Pharmacology Biochemistry & Behavior, Vol. 13, Suppl. 1, pp. 139–143. Printed in the U.S.A.

Acute Ethanol Effects on Rat Liver Tryptophan Oxygenase and Tyrosine Aminotransferase

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ROUACH, H., C. RIBIERE, J. NORDMANN AND R. NORDMANN. *Acute ethanol effects on rat liver tryptophan oxygenase and tyrosine aminotransferase*. PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 139–143, 1980.—In starved rats, ethanol administered acutely enhances tryptophan oxygenase (TO) and tyrosine aminotransferase (TAT) activities. Ethanol also inhibits the early phase of the cortisol-mediated TO and TAT induction. Ethanol administered at the same time as tryptophan does not modify the tryptophan-mediated TO and TAT induction. In cortisol-pretreated rats, ethanol enhances the subsequent TO and TAT induction whereas no additive effects are observed when ethanol is injected together with tryptophan. These results suggest that ethanol mimics the effects of tryptophan on TO and TAT activities. In fed animals, ethanol alone does not result in increased TO and TAT activities, but inhibits their cortisol induction. It increases TO activities when given together with a tryptophan dose which, when given alone, does not enhance these activities. It is suggested that the observed inhibitory effects of ethanol on cortisol-mediated TO and TAT induction in starved and fed animals are related to a defective cortisol transport in the liver cells.

Rats Ethanol Liver Tryptophan oxygenase Tyrosine aminotransferase Cortisol Tryptophan Induction

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TRYPTOPHAN oxygenase (TO) (EC 1.13.11.11) is the primary hepatic enzyme catabolizing tryptophan. Regulation of its activity may influence blood and brain tryptophan, which plays a role in cerebral 5-hydroxytryptamine biosynthesis [3,5]. This enzyme is inducible by glucocorticoids and by tryptophan [10]. Several investigators [1, 13, 18] have previously shown that an acute ethanol load results in increased hepatic TO activities. The mechanism of this increase is however still controversial.

As the hepatic regulation of tyrosine aminotransferase (TAT) (EC 2.6.1.5) shares many points in common with that of TO, and as TAT controls the degradation of tyrosine which is the precursor of catecholamines, it seemed of interest to test the effects of acute ethanol administration together on TO and TAT activities.

The present paper reports the acute ethanol effects on the basal TO and TAT activities as well as on the enzyme induction following either cortisol or tryptophan administration. These studies have been conducted both in starved and in fed rats.

METHOD

Animals

Female Wistar rats (150 ± 5 g body weight) were maintained on a standard laboratory diet (Iffa-Rat) containing 58% carbohydrates, 3% lipids and 17% proteins. Food was removed either 16 hr before ethanol treatment (starved rats) or just before ethanol treatment (fed rats).

Procedures

Ethanol (as an aqueous solution, 25%, v/v) was administered by stomach tube at 4 g/kg body weight. Cortisol 21-acetate (Sigma) (10 mg/rat) and/or L-tryptophan (Sigma) (200 or 500 mg/kg body weight depending upon experimental conditions) were injected intraperitoneally. Control rats received equivalent volumes of saline. The rats were sacrificed by decapitation under light ether anesthesia and the liver was exposed and frozen. In order to obtain a better reproducibility of the results, a lag period of exactly one minute was observed between decapitation and liver freezing.

Tryptophan oxygenase was determined according to Knox and Ogata [11] as modified by Magus and Fouts [12]. Before using this method, we ensured that ascorbate addition is necessary for maximal enzyme activity and that this supplementation does not interfere with kynurenine determination [20]. We found (results not shown) that ascorbate added to homogenates containing no tryptophan during incubation results only in a negligible increase in optical density at 365 nm. The enzyme activity 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 0.1 N sodium hydroxide) and was

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TABLE 1

EFFECTS OF ETHANOL ADMINISTRATION ON LIVER TRYPTOPHAN OXYGENASE (TO) AND TYROSINE AMINOTRANSFERASE (TAT) ACTIVITIES IN STARVED RATS

Hours after treatment	Treatment	TO activities Holoenzyme	TO activities Total enzyme	TAT activity
4	Saline	6.00 ± 0.84	9.36 ± 1.70 (7)	187 ± 46 (6)
	Ethanol	7.46 ± 0.98†	10.06 ± 1.02* (9)	310 ± 32‡ (6)
8	Saline	4.40 ± 0.35	7.85 ± 0.79 (13)	174 ± 50 (7)
	Ethanol	8.50 ± 1.28‡	13.00 ± 1.11‡ (13)	387 ± 56‡ (7)

Animals were given ethanol (4 g/kg body wt. by stomach tube). The enzyme activities were determined after 4 hr or 8 hr and are expressed as micromoles kynurenine formed per hour and per g liver wet weight for TO and as micromoles p-hydroxyphenylpyruvate formed per four and per g liver wet weight for TAT. The reported values are means ± SEM, with the number of animals in parentheses.

Statistical comparison versus saline: $^*p>0.05$, $^\dagger 0.02<p<0.05$, $^\ddagger p<0.01$.

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 micromoles kynurenine formed per hour and per gram liver wet weight.

Tyrosine aminotransferase was determined according to Rosen *et al.* [17] and was expressed as micromoles p-hydroxyphenylpyruvate formed per hour and per gram liver wet weight.

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

RESULTS

In starved rats (Table 1), acute ethanol administration increases both TO and TAT activities. Four hours after ethanol administration, TAT activity is significantly increased; the same holds true for the holoenzyme TO activity, but not for the total TO enzyme activity. Eight hours after ethanol treatment, both holoenzyme and total enzyme activities of TO as well as TAT activity are increased.

The effects of ethanol on the cortisol-mediated induction of TO and TAT are shown in Table 2. Four hours after ethanol administration the increase in TO and TAT activities related to cortisol was significantly inhibited; this inhibition is no longer apparent 4 hr later.

In order to test whether the inhibitory effect of ethanol administration is selective for steroid induction, experiments were performed using tryptophan as the inducer. Ethanol administered together with tryptophan (200 mg/kg body weight) did not modify the tryptophan-induced TO and TAT levels (Table 3). The present results show that ethanol does not interfere with TO and TAT induction by tryptophan in our experimental conditions.

Additional studies were designed to investigate the inhibitory effect of ethanol on the steroid-mediated induction of TO and TAT. This ethanol effect could result from an inhibition of protein synthesis. To test this possibility, ethanol was injected 3 hr after cortisol. In such conditions, ethanol increased the subsequent induction of TO and TAT (Fig. 1), whereas protein synthesis inhibitors are known to suppress it [6, 7, 19]. Such a discrepancy argues against a role of protein synthesis inhibition in the observed effect of ethanol on the cortisol-mediated induction of TO and TAT.

As ethanol seems to mimic the effects of tryptophan on TO and TAT 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 and TAT of ethanol and tryptophan when injected together suggest that ethanol shares the effects of tryptophan in starved rats.

In fed rats (Table 4), ethanol administered alone does not result in increased TAT activity. Concerning TO, whereas the holoenzyme is unaltered, the total enzyme activity is decreased 4 hr as well as 8 hr after ethanol administration. Ethanol inhibited the cortisol-mediated TO and TAT induction 4 hr after its administration (Table 5); it increased on the contrary significantly TO activities when given in combination with tryptophan (200 mg/kg body weight); it must be pointed out that this dose of tryptophan, given alone to fed rats, does not by itself enhance significantly the TO and TAT activities. A larger dose of tryptophan (500 mg/kg body weight) given alone increased significantly TO activities 4 hr after its administration. Ethanol, in combination with this high tryptophan dose, did not interfere with the enzyme induction (results not shown).

DISCUSSION

Data presented here suggest that ethanol shares the effects of tryptophan on TO and TAT activities in starved rats; they suggest furthermore that glucocorticoids are not involved in the TO and TAT changes following acute ethanol administration.

The results of three different experiments support this assumption. Ethanol, 4 hr after its administration, increases the holoenzyme but not the total TO enzyme; this indicates that ethanol increases the saturation of the apoenzyme by its haem cofactor, an effect that is also observed after administration of the enzyme substrate, L-tryptophan, but not of cortisol [8]. The inductive capacities of ethanol and tryptophan when administered together (both in non-pretreated rats and subsequently to cortisol treatment) were not additive on TO as well as on TAT activities. When ethanol is administered 3 hr after steroid treatment the enzyme induction of TO and TAT is enhanced; if ethanol acted on TO and TAT by glucocorticoids released following its administra-

TABLE 2

EFFECTS OF ETHANOL ADMINISTRATION ON CORTISOL INDUCTION OF LIVER TRYPTOPHAN OXYGENASE AND TYROSINE AMINOTRANSFERASE IN STARVED RATS

Hours after treatment	Treatment	TO activities: Holenzyme	TO activities: Total enzyme	TAT activity
4	Saline	6.00 ± 0.84	9.36 ± 1.70 (7)	243 ± 75 (6)
	Cortisol	14.60 ± 2.15	21.83 ± 2.68 (12)	530 ± 48 (6)
	Cortisol + ethanol	9.75 ± 1.62†	13.06 ± 2.18† (12)	414 ± 25† (6)
8	Saline	4.14 ± 0.52	6.30 ± 0.99 (5)	174 ± 50 (7)
	Cortisol	11.40 ± 1.76	20.56 ± 2.77 (7)	563 ± 85 (11)
	Cortisol + ethanol	13.71 ± 2.04*	22.06 ± 2.36* (7)	579 ± 34* (10)

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

Statistical comparison versus cortisol: *$p>0.05$, †$p<0.01$.

TABLE 3

EFFECTS OF ETHANOL ADMINISTRATION ON TRYPTOPHAN INDUCTION OF LIVER TRYPTOPHAN OXYGENASE AND TYROSINE AMINOTRANSFERASE IN STARVED RATS

Hours after treatment	Treatment	TO activities: Holoenzyme	TO activities: Total enzyme	TAT activity
4	Saline	4.41 ± 0.35	7.85 ± 0.79 (6)	102 ± 18 (4)
	Tryptophan	12.66 ± 1.61	20.42 ± 3.12 (6)	274 ± 39 (4)
	Tryptophan + ethanol	13.83 ± 1.23*	18.49 ± 2.03* (7)	243 ± 11* (4)
8	Saline	3.47 ± 0.80	6.12 ± 1.60 (4)	121 ± 23 (5)
	Tryptophan	7.13 ± 0.76	10.60 ± 2.15 (4)	290 ± 43 (6)
	Tryptophan + ethanol	8.09 ± 1.16*	11.81 ± 2.35* (4)	334 ± 50* (7)

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

Statistical comparison versus tryptophan: *$p>0.05$.

TABLE 4

EFFECTS OF ETHANOL ADMINISTRATION ON LIVER TRYPTOPHAN OXYGENASE AND TYROSINE AMINOTRANSFERASE ACTIVITIES IN FED RATS

Hours after treatment	Treatment	TO activities: Holoenzyme	TO activities: Total enzyme	TAT activity
4	Saline	5.96 ± 0.63	12.20 ± 1.34 (11)	117 ± 25 (5)
	Ethanol	5.07 ± 0.56*	9.73 ± 1.02‡ (11)	88 ± 14* (5)
8	Saline	4.27 ± 0.76	8.52 ± 1.20 (5)	103 ± 31 (5)
	Ethanol	3.25 ± 0.49*	6.08 ± 0.83† (5)	108 ± 15* (5)

Animals were given ethanol (4 g/kg body wt., by stomach tube). The enzymes activities were determined after 4 hr or 8 hr and expressed as in Table 1.

Statistical comparison versus saline: *$p>0.05$, †$0.02<p<0.05$, ‡$p<0.01$.

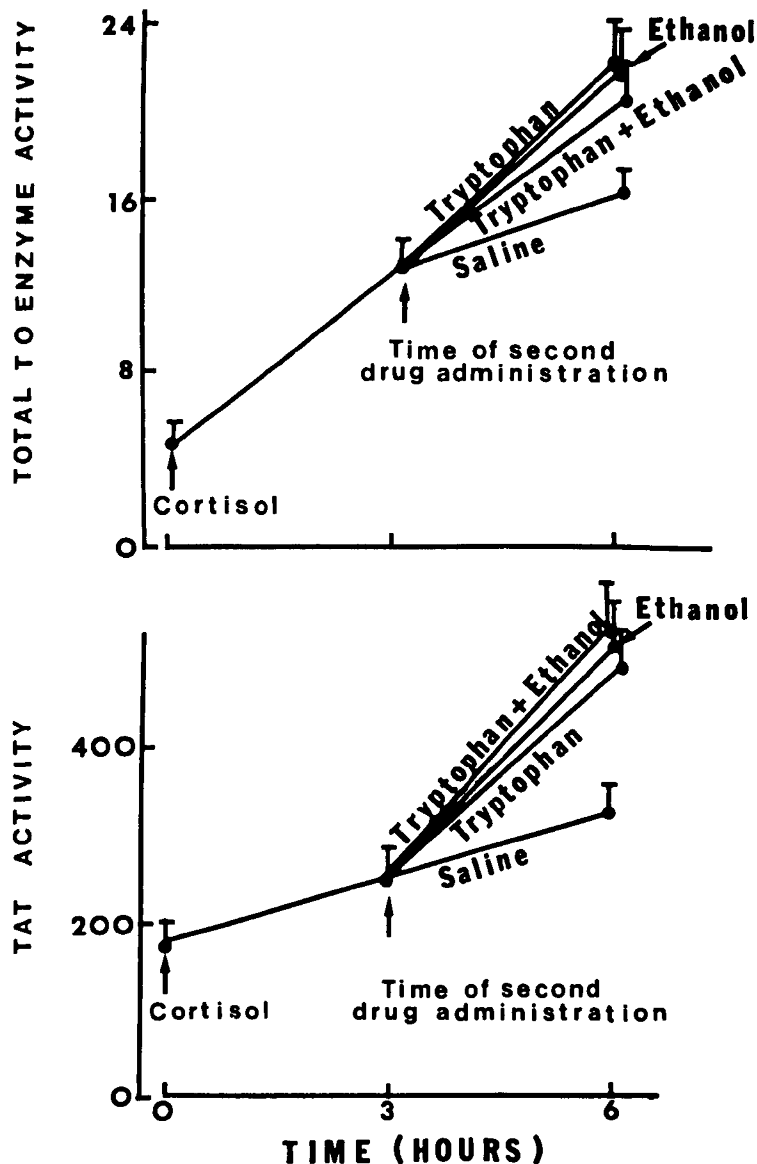

FIG. 1. Effects of ethanol on tryptophan oxygenase and tyrosine aminotransferase in starved cortisol-pretreated rats. At zero time animals were given cortisol (10 mg/rat, IP). After 3 hr, groups of rats were given ethanol (4 g/kg body weight by stomach tube), or tryptophan (200 mg/kg body weight, IP) or saline. Animals were sacrificed 3 hr later and hepatic enzyme activities were determined and expressed as in Table 1. Five animals in each group.

tion, as has been suggested [2,13], it would have been inactive in this experiment. The results of some previous investigators [9,14] were also interpreted against the role of glucocorticoids in the acute ethanol effect on TAT activity.

The lack of effect of acute ethanol administration on the basal TO and TAT activities in fed rats seems related to the lack of inducing effects of a small dose of tryptophan in such rats.

The fact that ethanol inhibits the steroid-mediated induction of TO and TAT only when administered at the same time as cortisol (and not subsequently to the steroid) argues against the role of protein synthesis inhibition at the translational level in the observed ethanol effects on TO and TAT activities.

The inhibitory effects of ethanol on the early phase of cortisol mediated induction of TO and TAT could however be related to either an interaction between ethanol and the inducer or an inhibition of protein synthesis at the transcriptional and/or pretranslational level. These possibilities have been suggested by Pösö and Pösö [15] during studies concerned with the effects of acute ethanol on ornithine decarboxylase activity during partial hepatectomy.

Our previous results [16] concerning TAT induction mediated by dibutyryl cAMP, which is known to increase the level of functional mRNA [4], have shown that ethanol does not modify the cAMP-induced TAT level during the two first hours following cAMP injection. These results argue against an ethanol inhibition of protein synthesis. It appears there-

TABLE 5

EFFECTS OF ETHANOL ADMINISTRATION ON CORTISOL OR TRYPTOPHAN INDUCTION OF LIVER TRYPTOPHAN OXYGENASE AND TYROSINE AMINOTRANSFERASE IN FED RATS

Treatment	TO activities		TAT activity
	Holoenzyme	Total enzyme	
Saline	5.96 ± 0.63	12.20 ± 1.34 (11)	117 ± 25 (5)
Cortisol	12.72 ± 0.92	23.68 ± 2.91 (11)	502 ± 52 (5)
Cortisol + ethanol	9.74 ± 1.15†	18.53 ± 2.83† (10)	314 ± 66† (5)
Saline	5.54 ± 0.42	11.20 ± 0.86 (5)	100 ± 38 (4)
Tryptophan	7.53 ± 1.33*	14.56 ± 2.69* (5)	144 ± 46* (4)
Tryptophan + ethanol	12.84 ± 1.86†	21.98 ± 2.83† (5)	146 ± 57* (4)

Animals were given cortisol (10 mg/rat, IP) or cortisol and ethanol (4 g/kg body wt., by stomach tube) or tryptophan (200 mg/kg, IP) or tryptophan and ethanol. The enzyme activities were determined 4 hr later and expressed as in Table 1.
Statistical comparison versus saline: $^*p>0.05$.
Statistical comparison versus cortisol or tryptophan: $^\dagger p<0.01$.

fore that the ethanol effect is 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. Further investigations (to be published) have shown that this ethanol effect is not mediated by disturbances in the α- or β-adrenergic receptors.

The present data suggest that the mechanisms of the effects of ethanol on both enzyme basal activities as well as on their cortisol-mediated induction are identical in the case of TO and TAT.

ACKNOWLEDGEMENTS

This work was supported by grants from the Université René Descartes (U E R Biomédicale des Saints-Pères), the Institut National de la Santé et de la Recherche Médicale and the Ecole Pratique des Hautes Etudes (3è Section).

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