RELATIONSHIP BETWEEN THE PHOSPHORYLATION STATE AND THE RATE OF ETHANOL ELIMINATION IN REGENERATING RAT LIVER

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1. Introduction

It has been suggested that ATP consumption by affecting the rate of NADH reoxidation in the respiratory chain may influence the rate of ethanol elimination [1,2]. On the other hand, the phosphorylation state ($ATP/ADP \times P_i$) of the liver cytoplasm rather than concentration of ATP or ADP, has been claimed to control the activity of the respiratory chain [3,4]. We made an experimental study of this supposed relationship between the phosphorylation state and the rate of ethanol elimination using partially hepatectomized rats. In regenerating rat liver the activity of the respiratory chain is enhanced [5] and ethanol elimination rate is higher than in unoperated control rats [6]. It was found that the changes in ethanol elimination rate corresponded to those in phosphorylation state. This suggests that the part played by phosphorylation state of the liver cytoplasm in regulation of the rate of ethanol elimination is important.

2. Experimental

Male rats of mixed strain [7] from Alko's laboratory were used and before starting the experiments the rats were fed commercial laboratory diet (Astra-Ewos, Södertälje, Sweden) and tap water ad libitum. The rats averaged 371 ± 49 g. In partial hepatectomy ~66% of the liver was removed under light diethylether anaesthesia [8], and the control rats were sham operated.

The hepatectomized rats were divided into two groups. Immediately after surgery, the sham-operated control group and 1 hepatectomized group were given 2 g ethanol/kg body wt in 15% (w/v) water solution by gastric intubation. After surgery (20 h) the other hepatectomized group received this ethanol dose in the same way. For the 3 groups, tail-blood ethanol concentrations were followed for 4 h. The elimination period for the entire dose was estimated from the linear part of the elimination curve and ethanol elimination rate was calculated.

Likewise, 3 additional groups of rats were given ethanol 2 g/kg body wt, perorally, 3 h and 23 h after partial hepatectomy or 3 h after sham-operation. After the administration (1 h) of ethanol liver specimens were taken with freeze-stop technique [9] under pentobarbital anaesthesia (Nembutal®, 40 mg/kg body wt, i.p.). Three further groups of rats, as control, received equal volume of water and the livers were similarly sampled 1 h after the gastric intubation of water. The liver specimens were homogenized in liquid nitrogen. Proteins were precipitated with 0.6 N perchloric acid. Metabolites were determined from the protein-free supernatant after neutralization.

Ethanol was determined gas-chromatographically (Perkin-Elmer F 40) using head-space analysis, at 65°C, in a water bath [10]. Lactate, pyruvate, ATP, ADP, and AMP were determined enzymatically with the methods in [11]. P_i was determined according to [12]. The Student's *t*-test was used to evaluate the statistical differences between the different groups.

3. Results and discussion

In this study the ethanol elimination rates were similar to those found [6] in regenerating rat liver.

Table 1
Effect of partial hepatectomy on the liver-to-body weight ratio and ethanol elimination rate

Time after hepatectomy (h)	(*n*)	Liver-to-body wt (%)	Ethanol elimination rate (μmol/100 g body wt/min)	(μmol/g liver wt/min)
Controls	6	3.37 ± 0.77	16.3 ± 2.60	4.94 ± 0.28
0	6	1.02 ± 0.13	7.88 ± 2.19	8.52 + 2.94
20	5	1.41 ± 0.21	7.00 ± 1.47	5.52 ± 0.34

Ethanol elimination rate was measured immediately after the partial hepatectomy, or sham operation and also 20 h after hepatectomy. Ethanol (2 g/kg body wt) was given by gastric intubation. The results are the mean ± SD

The hepatectomized groups showed a significantly higher ethanol elimination rate compared to the controls at 4 h postoperatively and slightly higher at 24 h (table 1).

Hepatectomy had no effect on the lactate-to-pyruvate (L/P) ratio in regenerating rat liver at 4 h after the operation, but a significant increase ($p < 0.01$) in L/P ratio was found at 24 h after the operation (table 2). The increasing effect of ethanol on the L/P ratio in regenerating rat liver was similar to that found in the control rats. Both the increase in lactate concentration and the decrease in pyruvate concentration were observed to contribute to the change in the L/P ratio. No correlation was found between ethanol elimination and the L/P ratio.

The cell content of adenine nucleotides was also unchanged both 4 h and 24 h after partial hepatectomy (table 2). In the control group, ethanol significantly increased the cell content of ATP, ADP, AMP and P_i, but this effect was not present in the partially hepatectomized rats. Both 4 h and 24 h after partial hepatectomy the phosphorylation state was significantly lower ($p < 0.01$ and $p < 0.05$, respectively) than in control rats. As found [13,14], ethanol had no effect on the phosphorylation state in the livers of fed control rats. Similarly, ethanol had no

Table 2
Effect of partial hepatectomy and ethanol on metabolite concentrations

Metabolite (μmol/g liver)	Control	Control +ethanol	4 h regeneration	4 h regeneration +ethanol	24 h regeneration	24 h regeneration +ethanol
n	10	5	8	5	5	5
Lactate	0.833 ± 0.225	1.626 ± 0.717[b]	0.871 ± 0.321	1.470 ± 0.360[b]	1.339 ± 0.361	2.758 ± 1.487
Pyruvate	0.150 ± 0.033	0.093 ± 0.010[b]	0.169 ± 0.037	0.087 ± 0.016[c]	0.104 ± 0.043	0.104 ± 0.043
ATP	2.087 ± 0.179	2.835 ± 1.053[a]	2.152 ± 0.325	2.292 ± 0.322	2.041 ± 0.350	2.731 ± 1.267
ADP	0.596 ± 0.079	0.809 ± 0.213[a]	0.752 ± 0.270	0.989 ± 0.185	0.713 ± 0.156	0.876 ± 0.436
AMP	0.390 ± 0.067	0.644 ± 0.188[b]	0.491 ± 0.106	0.463 ± 0.136	0.473 ± 0.175	0.517 ± 0.256
P_i	3.35 ± 0.35	3.86 ± 0.43[a]	3.64 ± 0.49	4.06 ± 0.57	4.16 ± 0.67	4.33 ± 1.93
L/P	5.8 ± 1.1	17.8 ± 8.7[b]	5.4 ± 2.8	17.4 ± 4.4[c]	11.4 ± 2.5	25.3 ± 5.9[b]
ATP/ADP × P_i (M^{-1})	1790 ± 340	1420 ± 450	1100 ± 570	1020 ± 370	1280 ± 510	1320 ± 920

[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.001$ from the corresponding control

Ethanol (2 g/kg body wt) was given by gastric intubation 3 h or 23 h after partial hepatectomy. Control rats were sham operated. Liver samples were taken 1 h after ethanol administration. The results are the mean ± SD

effect on the phosphorylation state in the operated rats. No correlation was found between the phosphorylation state and the L/P ratio.

When compared to fed rats the phosphorylation state in the liver has been reported to be lowered also in fasted rats [15], in rats treated with thyroxine [16] and in rats chronically treated with ethanol [17]. If, as suggested [3,4], the activity of the respiratory chain depends on the cytoplasmic phosphorylation state, a higher rate of ethanol elimination should be demonstrable in these rats. In fact, this has been found in thyroid hormone [18] and ethanol-treated rats [19], as well as after partial hepatectomy [6], but not in fasted rats [20]. A comparison of the ethanol elimination rates and the phosphorylation states measured in the different present groups shows that in the 4 h regeneration group with the highest rate of ethanol elimination the phosphorylation state is lowest (tables 1, 2). On the other hand, in controls the rate of elimination is lowest while the phosphorylation state in these rats is higher than in the partially hepatectomized rats. The relation between the phosphorylation state and the rate of ethanol elimination found in the present study is in accordance with the [1–4,14,17] and suggests that the phosphorylation state of the liver cytoplasm is an important regulator of ethanol elimination rate.

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