Inhibition of rat brain tryptophan metabolism by ethanol withdrawal and possible involvement of the enhanced liver tryptophan pyrrolase activity

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1. Chronic ethanol administration to rats was previously shown to enhance brain 5-hydroxytryptamine synthesis by increasing the availability of circulating tryptophan to the brain secondarily to the NAD(P)H-mediated inhibition of liver tryptophan pyrrolase activity. 2. At 24 h after ethanol withdrawal, all the above effects were observed because liver [NAD(P)H] was still increased. By contrast, all aspects of liver and brain tryptophan metabolism were normal at 12 days after withdrawal. 3. At 7–9 days after withdrawal, brain 5-hydroxytryptamine synthesis was decreased, as was tryptophan availability to the brain. Liver tryptophan pyrrolase activity at these time-intervals was maximally enhanced. 4. Administration of nicotinamide during the withdrawal phase not only abolished the withdrawal-induced enhancement of tryptophan pyrrolase activity on day 8, but also maintained the inhibition previously caused by ethanol. Under these conditions, the withdrawal-induced decreases in brain 5-hydroxytryptamine synthesis and tryptophan availability to the brain were abolished, and both functions were enhanced. Nicotinamide alone exerted similar effects in control rats. 5. It is suggested that ethanol withdrawal inhibits brain 5-hydroxytryptamine synthesis by decreasing tryptophan availability to the brain secondarily to the enhanced liver tryptophan pyrrolase activity. 6. The results are discussed in relation to the possible involvement of 5-hydroxytryptamine in dependence on ethanol and other drugs.

We have previously reported (Badawy & Evans, 1973, 1975a) that chronic ethanol administration to rats inhibits the activity of liver tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.11.11), whereas subsequent withdrawal causes an enhancement. An inverse relationship exists between liver tryptophan pyrrolase activity and brain 5-hydroxytryptamine metabolism in which the former determines the availability of circulating tryptophan to the brain (for a review, see Badawy, 1977), and it has been reported (Badawy *et al.*, 1979; Punjani *et al.*, 1979) that, by decreasing the hepatic catabolism of tryptophan, the chronic ethanol-induced inhibition of liver tryptophan pyrrolase activity increases the availability of circulating tryptophan to the brain and enhances brain 5-hydroxytryptamine synthesis. On the other hand, decreases in brain 5-hydroxytryptamine synthesis and turnover and in the availability of circulating tryptophan to the brain associated with an enhancement of liver tryptophan pyrrolase activity are observed after acute administration of cortisol (Green *et al.*, 1975) or ethanol (Badawy & Evans, 1976b). It was therefore decided to find out if the ethanol-withdrawal-induced

enhancement of liver tryptophan pyrrolase activity will also decrease brain 5-hydroxytryptamine synthesis by limiting tryptophan availability to the brain. Such an investigation is also of interest in relation to the possible involvement of 5-hydroxytryptamine in the mood disorders frequently encountered clinically in conditions of alcohol dependence and alcohol-withdrawal states. The results of this investigation form the subject of the present paper.

Materials and methods

Chemicals

Chemicals were purchased from Sigma (London) Chemical Co. and BDH Chemicals (both of Poole, Dorset, U.K.) and were of the purest commercially available grades.

Animals and treatments

Male Wistar rats were purchased from Hacking and Churchill, Wyton, Huntingdon, Cambs., U.K. The animals were housed three per cage, were maintained on cube diet 41B (Oxoid, Basingstoke,

Hants., U.K.) and water for at least 1 week before the start of experiments and were killed by decapitation between 10:00 and 11:00 h.

Ethanol was freely administered in drinking water in concentrations (v/v) of 5% for 2 days, 7.5% for 2 days more and finally 10% for the remainder of the experimental period. Average ethanol intake after the first 4 days of administration was 9.6 g/kg per day. Withdrawal of ethanol was performed after 3 weeks of chronic administration by replacing the drug solution with drinking water. When the effects of withdrawal were compared with those of chronic administration, the chronic ethanol-treated rats continued to receive the drug in their drinking fluid after the original 3-week period and up until the time at which the ethanol-withdrawn animals were killed. Some ethanol-withdrawn rats received, instead of drinking water, a solution of nicotinamide in water (5 g/l) for 8 days, and control animals for this particular treatment received a similar solution of nicotinamide at the same time (3 weeks after the start of experiment) and for the same duration (8 days). The average body weight of control rats at the start of experiments was 175 g, whereas the corresponding weight of ethanol-treated animals was deliberately 8–13% higher. At the end of the experimental period, the average weight gain by ethanol-treated rats was 8–20% lower than that achieved by control animals.

Chemical, enzymic and other determinations

Tryptophan pyrrolase activity was determined in fresh liver homogenates (Badawy & Evans, 1975c) either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added (2 μ M) haematin. The apoenzyme activity was obtained by difference.

Free (ultrafiltrable) serum, total (acid-soluble) serum, liver and brain tryptophan and brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid concentrations were determined by standard procedures (for references, see Badawy *et al.*, 1979). Analyses were performed with livers isolated by a locally manufactured freeze-clamp and with brains that had been rapidly removed (within 30 s of the death of the animals), immersed in liquid nitrogen for 3 min and stored at -20°C for 24 h.

The above frozen livers were also used for the direct determination of concentrations of oxidized and reduced nicotinamide-adenine dinucleotide (phosphate) by the method of Slater *et al.* (1964) with the minor modifications described by Punjani *et al.* (1979).

Fluorimetric determination of concentrations of serum glucose and corticosterone was performed by the methods of Slein (1963) and Glick *et al.* (1964) respectively, whereas blood ethanol concentration

was determined by a routine g.l.c. method at a nearby hospital laboratory.

Statistical analysis of results was performed by using Student's *t* test.

Ethological techniques were used to assess aggressive and social behaviour of rats (Mackintosh *et al.*, 1977) and the activities of the animals in the open-field test (Candland & Nagy, 1969).

Results and discussion

Mechanisms of the effects of chronic ethanol administration and subsequent withdrawal on rat liver tryptophan pyrrolase activity

We have previously reported (Badawy & Evans, 1973) that chronic ethanol administration to rats does not significantly alter the holoenzyme activity of liver tryptophan pyrrolase, but decreases that of the total enzyme. The apoenzyme is therefore the form specifically inactivated by ethanol, by a mechanism not involving a defective apoenzyme synthesis or a decreased cofactor availability. It was also shown that subsequent withdrawal did not reverse the above decreases at 1–3 days, but that, on day 5, the enzyme activity returned to the basal value. There then followed an enhancement of both holoenzyme and total pyrrolase activities that reached 2.5-fold the basal values on day 8, and this was followed by a final return to normal enzyme activity on day 11 after withdrawal.

In the present work, experiments were performed at 1, 7, 8, 9 and 12 days after ethanol withdrawal to investigate brain tryptophan and 5-hydroxytryptamine metabolism in relation to the above changes in liver tryptophan pyrrolase activity. The results in Fig. 1 show that, under the present experimental conditions, chronic ethanol administration did not alter the pyrrolase holoenzyme activity, but caused significant decreases in those of the total enzyme (31–39%; $P < 0.001$) and the apoenzyme (64–81%; $P = 0.005$ – 0.001), whereas subsequent withdrawal of the drug after 3 weeks of chronic administration did not influence these decreases after 1 day, but caused an enhancement of all pyrrolase activities on days 7–9 and was associated with a normal enzyme activity on day 12. This withdrawal-induced enhancement of pyrrolase activity was not associated with an altered saturation of the apoenzyme with haem (as assessed by the ratio of holoenzyme activity/apoenzyme activity). This type of enhancement is characteristic of the hormonal induction mechanism (see also Badawy, 1979), and this is further supported by the correlation between the changes in pyrrolase activity with those in serum corticosterone concentration, which are also shown in Fig. 1. These results also show that chronic ethanol administration caused only moderate

Fig. 1. Effects of chronic ethanol administration and subsequent withdrawal on rat liver tryptophan pyrrolase activity and serum corticosterone concentration

Ethanol was withdrawn after 3 weeks of chronic administration in drinking water. Some chronically treated rats continued to receive the drug after the initial 3-week period and up till the times indicated after withdrawal. The holoenzyme (a), total enzyme (b) and apoenzyme (c) activities of liver tryptophan pyrrolase and the concentration of serum corticosterone (d) were determined as described in the Materials and methods section. Pyrrolase activities were in μmol of kynurenine formed/h per g wet wt. of liver, whereas serum corticosterone concentration was in $\mu\text{g/l}$. The results are presented as percentage differences from control values (those in untreated rats). These percentages are based on the mean values for each group of four (pyrrolase activities) or six (corticosterone concentration) rats. The control values for each time-interval varied between 1.5 and 2.1 for holo-(tryptophan pyrrolase), 2.9 and 4.6 for total tryptophan pyrrolase, 1.4 and 2.5 for apo-(tryptophan pyrrolase) and 60 and 75 for serum corticosterone. Symbols: ●, chronic ethanol administration; ○, ethanol withdrawal.

increases in serum corticosterone concentration and that no further increase in the latter occurred at 24 h after ethanol withdrawal. These latter findings confirm those reported by Pohorecky *et al.* (1978) from experiments in which ethanol was administered in a liquid diet. We have also found in the present work that neither chronic administration nor subsequent withdrawal of ethanol caused any significant changes in serum glucose concentration (results not shown). The absence of changes in serum glucose concentration during chronic ethanol administration has previously been reported (Pohorecky *et al.*, 1978; Badawy *et al.*, 1979) and suggests that this treatment does not inhibit liver

tryptophan pyrrolase activity or increase brain tryptophan concentration by mechanisms involving glucose or insulin respectively.

That the chronic ethanol-induced decrease in liver tryptophan pyrrolase activity is mediated by increased hepatic concentrations of the allosteric inhibitors NAD(P)H is suggested by several findings (Badawy & Evans, 1975a; Badawy *et al.*, 1979; Punjani *et al.*, 1979). Because the above decrease in pyrrolase activity was not reversed at 24 h after ethanol withdrawal (Badawy & Evans, 1973; Fig. 1 in the present work), it was decided to find out if liver [NAD(P)H] was still increased at this time-interval. Liver [NADH] and [NADPH] (in $\mu\text{g/g}$ wet wt.,

$\pm$ S.E.M. for each group of six rats) were as follows: control rats 145 ± 2 and 232 ± 7 ; chronic ethanol-treated rats 219 ± 5 and 288 ± 7 ; ethanol-withdrawn rats 212 ± 9 and 282 ± 4 . $[\text{NAD}^+]$ of these three groups of rats was 274 ± 4 , 209 ± 4 and 201 ± 4 respectively. $[\text{NADP}^+]$ was not significantly altered by chronic ethanol administration or subsequent withdrawal. Gordon (1973) also reported that the chronic ethanol-induced increase in rat liver $[\text{NADH}]$ observed 1 month after administration of the drug in a liquid diet was not reversed at 24 h after withdrawal. These results therefore suggest that the chronic ethanol-induced changes in the redox states of the hepatic NAD(P) couples are not readily reversible at 24 h after withdrawal, and further work is required to examine the possible reasons for this. Since ethanol disturbs the above redox states via its own metabolism by alcohol dehydrogenase (Punjani *et al.*, 1979), the possibility was considered that blood ethanol concentration might have been high at 24 h after withdrawal. In experiments not shown here, it was found that this is not so.

Effects of chronic ethanol administration and subsequent withdrawal on rat brain tryptophan and 5-hydroxytryptamine metabolism

We have previously reported (Badawy *et al.*, 1979; Punjani *et al.*, 1979) that chronic ethanol administration to rats enhances brain 5-hydroxytryptamine synthesis by increasing the availability of circulating tryptophan to the brain, and that this increased availability is not insulin-mediated or lipolysis-dependent. Evidence was also presented from experiments with regenerators of liver NAD(P)^+ and with pyrazole that strongly suggested that the above effects of ethanol are caused by the decrease in liver tryptophan pyrrolase activity.

The effects of chronic ethanol administration and subsequent withdrawal on free serum and total serum tryptophan concentrations are shown in Fig. 2. Because similar changes were also observed in concentrations of liver and brain tryptophan and brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid, the results on these latter changes are not included in Fig. 2, but are described below in the text.

Chronic ethanol administration caused increases of 19–37% ($P = 0.01$ – 0.001) in concentrations of free serum, total serum, liver and brain tryptophan and brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid. At 24 h after withdrawal, the concentrations of the above substances were still increased (by 16–29%; $P = 0.05$ – 0.001), and in fact these increases were not significantly different from those caused by chronic ethanol administration ($P > 0.10$). By contrast, the concentrations of tryptophan and its indole metabolites were decreased on days 7–9 after withdrawal of ethanol by 13–32% ($P = 0.005$ – 0.001) in comparison with control values. On day 12 after withdrawal, all concentrations of tryptophan and hydroxyindoles were similar to those observed in control rats.

It is clear from the results in Figs. 1 and 2 that a reciprocal relationship exists between liver tryptophan pyrrolase activity and other aspects of tryptophan metabolism. The increases in tryptophan and hydroxyindole concentrations at 24 h, and their return to control values at 12 days, after ethanol withdrawal may simply be explained by liver tryptophan pyrrolase activity being decreased at the former time and normal at the latter. By contrast, the decreases in brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid concentrations observed at 7–9 days after ethanol withdrawal are consistent with defective 5-hydroxytryptamine

Fig. 2. Effects of chronic ethanol administration and subsequent withdrawal on the concentrations of free and total serum tryptophan in rats

Experimental details and expression of results are as described in Fig. 1, except that the percentage differences from control values are based on the mean for each group of six rats. Tryptophan concentrations (in $\mu\text{g/ml}$ of serum) were determined as described in the Materials and methods section. The control values for each group varied between 1.10 and 2.02 for free serum tryptophan (a) and 23.48 and 29.47 for total serum tryptophan (b). Symbols: ●, chronic ethanol administration; ○, ethanol withdrawal.

synthesis. Since brain tryptophan concentration was not unaltered or increased at these time-intervals, it may be suggested that the defective 5-hydroxytryptamine synthesis is not caused by the failure of the brain to convert adequate amounts of tryptophan into 5-hydroxytryptamine, but that it is most probably produced by a decreased brain tryptophan concentration secondarily to decreased availability of the circulating amino acid to the brain. That this is so is suggested by the observed (Fig. 2) decreases in free serum and total serum tryptophan concentrations at 7–9 days after withdrawal. That the enhanced liver tryptophan pyrrolase activity increasing the hepatic catabolism of tryptophan is the cause of the decreased availability of the circulating amino acid to the brain is suggested by the observed decrease in liver tryptophan concentration (see the text). It has also been shown previously that the enhancement of liver tryptophan pyrrolase activity caused by acute administration of cortisol (Green *et al.*, 1975) or ethanol (Badawy & Evans, 1976b) is associated with decreased concentrations of tryptophan in liver, serum (or plasma) and brain and of brain hydroxyindoles, and that, with ethanol, all these decreases can be blocked by prevention of the enhancement of pyrrolase activity by the pyrrolase inhibitor allopurinol and the α -adrenoceptor-blocking agent ergotamine.

More direct evidence for the possible involvement

of liver tryptophan pyrrolase activity in the ethanol-withdrawal-induced decrease in tryptophan availability to the brain and brain 5-hydroxytryptamine metabolism has been obtained from experiments (Table 1) in which the enhancement of pyrrolase activity observed at 8 days after ethanol withdrawal has been prevented by chronic administration of nicotinamide during the 8-day withdrawal period. Chronic nicotinamide administration has previously been shown to inhibit rat liver tryptophan pyrrolase activity by increasing hepatic [NAD(P)H] (Badawy & Evans, 1976a) and to increase the endogenous concentration of brain tryptophan and that elevated by administration of a tryptophan load (Badawy & Evans, 1975b). In the results in Table 1, nicotinamide alone decreased the holoenzyme, total enzyme and apoenzyme activities of liver tryptophan pyrrolase by 14, 39 and 54% respectively ($P = 0.01$ – 0.001) and increased the concentrations of liver, free serum, total serum and brain tryptophan and those of brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid by 25–39% ($P = 0.005$ – 0.001). These effects of nicotinamide are very similar to those exerted by chronic ethanol administration and strongly suggest that the former also enhances brain 5-hydroxytryptamine synthesis by increasing tryptophan availability to the brain secondarily to the inhibition of liver tryptophan pyrrolase activity. Nicotinamide did not significantly alter serum glucose concentration

Table 1. *Effects of chronic administration of nicotinamide to control rats and during ethanol withdrawal on liver and brain tryptophan metabolism and on serum glucose and corticosterone concentrations*

The above aspects were determined, as described in the Materials and methods section, in control rats and in those to which nicotinamide was administered in drinking water (5g/l) for 8 days either alone or immediately after withdrawal of ethanol previously administered for 21 days. Values are means $\pm$ S.E.M. for each group of four rats (liver tryptophan pyrrolase activity) or of six rats (all other determinations). Pyrrolase activities are expressed in μ mol of kynurenine formed/h per g wet wt. of liver, whereas serum glucose and corticosterone concentrations are in mg/100ml and μ g/l respectively. All other values (except the percentage free serum tryptophan) are in μ g/ml or μ g/g wet wt. of tissue. The values observed in nicotinamide-treated rats have been compared with those in control animals and the significance of differences is indicated as follows: $\dagger P < 0.025$; $*P < 0.01$; $**P < 0.005$; $***P < 0.001$.

Glucose, corticosterone and tryptophan-metabolism parameters

Determination	Treatment	Nil	Nicotinamide	Nicotinamide after ethanol withdrawal
Holo-(tryptophan pyrrolase)		2.10 $\pm$ 0.05	1.80 $\pm$ 0.06*	1.90 $\pm$ 0.13
Total tryptophan pyrrolase		5.10 $\pm$ 0.29	3.10 $\pm$ 0.16***	2.60 $\pm$ 0.14***
Apo-(tryptophan pyrrolase)		3.00 $\pm$ 0.26	1.30 $\pm$ 0.13**	0.70 $\pm$ 0.08***
Serum glucose		142.00 $\pm$ 3.00	136.00 $\pm$ 1.00	132.00 $\pm$ 2.00†
Serum corticosterone		63.20 $\pm$ 2.70	79.50 $\pm$ 2.50**	76.30 $\pm$ 2.00**
Free serum tryptophan		1.23 $\pm$ 0.06	1.57 $\pm$ 0.06**	1.55 $\pm$ 0.05**
Total serum tryptophan		23.83 $\pm$ 0.69	30.42 $\pm$ 0.97***	30.08 $\pm$ 1.06***
Free serum tryptophan (%)		5.16 $\pm$ 0.38	5.16 $\pm$ 0.60	5.15 $\pm$ 0.23
Liver tryptophan		4.65 $\pm$ 0.21	5.80 $\pm$ 0.07***	5.87 $\pm$ 0.20**
Brain tryptophan		2.45 $\pm$ 0.06	3.41 $\pm$ 0.05***	3.18 $\pm$ 0.07***
Brain 5-hydroxytryptamine		0.58 $\pm$ 0.007	0.71 $\pm$ 0.014***	0.71 $\pm$ 0.013***
Brain 5-hydroxyindol-3-ylacetic acid		0.42 $\pm$ 0.007	0.54 $\pm$ 0.013***	0.54 $\pm$ 0.014***

or tryptophan binding to serum proteins. The absence of changes in tryptophan binding is in contrast with the ability of acute nicotinic acid administration to increase this binding by inhibiting lipolysis (Curzon & Knott, 1974).

When administered chronically immediately after ethanol withdrawal (Table 1), nicotinamide was effective not only in preventing the withdrawal-induced enhancement of liver tryptophan pyrrolase activity, but also in maintaining the inhibition previously caused by chronic ethanol administration. It could be argued that the ability of nicotinamide to prevent the withdrawal-induced increase in serum corticosterone concentration (see Fig. 1) is responsible for the prevention of enhancement of pyrrolase activity. However, the observed inhibition of the latter by nicotinamide cannot be explained along these lines, and must therefore be caused by the NAD(P)H-mediated mechanism mentioned above. The ethanol-withdrawal-induced decreases in concentrations of liver, free serum, total serum and brain tryptophan and in those of brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid (see Fig. 2 and the text) were all prevented by nicotinamide (Table 1), and in fact the observed increases in all these concentrations (except that of brain tryptophan) were similar to, and could not be distinguished from, those produced by nicotinamide in control rats. It may therefore be concluded that the ethanol-withdrawal-induced decreases in tryptophan availability to the brain and in brain 5-hydroxytryptamine synthesis are caused by the enhancement of liver tryptophan pyrrolase activity. The present findings with nicotinamide and ethanol withdrawal also provide further support to the inverse relationship between liver tryptophan pyrrolase activity and brain 5-hydroxytryptamine synthesis.

Although many investigations of the effects of ethanol on brain 5-hydroxytryptamine metabolism have been performed, very little work has been done in relation to ethanol withdrawal. In the very few studies reported (Griffiths *et al.*, 1974; Pohorecky *et al.*, 1978), experiments were carried out up until only 30h after ethanol withdrawal, and no significant changes were observed. The findings of the present paper clearly illustrate the importance of performing studies at longer time-intervals after ethanol withdrawal.

Behavioural studies

Chronic ethanol administration for 3 weeks or subsequent withdrawal did not cause any significant changes in social behaviour of rats or in their activities in the open-field test. Only the home-cage aggression score was decreased by chronic ethanol administration, and this decrease was rapidly reversed after withdrawal. Taken together, these

findings suggest that, under the present experimental conditions, chronic ethanol administration or subsequent withdrawal do not cause remarkable changes in animal behaviour despite the observed changes in tryptophan metabolism. This is in contrast with the reported (Liljequist *et al.*, 1977) changes in behaviour during withdrawal after chronic administration of similar doses of ethanol for longer time-intervals, and the occurrence of gross behavioural abnormalities after withdrawal of ethanol previously administered in much larger doses in a liquid diet (Freund, 1969). It remains to be seen whether chronic administration of ethanol in larger doses and/or for longer periods will also be associated with similar or more pronounced biochemical changes in tryptophan metabolism, and whether these changes could be correlated with those in behaviour.

Possible involvement of 5-hydroxytryptamine in ethanol and drug dependence

Mood disorders are important clinical features of alcohol and drug dependence. It is also clinically recognized that depression and other aspects of lowered mood are associated with withdrawal. The possible role of 5-hydroxytryptamine in mood regulation and in depressive illness and the possible involvement of liver tryptophan pyrrolase activity in the regulation of brain 5-hydroxytryptamine synthesis are well documented (see Curzon, 1969; Lapin & Oxenkrug, 1969; Badawy, 1977; the present work and references cited therein). If changes in 5-hydroxytryptamine metabolism also occur in ethanol dependence and withdrawal in man, it may be useful to examine the possible role of this amine in the dependence process by modification of liver tryptophan metabolism by agents such as nicotinamide.

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