.[21] Statistical differences between different groups were calculated by Student's *t* test.

RESULTS

Rate of ethanol elimination. The rate of ethanol elimination was 4.6 μmol/[(g of liver wet weight) min] in the intact rat (Table 1). This value was similar to that obtained by Veech *et al.*[6] Inhibition of ethanol oxidation by pyrazole was linear with increasing doses and was 59 % with a dose of 2 mmol/(kg body weight). The amount needed for inhibition was much larger than used by Lester and Benson[22] and by Goldberg and Rydberg,[23] but of about the same order as that used by Papenberg *et al.*[24] and by Grunnet and Thieden.[25] Strain differences have been assumed to be responsible for the differences in sensitivity to pyrazole.[26]

Liver lactate/pyruvate and glyceraldehyde-3 phosphate/3-phosphoglycerate ratios. Pyrazole alone had no effect on the liver content of lactate, pyruvate, glyceraldehyde-3-phosphate, and 3-phosphoglycerate, and no change was found in the lactate/pyruvate or glyceraldehyde-3-phosphate/3-phosphoglycerate ratios (Table 2).

Administration of ethanol did not cause any significant change in the liver content of lactate (Table 2), nor was any change found when ethanol and various doses of pyrazole were administered together. There was a significant decrease ($p < 0.05$) in the liver content of pyruvate after administration of ethanol alone (Table 2) and also when ethanol and 0.1 – 1.0 mmol of pyrazole per kg body weight were administered together. With the highest dose of pyrazole [2 mmol/(kg body weight)] the difference to the control group was not significant ($p > 0.05$). The lactate/pyruvate ratio was significantly elevated ($p < 0.05$) when ethanol oxidation was not inhibited and when in-

Table 1. Rate of elimination of ethanol in the intact fed rat. Each figure represents the mean ± standard deviation of 8 – 10 animals. The groups receiving pyrazole were compared with the control group (pyrazole concentration 0) by Student's *t*-test.

Pyrazole administered [mmol/(g body weight)]	Rate of ethanol oxidation μmol/[(g liver wet wt)min]	*p*
0 (Control)	4.6(5)	–
0.1	4.1(5)	<0.050
0.5	3.6(11)	<0.050
1.0	3.4(13)	<0.050
2.0	2.3(13)	<0.001

Table 2. Effects of ethanol and pyrazole on the liver lactate – pyruvate and glyceraldehyde-3-phosphate – 3-phosphoglycerate couples in the intact fed rat. Each figure represents the mean ± standard deviation of 6 animals. Contents expressed in μmol/g of liver fresh weight. Groups receiving ethanol were compared with the corresponding control groups by Student's *t*-test.

Metabolite	Presence of ethanol	Amount of pyrazole administered [mmol/(kg body weight)] 0	0.1	0.5	1.0	2.0
Lactate	–	1.8(5)	1.8(7)	1.7(4)	1.7(7)	1.6(5)
	+	1.9(4)	1.5(4)	1.4(3)	1.7(3)	2.1(3)
Pyruvate	–	0.13(5)	0.14(3)	0.13(5)	0.13(4)	0.13(6)
	+	0.06(2)[a]	0.06(4)[b]	0.07(3)[a]	0.06(5)[a]	0.11(4)
Lactate/pyruvate	–	15(3)	12(4)	14(5)	14(5)	14(5)
	+	36(17)[a]	29(21)[a]	32(21)[a]	30(14)[a]	22(9)
3-Phosphoglycerate	–	0.30(12)	0.25(6)	0.19(5)	0.23(10)	0.19(4)
	+	0.09(3)[c]	0.14(6)[a]	0.13(6)	0.15(4)	0.15(3)
Dihydroxyacetone phosphate	–	0.05(3)	0.05(3)	0.05(3)	0.04(3)	0.05(3)
	+	0.05(2)	0.05(2)	0.04(1)	0.05(2)	0.06(2)
Glyceraldehyde-3-phosphate/3-phosphoglycerate	–	0.02(2)	0.02(1)	0.03(2)	0.02(2)	0.03(2)
	+	0.07(4)	0.04(2)	0.04(2)	0.03(1)	0.04(2)

[a] $p<0.050$. [b] $p<0.010$. [c] $p<0.001$.

hibition by pyrazole was slight [pyrazole doses 0.1 – 1.0 mmol/(kg body weight)]. A pyrazole dose of 2 mmol per kg body weight strongly depressed the rise in the liver lactate/pyruvate ratio caused by ethanol. A significant positive correlation ($r=0.90$, $p<0.05$) was found to exist between the lactate/pyruvate ratio and the rate of ethanol oxidation (Fig. 1).

A highly significant decrease ($p<0.001$) in the content of liver 3-phosphoglycerate was observed after ethanol administration (Table 2). This has been previously found by Williamson *et al.*[27] and also by Veech *et al.*[6] With simultaneous administration of 0.1 mmol of pyrazole per kg body weight the change was less marked and was not significant when 0.5 mmol or higher doses of pyrazole were given together with ethanol. No change in the glyceraldehyde-3-phosphate content was recorded. This may have been due to the very small concentration of this metabolite, which made an accurate determination very difficult. However, ethanol changed the glyceraldehyde-3-phosphate/3-phosphoglycerate ratio significantly ($p<0.05$). The change was due to the decrease in the 3-phosphoglycerate concentration. Administration of 0.1 mmol of pyrazole per kg body weight corrected this shift because there was an increase in the 3-phosphoglycerate concentration. No significant correlation was found between the lactate/pyruvate and glyceraldehyde-3-phosphate/3-phosphoglycerate ratios. This probably depends on the inaccurate ratio for the latter couple.

Liver phosphorylation state. Pyrazole alone had no influence on the contents of liver ATP, ADP, AMP and inorganic phosphate (Table 3). Ethanol administration caused a slight increase ($p<0.05$) in the liver content of ATP,

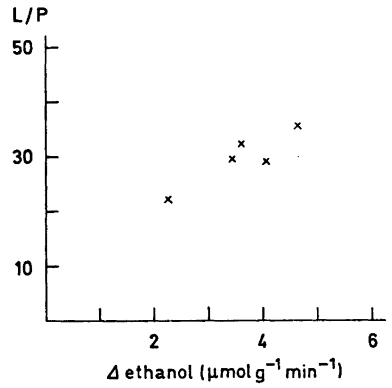

Fig. 1. Variation in the lactate/pyruvate ratio as a function of the ethanol oxidation rate. The scatter plot (with $r=0.90$; $p<0.05$) is drawn from the results of Tables 1 and 2.

Table 3. Effects of ethanol and pyrazole on the ATP, ADP, AMP, and P_i contents of the liver in the intact fed rat. Figures represent the mean ± standard deviation of 6 animals. Contents are expressed as μmol/g of liver fresh weight. Groups receiving ethanol were compared with the corresponding control groups by Student's *t*-test.

Metabolite	Presence of ethanol	Amount of pyrazole administered [mmol/(kg body weight)] 0	0.1	0.5	1.0	2.0
ATP	−	2.5(3)	2.6(3)	2.5(2)	2.5(5)	2.5(2)
	+	2.8(1)[a]	2.7(2)	2.6(3)	2.8(4)	2.8(2)[a]
ADP	−	0.7(1)	0.7(1)	0.7(1)	0.67(4)	0.7(1)
	+	0.7(1)	0.7(1)	0.7(1)	0.7(1)	0.7(1)
AMP	−	0.3(2)	0.3(1)	0.3(1)	0.4(2)	0.3(2)
	+	0.3(1)	0.2(1)	0.3(2)	0.2(1)	0.3(1)
P_i	−	3.6(11)	3.9(8)	3.6(8)	3.6(7)	3.7(8)
	+	3.5(7)	3.5(6)	3.4(7)	3.4(7)	3.5(7)
ATP/	−	1.8(7)	1.7(7)	1.8(5)	1.8(5)	1.6(4)
$ADP \times HPO_4^{2-}$	+	2.0(9)	2.0(6)	1.9(7)	2.0(6)	2.1(6)

[a] $p<0.050$.

but after 0.1 mmol of pyrazole per kg body weight no difference existed between the ethanol-treated and control groups. The concentration of other adenine nucleotides and inorganic phosphate did not change (Table 3). Ethanol did not influence the phosphorylation state of the fed rat liver, nor did the rate of ethanol oxidation influence this state.

DISCUSSION

Alcohol dehydrogenase (EC 1.1.1.1) plays a major role in the oxidation of alcohol in the liver.[28] During normal conditions more of the enzyme is present in the liver than is necessary for the rate of alcohol oxidation and it is, therefore, assumed that the rate of ethanol metabolism is not limited by the amount of this enzyme.[20,28,29] The excess of alcohol dehydrogenase may also explain our finding that rather large doses of pyrazole were needed before an effect on the rate of ethanol oxidation was observed.

Ethanol, when present, is the prime substrate for liver metabolism. The oxidation of ethanol to acetaldehyde takes place in the cytosol and the second reaction, oxidation of acetaldehyde to acetate, is evidently a mitochondrial reaction.[30,31] Only the first reaction is responsible for the change in the lactate/pyruvate ratio which takes place in the cytosol.[20,32] Accordingly, in the present study we found a positive linear correlation between the ethanol oxidation rate and the liver lactate/pyruvate ratio. In this case alcohol dehydrogenase was inhibited, and the decreased rate of ethanol oxidation was responsible for the decrease in the redox state. However, in rats fed with different substances[7] or fasted,[9] a negative correlation between the rate of ethanol oxidation and the lactate/pyruvate ratio was found, *i.e.*, when more ethanol was oxidized, the lactate/pyruvate ratio changed less. In these cases the amount of alcohol dehydrogenase is not rate limiting but rather the redox level of the $NAD^+/NADH$ system or the amount of either NAD^+ or NADH influences the oxidation rate of ethanol.[33] The ethanol oxidation rate can thus be influenced either by directly inhibiting alcohol dehydrogenase or by influencing the processes which affect the redox level of the cytosol.

Measurements of the overall levels of ATP, ADP and P_i in the liver give values which correspond closely to those in the cytosol, since the amounts of these compounds within the mitochondria are small and the mitochondrial matrix space is only about 6 % of the total liver space.[5,34] In the cytosol the phosphorylation state can be influenced by the redox state *via* the glyceraldehyde-3-phosphate – 3-phosphoglycerate couple,[5] by the rate of ATP utilization[35] and also by the interchange of ATP and ADP between cytosol

and mitochondria.[34] The amount of ATP formed by glycolysis in the cytoplasm is so small that it does not need to be taken into account.[36] When this work was begun, the shift in the redox state of the liver cytosol was expected to influence also the phosphorylation state, and we hoped that both of these changes could be correlated with the rate of ethanol oxidation. It was found, however, that only the redox state was influenced and this correlated well with the ethanol oxidation rate. The phosphorylation state was not influenced.

This result differs from those obtained with rats starved for 48 h.[5,6] In the liver of fasted rats ethanol has very strong effects on the lactate/pyruvate ratio [8] and a significant effect on liver phosphorylation state is also seen.[5] During starvation there is a partial shortage of substrate and at the same time intensive utilization of ATP for gluconeogenesis. When ethanol is available, this readily-oxidizable substrate is metabolized first, and, at the same time, ATP utilization for gluconeogenesis is depressed.[35] These effects of ethanol may contribute to the influence on the phosphorylation state of the liver in the fasted rat. In the fed rat the situation is different. Ethanol is utilized instead of other freely available substrates, and the rate of gluconeogenesis is small and not depressed by ethanol.[37] So only the change in the redox state or the interchange of adenosine phosphates with mitochondria can be expected to alter the phosphorylation state of the cytosol. There is no pressure on the adenosine phosphate system and, therefore, no effect of ethanol on the state of phosphorylation as demonstrated by Veech *et al.*[6] and confirmed in the present study. Apparently, in the liver of the fed rat the phosphorylation state is so well stabilized that the oversupply of reducing equivalents and the accompanying alteration of the cytosolic redox state cannot influence the phosphorylation state.

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