EFFECTS OF ACUTE ETHANOL ADMINISTRATION ON TYROSINE AMINOTRANSFERASE ACTIVITY

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1. Introduction

Whereas the effects of acute ethanol administration on tryptophan oxygenase (EC 1.13.11.11) activity have been extensively studied [1–4], less information is available on its action on tyrosine aminotransferase activity (TAT) (EC 2.6.1.5) [5,6].

TAT activity has been found [5] to be increased after acute ethanol treatment in adrenalectomized rats and [6] during ethanol perfusion. It seemed therefore of interest to test the effects of acute ethanol administration on TAT activity in the intact rat.

The present results show that ethanol induces tyrosine aminotransferase activity only in starved rats and, by comparing the response of this enzyme to different hormones, protein synthesis inhibitors or substrates in combination with ethanol, it is suggested that ethanol enhances the tyrosine aminotransferase activity by a substrate-type mechanism caused by tryptophan.

2. Materials and methods

Female Wistar rats (150 ± 5 g) maintained on a standard laboratory diet were used. Food was removed either 16 h before ethanol treatment (starved rats) or just before ethanol treatment (fed rats).

Actinomycin D, cortisol 21-acetate, N^6, $O^{2'}$-dibutyryl cyclic adenosine 3′:5′-monophosphate and L-tryptophan were purchased from Sigma (St Louis).

Ethanol was administered by stomach tube at 4 g/kg body wt (as an aqueous solution, 25% by vol.). Actinomycin D (0.7 mg or 6.6 mg/kg body wt depending upon experimental conditions), cortisol (10 mg/rat), dibutyryl cAMP (40 mg/kg body wt), L-tryptophan (200 mg/kg body wt) were injected intraperitoneally and the animals killed at various times afterwards, as indicated in section 3. Control rats received equivalent volumes of saline.

The whole liver was exposed and, for a better reproducibility of results, frozen exactly 1 min after decapitation. Tyrosine aminotransferase was determined according to [7] and was expressed as micromoles *p*-hydroxyphenyl pyruvate formed per hour and per gram liver wet weight.

All results are given as mean values ± SE and Student's *t*-test was used for statistical interpretation.

Table 1
Effect of ethanol administration on liver tyrosine aminotransferase activity of starved or fed rats

Hours after treatment	Treatment	TAT activity	
		Starved rats	Fed rats
4	Saline	187 ± 46 (6)	117 ± 25 (5)
	Ethanol	310 ± 32[b] (6)	88 ± 14[a] (5)
8	Saline	174 ± 50 (7)	103 ± 31 (5)
	Ethanol	387 ± 56[b] (7)	108 ± 15[a] (5)

[a] $p > 0.05$, [b] $p < 0.01$ versus saline

At zero time, animals were given ethanol (4 g/kg body wt, by stomach tube). The enzyme activities were determined after 4 h or 8 h and are expressed as micromoles *p*-hydroxyphenylpyruvate formed per hour and per gram liver wet weight. The reported values are means ± SE, with the number of animals in each group in parentheses

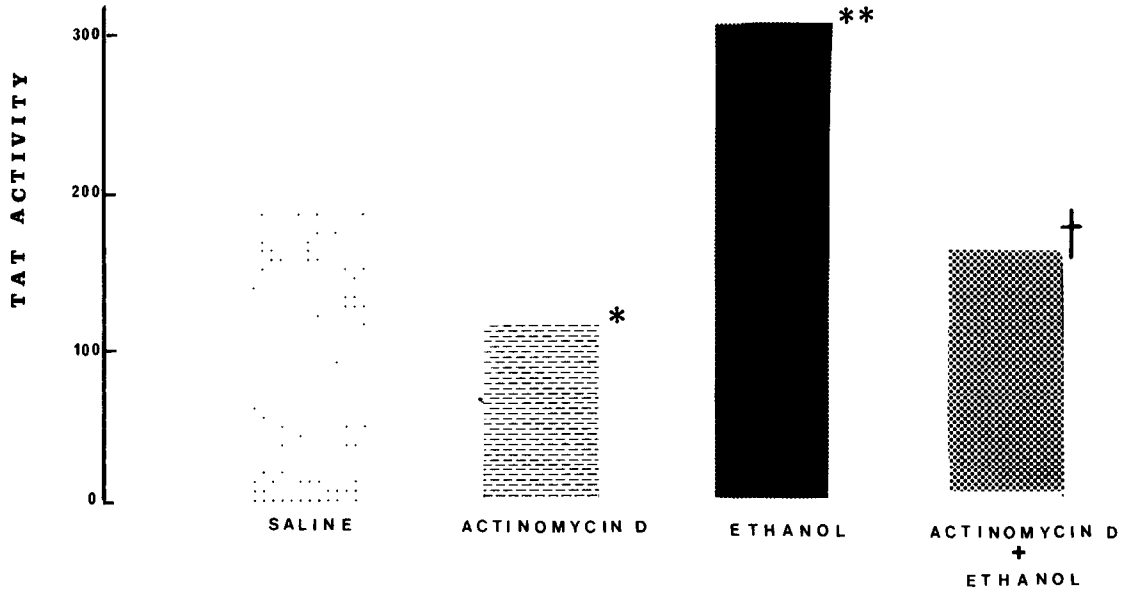

Fig.1. Effect of actinomycin D on the enhancement of rat liver tyrosine aminotransferase activity following acute ethanol administration. Fasted rats were given actinomycin D (0.7 mg/kg body wt, i.p.) and ethanol (4 g/kg body wt, by stomach tube) 4 h prior to sacrifice. The enzyme activity is expressed as micromoles *p*-hydroxyphenylpyruvate formed per hour and per gram liver wet weight. Six animals in each group. Statistical significance: † $p > 0.05$; * $0.01 < p < 0.02$; ** $p < 0.01$ versus saline.

3. Results

The effects of ethanol administration on the basal tyrosine aminotransferase activity are shown in table 1. Whereas the enzyme activity is unaltered in fed rats, a significant increase appears 4 h (65%) and 8 h (122%) after ethanol administration in starved rats.

In order to test whether the ethanol effect found in starved rats is mediated by changes in TAT effectors, we used the mixed homogenate technique [8]. This possibility was excluded, as the enzyme activity experimentally determined in the mixed homogenates was found identical to the theoretical value (results not shown).

Figure 1 shows that actinomycin D (0.7 mg/kg body wt) injected at the same time as ethanol, inhibits the marked rise of TAT activity produced by ethanol. This suggests that ethanol is an inducer of the enzyme.

A stimulation by ethanol of glucocorticoid secretion could explain this enzyme induction. We tested therefore the effects of ethanol on the cortisol-mediated TAT induction (table 2). Ethanol in combination with cortisol (10 mg/rat) inhibited this induction by 40% in starved rats. In fed rats ethanol, although not modifying the basal TAT activity, inhibited the cortisol-mediated TAT induction by 50%.

In order to test whether the inhibitory effect was selective for steroid induction, experiments were performed in starved rats using dibutyryl cAMP as the inducer. When ethanol was injected together with cAMP, it did not modify the cAMP induced TAT level

Table 2
Effects of ethanol administration on the induction of liver tyrosine aminotransferase by cortisol

Treatment	TAT activity	
	Starved rats	Fed rats
Saline	243 ± 75 (6)	117 ± 25 (5)
Cortisol	530 ± 48 (6)	502 ± 52 (5)
Cortisol + ethanol	414 ± 25[a] (6)	314 ± 66[a] (5)

[a] $p < 0.01$ versus cortisol

Animals were given cortisol (10 mg/rat, i.p.) or cortisol + ethanol (4 g/kg body wt, by stomach tube). The enzyme activity was determined 4 h after the treatment and expressed as in table 1

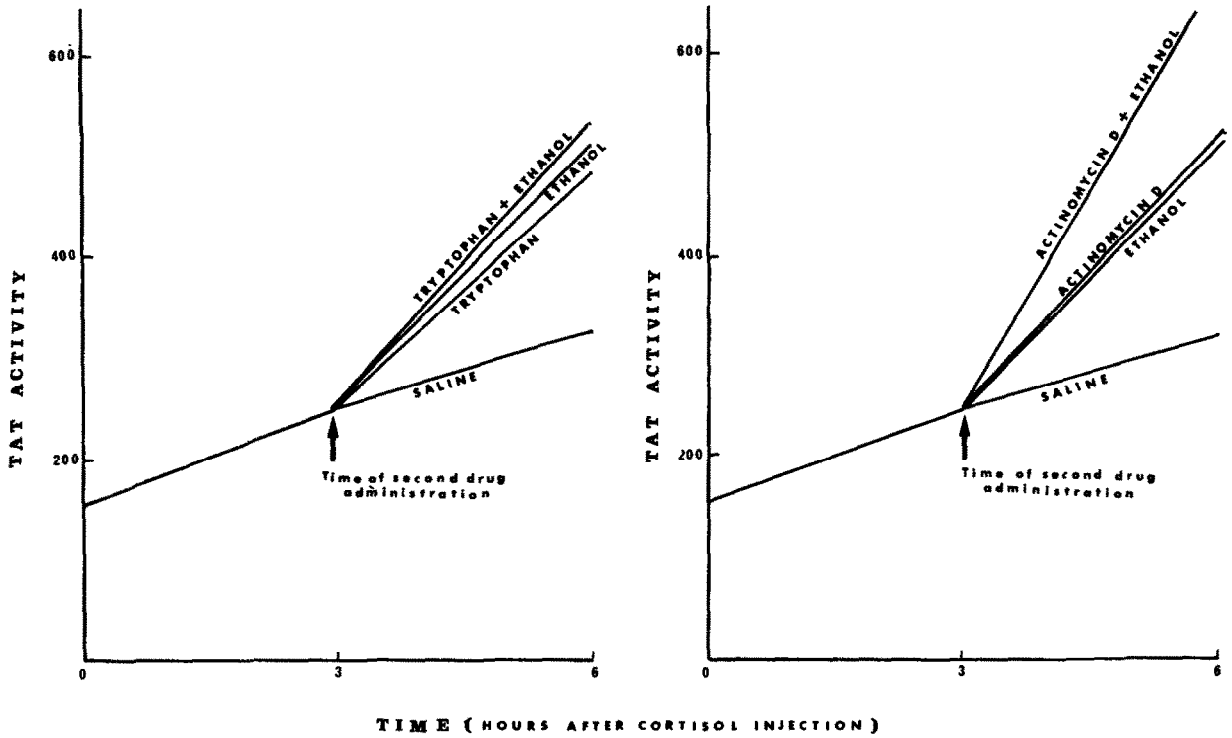

Fig.2. Effects of ethanol on tyrosine aminotransferase induction in cortisol-pretreated rats. At zero time animals were given cortisol (10 mg/rat, i.p.). After 3 h, groups of rats were given ethanol (4 g/kg body wt, by stomach tube), tryptophan (200 mg/kg body wt, i.p.), actinomycin D (6.6 mg/kg body wt, i.p.) or saline. Animals were sacrificed 3 h later and hepatic tyrosine aminotransferase was determined and expressed as in fig.1. Five animals in each group.

2 h after its injection but potentiated the dibutyryl cAMP effect at 4 h (table 3).

The effects of ethanol on the TAT level following tryptophan injection (200 mg/kg body wt) were further investigated. In fed rats (results not shown), it was observed that this dose of tryptophan, given alone or in combination with ethanol, does not modify the TAT activity. In starved rats, tryptophan administration gives an increase in TAT activity, increase which is not altered 4 h or 8 h after ethanol (table 4).

If tryptophan and ethanol were not acting at the same site, an additive effect would have been expected when the compounds were administered in combination to starved rats. When the administration of tryptophan was delayed until 3 h after the initiation of induction by cortisol, the subsequent induction of TAT was increased [9,10]. The same result could be obtained after actinomycin D treatment [10].

Table 3
Effects of ethanol administration on the induction of tyrosine aminotransferase by dibutyryl cAMP in liver of starved rats

Treatment	TAT activity (hours after treatment)	
	2	4
Saline	103 ± 15 (4)	120 ± 13 (4)
Dibutyryl cAMP	316 ± 20 (4)	184 ± 43 (4)
Dibutyryl cAMP + ethanol	304 ± 29[a] (4)	321 ± 40[b] (4)

[a] $p > 0.05$; [b] $p < 0.01$ versus dibutyryl cAMP

At zero time, animals were given dibutyryl cAMP (40 mg/kg body wt, i.p.) or dibutyryl cAMP + ethanol (4 g/kg body wt, by stomach tube). The enzyme activity was determined after 2 h or 4 h and expressed as in table 1

Table 4
Effects of ethanol administration on tryptophan induction of tyrosine aminotransferase in liver of starved rats

Treatment	TAT activity (hours after treatment)	
	4	8
Saline	102 ± 18 (4)	121 ± 23 (5)
Tryptophan	274 ± 39 (4)	290 ± 43 (6)
Tryptophan + ethanol	243 ± 11[a] (4)	334 ± 50[a] (7)

[a] $p > 0.05$ versus tryptophan

At zero time, animals were given tryptophan (200 mg/kg body wt, i.p.) or tryptophan + ethanol (4 g/kg body wt, by stomach tube). The enzyme activity was determined 4 h or 8 h later and expressed as in table 1

In order to check whether ethanol acts at the same sites or steps as tryptophan or actinomycin D, ethanol was administered either alone or in combination with tryptophan or actinomycin D (6.6 mg/kg body wt) (fig.2). The results show that ethanol increased the subsequent induction of TAT by cortisol. Furthermore the effects of ethanol and tryptophan either injected alone or in combination are similar while the effects of ethanol and actinomycin D are additive when both are administered together.

4. Discussion

These results show that ethanol induces TAT activity in starved rats. This induction does not appear steroid-mediated since ethanol inhibits the TAT induction by a saturating dose of cortisol. This is in accordance with the results [5] on basal TAT activity in ethanol treated adrenalectomized rats.

The fact that ethanol inhibits steroid induction only when administered at the same time and not subsequent to steroid led us to think that ethanol may interact with either the steroid receptor or the nuclear acceptor. This inhibition seems specific to steroid since ethanol does not inhibit the TAT induction by dibutyryl cAMP. The fact that additive activities were observed 4 h after administration of ethanol and dibutyryl cAMP in combination seems to show that the TAT induction following acute ethanol administration is not mediated by changes in hormonal secretions which enhance the hepatic cAMP level.

Data presented here suggest that ethanol shares the effects of tryptophan in starved rats. Evidence in support of this hypothesis is that the inductive capacities of ethanol and tryptophan when administered together (both in non-pretreated rats and subsequently to cortisol treatment) were not additive.

It was also observed here that a small dose of tryptophan does not alter TAT activity in fed rats. This observation could explain the lack of effect of ethanol treatment in fed rats.

These results on TAT activity and those in [3] on tryptophan oxygenase show that ethanol enhances the activities of both enzymes by the same compound, i.e., tryptophan, although the sites and the mechanisms of actions of tryptophan are different for the two enzymes [9,11].

Acknowledgements

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