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ENHANCEMENT OF RAT BRAIN METABOLISM OF A TRYPTOPHAN LOAD BY CHRONIC ETHANOL ADMINISTRATION

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We have previously shown that chronic ethanol administration enhances brain 5-hydroxytryptamine synthesis by increasing the availability of circulating tryptophan to the brain secondary to the decreased liver tryptophan pyrrolase activity. We now find that ethanol enhances the brain metabolism of a tryptophan load by the same mechanism. The results are discussed in relation to ethanol preference and the need for further clinical work on the effects of alcoholism on tryptophan metabolism.

Introduction Tryptophan loads have been administered to chronic alcoholics (Olson, Gursey & Vester, 1960; Murphy, Guze & King, 1962; Walsh, Howorth & Marks, 1966) to assess the influence of ethanol consumption on tryptophan metabolism. However, measurement of urinary 5-hydroxyindol-3-ylacetic acid (5-HIAA) concentration does not reflect changes in cerebral tryptophan metabolism because of the wide distribution of the 5-hydroxytryptamine (5-HT) pathway in the body. The way in which the brain of a chronic alcoholic handles tryptophan remains therefore unknown. One way of examining this point is, to administer a tryptophan load to a chronic ethanol-treated rat. This approach may also assist in clarifying the reported (Sprince, Parker, Smith & Gonzales, 1972) enhancement of ethanol preference by tryptophan. It has previously been shown that inhibitors of liver tryptophan pyrrolase activity such as allopurinol, glucose or nicotinamide (Badawy & Evans, 1975a) and carbidopa (Joseph & Hall-Tipping, 1978) enhance the rise in rat brain tryptophan concentration caused by a tryptophan load. Furthermore we have found that the decreased liver pyrrolase activity observed in rats chronically treated with ethanol (Badawy & Evans, 1975*b*) is involved in the concomitant enhancement (Badawy, Punjani & Evans, 1979) of brain 5-HT synthesis that is associated with an elevated brain tryptophan concentration. We have therefore examined the effects of chronic ethanol administration to rats on the brain metabolism of a tryptophan load, and found that it is enhanced.

Methods All details concerning animals, ethanol administration (for two weeks in drinking water) and analytical procedures have been described or referred to by Badawy *et al.* (1979).

Results Chronic ethanol administration decreased the activities of the pyrrolase holoenzyme, total enzyme and apoenzyme by 20, 51 and 75% respectively. The tryptophan load did not exert any significant effects on pyrrolase activities in control rats nor did it influence the inhibitory effects of ethanol (Table 1).

Chronic ethanol administration increased the concentrations of liver, free serum, total serum and brain tryptophan and those of brain 5-HT and 5-HIAA by 26, 21, 22, 21, 17.5 and 24% respectively. The binding of tryptophan to serum proteins (expressed as free serum tryptophan (%)) was not affected by ethanol (Table 1).

As expected, a tryptophan load increased all concentrations of the above parameters in control rats. In those treated with ethanol, the load-induced increases were potentiated by 22 to 33%, except with 5-HT concentration which was decreased by 19% by ethanol (Table 1).

Serum glucose concentration was not altered by the tryptophan load alone or by ethanol treatment alone. The two treatments together caused a small decrease in comparison with the value after the load alone (Table 1).

Discussion We have previously shown that chronic ethanol administration inhibits rat liver tryptophan pyrrolase activity by increasing the hepatic concentrations of the allosteric inhibitors NADH and NADPH (Badawy & Evans, 1975b) and that this inhibition increases the availability of circulating tryptophan to the brain, thus enhancing 5-HT synthesis (Badawy, *et al.*, 1979). It was also found in the latter work that the ethanol effects on brain tryptophan metabolism were not insulin-mediated or lipolysis-dependent.

The detailed examination of the distribution of a tryptophan load between liver, serum and brain under conditions of inhibited pyrrolase activity (Table 1) shows that the effects of the load on tryptophan concentration and that of brain 5-HIAA are potentiated by the ethanol treatment, presumably because of the inhibited pyrrolase activity diverting administered tryptophan to the brain. Ethanol, however, decreases the load-induced rise in brain 5-HT and this may be

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Table 1 Effects of chronic ethanol administration with or without a tryptophan load on various aspects of tryptophan (Trp) metabolism and on serum glucose concentration in the rat

	Control (no ethanol) rats		*Chronic ethanol-treated rats*	
Determination	*Saline*	*Tryptophan*	*Saline*	*Tryptophan*
Pyrrolase activity:				
Holoenzyme	2.50 ± 0.09	2.90 ± 0.26	2.00 ± 0.09††	2.10 ± 0.09**
Total enzyme	5.70 ± 0.35	5.80 ± 0.59	2.80 ± 0.20††††	3.30 ± 0.17††
Apoenzyme	3.20 ± 0.25	2.90 ± 0.35	0.80 ± 0.19††††	1.20 ± 0.13†††
Liver Trp	6.90 ± 0.38	23.40 ± 1.81	8.70 ± 0.19†††	30.05 ± 1.40†††
Free serum Trp	3.35 ± 0.23	30.60 ± 0.91	4.05 ± 0.26*	37.38 ± 3.31*
Total serum Trp	35.84 ± 1.05	71.50 ± 5.55	43.82 ± 1.60†††	91.00 ± 2.46†††
Free serum Trp (%)	9.35 ± 0.39	42.80 ± 3.63	9.24 ± 0.31	41.08 ± 3.20
Brain Trp	4.96 ± 0.27	22.42 ± 0.69	6.02 ± 0.20†	27.38 ± 1.59***
Brain 5-HT	0.40 ± 0.007	0.88 ± 0.03	0.47 ± 0.013††††	0.71 ± 0.03†††
Brain 5-HIAA	0.67 ± 0.014	0.88 ± 0.024	0.83 ± 0.017††††	1.17 ± 0.03††††
Serum glucose	132.00 ± 6.00	140.00 ± 5.00	124.00 ± 3.00	128.00 ± 1.00††

Ethanol was administered in drinking water for two weeks. Control and ethanol-treated rats received, at 0.5 h before death, an intraperitoneal injection of either tryptophan (50 mg/kg) or an equal volume (2 ml/kg) of 0.9% w/v NaCl solution (saline). Values are means ± s.e. mean for each group of 4 rats (pyrrolase activity) or of 6 rats (all other determinations). Pyrrolase activities are quoted in μmol of kynurenine formed/h per g wet wt. of liver, whereas serum glucose concentrations are in mg/dl. All other values are in μg/ml of serum or per g wet wt. of tissue (except the percentage free serum tryptophan). The effects of the tryptophan load in control (no ethanol) rats and in those treated with ethanol were similar and were all significant at a P level of <0.0005, except for the absence of change in serum glucose concentration. The values in column 3 are compared with those in column 1, whereas those in column 4 are compared with those in column 2, and the differences are signified as follows: *$P < 0.05$; **$P < 0.025$; ***$P < 0.0125$; †$P < 0.01$; ††$P < 0.005$; †††$P < 0.0025$; ††††$P < 0.0005$.

man, 1978) that, although brain tryptophan concentration rises in proportion to the dose of tryptophan administered (in the range of 0 to 100 mg/kg), those of 5-HT and 5-HIAA decline with doses above 25 mg/kg. However, when the rise in brain tryptophan produced by our load (50 mg/kg) is potentiated by ethanol by 22%, brain 5-HIAA concentration is further elevated by 33%. This suggests that ethanol may enhance 5-HT conversion into 5-HIAA by stimulating either 5-HT turnover or the 5-hydroxyindolepyruvate pathway. Further work is required to examine these possibilities.

The present results suggest that chronic ethanol administration alters the handling of tryptophan such that kynurenine metabolism is suppressed and that of 5-HT is enhanced. Evidence for decreased hepatic (kynurenine) tryptophan metabolism has been obtained in man (Walsh *et al.*, 1966) within 24 h of cessation of alcohol intake. By contrast, studies of urinary 5-HIAA excretion produced conflicting results (see the introduction) because of methodological and technical differences. Further work is therefore required to assess the effects of alcoholism on tryptophan metabolism under well-defined experimental and clinical conditions.

Tryptophan enhances ethanol preference (Sprince *et al.*, 1972) and it is therefore possible that it may do so by modifying brain 5-HT synthesis as the present results suggest. Alternatively, since L-DOPA enhances ethanol preference (Sprince *et al.*, 1972) and since tryptophan administration enhances rat brain catecholamine metabolism (Eccleston & Nicolaou, 1978), it is possible that tryptophan may enhance ethanol preference by acting on catecholamine synthesis. An assessment of changes in biogenic amine metabolism under conditions of tryptophan administration to ethanol-drinking animals may throw light on the mechanism(s) of ethanol preference.

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