Enhancement of Rat Brain Tryptophan Metabolism by Chronic Ethanol Administration and Possible Involvement of Decreased Liver Tryptophan Pyrrolase Activity

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1. Chronic ethanol administration enhances rat brain 5-hydroxytryptamine synthesis by increasing the availability of circulating tryptophan to the brain. This increased availability is not insulin-mediated or lipolysis-dependent. 2. Under these conditions, tryptophan accumulates in the liver and apo-(tryptophan pyrrolase) activity is completely abolished, but could be restored by administration of regenerators of liver NAD⁺ and/or NADP⁺. 3. All four regenerators used (fructose, Methylene Blue, phenazine methosulphate and sodium pyruvate) prevented the ethanol-induced increase in liver tryptophan concentration and the increased availability of tryptophan to the brain. 4. It is suggested that the enhancement of brain tryptophan metabolism by chronic ethanol administration is caused by the decreased hepatic tryptophan pyrrolase activity. The results are briefly discussed in relation to previous work with ethanol. 5. Fructose enhances the conversion of tryptophan into 5-hydroxyindol-3-ylacetic acid in brains of ethanol-treated rats, whereas Methylene Blue inhibits this conversion in both control and ethanol-treated animals.

Studies of the effects of chronic ethanol administration on animal brain 5-hydroxytryptamine concentration, synthesis or turnover have led to 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. Thus the above neurochemical aspects have been reported to be decreased (Yamanaka & Kono, 1974), unaltered (Frankel *et al.*, 1974; Rawat, 1974; Moscatelli *et al.*, 1975) or increased (Griffiths *et al.*, 1974) by chronic ethanol administration.

Our previous work with rat liver tryptophan pyrrolase (EC 1.13.11.11) suggests that chronic ethanol administration decreases the activity of the haem-free predominant form or apoenzyme by increasing liver [NADH] and [NADPH] (Badawy & Evans, 1973*a,b*, 1975*b*). Liver tryptophan pyrrolase is one of the peripheral factors that affect brain 5-hydroxytryptamine synthesis by altering the availability of circulating tryptophan to the brain (for a review, see Badawy, 1977), and we (Badawy *et al.*, 1978) have found that chronic ethanol administration for 7 days enhances brain 5-hydroxytryptamine synthesis (and possibly also turnover) by increasing tryptophan availability to the brain. We now report the results of a detailed study of this enhancement, and suggest that it is caused by the decreased liver tryptophan pyrrolase activity.

Materials and Methods

Chemicals

The sources of various chemicals and the methods of preparation and administration of some of them have been described (Badawy & Evans, 1976*a*). ATP, bovine serum albumin (30%, w/v, solution) and yeast hexokinase [500 units/mg of protein; one unit will phosphorylate 1.0 μ mol of glucose/min at pH 8.5 (± 0.5) and at 25°C] were from Sigma (London) Chemical Co., Poole, Dorset, U.K. Methylene Blue (1%, w/v) was from Harvey Laboratories, Philadelphia, PA 19144, U.S.A.

Animals and treatments

Locally bred male Wistar rats (150 g $\pm$ 7% at the start of each experiment) were housed three per cage and were maintained on cube diet 41B (Oxoid, Basingstoke, Hants., U.K.) and water. Ethanol was administered freely in drinking water in concentrations (v/v) of 5% for 2 days, 7.5% for 2 more days and finally 10% for the remainder of the experimental period(s). Some control and ethanol-treated rats also received, 2 h before death, an intraperitoneal injection of 0.9% (w/v) NaCl (2 ml/kg) or an equal volume of a solution of fructose (200 mg/kg), Methylene Blue (20 mg/kg), phenazine methosulphate (10 mg/kg) or sodium pyruvate (100 mg/kg).

Chemical and enzymic determinations

Tryptophan pyrrolase activity was determined in liver homogenates (Badawy & Evans, 1975a) in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin. The apoenzyme activity was calculated by difference. Free (ultrafiltrable) serum, total (acid-soluble) serum, liver and brain tryptophan concentrations were determined by a modification (Bloxam & Warren, 1974) of the method of Denckla & Dewey (1967) as described previously (Badawy & Evans, 1976b); brain tryptophan was present in the extract containing 5-hydroxytryptamine. This and the extract containing 5-hydroxyindol-3-ylacetic acid were prepared and analysed by the method of Curzon & Green (1970). Ultrafiltration of serum and rapid removal and cooling of brain were as described by Badawy & Evans (1976b,c). Serum glucose concentration was determined fluorimetrically by the method of Slein (1963). Student's *t* test was used to assess the differences between control and test results.

Results

Effects of chronic ethanol administration on rat liver tryptophan pyrrolase activity

The time-course of the effects of chronic ethanol administration on the enzyme activity has previously been presented (Badawy & Evans, 1973b). Whereas the holoenzyme activity was not significantly altered, that of the total enzyme was decreased within 1 week, reaching that of the holoenzyme 1 week later. The apoenzyme was therefore the form specifically decreased and remained so for 80 days (see also Badawy & Evans, 1975b). We also found that this decrease was associated with increased liver [NADH] and [NADPH] and suggested that these dinucleotides are

involved in the decrease because the latter was reversed by addition *in vitro* of NAD⁺ or NADP⁺ or by the administration of their regenerators fructose, pyruvate and phenazine methosulphate. These compounds were given in doses of 400, 400 and 10 mg/kg at 4, 4 and 1 h respectively before death. We now find that their administration in doses of 200, 100 and 10 mg/kg respectively at 2 h before death of rats given ethanol for 2 weeks also reverses the decrease in enzyme activity (results not shown). Similarly, another NAD⁺ regenerator, Methylene Blue (20 mg/kg, 2 h before death), reverses the decrease. The holoenzyme, total enzyme and apoenzyme activities (in μmol of kynurenine formed/h per g wet wt. of liver, $\pm$ S.E.M. for each group of four rats) were as follows: control rats, 1.4 ± 0.05 , 3.2 ± 0.13 and 1.8 ± 0.12 ; ethanol-treated rats, 1.4 ± 0.05 , 1.6 ± 0.07 and 0.2 ± 0.08 ; Methylene Blue-treated rats, 1.4 ± 0.06 , 3.0 ± 0.04 and 1.6 ± 0.06 ; Methylene Blue-plus-ethanol-treated rats, 1.4 ± 0.01 , 3.2 ± 0.15 and 1.8 ± 0.10 .

Effects of chronic ethanol administration on liver, serum and brain tryptophan and brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid concentrations

The time-course of these effects is shown in Table 1. Liver tryptophan concentration was increased by 18, 17, 12 and 9% ($P = 0.01-0.005$) respectively after 1, 2, 3 and 4 weeks of ethanol administration. At the same time-intervals, free serum tryptophan concentration was increased by 28, 27, 19 and 20% ($P = 0.05-0.0005$), and that of total serum tryptophan was raised by 26, 19, 17 and 20% ($P = 0.05-0.01$) respectively. The binding of tryptophan to serum proteins was not affected by the ethanol treatment, since the percentage of free tryptophan was not altered

Table 1. *Time-course of the effects of chronic ethanol administration on rat liver, serum and brain tryptophan metabolism*
Ethanol was administered chronically as described in the Materials and Methods section. The results are expressed in $\mu\text{g}/\text{ml}$ or $\mu\text{g}/\text{g}$ wet wt. of tissue and are means $\pm$ S.E.M. for each group of five to six rats. * $P < 0.05$; ** $P < 0.025$; *** $P < 0.01$; † $P < 0.005$; †† $P < 0.0025$; ††† $P < 0.0005$.

Determination	Treatment (weeks)	1	2	3	4
Liver tryptophan	Nil	6.33 ± 0.21	5.77 ± 0.16	6.60 ± 0.08	6.73 ± 0.16
	Ethanol	$7.50 \pm 0.22^{\dagger\dagger}$	$6.73 \pm 0.22^{***}$	$7.40 \pm 0.21^{\dagger}$	$7.33 \pm 0.02^{\dagger\dagger}$
Free serum tryptophan	Nil	1.45 ± 0.05	1.05 ± 0.13	1.93 ± 0.07	1.99 ± 0.12
	Ethanol	$1.85 \pm 0.06^{\dagger\dagger\dagger}$	$1.33 \pm 0.04^*$	$2.30 \pm 0.04^{\dagger\dagger\dagger}$	$2.39 \pm 0.11^{**}$
Total serum tryptophan	Nil	18.90 ± 0.88	18.75 ± 1.24	27.75 ± 1.68	28.20 ± 0.37
	Ethanol	$23.80 \pm 2.34^*$	$22.25 \pm 1.05^*$	$32.58 \pm 1.67^*$	$33.87 \pm 1.78^{***}$
Brain tryptophan	Nil	1.97 ± 0.04	2.43 ± 0.11	2.89 ± 0.06	2.47 ± 0.10
	Ethanol	$2.58 \pm 0.04^{\dagger\dagger\dagger}$	$2.93 \pm 0.09^{\dagger}$	$3.57 \pm 0.09^{\dagger\dagger\dagger}$	$3.17 \pm 0.08^{\dagger\dagger\dagger}$
Brain 5-hydroxytrypt- amine	Nil	0.69 ± 0.03	0.47 ± 0.02	0.76 ± 0.02	0.56 ± 0.01
	Ethanol	$0.85 \pm 0.03^{\dagger\dagger}$	$0.56 \pm 0.01^{\dagger\dagger}$	$0.85 \pm 0.03^{**}$	$0.64 \pm 0.02^{\dagger}$
Brain 5-hydroxyindol-3-yl- acetic acid	Nil	0.40 ± 0.01	0.43 ± 0.02	0.46 ± 0.01	0.40 ± 0.00
	Ethanol	$0.50 \pm 0.01^{\dagger\dagger\dagger}$	$0.49 \pm 0.02^*$	$0.54 \pm 0.00^{\dagger\dagger\dagger}$	$0.51 \pm 0.01^{\dagger\dagger\dagger}$

Table 2. *Effects of phenazine methosulphate on the chronic ethanol-induced changes in tryptophan metabolism*

Ethanol was administered chronically as described in the Materials and Methods section for 2 weeks. Control and ethanol-treated rats were given, 2h before death, an intraperitoneal injection of phenazine methosulphate (10mg/kg) or an equal volume of 0.9% (w/v) NaCl (2ml/kg). Values (means $\pm$ s.e.m. for each group of five to six rats) are in μ g/ml or μ g/g wet wt. of tissue. The results in columns 2 and 3 were statistically compared with those in column 1, whereas those in column 4 were compared with those in column 3: * P < 0.05; ** P < 0.025; *** P < 0.01; † P < 0.0025; †† P < 0.001; ††† P < 0.0005. Abbreviation: N.D., not determined.

Determination	Control rats		Chronic ethanol-treated rats	
	0.9% NaCl	Phenazine methosulphate	0.9% NaCl	Phenazine methosulphate
Liver tryptophan	7.20 $\pm$ 0.15	7.66 $\pm$ 0.17*	9.57 $\pm$ 0.19†††	7.25 $\pm$ 0.12†††
Free serum tryptophan	1.40 $\pm$ 0.04	1.43 $\pm$ 0.11	1.87 $\pm$ 0.22*	1.34 $\pm$ 0.17*
Total serum tryptophan	18.70 $\pm$ 0.37	18.00 $\pm$ 0.35	23.70 $\pm$ 1.46***	18.50 $\pm$ 0.34***
Brain tryptophan	2.58 $\pm$ 0.04	N.D.	3.13 $\pm$ 0.05†††	2.58 $\pm$ 0.14†
Brain 5-hydroxytryptamine	0.60 $\pm$ 0.07	0.60 $\pm$ 0.00	0.82 $\pm$ 0.05**	0.56 $\pm$ 0.02†††
Brain 5-hydroxyindol-3-yl-acetic acid	0.48 $\pm$ 0.01	0.49 $\pm$ 0.01	0.59 $\pm$ 0.01†††	0.49 $\pm$ 0.01††

Table 3. *Effects of sodium pyruvate on the chronic ethanol-induced changes in tryptophan metabolism*

Ethanol was administered chronically as described in the Materials and Methods section for 2 weeks. Control and ethanol-treated rats were given, 2h before death, an intraperitoneal injection of sodium pyruvate (100mg/kg) or an equal volume (2ml/kg) of 0.9% (w/v) NaCl. Values (means $\pm$ s.e.m. for each group of five to six rats) are in μ g/ml or μ g/g wet wt. of tissue. The results in column 3 are statistically compared with those in column 1, whereas those in column 4 are compared with those in column 3: * P < 0.025; ** P < 0.0125; *** P < 0.01; † P < 0.005; †† P < 0.0025; ††† P < 0.0005.

Determination	Control rats		Chronic ethanol-treated rats	
	0.9% NaCl	Pyruvate	0.9% NaCl	Pyruvate
Liver tryptophan	7.00 $\pm$ 0.19	7.10 $\pm$ 0.09	8.50 $\pm$ 0.20†††	7.00 $\pm$ 0.13†††
Free serum tryptophan	1.34 $\pm$ 0.04	1.39 $\pm$ 0.04	1.65 $\pm$ 0.09***	1.24 $\pm$ 0.03††
Total serum tryptophan	18.50 $\pm$ 0.64	18.44 $\pm$ 0.57	22.75 $\pm$ 1.23**	17.80 $\pm$ 0.56†
Brain tryptophan	2.21 $\pm$ 0.06	2.31 $\pm$ 0.10	2.91 $\pm$ 0.07†††	2.50 $\pm$ 0.08†
Brain 5-hydroxytryptamine	0.69 $\pm$ 0.03	0.73 $\pm$ 0.02	0.84 $\pm$ 0.03*	0.67 $\pm$ 0.04†
Brain 5-hydroxyindol-3-yl-acetic acid	0.40 $\pm$ 0.00	0.40 $\pm$ 0.01	0.49 $\pm$ 0.02††	0.42 $\pm$ 0.01***

(P > 0.10). Chronic ethanol administration caused insignificant (P > 0.05) increases (of up to 8%) in serum glucose concentrations (results not shown).

Brain tryptophan concentration was increased at 1, 2, 3 and 4 weeks after ethanol treatment by 31, 21, 23 and 28% respectively (P = 0.005–0.0005) (Table 1). There were also simultaneous increases in brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid concentrations; the rises in the former were 23, 29, 12 and 14% (P = 0.025–0.0025) and those in the latter were 25, 14, 17 and 27% (P = 0.05–0.0005).

The effects of an injection of phenazine methosulphate, sodium pyruvate, fructose and Methylene Blue in control rats and in those chronically treated with ethanol for 2 weeks are shown in Tables 2, 3, 4 and 5 respectively. All four compounds prevented the ethanol-induced increases in liver, free serum and

total serum tryptophan concentrations without altering those of control rats.

In addition, phenazine methosulphate (Table 2) reversed the ethanol-induced increases in brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid concentrations without exerting any significant effects in control rats. Pyruvate gave similar results, except that the reversal of the ethanol-induced rise in brain tryptophan concentration was only partial (Table 3).

The effects of fructose and Methylene Blue on brain tryptophan metabolism were different. Fructose (Table 4) caused small (7–12%) but significant increases in concentrations of all three brain indoles in control rats. In chronic ethanol-treated rats, fructose decreased brain tryptophan and 5-hydroxytryptamine concentrations by 37 and 50% respectively and increased that of 5-hydroxyindol-3-ylacetic acid by 63%.

Table 4. *Effects of fructose on the chronic ethanol-induced changes in tryptophan metabolism*

Ethanol was administered chronically as described in the Materials and Methods section for 2 weeks. Control and ethanol-treated rats received, 2h before death, an intraperitoneal injection of fructose (200mg/kg) or an equal volume (2ml/kg) of 0.9% (w/v) NaCl. Values (means $\pm$ S.E.M. for each group of six rats) are in $\mu\text{g/ml}$ or $\mu\text{g/g}$ wet wt. of tissue. The results in columns 2 and 3 are statistically compared with those in column 1, whereas those in column 4 are compared with those in column 3: $\dagger P < 0.05$; $*P < 0.005$; $**P < 0.0025$; $***P < 0.0005$.

Determination	Control rats		Chronic ethanol-treated rats	
	0.9% NaCl	Fructose	0.9 %NaCl	Fructose
Liver tryptophan	5.77 $\pm$ 0.16	5.80 $\pm$ 0.22	6.73 $\pm$ 0.22*	5.30 $\pm$ 0.28**
Free serum tryptophan	1.15 $\pm$ 0.04	1.22 $\pm$ 0.07	1.54 $\pm$ 0.08**	1.15 $\pm$ 0.06**
Total serum tryptophan	16.53 $\pm$ 0.37	16.13 $\pm$ 1.18	20.20 $\pm$ 0.44***	15.83 $\pm$ 0.34***
Brain tryptophan	1.63 $\pm$ 0.02	1.82 $\pm$ 0.03***	2.06 $\pm$ 0.05***	1.30 $\pm$ 0.05***
Brain 5-hydroxytryptamine	0.87 $\pm$ 0.02	0.93 $\pm$ 0.01†	1.07 $\pm$ 0.04**	0.53 $\pm$ 0.02***
Brain 5-hydroxyindol-3-yl-acetic acid	0.41 $\pm$ 0.01	0.45 $\pm$ 0.01**	0.51 $\pm$ 0.00***	0.83 $\pm$ 0.02***

Table 5. *Effects of Methylene Blue on the chronic ethanol-induced changes in tryptophan metabolism*

Ethanol was administered chronically as described in the Materials and Methods section for 2 weeks. Control and ethanol-treated rats received, 2h before death, an intraperitoneal injection of Methylene Blue (20mg/kg) or an equal volume (2ml/kg) of 0.9% (w/v) NaCl. Values (means $\pm$ S.E.M. for each group of five to six rats) are in $\mu\text{g/ml}$ or $\mu\text{g/g}$ wet wt. of tissue. The results in columns 2 and 3 are statistically compared with those in column 1, whereas those in column 4 are compared with those in column 3. $\dagger P < 0.05$; $*P < 0.005$; $**P < 0.0025$; $***P < 0.0005$.

Determination	Control rats		Chronic ethanol-treated rats	
	0.9% NaCl	Methylene Blue	0.9% NaCl	Methylene Blue
Liver tryptophan	7.78 $\pm$ 0.23	8.20 $\pm$ 0.32	9.73 $\pm$ 0.29***	7.57 $\pm$ 0.11***
Free serum tryptophan	1.79 $\pm$ 0.08	1.90 $\pm$ 0.04	2.48 $\pm$ 0.16**	1.75 $\pm$ 0.07**
Total serum tryptophan	25.40 $\pm$ 0.75	24.80 $\pm$ 0.75	31.00 $\pm$ 1.22**	24.70 $\pm$ 0.49**
Brain tryptophan	2.18 $\pm$ 0.04	2.89 $\pm$ 0.16**	2.61 $\pm$ 0.03***	3.43 $\pm$ 0.20**
Brain 5-hydroxytryptamine	0.61 $\pm$ 0.07	1.00 $\pm$ 0.03***	0.80 $\pm$ 0.07†	1.08 $\pm$ 0.04*
Brain 5-hydroxyindol-3-yl-acetic acid	0.45 $\pm$ 0.01	0.20 $\pm$ 0.01***	0.55 $\pm$ 0.01***	0.23 $\pm$ 0.01***

Methylene Blue, on the other hand, increased the concentrations of brain tryptophan and 5-hydroxytryptamine and decreased that of 5-hydroxyindol-3-ylacetic acid irrespective of whether or not the rats had been chronically pretreated with ethanol (Table 5). The brain and liver extracts of Methylene Blue-treated rats exhibited the colour of the drug, but it was demonstrated that there was no interference by the drug in the determination of brain indoles.

The above four compounds did not exert any significant effects on the concentration of serum glucose in control or ethanol-treated rats (results not shown).

Discussion

The present paper is concerned with the decrease in rat liver tryptophan pyrrolase activity caused by chronic ethanol administration, and this should not be confused with the enhancement of the enzyme activity by acute ethanol treatment (see Badawy & Evans, 1976c).

Effects of chronic ethanol administration on rat liver tryptophan pyrrolase activity

We have previously reported (Badawy & Evans, 1973a,b, 1975b) that chronic ethanol administration decreases the activity of the predominant form of rat liver tryptophan pyrrolase (the apoenzyme) by a mechanism excluding a defective apoenzyme synthesis or a decreased cofactor availability, but associated with increased liver concentrations of the allosteric inhibitors NADH and NADPH. That these are involved in the decreased pyrrolase activity is suggested by the reversal of the decrease by NAD⁺ or NADP⁺ *in vitro* and by administration of regenerators of these oxidized dinucleotides. The reversal of the decrease in enzyme activity achieved by Methylene Blue is reported here for the first time (see the Results section).

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

We have reported the effects of chronic ethanol

administration for 1 week on rat brain tryptophan and 5-hydroxytryptamine metabolism (Badawy *et al.*, 1978). The results in Table 1 suggest that the ethanol effects persist for longer time-intervals. The rises in brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid concentrations are consistent with chronic ethanol administration enhancing the rate of synthesis of brain 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 free serum (or plasma) tryptophan concentration and that of brain tryptophan (see Curzon & Knott, 1974, and references cited therein; Badawy & Evans, 1976c). The ethanol-induced increases in brain hydroxyindoles may therefore be explained by the increased brain tryptophan concentration. This, however, does not exclude the possibility that the above increases could also be caused, at least in part, by an enhanced brain tryptophan hydroxylase activity that has been reported to occur in mice chronically treated with ethanol (Kuriyama *et al.*, 1971). However, it is not clear at present whether an enhancement of hydroxylase activity could lead to an increased 5-hydroxytryptamine synthesis in the absence of an elevated brain tryptophan concentration.

Since ethanol does not block the formation of hydroxyindoles from tryptophan, it may be concluded that the increased brain tryptophan concentration is due to an increased availability of the circulating amino acid to the brain (Table 1). This increased availability is not due to an increased release of protein-bound serum tryptophan, since the percentage of free tryptophan is not altered by ethanol (see the text and Table 1). This is further suggested by the absence of an effect of ethanol on the concentration of serum non-esterified fatty acids (Badawy *et al.*, 1978). The increased availability of circulating tryptophan to the brain is also unlikely to be insulin-mediated, since this hormone increases the binding of tryptophan to plasma proteins, an effect not observed here (see Curzon & Knott, 1974), and also because ethanol does not affect serum glucose concentration (see the text).

The exclusion of lipolysis and insulin as mediators of the ethanol-induced increase in tryptophan availability to the brain leaves us with the only known alternative, that of diminished liver tryptophan pyrrolase activity decreasing the hepatic catabolism of tryptophan, thus increasing its availability to the brain. This suggestion is supported by the observed (Table 1) increase in tryptophan concentrations in

liver, serum and brain. The decreased liver tryptophan pyrrolase activity caused by chronic diethylrin administration is also associated with increased tryptophan concentrations in plasma and brain and with an increased brain 5-hydroxytryptamine metabolism (Kohli *et al.*, 1977). Further evidence for the involvement of decreased liver tryptophan pyrrolase activity in the ethanol-induced enhancement of tryptophan availability to the brain and the increased brain 5-hydroxytryptamine metabolism has been obtained in experiments in which the decreased pyrrolase activity has been prevented by phenazine methosulphate, sodium pyruvate, fructose and Methylene Blue (Tables 2–5). All four compounds prevented the increase in liver tryptophan concentration and in the availability of serum tryptophan to the brain. Furthermore, phenazine methosulphate and pyruvate also prevented the rises in brain indole concentrations. These results therefore strongly support the possibility that decreased liver tryptophan pyrrolase activity is involved in the chronic ethanol-induced enhancement of brain tryptophan and 5-hydroxytryptamine metabolism, and provide further evidence in favour of the inverse relationship between liver pyrrolase activity and brain 5-hydroxytryptamine synthesis.

The effects of fructose and Methylene Blue on brain tryptophan and 5-hydroxytryptamine metabolism deserve further comment. The fructose-induced decreases in brain tryptophan and 5-hydroxytryptamine concentrations and the concomitant rise in that of brain 5-hydroxyindol-3-ylacetic acid in ethanol-treated rats (Table 4) are consistent with an enhanced brain tryptophan hydroxylase and possibly also monoamine oxidase activity. Although fructose is not normally taken up by the brain (see Bachelard, 1974), the possibility cannot be ignored that pretreatment of rats with ethanol could alter the permeability of the brain to the sugar, thus leading to the observed effects. The alternative possibility, that of fructose accelerating ethanol metabolism in the liver, presumably by regenerating liver NAD^+ (see Slater, 1972), cannot explain the present effects, particularly in view of the fact that other NAD^+ regenerators do not cause similar changes. With Methylene Blue, there are direct effects suggesting an inhibition of activity of monoamine oxidase and possibly also tryptophan hydroxylase. Further work is clearly required to examine these possibilities.

Previous studies of the effects of chronic ethanol administration on brain 5-hydroxytryptamine metabolism

It is noteworthy that, in all studies suggesting that chronic ethanol administration decreases or exerts no effects on brain 5-hydroxytryptamine synthesis or turnover (see the introduction), control animals have

been treated with sucrose. Sucrose decreases liver tryptophan pyrrolase activity (Badawy & Evans, 1976a) and is not suitable in studies of the chronic effects of ethanol on this enzyme (Evans & Badawy, 1977) nor in those of the acute effects of ethanol on brain 5-hydroxytryptamine metabolism (Badawy & Evans, 1976c). Sucrose (and also glucose) could increase brain tryptophan concentration by decreasing pyrrolase activity, by acting through the insulin-mediated effects on plasma neutral amino acids (see Madras *et al.*, 1973), or both, and caution should therefore be exercised in interpreting the results of experiments with these sugars in relation to the actions of ethanol on tryptophan metabolism. In addition, some of the above studies have used monoamine oxidase inhibitors to measure the turnover rate of brain 5-hydroxytryptamine. The possible disadvantages of this method have been discussed (Badawy & Evans, 1976c).

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