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ETHANOL-INDUCED INCREASE IN LIVER TRYPTOPHAN OXYGENASE ACTIVITY IN THE STARVED RAT: EVIDENCE AGAINST TRYPTOPHAN MEDIATION

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Abstract—A single oral dose of ethanol (4.0 g/kg) increased the activity of liver tryptophan oxygenase in starved male rats. The peak increase of 340% for the total activity and 400% for the holoenzyme activity occurred 6 hr after ethanol administration. At or after these peaks, the levels of tryptophan in plasma and brain but not in liver, decreased significantly. Plasma total tryptophan and brain tryptophan started to decrease significantly as early as 0.5–1.0 hr after the ethanol treatment, while the activity of liver tryptophan oxygenase was still at the control level. These findings suggest that not all the changes in tissue tryptophan concentrations seen after acute ethanol treatment are caused by increased liver tryptophan oxygenase activity. Prior to the increase in liver tryptophan oxygenase activity, an increase of 104 and 50% in plasma corticosterone and free tryptophan, respectively, were seen 15 min after ethanol treatment. However, the increase in liver tryptophan at this time appeared to be small (13%) and statistically insignificant. With tryptophan treatment, the initial peak levels of liver tryptophan and plasma free tryptophan required to stimulate an increase in tryptophan oxygenase activity were 170 times higher than those caused by ethanol. It was therefore concluded that increases in plasma and liver tryptophan after acute ethanol ingestion, probably mediated by the lipolytic action of ethanol, are too small to cause the increase in liver tryptophan oxygenase activity seen after ethanol administration. However, experiments with different corticosterone doses showed that ethanol-induced increases in plasma corticosterone concentrations are high enough to cause an increase in liver tryptophan oxygenase activity.

It was first shown by Brodie and coworkers [1] and later confirmed by others [2–7] that acute ethanol treatment increased the activity of hepatic tryptophan oxygenase (TO) (EC 1.13.11.11.). Our earlier time– and dose–response study showed that this effect can be achieved only with relatively high ethanol doses, which produce blood ethanol concentrations high enough to trigger the activation of TO [7]. This phenomenon may be very important because it is possible that some ethanol-related changes in peripheral and central tryptophan metabolism may be caused by changes in liver TO activity. Liver TO is a rate-limiting enzyme in the most important pathway for tryptophan degradation in the body and changes in its activity can affect the amount of circulating tryptophan [4, 8, 9]. Therefore, these changes can alter tryptophan metabolism in other tissues, such as the synthesis of the neurotransmitter serotonin (5-hydroxytryptamine) in brain [4, 10, 11], which could be involved in some of the central nervous system effects of ethanol [for a review, see 12].

Well-known factors which can increase liver TO activity are corticosteroids, which increase synthesis of TO enzyme [13, 14], and tryptophan, which decreases the rate of degradation of TO [15, 16]. Since acute ethanol ingestion is known to increase the blood level of corticosteroids [17] it has been suggested that acute ethanol increases TO activity by this mechanism [3]. In addition, acute ethanol has also been shown to increase the level of free tryptophan in blood as well as in the liver, and this has been suggested to be another mechanism by which ethanol could increase TO activity [4]. Thus two different mechanisms for the ethanol mediated increase of liver TO have been proposed. When corticosteroid and tryptophan treatments are used to increase TO activity, the doses needed to achieve significant effects are rather high [10, 18] and it seemed to us unclear whether the increases in blood tryptophan and corticosteroid levels caused by ethanol were high enough to affect liver TO activity. The present study was an attempt to clarify mechanisms by which acute ethanol ingestion could increase liver TO activity. This was done by determining liver TO activity, the response of plasma, liver and brain tryptophan and plasma corticostrone to acute ethanol ingestion and comparing these results with those achieved after treatment with different doses of tryptophan or corticosterone.

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MATERIALS AND METHODS

Chemicals. Hemoglobin, corticosterone and silicic acid were purchased from Sigma (St. Louis, MO, U.S.A.). Nembutal was obtained from Abbot (Saint-Remy-sur-Avre, France). (1,2,6,7-H^3)-Corticosterone was purchased from Amersham (Buckinghamshire, U.K.) and scintillation fluid was from Hydro-Luma (Schaesberg, Netherlands). All other

chemicals were analytical grade and purchased from Merck (Darmstadt, Germany).

Animals. Adult male Wistar rats (Møllegaard, Ejby, Denmark) weighing 200–300 g were used. The animals were exposed to a 12 hr light/12 hr dark cycle with the light being switched off at 19:00 and on at 7:00 hr. The rat diet (Gesti & Son, Oslo, Norway) contained 50 g of carbohydrate, 25 g of protein and 2 g of fat per 100 g fresh weight. The animals were starved 20–24 hr before the experiments.

Treatments. Ethanol and tryptophan were intubated using plastic feeding tubes (ERU nr 3; W. Rusch, Stuttgart, Germany). Tryptophan was dissolved in saline (0.9% (w/v) NaCl) by heating, cooled to the room temperature and doses of 10, 25, 50, 100, 200 and 500 mg/kg body weight were intubated in 20 ml/kg body weight. A dose of 1000 mg/kg was intubated in 40 ml/kg. Corticosterone doses of 0.5, 1.0, 2.5, 5.0 and 15.0 mg/kg were injected intraperitoneally in 1 ml/kg as suspensions in saline. For the dose–response curves, the animals were killed between 12:00 and 17:00 hr so that all experimental groups were spread evenly over this time interval. For the time-course curves, the animals were killed between 12:00 and 17:00 hr in chronological order starting with the animals for the zero-time point (untreated animals) which were killed at 12:00 hr. With untreated animals killed at 12:00, 15:00 and 18:00 hr, it was found that none of the variables measured in the present study had any significant variation within this time interval.

Sampling procedure. Blood samples were taken into heparinized syringes from the hearts of Nembutal-anesthetized animals (60 mg/kg intraperitoneally). Thereafter livers were purged of blood by perfusing an ice-cold saline–heparin mixture (1000 IU heparin/100 ml of saline) through the portal vein for 30 sec and liver samples were taken as explained earlier [7] and frozen in liquid nitrogen. Immediately after the perfusion the brains (*cerebrum*) were taken, halved and frozen in liquid nitrogen. Frozen liver and brain tissues were stored at −80° until analysis. Blood samples were protected from light, cooled on ice for 1–2 min and centrifuged at 4° and 1000 *g* for 10 min. Plasma was separated and either analysed on the same day or stored at −80°.

Liver TO activity. TO activities were determined spectrophotometrically, measuring the formation of kynurenine from tryptophan during the incubation of crude liver homogenates in the presence (total activity) and in the absence (holoenzyme activity) of added hemoglobin as a source of heme-cofactor [19].

Plasma ultrafiltration. Samples of 1 ml of fresh plasma, kept in an ice bath, were centrifuged in CF50A Centriflo membrane cones (Amicon, Oosterhout, Holland) at 10° and 900 *g* for 30 min. In this procedure, approximately 100 μl of filtrate was collected. The filtrate was stored at −80° until analysis.

Tryptophan determinations. Samples of 20 μl of thawed plasma and 50–60 μl of plasma ultrafiltrate were used for tryptophan measurements by the fluorometric method of Denckla and Dewey [20]. The following modification described by Bloxam and Warren [21] was made to the original method: $FeCl_3$ (0.1 ml as 6 mM solution) was added to the reaction mixture just before it was boiling, in order to avoid destruction of tryptophan by ferric ions. For tryptophan determinations in brain and liver, the frozen tissue samples were homogenised in 5 and 7 parts of ice-cold saline, respectively, and 100 μl of brain homogenate or 80 μl of liver homogenate were pipetted into 2.5 ml of 0.1 N ice-cold HCl and rapidly mixed. After adding 0.3 ml of ice-cold 75% trichloracetic acid and mixing, the tubes were centrifuged at 4° and 1000 *g* for 10 min and the supernatants were treated according to the method of Denckla and Dewey [20]. Relative fluoresence values were read with a Farrand MK1 spectrofluorometer which was calibrated with a 2.5 μM norharman solution. However, when plasma from tryptophan-treated rats was assayed, twenty five micromolar norharman was used. Deionised distilled water was used for all reagents and all glassware was acid washed. The recoveries of tryptophan added to plasma and brain and liver homogenates were 90%.

Plasma corticosterone determination. A competitive protein binding radioassay was used for plasma corticosterone determinations [22]. Human plasma (diluted 20-fold) obtained during the last trimester of pregnancy was used as a source of corticosterone-binding protein. Unbound (1,2,6,7-H^3)-corticosterone was counted in Hydro Luma solution using a Wallac 1215 Rackbeta liquid scintillation counter. Recovery of corticosterone added to plasma was quantitative.

Plasma unesterified fatty acid determination. Unesterified fatty acids were determined by the colorimetric method of Laurell and Tibbling [23] using the modification of Itaya [24] which involved using a mixture of diphenylcarbazone and diphenylcarbazide as a color agent. Fresh plasma was extracted with a silicic acid–chloroform–methanol-mixture and the organic phase was kept at 4° overnight and analysed the next day. Recovery of palmitic acid added to plasma was quantitative.

Blood ethanol determination. Whole blood samples of 0.05 ml were pipetted into 0.45 ml of ice-cold distilled water and concentrations of ethanol in the resultant hemolysates were determined by headspace gas-chromatography as described by Eriksson and coworkers [25].

Statistics. The significance of the effects of different treatments was determined using one-way analysis of variance. Student's *t*-test for independent variables was used to investigate the significance of differences between the experimental and control groups. Since the number of *t*-tests was not greater than the number of groups minus one, no correction for the confidence levels was deemed to be necessary. Differences accompanied by P-values above 0.05 were considered insignificant.

RESULTS

Effects of treatment with a single dose of ethanol

Time course of changes in TO activity. Analysis of variance showed that, during the 8-hr period studied, the treatment with ethanol markedly altered both the holoenzyme ($F(8, 28) = 15.5$, $P < 0.001$) and the total activity of TO ($F(8, 28) = 27.0$, $P < 0.001$). Saline treatment had no significant effect on the

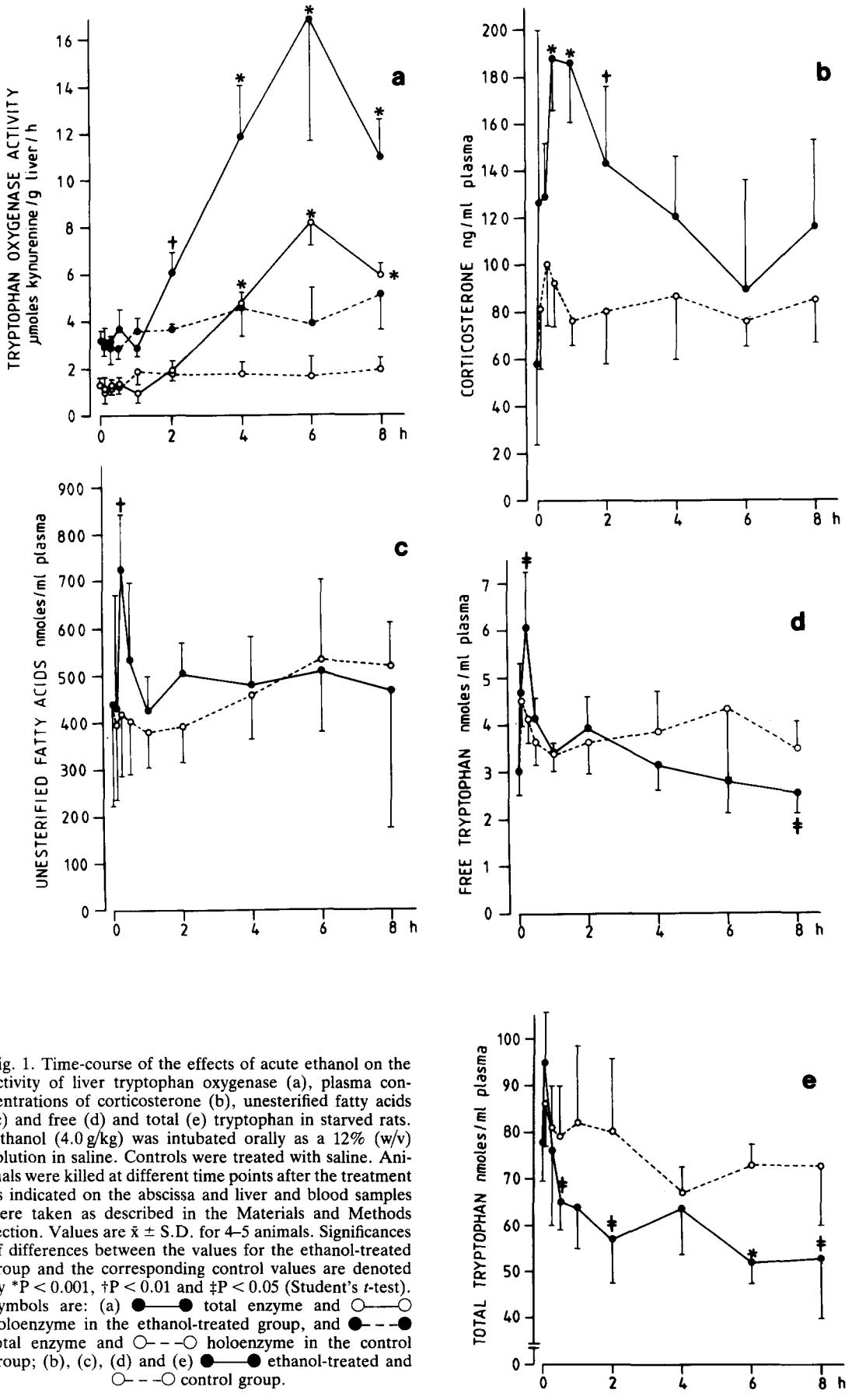

Fig. 1. Time-course of the effects of acute ethanol on the activity of liver tryptophan oxygenase (a), plasma concentrations of corticosterone (b), unesterified fatty acids (c) and free (d) and total (e) tryptophan in starved rats. Ethanol (4.0 g/kg) was intubated orally as a 12% (w/v) solution in saline. Controls were treated with saline. Animals were killed at different time points after the treatment as indicated on the abscissa and liver and blood samples were taken as described in the Materials and Methods section. Values are $\bar{x} \pm$ S.D. for 4–5 animals. Significances of differences between the values for the ethanol-treated group and the corresponding control values are denoted by *$P < 0.001$, †$P < 0.01$ and ‡$P < 0.05$ (Student's t-test). Symbols are: (a) ●——● total enzyme and ○——○ holoenzyme in the ethanol-treated group, and ●- - -● total enzyme and ○- - -○ holoenzyme in the control group; (b), (c), (d) and (e) ●——● ethanol-treated and ○- - -○ control group.

holoenzyme activities ($F(8, 27) = 0.2$). The total activity of TO, however, was slightly affected by saline treatment ($F(8, 27) = 2.7$, $P < 0.05$), probably due to a tendency of TO activity to increase towards the end of the light period of the day [7]. However, compared to the saline-treated control animals, the total and holoenzyme activities of TO in the ethanol-treated animals started to increase significantly after a lag of 1 and 2 hr, respectively (Fig. 1a). The peak activities (a 4-fold increase in the total activity, $P < 0.001$, and a 5-fold increase in the holoenzyme activities, $P < 0.001$) were reached 6 hr after the ethanol treatment (Fig. 1a).

Time course of changes in plasma corticosterone and unesterified fatty acid concentrations. During the 8-hr period after acute ethanol treatment, plasma corticosterone concentration was significantly affected ($F(8, 30) = 5.1$, $P < 0.001$) while remaining unaltered by the corresponding treatment with saline ($F(8, 30) = 1.1$). The peak concentrations of corticosterone were reached at 0.5–1.0 hr after ethanol administration (Fig. 1b) and were approximately twice as high as the levels in the saline-treated control rats ($P < 0.001$). For unesterified fatty acids, a significant 1.7-fold increase was seen 15 min after ethanol, compared to the saline-treated rats (Fig. 1c, $P < 0.01$, *t*-test), even though analysis of variance showed that the ethanol treatment did not have any significant effect ($F(8, 30) = 1.8$; with saline treatment, $F(8, 30) = 0.1$). However, in a separate independent study (unpublished results), measurement of plasma unesterified fatty acid concentrations 15 min after similar ethanol and saline treatments resulted in a level of significance as high as $P < 0.001$ (ethanol-group, $\bar{x} \pm$ S.D. nmoles/ml, $N = 6$, 734 ± 22; controls, $N = 5$, 391 ± 25). Therefore, it seems probable that in the present study, a real ethanol-induced increase in plasma unesterified fatty acids also occured (Fig. 1c).

Time course of changes in plasma concentrations of free and total tryptophan. Ethanol treatment significantly affected plasma concentrations of free ($F(8, 35) = 15.3$, $P < 0.001$) and total tryptophan ($F(8, 36) = 8.8$, $P < 0.001$) while the saline treatment was ineffective (for free tryptophan, $F(8, 29) = 1.5$; for total tryptophan, $F(8, 29) = 1.4$). Plasma free tryptophan concentration was increased significantly 15 min after the ethanol treatment (1.5-fold increase; $P < 0.05$) and was decreased significantly 8 hr after ethanol treatment ($P < 0.05$) compared to the controls (Fig. 1d). However, no significant initial increase in plasma total tryptophan concentration was seen after acute ethanol ingestion (Fig. 1e). The concentration of total tryptophan actually started to decrease significantly 30 min after ethanol ingestion ($P < 0.05$) compared to the controls, and with the exception of the 1 and 4 hr time points, the decrease was significant up to 8 hr after ethanol treatment (Fig. 1e: $P < 0.05$–0.001).

Time course of changes in tryptophan concentrations in the brain and liver. The shape of the time curve of liver tryptophan concentration after acute ethanol treatment (Fig. 2a) was similar to that of plasma free tryptophan (Fig. 1d). However,

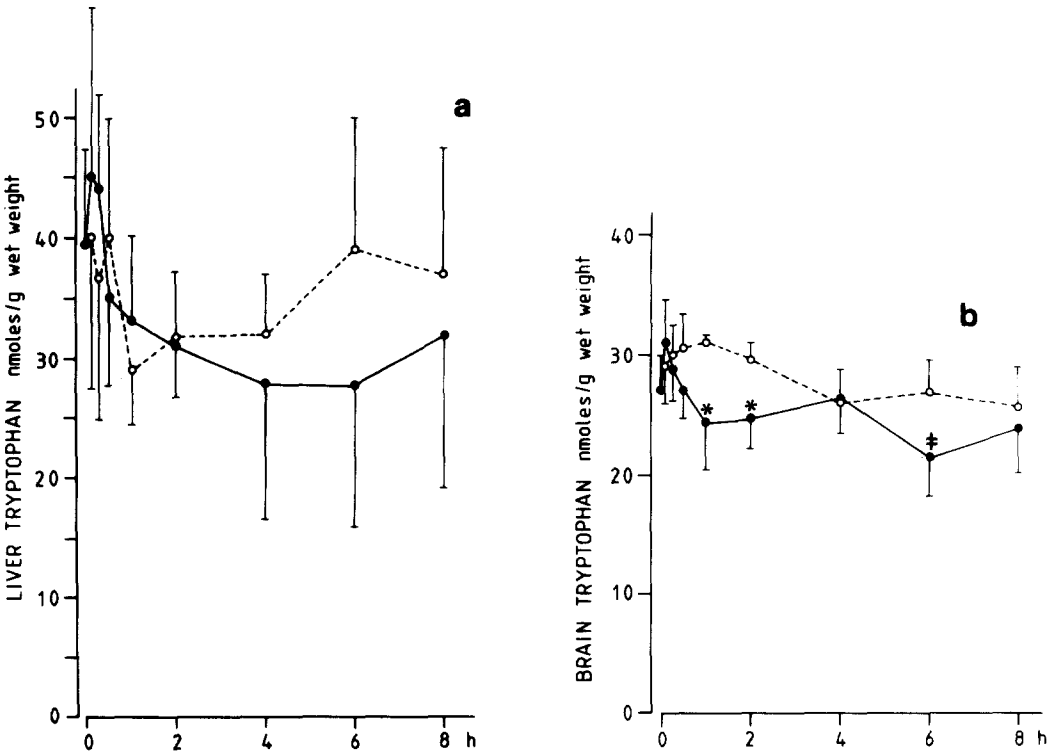

Fig. 2. Time-course of the effects of acute ethanol on the concentrations of tryptophan in liver (a) and in brain (b) in starved rats. The data are from the same experiment as described in Fig. 1. Values are $\bar{x} \pm$ S.D. for 4–5 animals. Significances of differences between the values for the ethanol-treated group (●——●) and the corresponding value in the control group (○- - -○) are denoted by $^*P < 0.01$, $^\dagger P < 0.05$ (Student's *t*-test).

Table 1. Dose-response of the activity of liver tryptophan oxygenase to tryptophan and corticosterone administration in starved rats*

	Tryptophan oxygenase activity (μmoles kynurenine/g liver/hr)	
	Total activity	Holoenzyme activity
mg tryptophan/kg body wt		
0 (saline)	4.7 ± 1.4	1.6 ± 0.6
10	4.0 ± 0.9	1.5 ± 0.7
25	5.6 ± 2.6	2.1 ± 1.0
50	5.7 ± 0.5	2.5 ± 0.4†
100	6.3 ± 0.8	2.7 ± 0.5†
200	7.9 ± 1.7†	3.7 ± 1.9†
500	13.0 ± 2.5§	7.1 ± 2.5‡
1000	22.3 ± 3.1§	16.9 ± 2.7§
mg corticosterone/kg body wt		
0 (saline)	3.9 ± 0.4	1.3 ± 0.1
0.5	6.4 ± 1.0‡	2.4 ± 0.3§
1.0	7.6 ± 1.1‡	3.0 ± 0.7‡
2.5	8.2 ± 0.5§	3.0 ± 0.5§
5.0	10.8 ± 1.8§	3.8 ± 1.5‡
15.0	11.1 ± 2.7‡	4.4 ± 1.4‡
50.0	20.0 ± 4.7§	6.7 ± 1.1§

* Animals were killed 5 hr after the oral intubation of tryptophan or intraperitoneal injection of corticosterone. Values are $\bar{x}$ ± S.D. for four animals.

† $P < 0.05$ with respect to the saline-treated animals by Student's *t*-test.

‡ $P < 0.01$ with respect to the saline-treated animals by Student's *t*-test.

§ $P < 0.001$ with respect to the saline-treated animals by Student's *t*-test.

analysis of variance showed no statistically significant change at any time point during the study ($F(8, 37) = 1.9$). A minor statistically insignificant increase of 13% was seen 15 min after ethanol treatment. Since liver samples for tryptophan analyses were from livers perfused with a saline–heparin-mixture (see Materials and Methods section) the determination of tryptophan after ethanol (4.0 g/kg to starved rats) or saline treatment was repeated in a separate experiment with liver samples taken *in situ* without perfusion, immediately after opening the abdominal cavity of anesthetized animals. The results were similar to those in Fig. 2(a) showing that the perfusion did not affect the tryptophan concentration in the liver. The results were (nmoles/g liver, $\bar{x}$ ± S.D. number of animals in parentheses): untreated animals, 40.8 ± 4.0 (3); 15 min and 2 hr after saline treatment, 48.6 ± 6.0 (5) and 35.5 ± 2.3 (3), respectively ($F(2, 8) = 0.7$); 15 min and 2 hr after ethanol treatment, 43.9 ± 10.6 (8) and 30.0 ± 5.1 (5), respectively ($F(2, 13) = 1.1$). Brain tryptophan concentration (Fig. 2b) was significantly altered by ethanol treatment ($F(8, 38) = 3.9$, $P < 0.01$): a significant decrease compared to the control rats was seen at 1 ($P < 0.01$), 2 ($P < 0.01$) and 6 hr ($P < 0.05$) after ethanol treatment. Treatment with saline had no effect either on liver ($F(8, 29) = 0.9$) or brain ($F(8, 30) = 1.3$) tryptophan concentrations.

Effects of treatment with a single dose of tryptophan

Dose–response of the activity of liver TO. In order to determine the plasma and liver tryptophan concentrations which were associated with an increase in TO activity of the same order of magnitude as that seen after acute ethanol, the dose–response of TO activity to tryptophan was studied (Table 1). The oral tryptophan doses up to 1000 mg of tryptophan per kg body weight caused a significant change 5 hr later in both the holoenzyme ($F(7, 24) = 45.7$, $P < 0.001$) and total activity ($F(7, 24) = 40.1$, $P < 0.001$) of TO. The total activity was increased significantly by a tryptophan dose of 200 mg/kg ($P < 0.05$) and a further increase was seen after doses of 500 ($P < 0.001$) and 1000 mg/kg ($P < 0.001$) compared to the saline-treated controls (Table 1). The activity of the holoenzyme started to increase significantly after a tryptophan dose as low as 50 mg/kg ($P < 0.05$).

Time course of changes in plasma and liver tryptophan concentrations. The time course of changes in plasma and liver tryptophan concentrations were measured after two doses of tryptophan: 25 mg/kg, which did not have any effect on TO activities 5 hr later (Table 1), and 500 mg/kg, which after 5 hr increased the TO activities by about the same extent as ethanol did (Table 1 and Fig. 1a). The results are presented in Table 2. During the 4-hr period studied, both the low and high dose of tryptophan had a marked effect on plasma free tryptophan concentration ($F(5, 16) = 7.1$, $P < 0.01$; $F(5, 12) = 13.3$, $P < 0.001$, respectively), total tryptophan concentrations ($F(5, 16) = 31.7$, $P < 0.001$; $F(5, 12) = 67.9$,

Table 2. Time course of the effects of two oral doses (25 and 500 mg/kg body weight) of tryptophan on the concentration of free and total tryptophan in plasma, liver tryptophan and activity of liver trytophan oxygenase in starved rats*

	min after tryptophan intubation						
	0	15	30	60	120	240	300
25 mg tryptophan/kg body wt							
Plasma free tryptophan nmoles/ml	4.5 ± 0.6	36.5 ± 17.8†	29.5 ± 14.0†	27.8 ± 2.9‡	10.9 ± 3.1†	6.8 ± 2.4§	
Plasma total tryptophan nmoles/ml	78.8 ± 6.1	221.4 ± 24.4‡	185.2 ± 42.6†	131.4 ± 12.6‡	92.5 ± 4.5†	73.9 ± 5.9	
Liver tryptophan nmoles/g wet wt	19.6 ± 2.7	64.9 ± 12.9‡	48.4 ± 18.7§	21.7 ± 0.4	22.8 ± 3.5	21.1 ± 3.9	
Tryptophan oxygenase μmoles of kynurenine/g liver/hr							
Total activity	3.9 ± 1.0	9.0 ± 1.4‡	6.1 ± 1.8	5.5 ± 2.9	5.4 ± 2.2	5.4 ± 2.0	5.6 ± 2.6
Holoenzyme	1.3 ± 0.3	3.7 ± 0.7‡	3.5 ± 0.9†	2.2 ± 1.5	2.2 ± 0.9	1.9 ± 0.8	2.1 ± 1.0
500 mg tryptophan/kg body wt							
Plasma free tryptophan nmoles/ml		335.9 ± 60.7‡	282.6 ± 92.0‡	370.7 ± 17.5‡	305.6 ± 83.3‡	87.4 ± 3.9‡	
Plasma total tryptophan nmoles/ml		825.2 ± 60.4‡	887.5 ± 37.2‡	944.7 ± 38.6‡	747.1 ± 146.2‡	263.6 ± 41.3‡	
Liver tryptophan nmoles/g wet wt		877.1 ± 101.0‡	688.5 ± 103.0‡	451.5 ± 207.7‡	436.2 ± 243.2‡	36.8 ± 4.6‡	
Tryptophan oxygenase μmoles of kynurenine/g liver/hr							
Total activity		7.8 ± 1.1‡	9.0 ± 2.5†	12.6 ± 3.2‡	16.5 ± 5.7†	21.1 ± 3.2‡	13.0 ± 2.5‡
Holoenzyme		3.8 ± 1.1†	5.1 ± 1.4‡	7.8 ± 3.9†	11.5 ± 5.1†	14.6 ± 1.3‡	7.1 ± 2.5†

* Values are $\bar{x} \pm$ S.D. for four animals.
† $P < 0.01$ with respect to zero-time values.
‡ $P < 0.001$ with respect to zero-time values.
§ $P < 0.05$ with respect to zero-time values.

$P < 0.001$, respectively) and liver tryptophan concentrations ($F(5, 16) = 13.1$, $P < 0.001$; $F(5, 12) = 26.6$, $P < 0.001$, respectively). In separate experiments (results not shown), control animals were used to confirm that in saline-treated animals there was no change either in TO activities or in plasma and liver tryptophan concentrations during the 5-hr period after the treatment (see also Figs. 1a, 1d, 1e and 2a).

The low dose of tryptophan (25 mg/kg) caused an 8.0 ($P < 0.01$), 2.8 ($P < 0.001$) and 3.3 ($P < 0.001$) fold increase in plasma free and total tryptophan and liver tryptophan concentrations, respectively, 15 min after the intubation, compared to the zero-time values (Table 2). Four hours after the treatment, the concentration of plasma free tryptophan was still 1.5 times higher than the zero-time value ($P < 0.05$). The plasma total tryptophan and liver tryptophan concentrations returned to their zero-time values 4 and 1 hr after tryptophan ingestion, respectively (Table 2).

Similarly with the higher dose of tryptophan (500 mg/kg), the peak concentrations of plasma free and total tryptophan and liver tryptophan were reached 15 min after tryptophan intubation, the respective peak concentrations being 75, 10 and 45 times higher than the zero-time values (Table 2: $P < 0.001$ for each). For free and total tryptophan in plasma, the peak concentrations were maintained up to 2 hr after tryptophan ingestion, and even after 4 hr the concentrations were 19 and 3 times higher, respectively, than the zero-time level ($P < 0.001$ for each). The peak concentration of liver tryptophan decreased more sharply but was still 2-fold higher than the zero-time value 4 hr after the treatment ($P < 0.001$).

Time course of changes in liver TO activity. For 5 hr after the treatment with a low (25 mg/kg) and a high (500 mg/kg) dose of tryptophan a marked change was seen both in the holoenzyme ($F(6, 19) = 6.5$, $P < 0.001$; and $F(6, 19) = 23.3$, $P < 0.001$, respectively) and in total activity of TO ($F(6, 19) = 12.0$, $P < 0.001$; and $F(6, 19) = 14.4$, $P < 0.001$, respectively). Fifteen minutes after the high dose, both the holoenzyme and total activity were about 2-fold higher than the zero-time values ($P < 0.01$ and $P < 0.001$, respectively). The peak activation was reached 4 hr after the tryptophan treatment, the holoenzyme activity being 11-fold ($P < 0.001$) and the total activity 5-fold ($P < 0.001$) higher than the control activities (Table 2). Surprisingly, the low dose of tryptophan, which did not cause any significant TO increase 4 or 5 hr after intubation, did cause a sharp short-lasting peak in both holoenzyme (2.8-fold; $P < 0.001$) and total activities (2.3-fold; $P < 0.001$) 15 min after the treatment (Table 2).

Effects of treatment with a single dose of corticosterone

Dose–response of the activity of liver TO. As with tryptophan, the dose- and time–response of liver TO activity and plasma corticosterone concentrations were determined after different doses of corticosterone in order to compare the responses with those achieved by ethanol. Both the total and holoenzyme activities of TO were significantly af-

Table 3. Time course of the effects of two doses of corticosterone (1 and 5 mg/kg body weight) on the concentration of corticosterone in plasma and the activity of liver tryptophan oxygenase*

	min after corticosterone injection				
	0	15	30	180	300
1 mg corticosterone/kg					
Plasma corticosterone ng/ml	80.5 ± 41.0	164.5 ± 11.6†	135.5 ± 30.3‡	93.0 ± 31.5	
Tryptophan oxygenase μ moles of kynurenine/g liver/hr					
Total activity	3.4 ± 0.7	4.1 ± 1.4	4.6 ± 1.5	4.8 ± 1.9	7.6 ± 1.1§
Holoenzyme	1.2 ± 0.3	1.4 ± 0.6	1.8 ± 0.4‡	2.1 ± 1.1	3.0 ± 0.7†
5 mg corticosterone/kg					
Plasma corticosterone ng/ml		392.0 ± 79.3§	226.3 ± 112.6‡	75.7 ± 8.1	
Tryptophan oxygenase μ moles of kynurenine/g liver/hr					
Total activity		4.3 ± 1.7	4.7 ± 1.9	8.0 ± 1.2§	10.8 ± 1.8§
Holoenzyme		1.4 ± 0.4	2.0 ± 0.8	3.9 ± 0.9§	3.8 ± 1.5†

* Corticosterone was injected intraperitoneally. Values are $\bar{x}$ ± S.D. for four animals.
† $P < 0.01$ with respect to zero-time values.
‡ $P < 0.05$ with respect to zero-time values.
§ $P < 0.001$ with respect to zero-time values.

fected by corticosterone doses up to 50 mg/kg when measured 5 hr later ($F(6, 21) = 16.7$, $P < 0.001$; and $F(6, 21) = 9.8$, $P < 0.001$, respectively). The results are presented in Table 1. The TO activities found after the 1 mg/kg dose were half the activities caused by ethanol (Table 1 and Fig. 1a) and the 5 mg/kg dose caused a TO activation similar to that caused by ethanol.

Time course of changes in plasma corticosterone concentration and liver TO activity. Table 3 shows the time course of plasma corticosterone concentration and liver TO activity after the administration of 1 and 5 mg/kg corticosterone. Both doses significantly altered the level of plasma corticosterone (for 1 mg/kg: $F(3, 12) = 6.5$, $P < 0.01$; for 5 mg/kg: $F(3, 12) = 24.3$, $P < 0.001$). Again, as in Fig. 1(b), saline treatment did not affect the plasma corticosterone levels during the 4-hr period studied (results not shown). The peak corticosterone concentration was reached 15 min after the treatment, the peak being 2-fold after 1 mg/kg ($P < 0.01$) and 5-fold after 5 mg/kg ($P < 0.001$) compared to the zero-time values. The former increase was 20% lower than that caused by ethanol (see Fig. 1b) and the latter was twice the peak concentration caused by ethanol. In both cases the control level was re-established 3 hr after the treatment. Similarly, for 5 hr after the treatment, both doses significantly affected the total activity (for 1 mg/kg: $F(4, 15) = 5.8$, $P < 0.01$; for 5 mg/kg: $F(4, 15) = 15.3$, $P < 0.001$) and holoenzyme activity (for 1 mg/kg: $F(4, 15) = 4.5$, $P < 0.05$; for 5 mg/kg: $F(4, 15) = 8.1$, $P < 0.01$) of TO. Lag periods of 3 and 2 hr after 1 and 5 mg/kg corticosterone, respectively, were seen before TO activities started to increase (Table 3). With 1 mg/kg the increase in the total TO activity was 2-fold ($P < 0.001$) and the holoenzyme activity 2.5-fold ($P < 0.01$) higher than the zero-time values, 5 hr after the treatment. With 5 mg/kg both the total and holoenzyme activities were 3-fold higher than the zero-time levels 5 hr after the treatment ($P < 0.001$ and $P < 0.01$, respectively).

DISCUSSION

Relevance of the ethanol-induced increase in liver TO activity to the regulation of tryptophan availability in the body. Earlier studies show that liver TO activity, which is induced by corticosteroids, is accompanied by decreased tryptophan concentrations in blood and brain [9, 26]. In some studies using ^{14}C-tryptophan *in vivo*, it seemed quite clear that these decreased tissue tryptophan levels resulted from increased tryptophan breakdown in the liver by higher TO levels [8, 9]. However, controversial results exist [27, 28] suggesting that the availability of substrate rather than the amount of enzyme may regulate TO activity *in vivo*. To our knowledge, only one study has been done previously in order to correlate ethanol-induced increases in liver TO activities with tissue tryptophan levels [4]. In that study, with fed rats, a biphasic response to ethanol was found in the concentration of free tryptophan in blood and total tryptophan in brain and liver. There was first an increase at 2–3 hr after ethanol treatment and then a decrease at 7–8 hr after ethanol. The initial increase was suggested by the authors to be a consequence of the lipolytic action of ethanol (see below) and the later decrease a result of increased liver TO activity. These results were only partly reproduced in the present study with starved rats. The biphasic response with the initial increase was seen only in plasma free tryptophan concentration but not in liver or brain tryptophan. Free and total tryptophan in blood, and brain tryptophan did decrease significantly at the time of peak liver TO activity (6–8 hr after ethanol treatment) but no significant decrease was seen in the liver tryptophan concentration. It was also surprising that the brain tryptophan curve followed the blood total tryptophan curve and not that of the free tryptophan. However, although it seems that in most cases, it is the blood free tryptophan fraction which determines brain tryptophan levels [29] there are some earlier studies suggesting that in some circumstances brain tryp-

tophan can change independently of blood free tryptophan [30, 31]. It is possible that some of the differences in the response of tryptophan to acute ethanol in our study with starved rats and Badawy's study with fed rats [4] may be due to the different feeding states of the animals since it is known that mobilization and uptake of tryptophan in different tissues is affected by feeding state [32]. However, the present study shows that the hepatic TO activity increase may not be responsible for all the changes in tryptophan concentrations seen after ethanol treatment. The finding that liver tryptophan was not decreased significantly when the amount of liver TO was increased suggests that: (1) in spite of the higher level of TO, the *in vivo*-degradation of tryptophan may not be affected in the liver of the starved ethanol-treated rat and the decreased levels of blood and brain tryptophan would then have to be caused by effects of ethanol other than the increased hepatic TO activity, or (2) in spite of an increased *in vivo*-degradation of tryptophan in the starved ethanol-treated liver, hepatic tryptophan concentrations are maintained at the expense of tryptophan in other tissues, such as blood and brain. The possibility that ethanol lowers tissue tryptophan levels by a mechanism other than the increase in liver TO, is further supported by the finding that blood total tryptophan and brain tryptophan started to decrease significantly as early as 0.5 hr (19% decrease) and 1 hr (23% decrease) after ethanol treatment, respectively, while liver TO activities were still at the control level. The same early decrease in blood total tryptophan concentration was also seen by Badawy [4].

The role of tryptophan in increasing liver TO activity after acute ethanol ingestion. Since tryptophan is known to decrease the degradation of liver TO [15, 16] the initial increase in liver tryptophan seen after acute ethanol ingestion has been suggested by Badawy and Evans [2, 4] to be the factor causing the later increase in TO activity. According to these authors, an increased supply of tryptophan to TO in the liver may be due to the catecholamine-mediated lipolytic action of ethanol. By increasing adipose tissue lipolysis via increased catecholamine production, a single large dose of ethanol increases circulating unesterified fatty acids [33], which compete with tryptophan for binding sites on plasma proteins and thus increase the level of free tryptophan in blood [34, 35]. Supporting their theory, Badawy and Evans [4] found in fed rats simultaneous increases of 255, 48 and 25% in plasma unesterified fatty acids, plasma free tryptophan and liver tryptophan, respectively, after ethanol treatment and prior to the main increase in TO activity. In agreement with this previous study, a 60% increase in plasma unesterified fatty acid concentration was seen in the present study with starved rats and this paralleled a significant increase (50%) in plasma free tryptophan accompanied by a smaller, but in our study insignificant, increase (13%) in liver tryptophan concentration. However, the present study shows that the increases in blood free tryptophan and liver tryptophan concentrations obtained in the present study as well as in the earlier study [4] after acute ethanol treatment are too small to affect hepatic TO activity. When tryptophan treatment alone was used to produce a peak of TO activity similar to that seen after acute ethanol ingestion, this treatment caused a 7400 and 4400% increase in blood free tryptophan and liver tryptophan, respectively, prior to the increase in TO activity. On the other hand, when early peak increases of 700 and 230% in blood free tryptophan and liver tryptophan, respectively, were produced by a lower tryptophan dose, they were not followed by any increase in TO activity. Therefore, it seems very unlikely that the small ethanol-induced elevations of the order of 50% and 13–25% in blood free tryptophan and liver tryptophan, respectively, can be responsible for the later increase in TO activity.

The reason for the sharp short-lasting increase in both the total TO activity and the holoenzyme activity during the first 0.5 hr after the treatment with the small tryptophan dose (Table 2), which did not cause any later increase in TO activities, is unclear. If the half-life of TO is 2–2.5 hr [36], the amount of TO can hardly be doubled in 15 min by decreased degradation only, and synthesis of new protein would be expected to take a longer time. This finding again raises the question of the existence of a possible inactive form of TO [19] or of the possibility that tryptophan changes the specific activity of TO.

Corticosterone as a possible inducer of liver TO after acute ethanol ingestion. It is well-known that pituitary-adrenal function is stimulated during acute ethanol intoxication [37], producing a rapid dose-dependent increase in blood corticosteroid concentration [17]. This has led to a suggestion that TO activation after acute ethanol ingestion could be corticosteroid-mediated [3] since corticosteroids are known to increase the synthesis of TO [13, 14]. Corticosteroids may also mediate the increase in heme-saturation of TO (holoenzyme activity) after ethanol treatment because steroids seem to increase the activity of the rate-limiting enzyme in heme synthesis, γ-aminolevulinate synthetase [38], an enzyme also shown to be activated by acute ethanol [39]. The evidence favouring the involvement of corticosteroids in TO activation after acute ethanol ingestion comes from two types of experiments. Firstly, no ethanol-induced increase in TO has been seen in isolated perfused liver [40], in adrenalectomized animals [5] or in hamsters and guinea pigs which lack the corticosteroid mechanism to induce TO [41, 42]. Secondly, when different ethanol treatments were used, the ethanol-induced increase in TO was seen only in rats with high levels of blood corticosterone [3]. The present study provides further evidence for a role of corticosterone in mediating TO activation after ethanol ingestion. We have shown that the TO activity increase caused by acute ethanol treatment is preceded by an increase in plasma corticosterone concentration. Furthermore, this concentration was similar to that which resulted from a parenteral corticosterone dose which by itself mimicked the effect of ethanol on TO activities.

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