REGULATION OF HEPATIC ELIMINATION OF ETHANOL *IN VIVO*

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1. Introduction

After ingestion of ethanol oxidation to acetaldehyde catalysed by alcohol dehydrogenase (ADH, alcohol – NAD oxidoreductase, EC 1.1.1.1), an enzyme located in the liver cytosol, is generally considered to be the main route of elimination *in vivo*. This reaction is dependent on the availability of the oxidized form of nicotinamide adenine dinucleotide (NAD^+) in the liver cytosol and therefore both the redox state of this compartment and the amount (maximum activity) of ADH must be regarded as rate-limiting factors in ethanol metabolism [1]. In fact, the rate of elimination of ethanol is found to be greatly reduced in protein-deficient rats, which have a very low liver ADH activity [2], and in rat livers perfused with pyrazole, which inhibits ADH [3]. However, the importance of the cytoplasmic redox state as a factor modifying the hepatic elimination of ethanol *in vivo* is not quite clearly established.

It has long been known that in isolated systems the dissociation of the ADH–NADH complex is the step limiting the rate of oxidation of ethanol [4]. *In vivo*, on the other hand, the most characteristic effect of ethanol metabolism in the liver is the shift of the redox state in a more negative direction, an effect which leads, among other things, to inhibition of other NAD^+-dependent reactions in the liver such as oxidation of sorbitol to fructose [5]. The recent demonstration that sorbitol also inhibits the oxidation of ethanol *in vivo* [6] prompted this study of the relationship between the hepatic elimination of ethanol and the redox state of the liver cytosol *in vivo*.

In this experiment animals were given drugs which are known to affect the elimination of ethanol *in vivo*. Propyl thiouracil [7] was used to decrease and promethazine [8] and clofibrate [9] to increase the hepatic elimination of ethanol. Since propyl thiouracil is known to increase liver ADH activity significantly [10], treatment with this drug and promethazine combined was given to one group of animals. The rate of elimination of ethanol was compared with the liver ADH activity and the lactate/pyruvate ratio, which serves as a measure of the redox state of the cytoplasmic NAD system [11]. The latter parameter, which was measured in hepatic venous blood, was found to be negatively correlated with the hepatic elimination of ethanol but no relationship was demonstrable between the rate of elimination of ethanol *in vivo* and liver ADH activity.

2. Materials and methods

Male Wistar rats, weighing 300–400 g and given ordinary laboratory food and tap water *ad libitum* were used for the experiments. The special treatments were given for three weeks. Daily injections of promethazine chloride, 0.25 mg/kg, were given intraperitoneally to two groups of animals. One of these groups and the third group received, in addition, propyl thiouracil 0.5 mg/kg/day via a stomach tube and the fourth group was injected daily with ethyl-α-*p*-chlorophenoxyisobutyrate (clofibrate) 2 mg/kg subcutaneously. The last group served as a control.

At the end of the third week the rate of elimination of ethanol was determined by giving a single intraperitoneal injection of ethanol, 1.5 g/kg, in saline as a 10% (w/v) solution. Blood samples were taken from the tip of the tail and the rate of elimination was calculated as described elsewhere [7]. The lactate/pyruvate ratio, which was previously demonstrated to be in

equilibrium between liver tissue and hepatic venous blood [5], was determined in the latter medium 15 min after a single intraperitoneal injection of ethanol, 1.0 g/kg. Thereafter the animals were killed, their livers weighed, and liver ADH activity assayed from the supernatant, which was incubated at 25° in a sodium pyrophosphate-semicarbazide-glycine buffer adjusted to pH 8.7 (for details, see [10]). Lactate and pyruvate concentrations were measured within a few hours after sampling by an enzymic method [12].

The results concerning liver ADH activity and the rate of elimination of ethanol were expressed against liver fresh weight, since all the experimental groups except the clofibrate-treated group had similar liver- to body weight ratios and since, as demonstrated recently [9], clofibrate treatment does not change the liver protein content.

3. Results and discussion

The effects of the various drugs on the parameters determined are summarized in table 1. The rate of elimination of ethanol was increased by both clofibrate and promethazine treatment, but the effect of the former drug was evidently attributable solely to an increase in liver weight, whereas the effect of the latter was still clear when the rate of elimination was expressed in relation to liver fresh weight. No significant difference in liver ADH activity was demonstrable in either of these groups as compared with the control group.

The marked decrease in the rate of elimination of ethanol induced by propyl thiouracil treatment was almost entirely prevented by simultaneous promethazine treatment but this combined treatment did not reduce the increase in liver ADH activity due to the propyl thiouracil treatment. In fact, liver ADH activity was markedly increased in both these groups as compared with the control group. This shows clearly that factors other than mere liver ADH activity interfere with the regulation of ethanol metabolism *in vivo*.

Altough no correlation was present between the rate of elimination of ethanol and liver ADH activity, a significant ($r = -0.577$, $p < 0.001$) negative correlation was demonstrable between the individual values for elimination of ethanol expressed in relation to liver fresh weight and those of the lactate/pyruvate ratio in the hepatic venous blood during ethanol metabolism. It appeared that the smaller the increase in this ratio the greater the rate of hepatic elimination of ethanol. A similar relationship was previously described between the rate of ethanol elimination and the cytoplasmic redox state of the liver in fed and starved animals [13].

The main conclusion to be drawn from the experiments reported above is that the cytoplasmic redox state of the liver plays an important part in the regulation of hepatic elimination of ethanol *in vivo*. This conclusion seems justified, since the extrahepatic

Table 1

Rate of elimination of ethanol in relation to the lactate/pyruvate ratio of hepatic venous blood and liver alcohol dehydrogenase activity.

Groups	Rate of elimination of ethanol (mg/100 g body wt/hr)	Rate of elimination of ethanol (mg/g liver wt/hr)	Lactate/pyruvate ratio (hepatic venous blood)	Liver ADH activity (U/g liver wt)
Promethazine-treated	37.3 ± 2.1*	11.6 ± 0.9*	20 ± 4*	2.1 ± 0.3
Controls	35.2 ± 1.8*	10.1 ± 0.6	29 ± 6	1.9 ± 0.3
Clofibrate-treated	39.6 ± 1.1*	10.0 ± 0.4	33 ± 5	1.9 ± 0.2
Promethazine + propyl thiouracil-treated	32.8 ± 2.8*	9.7 ± 1.1	37 ± 14	2.8 ± 0.3*
Propyl thiouracil-treated	27.6 ± 2.4*	8.6 ± 1.0	61 ± 13	2.7 ± 0.2*

* $p < 0.05$ for difference from corresponding controls. Each figure represents the mean of nine experiments ±S.D.

elimination of ethanol is very slight (less than 10%) and thus of minor importance [14] and since the microsomal ethanol-oxidizing system (MEOS) has quite recently been shown to play only an insignificant role in the metabolism of ethanol in the intact liver [15]. The role of liver ADH activity as a factor regulating the hepatic elimination of ethanol seems to be of minor importance at least in conditions when the activity is at a normal level or increased. However, when the liver ADH activity is markedly decreased, as in protein-deficient [2] or tri-iodothyronine-treated [10] rats or when it is inhibited by pyrazole [3], it may fairly well influence the rate of elimination of ethanol *in vivo*.

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