STABILIZATION OF TYROSINE AMINOTRANSFERASE AND ORNITHINE DECARBOXYLASE IN REGENERATING RAT LIVER BY ETHANOL TREATMENT

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1. Introduction

Changes in the activity of enzymes possessing rapid turnover rates can be used as markers of protein synthesis. One such enzyme is tyrosine aminotransferase (TAT; EC 2.6.1.5) which has a biological half-life of 1.5–3 h in normal rat liver [1,2]. The synthesis of TAT in rats can be induced by glucocorticoids both in vivo [1] and in vitro [3] and it is also induced after partial hepatectomy [4,5]. The induction of TAT is accompanied by an increased synthesis of immunoreactive protein [2] and is preceeded by an increase in the concentration of mRNA for TAT [3].

Since the induction of TAT is based on the synthesis of new protein, we have used here the activity of TAT to elucidate the effect of ethanol on protein synthesis during liver regeneration. Recent reports on the effect of ethanol on the activity of TAT have been contradictory. It has been suggested that ethanol may inhibit the synthesis of the enzyme [6,7], induce the enzyme [8,9] or stabilize the enzyme by slowing down its intracellular degradation [10]. In order to differentiate the effects of ethanol on the synthesis and degradation of TAT we compared the acute and chronic effects of ethanol on the activity of TAT in regenerating rat liver after partial hepatectomy.

Our results indicate that, under appropriate conditions, ethanol can inhibit both the synthesis and the intracellular degradation of TAT. Thus, the stimulation of TAT activity during liver regeneration was diminished by acute ethanol administration before the partial hepatectomy; but both the activity and the half-life in regenerating liver of TAT, and also of ornithine decarboxylase (ODC; EC 4.1.1.17), another enzyme with a very short half-life [12], were increased by chronic ethanol treatment.

2. Experimental

D,L-[1-^{14}C]Ornithine (spec. radioact. 53 mCi/mmol) was purchased from the Radiochemical Centre (Amersham). Cycloheximide (Actidione®) was the product of Boehringer (Mannheim).

Female (tables 1,2, fig.2) and male (fig.1) rats from the mixed strain bred at the laboratories of Alko (Helsinki) were used [13]. The weight of females was 245 ± 27 g and that of males 337 ± 32 g. In acute experiments rats were fed ad libitum with Astra-Ewos chow (Södertälje) and tap water, and ethanol was given as a single peroral dose (3 g/kg body wt, 10% (w/v) solution in water) 1 h before the partial hepatectomy. In chronic experiments forced feeding with a nutritionally adequate liquid diet was used [14]. Ethanol treatment was started either 24 h or 3 h before the operation and continued for 1–5 days after it, and the constant presence of ethanol was verified with measurements of blood ethanol concentration, on the basis of which the dose of ethanol in the liquid diet was adjusted. Partial hepatectomy was performed under light ether anaesthesia as in [15].

Activities of TAT, ODC, alanine aminotransferase (ALAT; EC 2.6.1.2) and lactate dehydrogenase (LDH; EC 1.1.1.27) were determined as in [16–18]. Protein was measured with bovine plasma albumin as standard [19]. Blood ethanol concentrations were measured gas chromatographically (Perkin-Elmer F 40) using head-space techniques from samples diluted with distilled water.

3. Results and discussion

As shown in table 1 a sham operation, otherwise

Table 1
Effect of ethanol on the activity of tyrosine aminotransferase (TAT) in regenerating rat liver 4 h after partial hepatectomy

Treatment	Presence of ethanol	Number of animals	TAT activity (nmol . min^{-1} . mg protein^{-1})
Unoperated controls	–	5	6.2 ± 1.5
Sham-operated controls	–	7	17.0 ± 9.2
Sham-operated	+	4	9.9 ± 2.9
Partial hepatectomy	–	5	53.9 ± 10.1
Partial hepatectomy	+	8	30.9 ± 13.3[b]
Partial hepatectomy + 4-MP	–	5	54.0 ± 9.6
Partial hepatectomy + 4-MP	+	5	39.3 ± 7.0[a]
Partial hepatectomy + CaCy	–	5	53.4 ± 11.9
Partial hepatectomy + CaCy	+	5	29.8 ± 3.0[c]

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

Ethanol (3 g/kg body wt) was given by gastric intubation 1 h before partial hepatectomy or sham operation. 4-Methylpyrazole (4-MP, 0.2 mmol/kg body wt) was given intraperitoneally 15 min before ethanol and calcium cyanamide (CaCy, 1 mg/kg body wt) perorally 2 h before the ethanol. The results are the means ± SD

like partial hepatectomy, stimulated the activity of TAT. However, a much greater stimulation occurred after partial hepatectomy, and was prevented by ethanol treatment. Also after sham operation ethanol diminished the stimulation of TAT, but the decrease did not reach the level of statistical significance. We also wanted to test if the effect of ethanol was direct or mediated via acetaldehyde, or the ethanol-induced change in the NADH/NAD$^+$ redox state. 4-Methylpyrazole was used to block ethanol metabolism [20] and calcium cyanamide to increase the hepatic concentration of acetaldehyde [21]. As can be seen from table 1, neither 4-methylpyrazole nor cyanamide alone had any effect on the activity of TAT in regenerating rat liver 4 h after partial hepatectomy. The combined effect of ethanol and 4-methylpyrazole was similar to that found when ethanol was used alone. The combination of ethanol and cyanamide caused slightly more inhibition than ethanol alone, indicating that acetaldehyde may participate in the inhibition.

We have shown that 4-methylpyrazole (0.2 mmol/kg body wt) totally prevented the ethanol-induced change in the lactate-to-pyruvate ratio, and no acetaldehyde could be detected in the tail-blood of treated animals [11,22]. Thus the effect of ethanol on the synthesis of TAT, like that on ODC synthesis [11], does not require oxidation of ethanol, and is probably a direct effect of ethanol itself.

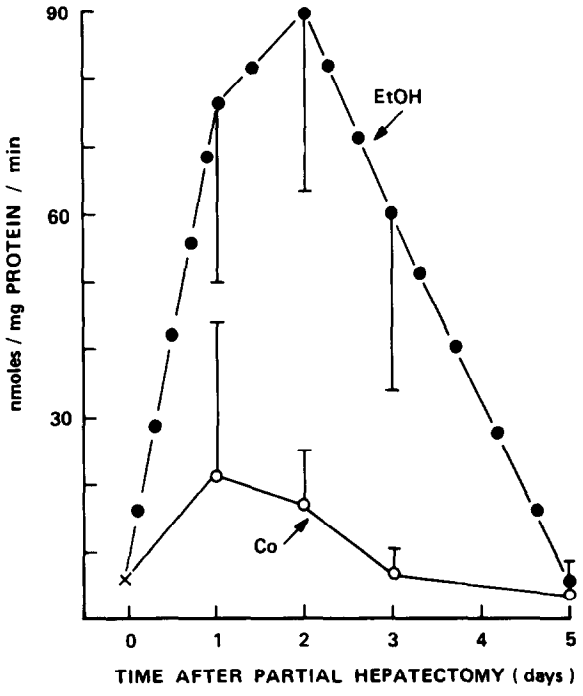

Fig.1. Effect of ethanol containing diet on the activity of TAT in liver after partial hepatectomy. Control diet (Co) or ethanol diet (EtOH) was intubated 4 times/day. The dose of ethanol was: at day 1, 10.0 g/kg body wt; at day 2, 3.7 g; at day 3, 5.4 g; at day 4, 9.6 g; at day 5, 11.4 g. Each group consisted of 5–8 animals. The vertical bars represent standard deviations.

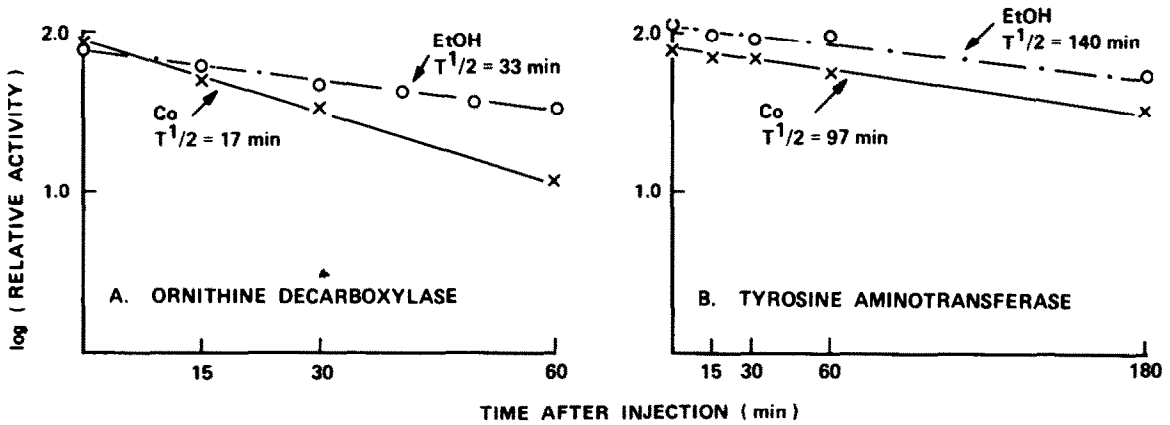

Fig.2. Effect of cycloheximide (1.5 mg/kg body wt) treatment on the activity of ODC (A) and TAT (B) in regenerating rat liver. Rats were partially hepatectomized 26 h earlier and intubated with control diet (Co) or with ethanol diet (EtOH) 4 times/day. The daily dose of ethanol was 5 g/kg body wt. Cycloheximide was injected at zero time. Each group consisted of 3–6 animals. The regression lines were computed by the least squares method.

Surprisingly, in spite of the inhibitory effect of acute ethanol-treatment on TAT, chronic treatment caused a clear stimulation of TAT in regenerating rat liver (fig.1), which was similar to the stimulation of ODC by chronic ethanol treatment during liver regeneration [14]. We therefore measured the half-lives of TAT and ODC in 26 h regenerating liver after cycloheximide treatment to see if the longer treatment stabilized the enzymes. As shown in fig.2A the half-life of ODC was changed from 17 min to 33 min ($p < 0.01$) and that of TAT (fig.2B) from 97 min to 140 min ($p < 0.01$) after 30 h treatment with ethanol.

These results apparently indicate that the longer treatment with ethanol produced a distinct stabilization of the enzymes. To exclude the possibility that ethanol treatment prevented the uptake of cycloheximide, we tested the effect of the cytosol obtained from partially hepatectomized rats treated with control diet, control diet and cycloheximide or ethanol-containing diet and cycloheximide, on the activity of ODC in 4 h regenerating rat livers in test animals. As shown in table 2 liver cytosols from regenerating con-

Table 2
Demonstration of the presence of cycloheximide in rat livers

Treatment	ODC activity (pmol . 30 min^{-1} . mg $protein^{-1}$)
Control + saline	1397 ± 355
Control + cytosol without treatment	1271 ± 165
Control + cytosol from cycloheximide treated rats	582 ± 127[a]
Control + cytosol from ethanol plus cycloheximide treated rats	310 ± 115[b]

[a] $p < 0.05$
[b] $p < 0.01$ from controls receiving saline

Pooled liver cytosols (100 000 × g supernatants) prepared from groups of 4 rats treated as in fig.2, and slaughtered 30 min after cycloheximide treatment, were injected intraperitoneally (2 ml/animal) into test rats 3.25 h after their partial hepatectomy. After 45 min the test animals were killed and ornithine decarboxylase (ODC) activity in their livers was determined. The results are the means ± SD for 3 test animals

trols did not inhibit the activity of ODC when injected into the test animals 45 min before their slaughter. Liver cytosols from control plus cycloheximide and ethanol plus cycloheximide treated rats both inhibited the activity of ODC, showing that cycloheximide was present in the livers of both groups.

We also checked the effect of ethanol on two hepatic enzymes possessing long half-lives, namely ALAT, half-life 3.5 days [23], and LDH, half-life 16 days [23]. The activity of these enzymes is not changed in acute experiments [11,24]. ALAT behaved similarly as TAT and ODC, though the stimulation was smaller than that for TAT and ODC, as might be expected from the longer half-life of ALAT. In contrast, LDH, which is still more stable, did not show any change in activity (results not shown).

Our results indicate that the acute inhibition of TAT and ODC is a direct effect of ethanol itself (table 1, [11]). However, longer treatment with ethanol (1–5 days) caused stabilization of both enzyme activities (fig.2A,B). This may overcome the inhibitory effect seen in the beginning of the regeneration, and so cause the accumulation of enzyme protein during regeneration (fig.1, [14]).

Our results raise the new possibility that inactivation of some proteolytic system(s) (lysosomal? [25]) may contribute to the pathological accumulation of protein after chronic ethanol consumption both in normal [26] and in regenerating rat liver [14,27]. Evidently the system responsible for rapid inactivation of TAT and ODC is more sensitive to ethanol than are TAT and ODC themselves. Interestingly, we have found earlier that other hepatotoxic agents such as thioacetamide and carbon-tetrachloride caused a striking stabilization of ODC in rat liver [28]. Thus the stabilization of proteins may be a common effect of chemicals that damage the liver.

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