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INHIBITION BY ALIPHATIC ALCOHOLS OF THE STIMULATED ACTIVITY OF ORNITHINE DECARBOXYLASE AND TYROSINE AMINOTRANSFERASE OCCURRING IN REGENERATING RAT LIVER

HANNU PÖSÖ* and A. REETA PÖSÖ†

*Research Laboratories of the State Alcohol Monopoly (Alko), Box 350, SF-00101 Helsinki 10, and †Department of Biochemistry, College of Veterinary Medicine, Helsinki, Finland

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Abstract—Inhibition of the synthesis of ornithine decarboxylase and tyrosine aminotransferase by ethanol, 1-propanol, 2-propanol and 2-methyl-2-propanol was studied in partially hepatectomized rats. The inhibition by ethanol appeared to have a direct dependence upon the dose. Other alcohols tested were even more efficient inhibitors than ethanol of the stimulation of ornithine decarboxylase and tyrosine aminotransferase, in regenerating liver remnant after partial hepatectomy. In addition, the stress of the operation was found to stimulate the activity of ornithine decarboxylase in the kidneys and to a lesser extent in the brain. In kidneys, but not in the brain, ethanol inhibited the increase in the activity of ornithine decarboxylase in a dose-dependent manner. 2-Methyl-2-propanol, similarly to the higher dose of ethanol, inhibited ornithine decarboxylase in the kidneys, whereas other alcohols appeared to have no effect. Since 2-propanol and 2-methyl-2-propanol do not produce aldehydes in the liver or change the NADH/NAD^+ redox state, the results suggest that the synthesis of ornithine decarboxylase and tyrosine aminotransferase was inhibited by ethanol molecules. The dose dependence of the ethanol inhibition, both in the liver and kidneys, also supports this suggestion.

The inhibition of protein synthesis in the liver caused by acute alcohol treatment is still unclear and remains a question of controversy. The *in vitro* incorporation of radioactive label into proteins is usually inhibited [1–4], although *in vivo* inhibition [5–7], unaffected [4] and even accelerated [5, 8] protein synthesis have been reported after acute ethanol treatment.

As the low rate of protein synthesis in normal liver could be one reason for the large variation in the results, we used regenerating rat liver as the model tissue, as suggested in our previous study [7]. After partial hepatectomy the rate of general protein synthesis, including the synthesis of specific proteins, is greatly enhanced and therefore the possible inhibition by ethanol is easier to detect. When the activity of ornithine decarboxylase (E.C. 4.1.1.17) was used as the marker of protein synthesis [9], we found at 4 hr after partial hepatectomy that a moderate dose of ethanol inhibited the synthesis of this enzyme by 50–70 per cent [7]. From the experiments with disulfiram and 4-methylpyrazole it was suggested that this effect was directly due to the ethanol molecules and not mediated via acetaldehyde, acetate or the changed NADH/NAD^+ redox state.

The aim of the present study was to test our hypothesis that ethanol itself inhibits protein synthesis, by studying the effect of other aliphatic alcohols. In addition to ethanol we tested 1-propanol, which is a primary alcohol and metabolized by alcohol dehydrogenase to propionaldehyde, 2-propanol, which is a secondary alcohol and is metabolized to acetone, and 2-methyl-2-propanol, which is not metabolized in the liver but is mainly eliminated by excretion [10]. The lactate/pyruvate ratio is increased in the liver during the elimination of ethanol and 1-propanol but not after treatment with 2-propanol and 2-methyl-2-propanol [11]. The activities of ornithine decarboxylase, tyrosine aminotransferase and alanine aminotransferase were measured. The two former enzymes have short half-lives, 15 min [12] and 3 hr [13], respectively, and therefore the activities of these enzymes can be used as specific markers of acceleration in the synthesis of proteins. Both enzymes are also induced after partial hepatectomy [14–16]. Alanine aminotransferase was measured as control, because it has a long biological half-life (3 days; [17]).

EXPERIMENTAL

Female rats, of the mixed strain from the laboratory of Alko [18], were used in this study. Rats were housed 6–8 per cage, at 24–25° and on a day–night schedule of 12:12 hr. Until the beginning of the experiments, rats received standard diet (from Astra-Ewos, Södertälje, Sweden) and tap water *ad lib.* Rats, which were 3 months of age and weighed 238 ± 22 g, were used.

DL-[1-^{14}C]ornithine (specific radioactivity 53 mCi/mmole) was purchased from The Radiochemical Centre (Amersham, U.K.). L-Ornithine, pyridoxal 5′-phosphate, EDTA, L-tyrosine, α-ketoglutaric acid, 1-propanol (p.a.), 2-propanol (p.a.) and 2-methyl-2-propanol (p.a.) were obtained from Merck

(Darmstadt, F.R.G.). Ethanol (94% w/v, grade A) was the product of the State Alcohol Monopoly (Alko, Helsinki, Finland). Dithiothreitol was purchased from Calbiochem (La Jolla, CA, U.S.A.).

On the morning of the day of the experiment, rats were partially hepatectomized under light diethyl-ether anaesthesia [19]. Control rats were similarly anaesthetized and their abdomen was opened. The pieces of liver removed during the operation weighed 4.7 ± 0.6 g, the liver remnant at the end of the experiment 2.6 ± 0.3 g, and the livers of sham-operated controls 8.6 ± 0.6 g (wet wt).

Immediately after partial hepatectomy, ethanol [1.8 g per kg body wt as a 15% (w/v) solution in water] was given to 6 rats by gastric intubation. An equimolar dose of 1-propanol [2.3 g per kg body wt as a 15% (w/v) solution in water], 2-propanol [2.3 g per kg body wt as a 15% (w/v) solution in water] and 2-methyl-2-propanol [2.8 g per kg body wt as a 15% (w/v) solution in water] were also given to groups of 6 rats immediately after the operation. Since equimolar doses of aliphatic alcohols produce different degrees of intoxication [10], a further group of 6 rats received a higher dose of ethanol which produced approximately the same degree of intoxication as the doses of the other alcohols used [10]. This higher dose of ethanol was 5 g per kg body wt and it was also given as a 15% (w/v) solution in water by gastric intubation after partial hepatectomy.

Blood samples for alcohol analysis were taken from the tip of the rat's tail at hourly intervals and placed in ampules which contained ice-cold distilled water. All the rats were decapitated 4 hr after partial hepatectomy. Livers, kidneys and brains were quickly removed, rinsed in ice-cold buffer (25 mM Tris–HCl, 0.1 mM EDTA and 1 mM dithiotreitol, pH 7.1), weighed and then homogenized in 2 vol. of the same buffer. Homogenates were centrifuged for 30 min at 105,000 g_{max} and +2°. The supernatant fractions were used in the enzyme assays.

The activities of ornithine decarboxylase, tyrosine aminotransferase (EC 2.6.1.5) and alanine aminotransferase (EC 2.6.1.2) were measured by methods described previously [20–22]. Alanine aminotransferase was measured using a Gilford 3500 computer directed analyzer (Oberlin, OH, U.S.A.).

Ethanol was measured gas chromatographically (Perkin–Elmer F 40) [23] using head-space analysis. The concentrations of other alcohols tested were similarly measured, with the corresponding alcohols as standard. The protein content was measured according to Lowry *et al.* [24], with bovine serum albumin as the standard.

Statistical differences were calculated using the Student's *t*-test and the correlations were calculated by the least-squares method.

RESULTS

Blood alcohol concentrations. Within 1 hr a constant level of ethanol, 1-propanol and 2-propanol in the blood was reached (Fig. 1). The concentration in the blood remained approximately constant during the remaining 3 hr of the experiment, i.e. until the decapitation (Fig. 1). The concentration of 2-methyl-2-propanol increased slightly during the whole experiment. The molar concentrations of blood ethanol (1.8 g per kg), 2-propanol and 2-methyl-2-propanol were equal but, as reported previously [10], 1-propanol gave a slightly lower blood concentration than the equimolar dose of ethanol (Fig. 1). The larger dose of ethanol produced a blood ethanol concentration of 95 mM, which was reached within 2 hr after the incubation (at 1 hr the concentration was 82 mM) and remained constant until decapitation.

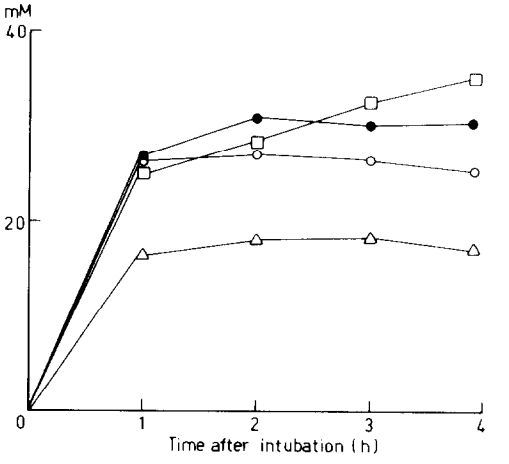

Fig. 1. Concentration of aliphatic alcohols in the tail blood of partially hepatectomized rats. Immediately after partial hepatectomy rats were given 1.8 g of ethanol (○——○), 2.3 g of 1-propanol (△——△), 2.3 g of 2-propanol (●——●) or 2.8 g of 2-methyl-2-propanol (□——□) per kg body wt by gastric intubation. Tail blood alcohol concentrations were followed for 4 hr. The results shown are the mean values obtained from groups of 3–6 rats. The standard deviation of alcohol concentrations shown in the figure was 31.1 per cent on average and alcohol concentrations did not differ significantly from each other.

Enzyme activities in the liver after partial hepatectomy. The activities of both ornithine decarboxylase and tyrosine aminotransferase were greatly stimulated 4 hr after partial hepatectomy (Table 1). Ethanol, as reported previously [7], inhibited the increase in the activity of ornithine decarboxylase. The inhibition of ornithine decarboxylase by ethanol appeared to be dose-dependent (Table 1). If the correlation coefficient between the dose of ethanol (0, 1.8 and 5.0 g per kg) and the activity of ornithine decarboxylase is calculated, a value of −0.765 (d.f. = 15, $P < 0.001$) is obtained. All other alcohols tested were even more powerful inhibitors of the increase in the activity of ornithine decarboxylase (Table 1). This is in agreement with the studies in which it was found that the biological activity of alcohols increases with increased length of the carbon chain [10]. The results also support our earlier finding [7] that the effects of ethanol on protein synthesis are not mediated via acetaldehyde, as neither 2-propanol nor 2-methyl-2-propanol produce aldehydes [10].

The degree of inhibition of the increase in activity of tyrosine aminotransferase was slightly smaller than that affecting the activity of ornithine decarboxylase (Table 1). This may be due to the longer half-life of the latter enzyme [17]. When tyrosine aminotransferase was studied, a dose-dependent

Table 1. The effect of acute treatment with aliphatic alcohols on the activity of ornithine decarboxylase (ODC), tyrosine aminotransferase (TAT) and alanine aminotransferase (ALAT) in regenerating rat liver 4 hr after partial hepatectomy*

Treatment and dose		ODC activity (pmoles/ 30 min per mg of protein)	TAT activity (nmoles/ min per mg of protein)	ALAT activity (μmoles/ min per mg of protein)
Sham + saline	(4)	65 ± 33§	5.68 ± 0.56§	0·75 ± 0.10
Hepatectomized + saline	(6)	1089 ± 235	30.66 ± 7.41	0.65 ± 0.11
Hepatectomized + ethanol (1.8 g/kg)	(5)	510 ± 283‡	20.38 ± 3.54†	0.52 ± 0.09
Hepatectomized + ethanol (5.0 g/kg)	(6)	307 ± 233§	19.31 ± 5.28†	0.62 ± 0.08
Hepatectomized + 1-propanol (2.3 g/kg)	(6)	188 ± 93§	21.47 ± 6.06†	0.64 ± 0.17
Hepatectomized + 2-propanol (2.3 g/kg)	(6)	320 ± 144§	21.75 ± 2.52†	0.65 ± 0.04
Hepatectomized + 2-methyl-2-propanol (2.8 g/kg)	(3)	238 ± 156§	15.81 ± 4.15‡	0.75 ± 0.04

* Ethanol, 1-propanol, 2-propanol, 2-methyl-2-propanol or saline was administered immediately after partial hepatectomy. Rats were decapitated 4 hr after the operation and the activities of the enzymes were assayed as described in the text. The number of the animals in each group is shown in parentheses. The results are given as means ± S.D.

† Significantly different from control, $P < 0.05$.

‡ Significantly different from control, $P < 0.01$.

§ Significantly different from control, $P < 0.001$.

inhibition by ethanol was found ($r = -0.596$, d.f. = 15, $P < 0.05$).

As shown earlier [25], the activity of alanine aminotransferase remained constant after partial hepatectomy (Table 1). Alanine aminotransferase [26], as well as ornithine decarboxylase [20] and tyrosine aminotransferase [27], is pyridoxal 5′-phosphate-dependent enzyme and therefore acetaldehyde and other aldehydes, as recently suggested [28], could inhibit its activity by interfering with the binding of the coenzyme to the apoenzyme. Since alanine aminotransferase has a long biological half-life, the possible changes in its activity should not be caused by the changes in the synthesis of new protein in acute experiments and thus if any changes were recorded they would be due to the action of acetaldehyde and other aldehydes. As Table 1 shows, the activity of alanine aminotransferase remained constant after all treatments. It should be mentioned in connection with these results that we also tested that acetaldehyde *in vitro*, between the concentration range of 0 and 500 μM, did not inhibit the activity of tyrosine aminotransferase (results not shown). These results thus suggest that the effect of acetaldehyde on pyridoxal 5′-phosphate binding *in vivo* was negligible.

Enzyme activities in the kidneys after partial hepatectomy. In our previous study [7] we found that the activity of ornithine decarboxylase was greatly stimulated in the kidneys 4 hr after partial hepatectomy. However, in that study we did not use sham-operated controls and, comparing the results of the present study with the data of unoperated rats [7], it is apparent that the induction of ornithine decarboxylase was caused by the stress of the surgical operation (Table 2; [29, 30]). It was, however, inter-

Table 2. The effect of acute treatment with aliphatic alcohols on the activity of ornithine decarboxylase (ODC) and alanine aminotransferase (ALAT) in kidney 4 hr after partial hepatectomy*

Treatment and dose		ODC activity (pmoles/ 30 min per mg of protein)	ALAT activity (nmoles/ min per mg of protein)
Sham + saline	(4)	994 ± 542	50.0 ± 10.8
Hepatectomized + saline	(6)	1471 ± 1184	61.6 ± 9.9
Hepatectomized + ethanol (1.8 g/kg)	(6)	1152 ± 325	54.5 ± 7.0
Hepatectomized + ethanol (5.0 g/kg)	(6)	504 ± 281†	56.3 ± 10.6
Hepatectomized + 1-propanol (2.3 g/kg)	(6)	1879 ± 1259	61.9 ± 13.4
Hepatectomized + 2-propanol (2.3 g/kg)	(6)	1164 ± 409	55.7 + 14.5
Hepatectomized + 2-methyl-2-propanol (2.8 g/kg)	(3)	460 ± 167‡	59.6 ± 2.9

* Experimental details as in Table 1. The number of the animals in each group is shown in parentheses. The results are given as means ± S.D.

† Significantly different from control, $P < 0.01$.

‡ Significantly different from control, $P < 0.001$.

Table 3. The effect of acute treatment with aliphatic alcohols on the activity of ornithine decarboxylase in the brain 4 hr after partial hepatectomy*

Treatment and dose		Activity of ornithine decarboxylase (pmoles/30 min per mg protein)
Sham + saline	(4)	1.36 ± 0.41†
Hepatectomized + saline	(6)	2.42 ± 0.71
Hepatectomized + ethanol (1.8 g/kg)	(6)	2.72 ± 0.85
Hepatectomized + ethanol (5.0 g/kg)	(6)	1.78 ± 0.44
Hepatectomized + 1-propanol (2.3 g/kg)	(5)	2.29 ± 0.69
Hepatectomized + 2-propanol (2.3 g/kg)	(5)	4.09 ± 1.26†
Hepatectomized + 2-methyl-2-propanol (2.8 g/kg)	(2)	3.81

* Experimental details as in Table 1. The number of the rats in each group is shown in parentheses. The results are given as means ± S.D.

† Significantly different from control, $P < 0.05$.

esting to observe that ethanol, at the higher dose (5 g/kg), decreased the activity of ornithine decarboxylase to a level lower than that found after sham-operation (Table 2). The inhibited activity measured in the present study was approximately of the same magnitude as the activity in the unoperated controls measured in the earlier study [7]. In kidneys, as in the liver, the inhibition of the activity of ornithine decarboxylase appeared to be dose-dependent ($r = -0.518$, d.f. = 16, $P < 0.05$). From the other alcohols tested, only 2-methyl-2-propanol had an inhibitory influence on this enzyme (Table 2). As seen in Table 2, treatment with various aliphatic alcohols did not significantly alter the activity of alanine aminotransferase.

Activity of ornithine decarboxylase in the brain after partial hepatectomy. In order to study further the inducibility of ornithine decarboxylase in tissues other than the liver, after partial hepatectomy, the activity of this enzyme was measured in the brain. The brain was chosen because it is surrounded by a blood–brain barrier which makes it more resistant to different types of stimuli. However, in comparison to the sham-operated controls, a slight increase in the activity of ornithine decarboxylase was found after the operation (Table 3). This increase was not inhibited by any of the alcohols tested. 2-Propanol even appeared to stimulate the activity. The results obtained from adult rat brain differ from those obtained from foetal brain, where ethanol, when given to the mothers, was found to inhibit the activity of ornithine decarboxylase [31].

DISCUSSION

The inhibition of protein synthesis occurring after ethanol treatment, both *in vivo* and *in vitro*, has been claimed to be due to ethanol itself [7], acetaldehyde [32], acetate [33], and the increased redox state of the NADH/NAD$^+$ couple [2, 4]. Ethanol or its metabolites have also been reported to interfere specifically with the synthesis of glycoproteins [3, 5], lipoproteins [5, 34] or with the excretion of plasma proteins from the liver [35]. The present results give further support to our suggestion [7] that, after acute ethanol treatment, the inhibition of protein synthesis in the liver is due to ethanol molecules.

The dose dependence of the inhibition of ornithine decarboxylase and tyrosine aminotransferase by ethanol can be taken as evidence of the inhibitory role of ethanol molecules *in vivo*, assuming that ethanol is eliminated at a constant rate [36]. Recently, however, it has been reported [37, 38] that the ethanol elimination rate is concentration-dependent. In addition, the enhanced rate of oxidation of ethanol, after a high dose, also produces more acetaldehyde, a compound which has been reported to inhibit protein synthesis [4, 32]. In our experiments, however, we found that ornithine decarboxylase was also inhibited in a dose-dependent manner in the kidneys, where the activity of alcohol dehydrogenase is far lower than that in the liver [39]. This result does not support the theory of an inhibitory role for acetaldehyde. 2-Propanol and 2-methyl-2-propanol, which do not produce aldehydes during their elimination, inhibited both the activity of ornithine decarboxylase and tyrosine aminotransferase (Table 1). These results also suggest that the production of aldehydes is not necessary for the inhibitory action of alcohols to be exhibited.

The lack of inhibition of ornithine decarboxylase in the kidney by alcohols other than ethanol and 2-methyl-2-propanol may be attributed to the difference in the induction of ornithine decarboxylase in this organ. The factor(s) that induces ornithine decarboxylase after partial hepatectomy is still unknown [9]. It appears, however, that the stimulation is not caused by stress, as prior removal of the thyroid gland, adrenals, ovaries and pituitary gland does not prevent the stimulation of ornithine decarboxylase after partial hepatectomy [12]. In contrast to liver, the induction of ornithine decarboxylase in the kidney, where it is much lower, is due to the stress of the operation (Table 2; [29, 30]).

It has been reported that administration of 2-propanol and 2-methyl-2-propanol does not alter the redox state of NADH/NAD$^+$ couple in the liver [11]. However, both of these alcohols, in addition to ethanol and 1-propanol which do increase the redox state [11], inhibited the synthesis of both ornithine decarboxylase and tyrosine aminotransferase. This result is in accordance with those results in which the administration of 4-methylpyrazole did not abolish the ethanol-induced inhibition of ornithine decarboxylase in regenerating rat liver [7]. Thus it is probable that, *in vivo*, the ethanol-induced inhibition

of protein synthesis is not mediated via the increased redox state. *In vitro*, however, the increase in the redox state of the NADH/NAD^+ couple after ethanol treatment [40] is usually greater than *in vivo* [41] and could therefore, as pointed out by Baraona *et al.* [4], have an effect on the protein synthesis.

As suggested previously, [7], regenerating rat liver appears to be a good experimental model for the study of the action of ethanol on the synthesis of proteins. Regenerating rat liver, being a fast growing tissue, in many respects resembles the growth of a foetus. It is therefore important to point out that the inhibition was caused by ethanol, which, in contrast to acetaldehyde [42], penetrates through the placenta [43]. Our results are in accordance with the findings of Thadani *et al.* [31], who reported that ornithine decarboxylase was inhibited in the hearts and brains of rat pups bred from ethanol-fed rats. Thus the detrimental effect of ethanol on protein synthesis becomes apparent more rapidly and clearly in fast growing systems such as foetal tissues.

In conclusion, our results suggest that the inhibition of protein synthesis in the liver after acute ethanol treatment is attributable directly to the ethanol molecules. Both ornithine decarboxylase and tyrosine aminotransferase, which were used as the markers of protein synthesis, are both proteins which are not excreted from the liver into the blood. It must be emphasized in this connection that there are reports which state that the primary site of the inhibitory action of ethanol is not at the transcriptional or translational level of protein synthesis, but rather an effect on the excretion of proteins [3–5, 39]. Thus it is obvious that ethanol, and/or its metabolites, can interfere with protein synthesis at more than one level, depending on the protein synthesized and also on the experimental system employed.

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