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Alteration in serum tyrosine and tryptophan concentrations associated with the induction of physical dependence on ethanol in mice

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Changes in central monoamine neurotransmitter metabolism have frequently been reported in association with the development of physical dependence on ethanol in laboratory animals [1–3]. During the physical syndrome of withdrawal from ethanol further changes in central metabolism of monoamines occur which play important roles in behavioural aspects of the withdrawal syndrome [4, 5, 1]. The reasons for changes in central monoamine metabolism during the induction of ethanol dependence are therefore of interest.

There is some evidence that concentrations of the amino acid precursors for monoamine biosynthesis in the brain are altered in ethanol-dependent animals [3, 6] and it has been suggested that altered peripheral handling of these precursors, notably by the liver, may contribute to changes in central amine metabolism induced by ethanol [7, 8]. We now report that the induction of ethanol dependence in mice is associated with alterations in concentrations of tyrosine and free and bound tryptophan in plasma. Such changes may influence central amine metabolism [9].

MATERIALS AND METHODS

Male T.O. Swiss strain mice of 20–25 g obtained from LAB Centre, Dagenham, Essex, were made physically dependent on ethanol in the way previously described [1]. This method involves administration of ethanol by inhalation in increasing concentration to groups of mice. After administration of ethanol by this regime for 5 days, there is little sign of physical dependence, whereas after 10 days all mice show signs of a physical syndrome (cf. [1]) when ethanol is removed. The withdrawal syndrome can be observed for 10–12 h in most mice, and is at its peak after 4–5 h.

Mice were killed by decapitation after administration of ethanol for 5 and 10 days or after 5 and 10 h of the ethanol withdrawal syndrome. Mixed arterial and venous blood was obtained from the neck and collected into heparinised glass vials. After centrifugation at 10,000 g for 30 sec. Serum was removed for estimation of amino acids. Serum free tyrosine was estimated by the method of Waalkes and Udenfriend [10], serum total tryptophan by the method of Denckla and Dewey [11]. Free tryptophan was separated from the protein-bound fraction by the method of Knott and Curzon [12]. Serum from the pooled blood of two mice was used for free tryptophan estimation. In all cases comparison between values obtained was made with those from control animals exposed to the same environmental conditions, but with the absence of ethanol in inspired air.

RESULTS

As shown in Table 1, administration of ethanol for 5 days causes no significant change in concentrations of tyrosine in serum, but significantly reduces serum total tryptophan. The administration of ethanol for 10 days, a period sufficient to induce physical dependence, is associated with a significant increase in serum tyrosine and in serum total tryptophan concentrations. Both were increased to about 50 per cent above control levels. When separation of total tryptophan into the free and bound fractions was achieved, it was observed that, in ethanol dependent mice, the increase in total tryptophan was reflected in a smaller, but significant ($P < 0.05$) increase in free tryptophan. Control animals showed free tryptophan concentrations in serum of 4.78 ± 0.28 μg/ml ($n = 5$) whereas the value for ethanol dependent mice was 5.92 ± 0.33 μg/ml ($n = 5$).

During the syndrome of withdrawal from ethanol concentrations of tyrosine in serum remained significantly elevated above control (approximately double control values when the withdrawal syndrome was at its height). Con-

Table 1. Concentrations of tyrosine and total tryptophan in serum during the induction of ethanol dependence and during the withdrawal syndrome

Treatment	Tyrosine μg/ml	Tryptophan μg/ml
Control	16.4 ± 1.64	18.5 ± 2.00
Ethanol 5 days	17.6 ± 1.38	13.2 ± 1.1*
Ethanol 10 days	24.9 ± 2.83*	29.1 ± 4.81*
Withdrawal 5 h	34.1 ± 1.18†	21.9 ± 2.53
Withdrawal 10 h	25.6 ± 0.86*	20.8 ± 2.53

This Table shows the concentrations of tyrosine and total tryptophan found in the serum of mice during the inhalation of ethanol or during its withdrawal (further details see text). Each value represents the mean S.E. of at least five determinations. Asterisks indicate a difference from control values significant at the $P < 0.05$ level* or the $P < 0.01$ level† in the Student's "t" test.

centrations of total tryptophan in serum were elevated, but this was not significant at any time studied. These results are shown in Table 1.

DISCUSSION

Results show that alterations in peripheral metabolism of amino acids may contribute to changes in central neurotransmitter metabolism associated with the induction of ethanol dependence. Increases in catecholamine concentrations [1] and synthesis [2], in brains of animals rendered ethanol-dependent, or undergoing the ethanol withdrawal syndrome, may be related to the increases in serum tyrosine concentration reported here. The situation is more complex with regard to tryptophan metabolism, but it seems that the small increase in free tryptophan observed in ethanol-dependent animals could contribute to alterations in 5-hydroxytryptamine metabolism in the brain [1, 2]. Certainly changes in peripheral handling of endogenous, or administered, amino acid precursors must be considered as contributory factors in assessing results on central monoamine metabolism obtained during chronic administration of ethanol. This factor may explain some discrepancies in the literature [3, 13].

The reasons for the changes in serum amino acid concentrations reported here remain obscure. It seems likely that hepatic metabolism of amino acids plays a role [7, 8]. The non-specific stress of ethanol administration and withdrawal could also be important.

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Possible mechanism of action of propranolol in hypertension

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In recent years, propranolol, a β-adrenoceptor-blocking drug, has been used in the management of essential hypertension. The mechanism by which this drug lowers blood pressure is still speculative. After intravenous administration of propranolol, cardiac output decreases in all hypertensive patients but arterial pressure remains unaltered. Chronic therapy with the drug is needed to lower the blood pressure. A fall in blood pressure is associated with a decrease in the initially elevated peripheral resistance. A big advantage of propranolol is that lying and standing pressures are equally lowered and there is no orthostatic hypotension.

Among the factors that may be involved in propranolol-induced lowering of blood pressure are: (1) decreased cardiac output, (2) inhibition of renin release, and (3) diminution of tonic sympathetic nerve outflow from the vasomotor center in the brain. However, all these factors do not explain the long time lag between the administration of propranolol and the fall in blood pressure.

The present study shows that prolonged treatment with propranolol causes a reduction in adrenal tyrosine hydroxylase (TH) activity of the spontaneously hypertensive rat (SHR), an animal model that closely resembles human essential hypertension [1, 2]. Since TH activity is related to impulse traffic in a sympathetic nerve, a reduction in TH activity would indicate a decrease in sympathetic nerve