The Role of Free Serum Tryptophan in the Biphasic Effect of Acute Ethanol Administration on the Concentrations of Rat Brain Tryptophan, 5-Hydroxytryptamine and 5-Hydroxyindol-3-ylacetic Acid

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1. Acute administration of ethanol exerts a biphasic effect on the concentrations of rat brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid. Both effects are associated with corresponding changes in the availability of circulating free tryptophan.
2. The initial increases in the above concentrations are prevented by ergotamine, are unaltered by allopurinol and are potentiated by theophylline, whereas the later decreases are prevented by both ergotamine and allopurinol.
3. It is suggested that the initial enhancement by ethanol of brain tryptophan metabolism is caused by catecholamine-mediated lipolysis followed by displacement of protein-bound serum tryptophan, whereas the activation of liver tryptophan pyrrolase, which is produced by the same mechanism, leads to the later decreases in the brain concentrations of tryptophan and its metabolites.
4. The initial effects of ethanol can be reproduced by an equicaloric dose of sucrose, and a comparison of the two treatments alone could therefore be misleading.
5. The effects of ethanol on liver and brain tryptophan metabolism have also been examined in mice, and a comparison of the results with those previously reported suggests that the ethanol effects are strain-dependent.

Mood disorders are well known clinical features of acute and chronic alcoholism and alcohol-withdrawal states. The possible involvement of brain 5-hydroxytryptamine in the regulation of mood has led several workers to examine the effects of ethanol on the concentration, synthesis or turnover of this biogenic amine in brains of animals. Studies of the acute effects of ethanol have produced contradictory results largely because of differences in species and/or strains of animals tested, doses of ethanol and routes of its administration, choice of control groups of animals and methods. Previous studies in the rat suggest that acute ethanol administration increases (Palaić *et al.*, 1971), decreases (Tyce *et al.*, 1970) or does not alter (Frankel *et al.*, 1974) the turnover rate of brain 5-hydroxytryptamine, and similar (contradictory) conclusions have also been reached from experiments with various mouse strains (Griffiths *et al.*, 1974; Rawat, 1974; Yamanaka & Kono, 1974; Tabakoff *et al.*, 1975).

Our previous work on the effects of acute ethanol administration on the activity of rat liver tryptophan pyrrolase [L-tryptophan-oxygen 2,3-oxidoreductase (decyclizing), EC 1.13.11.11] suggests that ethanol enhances the enzyme activity by increasing the availability of circulating tryptophan to the liver by a sequence of interactions involving catecholamines, cyclic AMP, adipose-tissue lipase, serum non-esterified fatty acids and free (ultrafiltrable) serum trypto-

phan (Badawy & Evans, 1973*a,c*, 1975*a*). An increased availability of the latter to tissues should also increase brain tryptophan concentration, which plays an important role in the synthesis of 5-hydroxytryptamine (Curzon & Knott, 1974, and references therein). We have therefore examined the effects of acute ethanol administration on the concentrations of rat brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid. The results presented in the present paper show that in addition to the initial increases in the above concentrations, ethanol causes subsequent decreases. Both aspects of this biphasic effect are associated with an altered availability of circulating free tryptophan. Experiments with two strains of mice have also been performed and the results are discussed in relation to previous work on the effects of ethanol in this species.

Materials and Methods

Chemicals

Allopurinol (4-hydroxypyrazolo[3,4-*d*]pyrimidine) was a gift from the Wellcome Foundation, London NW1 2BP, U.K. Cortisol 21-acetate, ergotamine tartrate, theophylline (1,3-dimethylxanthine) and L-tryptophan were from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. Ethanol (99.7-100%), formaldehyde solution (37-40%, w/v) and all other chemicals (from BDH Chemicals, Poole,

Dorset, U.K.) were of AnalaR grade except for acetaldehyde (99.5%), adrenaline (hydrogen tartrate), L-cysteine, dimethylformamide, haemin (haematin hydrochloride), L-noradrenaline, *o*-phthalaldehyde and linoleic acid, oleic acid or palmitic acid, which were of reagent grade.

Animals and treatments

Adult male Wistar rats (110–140 g) and CBA/CA mice (25–35 g) were locally bred, whereas mice of the C57BL strain (20–25 g) were from Bantin and Kingman, Grimston, Aldborough, Hull, U.K. All animals were allowed free access to cube diet 41B (Oxoid, Basingstoke, Hants., U.K.) and water. The diet was removed from all cages during each experiment. Ethanol was administered intraperitoneally to mice and to most rats. In experiments on tryptophan pyrrolase, the dose was 5 ml of ethanol/kg body wt. in the form of a 25% (v/v) solution in 0.9% (w/v) NaCl. Control animals received equal volumes of 0.9% NaCl. In experiments involving measurement of the serum and brain concentrations of tryptophan and/or its indole metabolites, rats received doses of 1, 3 or 5 ml of ethanol/kg, whereas mice were given a dose of 3.8 ml/kg body wt. Additional rats received oral intubations of water (25 ml/kg), sucrose [7.2 g/kg, as a 28.8% (w/v) solution in water] or ethanol [5 ml/kg, as a 20% (v/v) solution in water]. Doses of the latter two compounds were isocaloric (i.e. of the same calorific value).

All other chemicals were given intraperitoneally to rats. Cortisol acetate (20 mg/kg) and linoleic acid, oleic acid or palmitic acid (100 mg/kg each) were dissolved in dimethylformamide; each rat received 1.0 ml/kg. Control rats received an equal volume of the solvent. Ergotamine tartrate (2.5 mg/kg) was dissolved in a mixture of 0.2 ml of dimethylformamide plus 0.8 ml of 0.9% NaCl; each rat received 1.0 ml/kg and control rats were given an equal volume of this solvent mixture. Allopurinol (20 mg), theophylline (100 mg) and L-tryptophan (200 mg) were each dissolved in the minimum amount of 1–4 M-NaOH and diluted to 4, 10 or 10 ml respectively with 0.9% NaCl after the pH had been adjusted to 7.3–7.8 with 1 M-HCl; each rat received one of the above volumes/kg body wt. Adrenaline or noradrenaline (2 mg of base/kg body wt. each) was dissolved in 0.9% NaCl with or without a little 0.1 M-HCl and adjusted to pH 7.3 with 0.1 M-NaOH; each rat received 2.0 ml/kg body wt. Acetaldehyde (156 mg/kg) was also dissolved in 0.9% NaCl and was given in a volume of 1.0 ml/kg.

For the determination of liver tryptophan pyrrolase activity, all animals were killed by stunning and cervical dislocation between 13:15 and 15:15 h, whereas analyses of serum and brain for tryptophan and/or its indole metabolites were performed with animals that had been killed by decapitation between 15:30 and 16:00 h.

Chemical and enzymic determinations

Tryptophan pyrrolase activity was determined in fresh liver homogenates by measuring the formation of kynurenine from L-tryptophan (Feigelson & Greengard, 1961) in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin (2 μ M). The apoenzyme activity was calculated by difference. The assay of the rat liver enzyme was performed as detailed previously (see, e.g., Badawy & Evans, 1973b). The same procedure was used to determine the activity of the mouse liver enzyme, except that the volume of incubation and of subsequent treatments was scaled down by one-half.

Serum was ultrafiltered (Badawy & Evans, 1976b) immediately after isolation. Ultrafiltrates and original sera were then stored at -20°C for 16 h before analysis. Free (ultrafiltrable) and total (acid-soluble) serum tryptophan concentrations were determined by a modification (Bloxam & Warren, 1974) of the method of Denckla & Dewey (1967) as detailed previously (Badawy & Evans, 1976b). Liver tryptophan concentration was determined by the same procedure, in samples of livers (1 g each) that had been stored at -20°C for 16 h beforehand.

Brains were rapidly removed (within 20 s of the death of the animals), immersed in liquid N_2 for 3 min and then stored at -20°C for 16 h before analysis. 5-Hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid were extracted from whole brains and their concentrations were determined by the method of Curzon & Green (1970). The concentration of tryptophan was determined, in portions of the extract containing 5-hydroxytryptamine, by the same procedure used for serum tryptophan determination. Recoveries of tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid, added to the butan-1-ol at the start of extraction of brains, were 90, 102.5 and 100% respectively, and corrections for complete recoveries of the first two compounds have been made. Rat brains were extracted and analysed individually, whereas two mouse brains were pooled for each extraction.

The concentration of serum non-esterified fatty acids was determined by the method of Mikac-Devic *et al.* (1973) with palmitic acid as standard.

Results

Effects of ethanol, acetaldehyde, catecholamines, non-esterified fatty acids and sucrose on rat liver tryptophan pyrrolase activity

The time-course and nature of the effect of a single intraperitoneal injection of ethanol (5 ml/kg) on the activity of the rat liver enzyme have previously been reported (Badawy & Evans, 1973a,c, 1975a). Ethanol has been found to enhance the pyrrolase activity by a

Table 1. *Effects of ethanol, acetaldehyde, catecholamines, non-esterified fatty acids and sucrose on the activity of rat liver tryptophan pyrrolase*

Each rat received an intraperitoneal injection of 0.9% NaCl, dimethylformamide (1 ml/kg), cortisol acetate (20 mg/kg), tryptophan (200 mg/kg), ethanol (5 ml/kg), acetaldehyde (156 mg/kg), adrenaline or noradrenaline (2 mg of base/kg each), linoleic acid, oleic acid or palmitic acid (100 mg/kg each) 4 h before death. Other rats received, 4 h before death, an oral intubation of water (25 ml/kg), ethanol [5 ml/kg, as a 20% (v/v) solution in water] or sucrose [7.2 g/kg, as a 28.8% (w/v) solution in water]. The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin (2 μ M). The apoenzyme activity was calculated by difference and was used to determine the ratio of holoenzyme/apoenzyme activity. Values are means $\pm$ S.E.M. for each group of four rats.

Method of administration	Treatment	Kynurenine formed (μ mol/h per g wet wt. of liver)		Ratio of holoenzyme/apoenzyme activity
		Holoenzyme activity	Total enzyme activity	
Intraperitoneal	0.9% NaCl	1.7 $\pm$ 0.2	4.6 $\pm$ 0.4	0.59
	Dimethylformamide	1.7 $\pm$ 0.2	4.7 $\pm$ 0.4	0.57
	Cortisol	6.4 $\pm$ 0.7	16.4 $\pm$ 1.7	0.64
	Tryptophan	6.2 $\pm$ 0.4	9.3 $\pm$ 0.8	2.00
	Ethanol	5.1 $\pm$ 0.5	8.3 $\pm$ 0.5	1.59
	Acetaldehyde	6.2 $\pm$ 0.7	9.8 $\pm$ 0.5	1.72
	Adrenaline	4.4 $\pm$ 0.6	6.0 $\pm$ 0.7	2.75
	Noradrenaline	4.8 $\pm$ 0.6	5.8 $\pm$ 0.7	4.80
	Linoleic acid	5.3 $\pm$ 0.2	7.0 $\pm$ 0.5	3.12
	Oleic acid	5.6 $\pm$ 0.2	7.0 $\pm$ 0.6	4.00
	Palmitic acid	6.1 $\pm$ 0.4	9.2 $\pm$ 1.0	1.97
Oral	Water	1.4 $\pm$ 0.1	4.0 $\pm$ 0.3	0.54
	Ethanol	4.6 $\pm$ 0.7	7.4 $\pm$ 0.6	1.64
	Sucrose	1.5 $\pm$ 0.1	1.7 $\pm$ 0.0	—

substrate-type, but not by a hormonal-type, mechanism, and, as shown in Table 1, ethanol resembled tryptophan in producing a largely haem-saturated apoenzyme and increasing the ratio of holoenzyme/apoenzyme activity, which indicates the extent of this saturation, to above 1. The results in Table 1 also show that a substrate-type enhancement of the enzyme activity was produced by the administration of acetaldehyde, adrenaline, noradrenaline and also linoleic acid, oleic acid or palmitic acid.

The effects of oral intubation of rats with ethanol (5 ml/kg) or with an equicaloric dose of sucrose on the pyrrolase activity are also shown in Table 1. At 4 h, ethanol increased both holoenzyme and total enzyme activities by 228 and 85% respectively ($P < 0.005$). These rises compare favourably with those observed at the same time-interval after intraperitoneal administration of the same dose of ethanol (200 and 80% respectively). Oral administration of ethanol also increased the ratio of holoenzyme/apoenzyme activity from a control value (in water-treated rats) of 0.54 to 1.64. At 4 h after oral administration of sucrose, the total pyrrolase activity was significantly ($P < 0.001$) decreased by 57%, whereas that of the holoenzyme was not ($P > 0.10$). The apoenzyme activity (calculated by difference) was therefore the form specifically inhibited by sucrose, by 92%.

Effects of ethanol on mouse liver tryptophan pyrrolase activity

The effects of an intraperitoneal injection of ethanol (5 ml/kg) on the activity of the mouse liver enzyme are shown in Table 2. In the C57BL strain, neither ethanol nor an equal volume of 0.9% NaCl had any significant effect at 3 h. There was, however, an enhancement of the holoenzyme activity of 83–100% ($P = 0.005$ – 0.001) at 1–3 h after administration of the same dose of ethanol to mice of the CBA/CA strain.

Effects of ethanol on the concentrations of rat liver and free and total serum tryptophan and brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid

The time-course of the effects of a single intraperitoneal injection of ethanol (5 ml/kg) on the concentrations of the above compounds is shown in Fig. 1. The results are expressed as percentages of control values observed with rats given equal volumes of 0.9% NaCl at each of the time-intervals examined. The concentration of free (ultrafiltrable) serum tryptophan (Fig. 1a) was significantly ($P < 0.001$) increased by 36 and 48% respectively at 2 and 3 h, and returned to control values at 4 h. There then followed a decrease of 43% ($P < 0.001$) at 7 h and of 20% ($P < 0.005$) at 8 h.

and a return to normal values at 10h after the injection of ethanol. By contrast, the concentration of total (acid-soluble) serum tryptophan (Fig. 1b) was significantly ($P < 0.001$) decreased by 51 at 2h. After a slight recovery, it decreased again by 52% at 7h before finally returning to the control value at 10h. The decrease in the concentration of free serum tryptophan at 7 or 8h was not due to a decreased binding to proteins, because the proportion of serum tryptophan present in the free state was not decreased by ethanol.

The changes in the concentrations of tryptophan in liver (Fig. 1c) or brain (Fig. 1d) followed a time-course similar to that observed with free serum tryptophan concentration. The increases (in liver and brain tryptophan concentrations) at 2 and 3h ranged between 11 and 40% ($P = 0.05-0.001$), whereas the decreases observed at 7-8h varied from 16 to 27% ($P = 0.01-0.001$). The concentrations of 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid in brains of ethanol-treated rats (Figs. 1e and 1f) were also altered in a similar fashion: the rises at 2-4h were 24-71% ($P = 0.02-0.001$), whereas the decreases at 7-8h were between 16 and 27% ($P = 0.01-0.001$).

The effects of smaller doses of ethanol were also examined (Table 3). At 2h, the 3ml/kg dose caused

significant changes ($P > 0.001$) that were similar in magnitude to those produced at the same time-interval after administration of the 5ml/kg dose (see Fig. 1), whereas the 1ml/kg dose caused significant ($P = 0.025-0.005$) changes in all but the concentration of brain 5-hydroxyindol-3-ylacetic acid. This small dose of ethanol did not exert any significant effects at 7h, whereas the 3ml/kg dose produced changes that were significant ($P = 0.01-0.001$), but less pronounced than those observed at 7h after administration of the 5ml/kg dose (see Fig. 1).

The effects of pretreatment of rats with ergotamine, allopurinol or theophylline on the changes observed at 2h after ethanol administration are shown in Table 4. The ethanol-induced increases in the concentrations of free serum tryptophan and brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid, as well as the decrease in that of total serum tryptophan (see Fig. 1), were all prevented by ergotamine, but not by allopurinol. Neither drug exerted any significant effects on its own. Theophylline, on the other hand, caused significant increases ($P < 0.001$) in the concentrations of all but total serum tryptophan. When theophylline-pretreated rats were given ethanol, the concentration of total serum tryptophan was decreased, as with ethanol alone (see Fig.

Table 2. *Effects of ethanol on mouse liver tryptophan pyrrolase activity*

Treated mice received an intraperitoneal injection of either ethanol [5ml/kg, as a 25% (v/v) solution in 0.9% NaCl] or equal volumes of 0.9% NaCl for the durations listed. The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin (2 μ M). Values are means $\pm$ S.E.M. for each group of four mice.

Strain of mice	Treatment	Duration (h)	Kynurenine formed (μ mol/h per g wet wt. of liver)	
			Holoenzyme activity	Total enzyme activity
C57BL	None	0	3.0 $\pm$ 0.3	6.1 $\pm$ 0.3
	0.9% NaCl	3	2.9 $\pm$ 0.3	6.0 $\pm$ 0.2
	Ethanol	3	3.1 $\pm$ 0.2	5.8 $\pm$ 0.6
CBA/CA	None	0	1.2 $\pm$ 0.1	2.8 $\pm$ 0.3
	0.9% NaCl	3	1.2 $\pm$ 0.1	2.7 $\pm$ 0.1
	Ethanol	3	2.4 $\pm$ 0.1	2.7 $\pm$ 0.1
	Ethanol	2	2.3 $\pm$ 0.1	2.7 $\pm$ 0.1
	Ethanol	1	2.2 $\pm$ 0.2	2.8 $\pm$ 0.2

Fig. 1. *Time-course of the effects of acute ethanol administration on the concentrations of rat liver, free and total serum, and brain tryptophan, and brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid*

Rats received single intraperitoneal injections of either ethanol [5ml/kg, as a 25% (v/v) solution in 0.9% NaCl] or an equal volume of 0.9% NaCl, and were killed at the times indicated. The concentrations of the above compounds were determined as described in the Materials and Methods section, and the ethanol effects have been expressed as percentage of control (0.9% NaCl-treated rats) values. The means of the latter (in μ g/ml or per g wet wt.) (with the lowest and highest results) for 42 control rats were as follows: (a) free serum tryptophan 1.52 (1.22, 1.81); (b) total serum tryptophan 18.36 (13.68, 22.90); (c) liver tryptophan 6.81 (6.60, 7.22); (d) brain tryptophan 2.61 (2.14, 2.97); (e) brain 5-hydroxytryptamine 0.54 (0.50, 0.58); (f) brain 5-hydroxyindol-3-ylacetic acid 0.37 (0.28, 0.41).

Table 3. *Effects of intraperitoneal administration of ethanol (1 or 3 ml/kg) on the concentrations of rat free and total serum tryptophan and of brain tryptophan 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid*

Rats received, at 2 or 7 h before death, an intraperitoneal injection of either 1 or 3 ml of ethanol/kg body wt. in the form of a 25% (v/v) solution in 0.9% NaCl. In the 2 h experiments, control rats also received equal volumes of 0.9% NaCl, whereas the 7 h-control animals received volumes of the solvent similar to that of the smaller dose of ethanol. The concentrations of the above serum and brain constituents were determined as described in the Materials and Methods section. Values are means $\pm$ S.E.M. for each group of six rats.

Duration (h)	Treatment	Dose (ml/kg)	Concentration of serum tryptophan (μ g/ml)		Concentration in brain (μ g/g wet wt.)		
			Free	Total	Tryptophan	5-Hydroxy-tryptamine	5-Hydroxy-indol-3-ylacetic acid
2	0.9% NaCl	1	1.37 $\pm$ 0.03	16.50 $\pm$ 0.77	2.38 $\pm$ 0.06	0.51 $\pm$ 0.02	0.47 $\pm$ 0.02
	Ethanol		1.64 $\pm$ 0.06	12.70 $\pm$ 0.44	2.77 $\pm$ 0.09	0.56 $\pm$ 0.01	0.47 $\pm$ 0.01
	0.9% NaCl	3	1.33 $\pm$ 0.07	15.27 $\pm$ 0.31	2.15 $\pm$ 0.02	0.51 $\pm$ 0.01	0.39 $\pm$ 0.01
	Ethanol		1.79 $\pm$ 0.01	7.48 $\pm$ 0.41	2.82 $\pm$ 0.09	0.68 $\pm$ 0.02	0.58 $\pm$ 0.01
7	0.9% NaCl	1	1.72 $\pm$ 0.09	17.47 $\pm$ 0.69	2.70 $\pm$ 0.08	0.50 $\pm$ 0.01	0.41 $\pm$ 0.01
	Ethanol		1.60 $\pm$ 0.08	16.55 $\pm$ 0.51	2.58 $\pm$ 0.05	0.49 $\pm$ 0.01	0.40 $\pm$ 0.01
	Ethanol	3	1.20 $\pm$ 0.04	12.18 $\pm$ 0.40	2.30 $\pm$ 0.03	0.39 $\pm$ 0.01	0.36 $\pm$ 0.01

Table 4. *Effects of pretreatment of rats with ergotamine, allopurinol or theophylline on the 2 h-ethanol-induced changes in concentrations of free and total serum tryptophan and brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid*

Three groups of 18 rats each were divided into three equal subgroups. Subgroups were injected with the compounds shown. Ethanol [5 ml/kg, as a 25% (v/v) solution in 0.9% NaCl] or an equal volume of 0.9% NaCl was injected intraperitoneally at 2 h before death. Ergotamine (2.5 mg/kg) and theophylline (100 mg/kg) were given 1 h before, whereas allopurinol (20 mg/kg) was injected 0.5 h before either ethanol or 0.9% NaCl. Control animals for the three pretreatments received equal volumes of 0.9% NaCl. The concentrations of the above serum and brain constituents were determined as described in the Materials and Methods section. Values are means $\pm$ S.E.M. for each subgroup of six rats.

Pretreatment	Treatment	Concentration of serum tryptophan (μ g/ml)		Concentration in brain (μ g/g wet wt.)		
		Free	Total	Tryptophan	5-Hydroxy-tryptamine	5-Hydroxy-indol-3-ylacetic acid
0.9% NaCl	0.9% NaCl	1.29 $\pm$ 0.06	13.85 $\pm$ 1.03	2.74 $\pm$ 0.03	0.52 $\pm$ 0.02	0.39 $\pm$ 0.04
Ergotamine	0.9% NaCl	1.44 $\pm$ 0.05	15.40 $\pm$ 0.52	2.69 $\pm$ 0.06	0.54 $\pm$ 0.01	0.32 $\pm$ 0.01
Ergotamine	Ethanol	1.29 $\pm$ 0.06	13.35 $\pm$ 0.56	2.51 $\pm$ 0.06	0.54 $\pm$ 0.01	0.33 $\pm$ 0.01
0.9% NaCl	0.9% NaCl	1.16 $\pm$ 0.04	12.37 $\pm$ 0.57	2.31 $\pm$ 0.03	0.51 $\pm$ 0.01	0.40 $\pm$ 0.01
Allopurinol	0.9% NaCl	1.17 $\pm$ 0.04	12.10 $\pm$ 0.35	2.39 $\pm$ 0.08	0.50 $\pm$ 0.01	0.41 $\pm$ 0.01
Allopurinol	Ethanol	1.73 $\pm$ 0.08	9.16 $\pm$ 0.57	3.13 $\pm$ 0.02	0.67 $\pm$ 0.02	0.61 $\pm$ 0.02
0.9% NaCl	0.9% NaCl	1.21 $\pm$ 0.03	13.67 $\pm$ 0.54	2.68 $\pm$ 0.02	0.52 $\pm$ 0.01	0.37 $\pm$ 0.01
Theophylline	0.9% NaCl	2.00 $\pm$ 0.09	13.40 $\pm$ 0.48	4.26 $\pm$ 0.06	0.66 $\pm$ 0.01	0.63 $\pm$ 0.01
Theophylline	Ethanol	2.65 $\pm$ 0.18	9.53 $\pm$ 0.64	5.35 $\pm$ 0.19	0.75 $\pm$ 0.01	0.84 $\pm$ 0.02

1b), whereas those of the remaining constituents were increased to values that were similar to the sums of the separate effects of ethanol and theophylline.

The decreases in the concentrations of free and total serum tryptophan and of brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid observed at 7 h after ethanol administration (see Fig. 1) were prevented by pretreatment of rats with either ergotamine or allopurinol (Table 5). Ergotamine alone did not exert any significant effects ($P > 0.10$), whereas allopurinol caused significant ($P = 0.05$ –

0.001) increases in the concentrations of all the above serum and brain constituents. There were, however, no significant differences between the effects of allopurinol in the absence or presence of subsequent ethanol administration. It has not been possible to test the effects of pretreatment of rats with theophylline on the 7 h-ethanol-induced changes in the concentrations of the above constituents, because the toxicity of the former has caused death before the time of testing (8 h).

The effects of oral intubation of rats with ethanol

Table 5. *Effects of pretreatment of rats with ergotamine or allopurinol on the 7 h-ethanol-induced decreases in concentrations of free and total serum tryptophan and brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid*

Design of experiments, injections, doses and duration of pretreatments are as described in Table 4. All animals were killed at 7 h after treatment with ethanol or an equal volume of 0.9% NaCl. The concentrations of the above serum and brain constituents were determined as described in the Materials and Methods section. Values are means $\pm$ S.E.M. for each subgroup of six rats.

Pretreatment	Treatment	Concentration of serum tryptophan ($\mu\text{g/ml}$)		Concentration in brain ($\mu\text{g/g}$ wet wt.)		
		Free	Total	Tryptophan	5-Hydroxy-tryptamine	5-Hydroxy-indol-3-ylacetic acid
0.9% NaCl	0.9% NaCl	1.27 $\pm$ 0.04	13.90 $\pm$ 0.41	2.51 $\pm$ 0.06	0.51 $\pm$ 0.01	0.42 $\pm$ 0.01
Ergotamine	0.9% NaCl	1.35 $\pm$ 0.04	14.60 $\pm$ 0.32	2.55 $\pm$ 0.05	0.50 $\pm$ 0.01	0.43 $\pm$ 0.01
Ergotamine	Ethanol	1.28 $\pm$ 0.04	13.96 $\pm$ 0.28	2.61 $\pm$ 0.10	0.53 $\pm$ 0.01	0.42 $\pm$ 0.01
0.9% NaCl	0.9% NaCl	1.16 $\pm$ 0.08	12.27 $\pm$ 1.14	2.42 $\pm$ 0.08	0.51 $\pm$ 0.02	0.41 $\pm$ 0.01
Allopurinol	0.9% NaCl	1.57 $\pm$ 0.09	15.50 $\pm$ 1.38	3.03 $\pm$ 0.15	0.56 $\pm$ 0.01	0.50 $\pm$ 0.01
Allopurinol	Ethanol	1.49 $\pm$ 0.09	14.82 $\pm$ 1.14	3.15 $\pm$ 0.08	0.57 $\pm$ 0.01	0.51 $\pm$ 0.01

Table 6. *Effects of oral intubation of ethanol or sucrose on the concentrations of rat free and total serum tryptophan and brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid*

Each rat received, at 2 or 7 h before death, an oral intubation of water (25 ml/kg), ethanol [5 ml/kg, as a 20% (v/v) solution in water] or sucrose [7.2 g/kg, as a 28.8% (w/v) solution in water]. The concentrations of the above serum and brain constituents were determined as described in the Materials and Methods section. Values are means $\pm$ S.E.M. for each group of six rats.

Time after dosing (h)	Treatment	Concentration of serum tryptophan ($\mu\text{g/ml}$)		Concentration in brain ($\mu\text{g/g}$ wet wt.)		
		Free	Total	Tryptophan	5-Hydroxy-tryptamine	5-Hydroxy-indol-3-ylacetic acid
2	Water	1.19 $\pm$ 0.03	12.47 $\pm$ 0.16	2.47 $\pm$ 0.04	0.51 $\pm$ 0.01	0.38 $\pm$ 0.01
	Ethanol	1.67 $\pm$ 0.06	8.13 $\pm$ 0.42	3.10 $\pm$ 0.07	0.66 $\pm$ 0.01	0.48 $\pm$ 0.01
	Sucrose	1.43 $\pm$ 0.05	12.32 $\pm$ 0.11	2.98 $\pm$ 0.06	0.67 $\pm$ 0.01	0.46 $\pm$ 0.01
7	Water	1.54 $\pm$ 0.07	16.05 $\pm$ 0.88	2.64 $\pm$ 0.04	0.50 $\pm$ 0.01	0.43 $\pm$ 0.01
	Ethanol	1.07 $\pm$ 0.05	10.82 $\pm$ 0.48	2.18 $\pm$ 0.05	0.40 $\pm$ 0.01	0.36 $\pm$ 0.01
	Sucrose	1.53 $\pm$ 0.04	15.60 $\pm$ 0.55	2.69 $\pm$ 0.09	0.49 $\pm$ 0.01	0.42 $\pm$ 0.01

or an equicaloric dose of sucrose (that has been used in experiments by Frankel *et al.*, 1974) are shown in Table 6. At 2 h, ethanol significantly ($P < 0.001$) decreased the concentration of total serum tryptophan and increased those of free serum tryptophan and brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid. At 2 h after oral intubation of sucrose, the concentration of total serum tryptophan was not altered, whereas those of the other constituents were increased by 20–31% ($P = 0.005$ – 0.001). A comparison of the 2 h effects of ethanol and sucrose suggested no significant differences ($P > 0.10$) in concentrations of brain indoles, whereas the effects of ethanol on serum tryptophan concentrations were different ($P = 0.02$ – 0.001) from those of sucrose. The 7 h effects of oral intubation of ethanol or sucrose are also shown in Table 6. Sucrose exerted no significant effects ($P > 0.10$), whereas

ethanol caused decreases of 16–33% ($P < 0.001$) in all concentrations. The proportion of serum tryptophan present in the free state was not altered by ethanol.

Effects of ethanol on the concentrations of mouse free and total serum tryptophan and brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid

The above effects are shown in Table 7. At 2 h after intraperitoneal administration of ethanol (3.8 ml/kg) to mice of the C57BL strain, the concentrations of all the above compounds were not significantly different ($P > 0.10$) from those observed in 0.9% NaCl-treated mice. By contrast, ethanol administration to mice of the CBA/CA strain caused significant decreases ($P = 0.01$ – 0.001) of 11–36% in all concentrations except that of brain 5-hydroxyindol-3-

Table 7. *Effects of intraperitoneal administration of ethanol on the concentrations of mouse free and total serum tryptophan and brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid*

Each treated mouse received an intraperitoneal injection of either ethanol [3.8 ml/kg, as a 19% (v/v) solution in 0.9% NaCl] or an equal volume of 0.9% NaCl at 2 h before death. The concentrations of the above brain and serum constituents were determined as described in the Materials and Methods section. Values for free and total serum tryptophan concentrations are means $\pm$ S.E.M. for each group of four determinations each of which has been made with pooled sera from three mice, whereas those for brain constituents are means $\pm$ S.E.M. for each group of six determinations each of which has been performed on two pooled brains. N.D., not determined.

Strain of mice	Treatment	Concentration of serum tryptophan (μ g/ml)		Concentration in brain (μ g/g wet wt.)		
		Free	Total	Tryptophan	5-Hydroxy-tryptamine	5-Hydroxy-indol-3-ylacetic acid
C57BL	None	N.D.	13.22 $\pm$ 0.92	2.20 $\pm$ 0.05	0.76 $\pm$ 0.01	0.27 $\pm$ 0.005
	0.9% NaCl	2.64 $\pm$ 0.13	14.55 $\pm$ 1.00	2.19 $\pm$ 0.06	0.76 $\pm$ 0.01	0.28 $\pm$ 0.005
	Ethanol	2.57 $\pm$ 0.12	13.50 $\pm$ 0.97	2.23 $\pm$ 0.04	0.76 $\pm$ 0.01	0.28 $\pm$ 0.007
CBA/CA	None	2.19 $\pm$ 0.06	15.22 $\pm$ 0.94	2.46 $\pm$ 0.06	0.86 $\pm$ 0.02	0.36 $\pm$ 0.01
	0.9% NaCl	2.23 $\pm$ 0.08	14.95 $\pm$ 0.57	2.46 $\pm$ 0.07	0.84 $\pm$ 0.02	0.37 $\pm$ 0.01
	Ethanol	1.63 $\pm$ 0.03	9.50 $\pm$ 0.23	2.19 $\pm$ 0.04	0.73 $\pm$ 0.01	0.38 $\pm$ 0.01

Table 8. *Effects of ethanol on the concentration of rat serum non-esterified fatty acids*

Each rat received, at 2 or 7 h before death, an intraperitoneal injection of either ethanol [5 ml/kg, as a 25% (v/v) solution in 0.9% NaCl] or an equal volume of 0.9% NaCl. The concentration of serum non-esterified fatty acids was determined as described in the Materials and Methods section. Values are means $\pm$ S.E.M. for each group of four to five rats.

Time after injection (h)	Injection	Concentration of serum non-esterified fatty acids (mmol/l)
2	0.9% NaCl	0.31 $\pm$ 0.04
	Ethanol	1.10 $\pm$ 0.09
7	0.9% NaCl	0.31 $\pm$ 0.01
	Ethanol	0.30 $\pm$ 0.02

ylacetic acid. The proportion of serum tryptophan present in the free state was not decreased by ethanol in this strain of mice.

Effects of ethanol on the concentration of rat serum non-esterified fatty acids

The 2 and 7 h effects of intraperitoneal administration of ethanol (5 ml/kg) on the above concentration are shown in Table 8. Whereas ethanol did not cause any significant change at 7 h, it produced a 255% ($P < 0.001$) increase at 2 h.

Discussion

Effects of acute ethanol administration on the concentrations of rat brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid

Ethanol exerts a biphasic effect on the above concentrations irrespective of whether it is given intra-

peritoneally (Fig. 1) or orally (Table 6). The early increases and the subsequent decreases in the concentrations of brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid are consistent with an increased and a decreased rate of both synthesis and turnover of 5-hydroxytryptamine.

Brain tryptophan hydroxylase, the rate-limiting enzyme of the 5-hydroxytryptamine biosynthetic pathway, exists unsaturated with its substrate tryptophan (Eccleston *et al.*, 1965; Friedman *et al.*, 1972). Brain tryptophan concentration may therefore play an important role in 5-hydroxytryptamine synthesis, and there is considerable evidence in favour of both this concept and the direct relationship between brain tryptophan concentration and that of free serum (or plasma) tryptophan (see Curzon & Knott, 1974, and references therein). The biphasic effect of ethanol on rat brain 5-hydroxytryptamine metabolism may therefore be due to the altered availability of free serum tryptophan to the brain (Fig. 1 and Table 6). The early decrease in the concentration of total serum tryptophan argues against a role for it in tryptophan uptake by the brain, and appears to occur after administration of agents that displace the protein-bound amino acid, such as salicylate (Badawy & Smith, 1972; Bourgoin *et al.*, 1974; Tagliamonte *et al.*, 1973) and catecholamines (Curzon & Knott, 1974).

The mechanism by which ethanol enhances the activity of rat liver tryptophan pyrrolase (see the introduction) may also be involved in the increased availability of free serum tryptophan to the brain observed early after acute ethanol administration (Fig. 1 and Table 6). This is suggested by the findings that pretreatment of rats with the α -adrenoceptor blocking agent ergotamine, which prevents the ethanol-induced increase in free serum tryptophan concentration, also prevents the enhancement of brain tryptophan metabolism, whereas the phospho-

diesterase inhibitor theophylline potentiates both changes (Table 4). Administration of catecholamines has previously been shown (Curzon & Knott, 1974) to increase the concentration of free plasma and brain tryptophan, and to enhance brain 5-hydroxytryptamine turnover.

The decreased availability of free serum tryptophan to the brain observed at a later time-interval after ethanol administration (Fig. 1 and Table 6) may be due to either the enhancement of liver tryptophan pyrrolase activity or a possible anti-lipolytic action by ethanol. Since the latter possibility can be excluded by the results in Table 8, it may be useful to consider the first. The decreases in tryptophan concentrations in liver, serum and brain observed at 7 h after ethanol administration (Fig. 1), i.e. at 2 h after maximum activation of the pyrrolase has occurred (Badawy & Evans, 1973a), are consistent with the possibility that an enhanced pyrrolase activity could divert circulating tryptophan to, and increase its catabolism in, the liver, thus decreasing its availability to the brain. Similar changes have been reported (Green *et al.*, 1975) after pyrrolase induction by cortisol. Further evidence in favour of pyrrolase involvement in the ethanol effects is provided by the following findings. (1) Administration of a 1 ml/kg dose of ethanol, which does not enhance the pyrrolase activity (Badawy & Evans, 1973a), fails to alter brain tryptophan metabolism at 7 h (Table 3). (2) Pretreatment of rats with allopurinol or ergotamine, which prevents the pyrrolase activation by ethanol (Badawy & Evans, 1973b, 1975a), reverses the decreases in the serum and brain concentrations of tryptophan and/or its indole metabolites observed at 7 h after ethanol treatment (Table 5). Allopurinol has also been shown (Green & Curzon, 1968) to prevent the cortisol-induced decreases in rat brain, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid concentrations.

Previous studies of the acute effects of ethanol on brain 5-hydroxytryptamine synthesis and turnover

Studies of the above effects in the rat and mouse have produced contradictory results (see the introduction). The use of a monoamine oxidase inhibitor to measure the turnover rate of 5-hydroxytryptamine (as has been the case in most of these studies) may render the results with ethanol difficult to interpret, because monoamine oxidase inhibitors may exert unknown effects on tryptophan metabolism, and also in view of the possible inhibition of tryptophan hydroxylase activity *in vivo* by either catecholamines (Koe, 1971; Karobath *et al.*, 1972) or 5-hydroxytryptamine (Hamon *et al.*, 1973, and references therein). Further studies with monoamine oxidase inhibitors are therefore required. Moreover, a comparison of the early effects of ethanol with those of an equicaloric dose of sucrose (see Frankel *et al.*, 1974)

may be misleading as the results in Table 6 suggest. Sucrose inhibits tryptophan pyrrolase activity (Table 1; see also Badawy & Evans, 1976a) and its effect on the concentrations of brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid is consistent with an increased availability of circulating tryptophan to the brain (Table 6), as is the case with glucose (Badawy & Evans, 1975b). Sugars (or carbohydrates) may, however, increase brain tryptophan concentration by an additional mechanism involving a decrease in the serum concentrations of amino acids that compete with tryptophan for uptake by the brain (see Madras *et al.*, 1973).

Our studies in the mouse show that the acute effects of ethanol on liver tryptophan pyrrolase activity (Table 2) and on the concentrations of serum and brain tryptophan and/or its indole metabolites (Table 7) are strain dependent. The failure of ethanol to alter brain tryptophan metabolism in the C57BL strain is consistent with the absence of either the activation of the pyrrolase or the release of protein-bound serum tryptophan, both phenomena occur in the rat by a catecholamine-mediated lipolytic mechanism. The ethanol inhibition of brain tryptophan metabolism in mice of the CBA/CA strain is consistent with a decreased availability of circulating tryptophan (Table 7). These changes resemble those observed at 7 h after administration of ethanol to rats (Fig. 1 and Table 6), and may therefore be pyrrolase-mediated. However, the rise in the latter in this strain of mouse, although more rapid (Table 2), is less pronounced than that in the rat (Table 1), but it is possible that mice may metabolize tryptophan more rapidly than do rats (Monroe, 1968). These results, and those previously published (see the introduction) suggest the need for further work to elucidate the mechanism(s) of strain differences in the acute effects of ethanol on mouse liver and brain tryptophan metabolism.

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References

- Badawy, A. A.-B. & Evans, M. (1973a) *Biochem. Soc. Trans.* **1**, 193-195
- Badawy, A. A.-B. & Evans, M. (1973b) *Biochem. J.* **133**, 585-591
- Badawy, A. A.-B. & Evans, M. (1973c) *Adv. Exp. Med. Biol.* **35**, 105-123
- Badawy, A. A.-B. & Evans, M. (1975a) *Adv. Exp. Med. Biol.* **59**, 229-251
- Badawy, A. A.-B. & Evans, M. (1975b) *Lancet* **ii**, 869
- Badawy, A. A.-B. & Evans, M. (1976a) *Biochem. J.* **156**, 381-390

- Badawy, A. A.-B. & Evans, M. (1976b) *Biochem. J.* **158**, 79–88
- Badawy, A. A.-B. & Smith, M. J. H. (1972) *Biochem. Pharmacol.* **21**, 97–101
- Bloxam, D. L. & Warren, W. H. (1974) *Anal. Biochem.* **60**, 621–625
- Bourgoin, S., Faivre-Bauman, A., Benda, P., Glowinski, J. & Hamon, M. (1974) *J. Neurochem.* **23**, 319–327
- Curzon, G. & Green, A. R. (1970) *Br. J. Pharmacol.* **39**, 653–655
- Curzon, G. & Knott, P. J. (1974) *Br. J. Pharmacol.* **50**, 197–204
- Denckla, W. D. & Dewey, H. K. (1967) *J. Lab. Clin. Med.* **69**, 160–169
- Eccleston, D., Ashcroft, G. W. & Crawford, T. B. B. (1965) *J. Neurochem.* **12**, 493–503
- Feigelson, P. & Greengard, O. (1961) *J. Biol. Chem.* **236**, 153–157
- Frankel, D., Khanna, J. M., Kalant, H. & LeBlanc, A. E. (1974) *Psychopharmacologia* **37**, 91–100
- Friedman, P. A., Kappelman, A. H. & Kaufman, S. (1972) *J. Biol. Chem.* **247**, 4165–4173
- Green, A. R. & Curzon, G. (1968) *Nature (London)* **220**, 1095–1097
- Green, A. R., Woods, H. F., Knott, P. J. & Curzon, G. (1975) *Nature (London)* **255**, 170
- Griffiths, P. J., Littleton, J. M. & Ortiz, A. (1974) *Br. J. Pharmacol.* **50**, 489–498
- Hamon, M., Bourgoin, S. & Glowinski, J. (1973) *J. Neurochem.* **20**, 1727–1745
- Karobath, M., Diaz, J.-L. & Huttunen, M. (1972) *Biochem. Pharmacol.* **21**, 1245–1251
- Koe, B. K. (1971) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **30**, 886–896
- Madras, B. K., Cohen, E. L., Fernstrom, J. D., Larin, F., Munro, H. N. & Wurtman, R. J. (1973) *Nature (London)* **244**, 34–35
- Mikac-Dević, D., Stanković, H. & Bošković, K. (1973) *Clin. Chim. Acta* **45**, 55–59
- Monroe, C. B. (1968) *Am. J. Physiol.* **214**, 1410–1414
- Palaić, D. J., Desaty, J., Albert, J. M. & Panisset, J. C. (1971) *Brain Res.* **25**, 381–386
- Rawat, A. K. (1974) *J. Neurochem.* **22**, 915–922
- Tabakoff, B., Ritzmann, R. F. & Boggan, W. O. (1975) *J. Neurochem.* **24**, 1043–1051
- Tagliamonte, A., Biggio, G., Vargiu, L. & Gessa, G. L. (1973) *J. Neurochem.* **20**, 909–912
- Tyce, G. M., Flock, E. V., Taylor, W. F. & Owen, C. A., Jr. (1970) *Proc. Soc. Exp. Biol. Med.* **134**, 40–44
- Yamanaka, Y. & Kono, S. (1974) *Jpn. J. Pharmacol.* **24**, 247–252