EFFECTS OF ACUTE ETHANOL ADMINISTRATION ON THE STEROID-MEDIATED INDUCTION OF HEPATIC TRYPTOPHAN OXYGENASE

Catherine RIBIERE, Hélène ROUACH, Joseph NORDMANN and Roger NORDMANN
Service de Biochimie de la Faculté de Médecine de Paris-Ouest et Groupe de Recherches U 72 de l'INSERM, 45 rue des Saints-Pères, 75270 Paris Cédex 06, France

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1. Introduction

The two hepatic enzymes tyrosine aminotransferase (TAT) (EC 2.6.1.5) and tryptophan oxygenase (TO) (EC. 1.13.11.11) are known to be induced by treatment of rats with corticosteroids or by stress conditions in which the circulating concentration of these hormones is increased.

Our studies [1] concerning the effects of ethanol on TAT activity have shown that ethanol increases basal TAT activity only in starved rats, while it inhibits the early phase of TAT cortisol induction in starved as well as in fed rats. It seems therefore of interest to test whether ethanol administration in the same conditions as those used for studying TAT affects also cortisol induction of TO in starved and in fed rats.

These results show that acute ethanol administration results in an increase in basal TO activity in starved rats and an inhibition of the early phase of TO induction in starved as well as in fed animals. In the experimental conditions used, the acute ethanol effects on TO and TAT appear thus similar.

2. Material 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).

Cortisol 21-acetate, L-tryptophan, D,L-propranolol–HCl, and actinomycin D were from Sigma (St Louis, MO). Phentolamine methanesulfonate was a gift from Ciba-Geigy (France).

Ethanol was administered by stomach tube at 4 g/kg body wt (as an aqueous solution, 25% by vol.) Cortisol 21-acetate, L-tryptophan (200 mg or 500 mg/kg body wt depending upon experimental conditions), phentolamine methanesulfonate (3 mg/rat), propranolol–HCl (2.5 mg/rat), actinomycin D (6.6 mg/kg body wt) were injected intraperitoneally (i.p.) 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. Tryptophan oxygenase was determined according to [2] as modified [3]. Before using this method, we have ensured that the addition of ascorbate is necessary for maximal enzyme activity and that this supplementation does not interfere with the kynurenine determination [4]. We found (not shown) that ascorbate added to homogenates containing no tryptophan determines only a negligible increase in A_{365} during incubation. The activity of the enzyme was determined in liver homogenates either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added hematin. The hematin solution was prepared immediately before use (by dissolving hemin in OIN NaOH) and was added to the incubation medium to give the final concentration which was found optimal for enzyme activation, i.e., 5 μM. The enzyme activity was expressed as μmol kynurenine formed . h^{-1} . g wet wt^{-1}.

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

3. Results and discussion

In starved rats (table 1), the holoenzyme but not

Table 1
Effects of ethanol administration on the induction of tryptophan oxygenase by cortisol in liver of starved rats

Hours after treatment	Treatment		Kynurenine formed (μmol . h^{-1} . g liver wet wt^{-1})	
			Holoenzyme act.	Total enzyme act.
4	Saline	(7)	6.00 ± 0.84	9.36 ± 1.70
	Ethanol	(9)	7.46 ± 0.98[b]	10.06 ± 1.02[a]
	Cortisol	(12)	14.60 ± 2.15[c]	21.83 ± 2.68[c]
	Cortisol + ethanol	(12)	9.75 ± 1.62[e]	13.06 ± 2.18[e]
8	Saline	(5)	4.14 ± 0.52	6.30 ± 0.99
	Ethanol	(5)	7.57 ± 2.15[c]	11.13 ± 3.63[c]
	Cortisol	(7)	11.40 ± 1.76[c]	20.56 ± 2.77[c]
	Cortisol + ethanol	(7)	13.71 ± 2.04[d]	22.06 ± 2.36[d]

[a]$p > 0.05$, [b]$0.02 < p < 0.05$, [c]$p < 0.01$ versus saline; [d]$p > 0.05$, [e]$p < 0.01$ versus cortisol
Animals were given ethanol (4 g/kg body wt, by stomach tube), cortisol (10 mg/rat, i.p.) or cortisol + ethanol. The enzymes activities were determined after 4 h or 8 h. The reported values are means ± SEM, with the no. animals in parentheses

the total enzyme activity is increased 4 h after ethanol administration whereas both the holoenzyme and the total enzyme activities are increased 4 h later. The effects of ethanol administration on the cortisol-induction of TO are also shown in table 1. Ethanol, 4 h after its administration, significantly inhibited the increase in the holoenzyme and total enzyme activities related to cortisol; this inhibition is no more apparent 4 h later. The mixed homogenate technique [5] was used to test whether the effects of ethanol on cortisol induction are mediated by changes in TO effectors. This possibility was excluded as the enzyme activities

Table 2
Effects of ethanol administration on the induction of tryptophan oxygenase by cortisol in liver of fed rats

Hours after treatment	Treatment		Kynurenine formed (μmol . h^{-1} . g liver wet wt^{-1})	
			Holoenzyme act.	Total enzyme act.
4	Saline	(11)	5.96 ± 0.63	12.20 ± 1.34
	Ethanol	(11)	5.07 ± 0.56[a]	9.73 ± 1.02[c]
	Cortisol	(11)	12.72 ± 0.92[c]	23.68 ± 2.51[c]
	Cortisol + Ethanol	(10)	9.74 ± 1.15[e]	18.53 ± 2.33[e]
8	Saline	(5)	4.27 ± 0.76	8.52 ± 1.20
	Ethanol	(5)	3.25 ± 0.49[a]	6.08 ± 0.83[b]
	Cortisol	(5)	10.99 ± 1.54[c]	18.94 ± 2.70[c]
	Cortisol + ethanol	(5)	7.37 ± 2.13[d]	12.35 ± 2.63[d]

[a]$p > 0.05$, [b]$0.02 < p < 0.01$, [c]$p < 0.01$ versus saline; [d]$0.02 < p < 0.01$, [e]$p < 0.01$ versus cortisol

Animals were given ethanol (4 g/kg body wt, by stomach tube), cortisol (10 mg/rat, i.p.) or cortisol + ethanol. The enzyme activities were determined after 4 h or 8 h and expressed as in table 1

Table 3
Effects of ethanol administration on tryptophan induction of tryptophan oxygenase in liver of starved rats

Hours after treatment	Treatment		Kynurenine formed (μmol . h^{-1} . g liver wet wt^{-1})	
			Holoenzyme act.	Total enzyme act.
4	Saline	(6)	4.40 ± 0.35	7.85 ± 0.79
	Tryptophan	(6)	12.66 ± 1.61[a]	20.42 ± 3.12[a]
	Tryptophan + ethanol	(7)	13.83 ± 1.23[a,b]	18.49 ± 2.03[a,b]
8	Saline	(3)	3.47 ± 0.80	6.12 ± 1.60
	Tryptophan	(3)	7.13 ± 0.76[a]	10.60 ± 2.15[a]
	Tryptophan + ethanol	(3)	8.09 ± 1.16[a,b]	11.81 ± 2.35[a,b]

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

Animals were given tryptophan (200 mg/kg body wt, i.p.) or tryptophan + ethanol (4 g/kg body wt, by stomach tube). The enzyme activities were determined after 4 h or 8 h and expressed as in table 1

experimentally determined in the mixed homogenates were found identical to the theoretical values (results not shown).

In fed rats, whereas the holoenzyme is unaltered, the total enzyme activity is decreased 4 h as well as 8 h after ethanol administration (table 2). Ethanol inhibited the cortisol-mediated TO induction 4 h as well as 8 h after its administration (table 2).

These results show that acute ethanol administration inhibits the cortisol-mediated TO induction both in starved and in fed rats; they disagree with data in [6,7] concerning male rats.

To test whether the inhibitory effect of ethanol administration is selective for steroid induction, experiments were performed using tryptophan as the inducer. In starved rats, ethanol administered together with tryptophan (200 mg/kg body wt) did not modify the tryptophan-induced TO level 4 h or 8 h after its administration (table 3). In fed rats, it was observed that this dose of tryptophan, given alone,

Table 4
Effects of ethanol administration on tryptophan induction of tryptophan oxygenase in liver of fed rats

Treatment		Kynurenine formed (μmol . h^{-1} . g liver wet wt^{-1})	
		Holoenzyme act.	Total enzyme act.
Saline	(5)	5.54 ± 0.42	11.20 ± 0.86
Tryptophan (200 mg/kg)	(5)	7.53 ± 1.33[a]	14.56 ± 2.69[a]
Tryptophan (200 mg/kg + ethanol	(5)	12.84 ± 1.86[b,d]	21.98 ± 2.83[b,d]
Saline	(4)	5.64 ± 1.20	10.18 ± 1.23
Tryptophan (500 mg/kg)	(5)	14.33 ± 3.33[b]	18.35 ± 5.01[b]
Tryptophan (500 mg/kg) + ethanol	(5)	10.45 ± 2.00[b,c]	15.56 ± 1.86[b,c]

[a] $p > 0.05$, [b] $p < 0.01$ versus saline; [c] $p > 0.05$, [d] $p < 0.01$ versus tryptophan

Animals were given tryptophan or tryptophan + ethanol (4 g/kg body wt, by stomach tube). The enzyme activities were determined 4 h after the treatment and expressed as in table 1

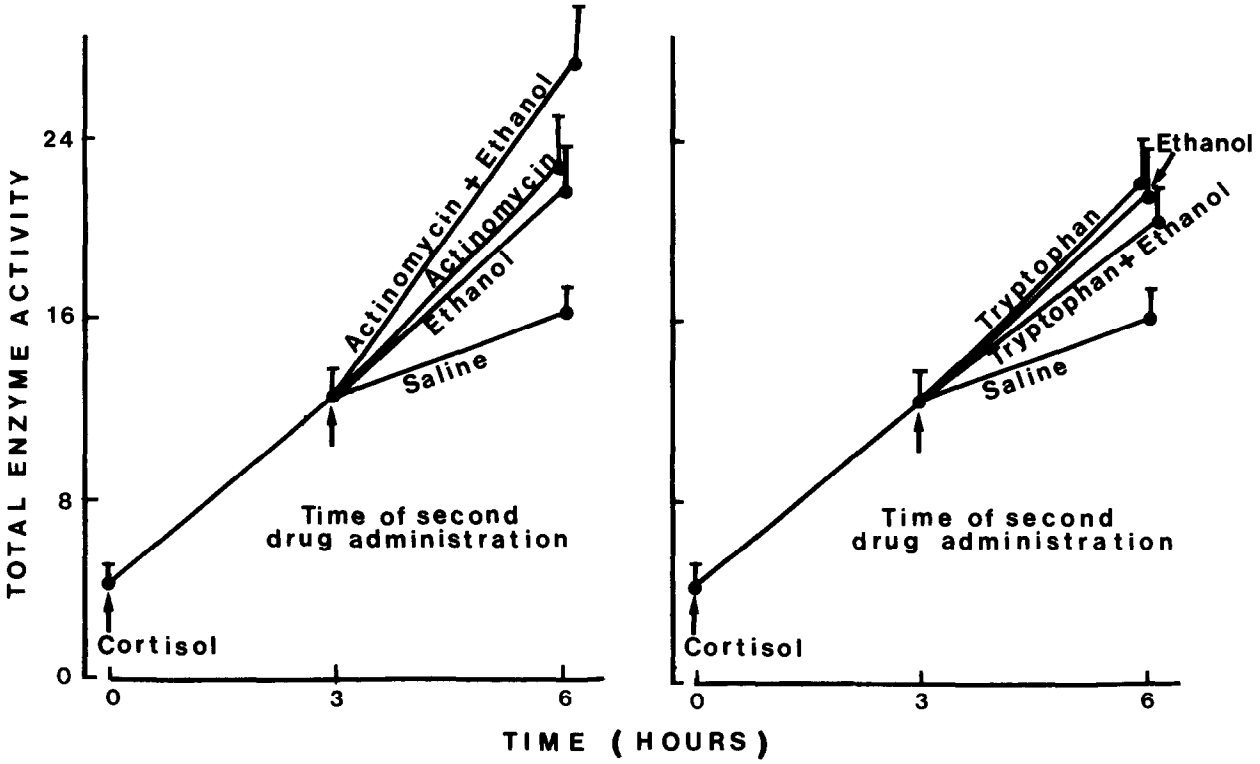

Fig.1. Effects of ethanol on tryptophan oxygenase in starved cortisol-pretreated rats. At zero time animals were given cortisol (10 mg/rat, i.p.). After 3 h groups of rats were given ethanol (4 g/kg body wt, by stomach tube), tryptophan (200 mg/kg body wt, i.p.), actinomycin D (6.6 mg/kg body wt, i.p.) or saline. Animals were sacrificed 3 h later and hepatic tryptophan oxygenase activity was determined and expressed as in table 1. Each group had 5 animals.

Table 5
Effects of α- or β-adrenergic blocking agents on the effects of ethanol on cortisol induction of tryptophan oxygenase in liver of starved rats

Treatment		Kynurenine formed (μmol . h^{-1} . g liver wet wt^{-1})	
		Holoenzyme act.	Total enzyme act.
Cortisol	(4)	14.34 ± 0.93	20.95 ± 1.57
Cortisol + phentolamine	(4)	13.39 ± 2.18[a]	20.36 ± 2.36[a]
Cortisol + phentolamine + ethanol	(4)	9.41 ± 2.08[b]	12.24 ± 2.40[b]
Cortisol	(4)	12.33 ± 1.58	20.85 ± 4.16
Cortisol + propranolol	(4)	10.54 ± 1.36[a]	17.80 ± 2.53[a]
Cortisol + phentolamine + ethanol	(5)	6.50 ± 1.50[b]	9.90 ± 1.73[b]

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

Animals were given cortisol (10 mg/rat, i.p.), phentolamine (3 mg/rat, i.p.), propranolol (2.5 mg/rat, i.p.) or ethanol (4 g/kg body wt, by stomach tube). The α- or β-adrenergic blocking agent was injected 10 min before cortisol or cortisol + ethanol. The enzymes activities were determined 4 h after steroid treatment and expressed as in table 1

does not modify the TO activities 4 h after its administration, but increases significantly TO activities when given in combination with ethanol (table 4). A larger dose of tryptophan (500 mg/kg body wt) given alone increased significantly TO activities 4 h after its administration; ethanol, in combination with this high tryptophan dose, does not interfere with the enzyme induction (table 4). These results show that ethanol does not interfere with TO induction by tryptophan in our experimental conditions.

Additional studies were designed to investigate the inhibitory effect of ethanol on the steroid-induction of TO. As noradrenaline produces a similar inhibition in the early phase of cortisol induction that can be reversed by simultaneous treatment with α-adrenergic blocking agents but not with β-blockers [8,9], we tested the influence of an α-blocker (phentolamine) and a β-blocker (propranolol) on the inhibitory effect of ethanol on cortisol TO induction. Phentolamine (3 mg/rat, i.p.) or propranolol (2.5 mg/rat, i.p.) was injected 10 min before cortisol or cortisol plus ethanol in starved rats and TO activities were determined 4 h later (table 5). Phentolamine, which does not by itself affect the cortisol-mediated TO induction, did not suppress the inhibitory effect of ethanol on this induction. The same holds true for propranolol. These results support the view that the ethanol effects are not mediated by disturbances in the α- or β-adrenergic receptors.

These ethanol effects could however result from an inhibition of protein synthesis. To test this possibility, ethanol was injected 3 h after cortisol. In such conditions, ethanol increased the subsequent induction of TO (fig.1), whereas protein synthesis inhibitors are known to suppress this subsequent induction [10,12]. Such a discrepancy argues against a role of protein synthesis inhibition in the observed effects of ethanol on the cortisol-mediated TO induction; this result can also be interpreted against the role of steroid in the basal TO enhancement after acute ethanol administration as suggested in [7]. The fact that ethanol injected together with a large dose of actinomycin D in cortisol pretreated rats results in a further increase in TO activities (fig.1) argues also against the role of protein synthesis inhibition at the translational level in the observed ethanol effects on TO activities.

As ethanol inhibits the steroid TO induction only when it is administered at the same time as cortisol and not subsequently to the steroid, the ethanol effects are likely to result from an interaction of ethanol with the steroid transport in the cell, interaction which could be located either at the steroid receptor or the nuclear acceptor level.

As ethanol seems to mimic the effects of tryptophan on TO activities, further experiments were performed to test whether ethanol acts also like tryptophan in cortisol-pretreated rats. The results (fig.1) show that the effects of ethanol and tryptophan either injected alone or in combination are similar. These results as well as the lack of additive effects on TO of ethanol and tryptophan when injected together suggest that ethanol shares the effects of tryptophan in starved rats.

The results found in fed animals can be related to the lower inducing ability of tryptophan on TO in such a nutritional condition.

These results show that acute ethanol administration to starved rats results together in an increase in basal TO activity (through a mechanism similar to that of tryptophan) and an inhibition of the early phase of cortisol TO induction (likely to be linked to an alteration of the steroid transport into the cell).

In discrepancy with [6,13] these results on TO activities as well as those concerning TAT [1] suggest that the mechanisms of the ethanol effects on these two enzymes are similar.

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