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Role of Tyrosine in the Acute Effects of Ethanol on Rat Brain Catecholamine Synthesis

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BADAWY, A. A.-B., D. L. WILLIAMS AND M. EVANS. *Role of tyrosine in the acute effects of ethanol on rat brain catecholamine synthesis.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 389–396, 1983.—Acute ethanol administration exerts multiple effects on rat brain catecholamine synthesis, associated with corresponding changes in cerebral tyrosine concentration. Catecholamine synthesis is enhanced at 1 hr by an increased availability of circulating tyrosine to the brain after inhibition of liver tyrosine aminotransferase activity. Tyrosine hydroxylation *in vivo* and tyrosine hydroxylase activity measured *in vitro* are also enhanced at 1 hr. Catecholamine synthesis is inhibited at 2–4 hr when tyrosine availability to the brain is decreased because of an enhancement of liver tyrosine aminotransferase activity. Serum neutral amino acid concentrations are decreased at 5 hr. This is followed 1 hr later by normalization of cerebral catecholamine synthesis. By 8 hr after ethanol administration, the latter becomes enhanced because of increased cerebral uptake of tyrosine. Catecholamine synthesis is inhibited at 12 hr because of enhanced transamination of brain tyrosine. Tyrosine metabolism finally returns to normal at 16 hr after ethanol administration. These results are discussed in relation to previous work with ethanol, and to central and peripheral mechanisms of regulation of brain catecholamine synthesis.

Ethanol Brain catecholamine synthesis Brain tyrosine concentration Liver tyrosine aminotransferase Transamination of brain tyrosine Cerebral tyrosine uptake Regulation of catecholamine synthesis Tyrosine metabolsim

THE effects of acute ethanol administration on mammalian brain catecholamine metabolism have been the subject of much investigation and controversy (for a review, see [15]). This controversy could be explained largely by differences in doses of the drug, time intervals after, and routes of its administration, and methods of assessment of catecholamine synthesis and turnover. Because of these considerations and the fact that workers have previously examined ethanol effects at only one or a few time intervals, it was considered important to perform a detailed investigation of the effects of various doses of ethanol on rat brain catecholamine concentrations and synthesis over a long time course. Also in view of evidence [4, 5, 8, 26, 27] that brain tyrosine concentration exerts an important influence on catecholamine synthesis, emphasis was placed on the role that any likely changes in tyrosine availability to, or within, the brain may play in the actions of ethanol. The results of these investigations form the subject of the present paper.

METHOD

Chemicals

The sources of various chemicals and the methods of preparation of solutions of some of them for administration have previously been described [2, 3, 4]. Doses are given with the relevant results.

Animals and Treatments

Locally bred male Wistar rats (150–170 g) were housed three per cage (at 22±1°C and under natural light-dark cycles) and were maintained on cube diet 41B (Oxoid, Basingstoke, Hants, U.K.) and water. The animals were killed by decapitation between 14:00 and 15:00 hr and their brains and livers were rapidly removed (within 10 or 20 sec of death, respectively). Livers were isolated by a freeze-clamp, whereas brains were immersed in liquid N_2 for 3 min; both organs were then stored at −20°C overnight before analysis. To minimise animal variations, each experimental treatment was accompanied by its own control group. Ethanol and other compounds were administered intraperitoneally.

Chemical, Enzymic and Other Determinations

Liver, serum and brain tyrosine concentrations and those of brain dopamine (3,4-dihydroxyphenethylamine), dopac (3,4-dihydroxyphenylacetic acid) and noradrenaline were determined fluorimetrically after separation by various tech-

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niques including ion-exchange column chromatography as described previously [4]. Brain tyrosine hydroxylase activity *in vitro* was also determined fluorimetrically [28] in the presence of a 1.6 mM final concentration of tetrahydropteridine and a 150 μM final concentration of *L*-tyrosine as described previously [4]. The rate of tyrosine hydroxylation *in vivo* was determined by measuring the accumulation of dopa (3,4-dihydroxyphenylalanine) at 30 min after intraperitoneal administration of the decarboxylase inhibitor compound NSD-1015 (3-hydroxybenzylhydrazine hydrochloride; 100 mg/kg body wt.). The concentrations of circulating amino acids were determined by an auto-analyser procedure used routinely in a nearby laboratory. Statistical analysis of the results was performed by using Student's *t*-test.

RESULTS

Time Course of the Effects of Administration of a 4 g/kg Dose of Ethanol on Concentrations of Liver, Serum and Brain Tyrosine and Brain Catecholamines

These effects, expressed as percentage differences from controls, are shown in Fig. 1. At 1 hr, the concentrations of liver, serum and brain tyrosine and those of brain dopamine, dopac and noradrenaline were all significantly increased by 25–111% ($p=0.01$–0.001). By contrast, decreases in all six concentrations of 26–35% ($p=0.02$–0.001) were observed at 2 hr. These decreases persisted for 2 hr more, being 31–59% at 3 hr and 30–57% at 4 hr ($p=0.005$–0.001). By 6 hr, all parameters returned to normal, except liver tyrosine concentration, which remained decreased (by 29%; $p<0.001$); it, however, returned to normal thereafter. At 8 hr, serum tyrosine concentration was 22% below normal ($p<0.001$), whereas those of brain tyrosine, dopamine, dopac and noradrenaline were increased by 22–74% ($p=0.05$–0.001). By contrast with these latter increases at 8 hr, decreases in these four parameters and in serum tyrosine concentration of 14–33% ($p=0.02$–0.001) were observed at 12 hr. All five parameters finally returned to normal at 16 hr. The changes observed at 12 hr (Fig. 1) have previously been reported [5].

Although control values (those observed in 0.9% NaCl-treated rats) varied at various time intervals (see Fig. 1), a comparison of such values obtained in several other experiments performed at specific time intervals throughout the present work revealed that animal variations, rather than the time interval of the 0.9% NaCl values, was the major, if not the only, factor responsible for such variations.

Effects of Administration of Various Doses of Ethanol at 1 hr on Tyrsoine Metabolism

The results in Fig. 2 show that the accumulation of tyrosine in liver and serum and the increases in brain tyrosine and catecholamine concentrations observed at 1 hr after ethanol administration were significant ($p=0.05$–0.001) with a 0.2 g/kg dose, and were maximum after treatment with a 1.6 g/kg dose, of the drug.

Effects of Administration of Various Doses of Ethanol at 1 hr on Brain Tyrosine Hydroxylation In Vivo *After Decarboxylase Inhibition by Compound NSD-1015*

As the results in Fig. 3 show, brain dopa concentration was significantly increased by a 0.2 g/kg dose of ethanol (by 19%; $p<0.01$) and maximally by a 1.6 g/kg dose of the drug (by 62%; $p<0.005$). Under these conditions, serum tyrosine concentration was also elevated in a dose-dependent fashion, with the first significant increase (18%; $p<0.05$) being caused by a 0.2 g/kg dose of ethanol and the maximum increase (49%; $p<0.02$) being achieved by a 1.6 g/kg dose of the drug. Brain tyrosine concentration was also significantly increased by the 0.2 g/kg dose (by 17%; $p<0.001$), but the maximum increase in this parameter was observed with the 4 g/kg, but not with the 1.6 g/kg, dose.

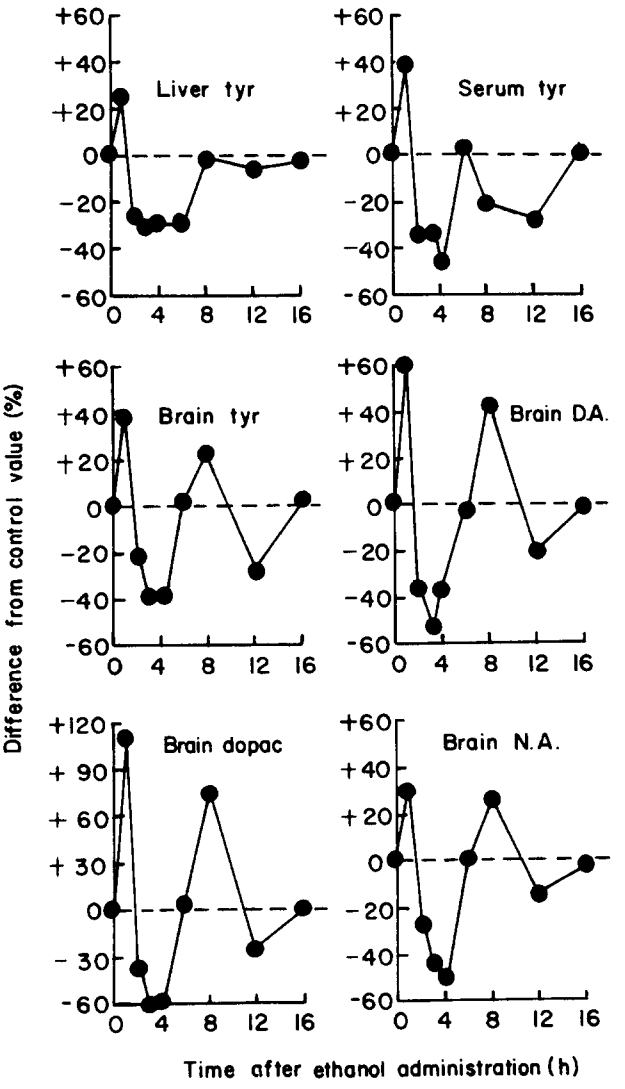

FIG. 1. Time course of effects of administration of a 4 g/kg dose of ethanol on concentrations of rat liver, serum and brain tyrosine (tyr) and brain dopamine (D.A.), dopac and noradrenaline (N.A.). Rats received an IP injection of either ethanol (4 g/kg) or an equal volume (20 ml/kg) of 0.9% NaCl and were killed at the times indicated. The results are expressed as percentage differences from control values (those in 0.9% NaCl-treated rats) and are based on means of 6 animals per group. Average control values (in μg/ml of serum or per g wet wt. of tissue) for 48 rats per parameter, with the lowest and highest six given in means±SEM were as follows: liver tyrosine (15.21, 12.51±0.62 and 20.47±0.56); serum tyrosine (16.90, 14.27±0.33 and 20.40±0.55); brain tyrosine (16.76, 13.14±0.72 and 23.14±1.15); brain dopamine (0.75, 0.61±0.02 and 1.07±0.04); brain dopac (0.35, 0.23±0.005 and 0.46±0.03); brain noradrenaline (0.46, 0.32±0.008 and 0.61±0.016).

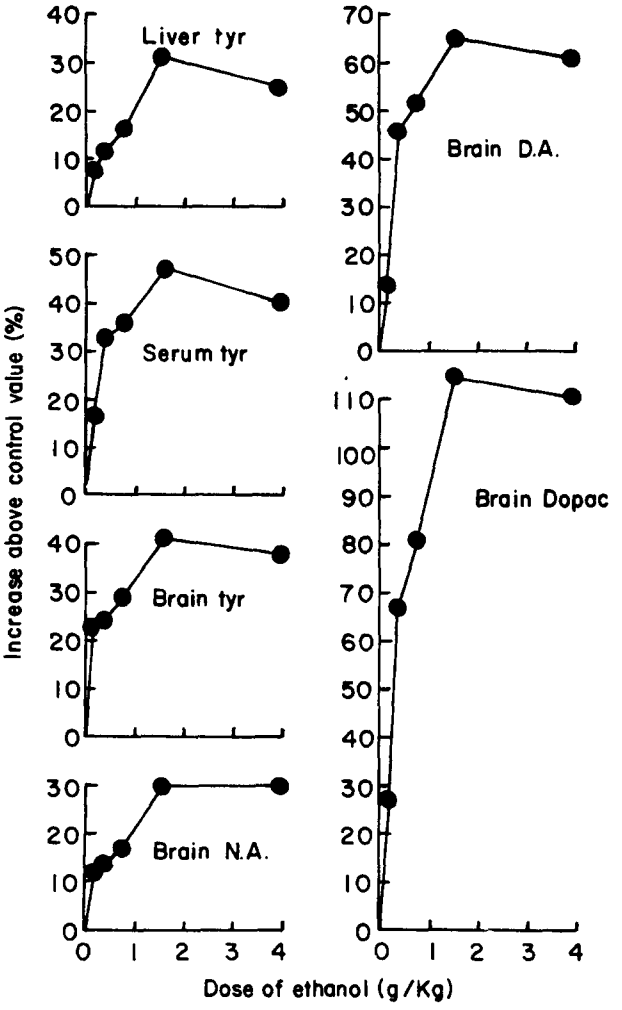

FIG. 2. Effects of various doses of ethanol at 1 hr on rat tyrosine metabolism. Rats received an IP injection of either ethanol (0.2–4 g/kg) or an equal volume (20 ml/kg) of 0.9% NaCl and were killed 1 hr later. The effects of ethanol are expressed as the percentage increases above control values (those in 0.9% NaCl-treated rats) and are based on means of 6 rats per group. The data are the outcome of 3 separate experiments, each of which had a control group, the values of which (in μg/ml of serum or per g wet wt. of tissue; means±SEM of 6 rats) were as follows: liver tyrosine (16.62±0.23, 14.28±0.56 and 13.43±0.32); serum tyrosine (14.10±0.34, 17.12±1.09 and 14.27±0.33); brain tyrosine (16.65±0.67, 14.06±0.48 and 14.68±0.39); brain dopamine (0.56±0.01, 0.63±0.02 and 0.62±0.01); brain dopac (0.26±0.01, 0.18±0.007 and 0.28±0.028); brain noradrenaline (0.42±0.005, 0.46±0.003 and 0.33±0.012).

Effect of Ethanol Administration at 1 hr on Brain Tyrosine Hydroxylase Activity In Vitro

When rats were treated 1 hr previously with a 4 g/kg dose of ethanol or an equal volume (20 ml/kg) of 0.9% NaCl, it was found that the ethanol treatment increased brain tyrosine hydroxylase activity *in vitro* by 29% ($p<0.005$). Thus hydroxylase activities (in nmol of dopa formed/min per g wet wt. of brain; means±SEM for each group of four rats) were as follows: 0.9% NaCl group (0.24±0.005); ethanol group (0.31±0.014).

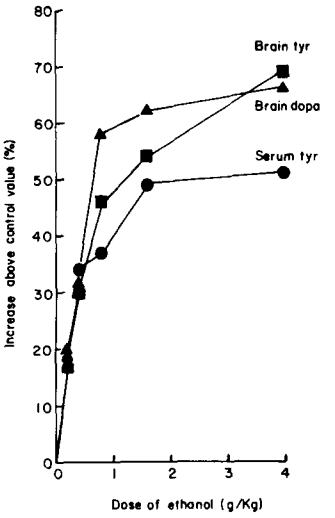

FIG. 3. Effects of various doses of ethanol at 1 hr on rat brain dopa synthesis. Rats received an IP injection of either ethanol (0.2–4 g/kg) or an equal volume (20 ml/kg) of 0.9% NaCl and were killed 1 hr later. The animals also received, at 30 min before death, a similar injection of compound NSD-1015 (100 mg/kg). The results are expressed as the percentage increases in serum and brain tyrosine and brain dopa concentrations above control values (those in 0.9% NaCl-treated rats) and are based on means of 5 rats per group. The data are the outcome of 2 separate experiments, each of which had its own control group, the values in which (in μg/ml of serum or per g wet wt. of brain; means±SEM for 5 rats) were as follows: serum tyrosine (17.68±1.09 and 17.60±1.08); brain tyrosine (15.40±0.12 and 14.75±0.69); brain dopa (0.123±0.006 and 0.156±0.009).

Prevention by Ergotamine of the Effects of Ethanol Administration at 1 hr on Tyrosine Metabolism

The results in Table 1 show that pretreatment of rats with the α-adrenoceptor-blocking agent ergotamine, which exerted no significant effects in control animals, prevented the increases in concentrations of liver, serum and brain tyrosine and brain dopamine, dopac and noradrenaline observed (see Fig. 1) at 1 hr after administration of a 4 g/kg dose of ethanol.

Effects of Administration of Various Doses of Ethanol at 4 hr on Tyrosine Metabolism

The results in Fig. 4 show that, whereas no significant effects were exerted by doses of ethanol of 0.4–0.8 g/kg, doses of 1.6–4 g/kg caused dose-dependent and significant ($p=0.05$–0.001 decreases in concentrations of liver (11–28%), serum (22–45%) and brain (12–32%) tyrosine and in those of brain dopamine (9–32%), dopac (19–57%) and noradrenaline (8–32%).

An attempt to find out if the synthesis of dopa (as determined by monitoring its concentration after decarboxylase inhibition by compound NSD-1015; 100 mg/kg 30 min before death) is also influenced at 4 hr after administration of a 4 g/kg dose of ethanol failed. It was found (results not shown) that compound NSD-1015 overcame the 4 hr-ethanol-induced decreases in serum and brain tyrosine concentrations with the result that the concentration of brain dopa was similar in both control and ethanol-treated rats.

TABLE 1

PREVENTION BY ERGOTAMINE OF THE 1 HR EFFECTS OF ETHANOL ON RAT TYROSINE METABOLISM

Pretreatment Treatment Determination	0.9% NaCl 0.9% NaCl	Ergotamine 0.9% NaCl	Ergotamine Ethanol
Liver tyrosine	14.28 ± 0.65	15.16 ± 0.33	14.80 ± 0.91
Serum tyrosine	14.73 ± 0.36	14.64 ± 0.75	15.37 ± 0.98
Brain tyrosine	15.70 ± 0.54	15.32 ± 0.72	16.80 ± 0.43
Brain dopamine	0.56 ± 0.028	0.58 ± 0.019	0.61 ± 0.020
Brain dopac	0.39 ± 0.026	0.40 ± 0.024	0.40 ± 0.027
Brain noradrenaline	0.48 ± 0.018	0.49 ± 0.018	0.50 ± 0.017

Rats received an IP injection of either ethanol (4 g/kg) or an equal volume (20 ml/kg) of 0.9% NaCl and were killed 1 hr later. The animals also received, at 0.5 hr before the above treatments, a similar injection of ergotamine tartrate (2.5 mg/kg). Additionally, a group of rats which had received 0.9% NaCl 1 hr before was given 0.5 hr earlier, an injection of 0.9% NaCl (2 ml/kg). These animals were killed 1 hr after saline treatment. Values (expressed in μg/ml of serum or per g wet wt. of tissue) are means ± SEM for each group of 5 rats. There were no significant differences among values in any of the 3 columns. The effects of ethanol alone (shown in Fig. 1) were not examined in the present experiments.

Effects of Administration of a 4 g/kg Dose of Ethanol on Serum Neutral and Other Circulating Amino Acids

The results in Table 2 show that ethanol significantly decreased, at 5 hr, the concentrations of all circulating amino acids that compete with tyrosine from the same cerebral uptake mechanism (by 20–32%; $p=0.05$–0.001), as well as that of serum tyrosine (by 23%; $p<0.05$). These decreases were not, however, specific to this group of amino acids, because concentrations of all other circulating amino acids (except that of histidine, which was increased by 13%; $p<0.05$) were also decreased at 5 hr after ethanol administration (results not shown). The 6 hr and 8 hr effects of ethanol administration were also examined, and it was found (results not shown) that the concentrations of competing amino acids were not significantly different from controls, neither was there any evidence of a generalized increase in the size of the circulating amino acid pool.

Effects of Administration of Various Doses of Ethanol at 12 hr on Tyrosine Metabolsim

The results in Fig. 5 show that the decreases in concentrations of serum and brain tyrosine and brain dopamine, dopac and noradrenaline which resulted at 12 hr after ethanol administration were dose-dependent and significant ($p=0.05$–0.001) only in the dose range of ethanol of 2.4–4 g/kg.

DISCUSSION

The results in Fig. 1 demonstrate that acute ethanol administration results in the following time-dependent multiple effects on rat brain catecholamine concentrations: (1) an increase at 1 hr associated with an increased availability of circulating tyrosine to the brain; (2) a decrease at 2–4 hr associated with a decreased availability of circulating tyrosine to the brain; (3) an increase at 8 hr associated with a decreased serum, and an increased brain, tyrosine concentration; (4) a decrease at 12 hr associated with decreased serum and brain tyrosine concentrations. These changes are consistent with ethanol influencing cerebral catecholamine synthesis (and possibly also turnover) by mechanisms involving appropriate changes in brain tyrosine concentration; this provides considerable support to the concept [4, 5, 8, 26, 27] that the latter plays an important role in cerebral catecholamine synthesis.

Mechanism of 1 hr-Ethanol-Induced Enhancement of Rat Brain Catecholamine Synthesis

The results in Fig. 1 suggest that the enhancement of rat brain catecholamine synthesis caused by ethanol results from an increased availability of circulating tyrosine in the brain. This latter effect is most likely the result of decreased hepatic tyrosine degradation (as is suggested by the observed increase in the hepatic concentration of the amino acid, see Fig. 1). This change may result from the previously reported [2] 1 hr-ethanol-induced catecholamine-mediated inhibition of activity of the major tyrosine-degrading enzyme, hepatic tyrosine aminotransferase (EC 2.6.1.5). Evidence supporting this hepatic mechanism is provided by the following additional findings: (1) the ability of the α-adrenoceptor-blocking agent ergotamine to prevent both the inhibition of liver tyrosine aminotransferase activity [2] and the enhancement of cerebral catecholamine synthesis (Table 1); (2) the ability of a 0.2 g/kg dose of ethanol to cause a significant inhibition of liver aminotransferase activity [2] and a significant enhancement of catecholamine synthesis (Fig. 2); (3) the ability of a 1.6 g/kg dose of ethanol to cause the maximum inhibition of liver aminotransferase activity [2] and the maximum enhancement of brain catecholamine synthesis (Fig. 2); (4) the ability of the above two doses of ethanol to cause respectively the first significant and the maximum enhancement of brain dopa synthesis (Fig. 3).

These results therefore provide strong support to the suggestion that cerebral catecholamine synthesis is enhanced at 1 hr after acute ethanol administration by an inhibition of liver tyrosine aminotransferase activity leading to an in-

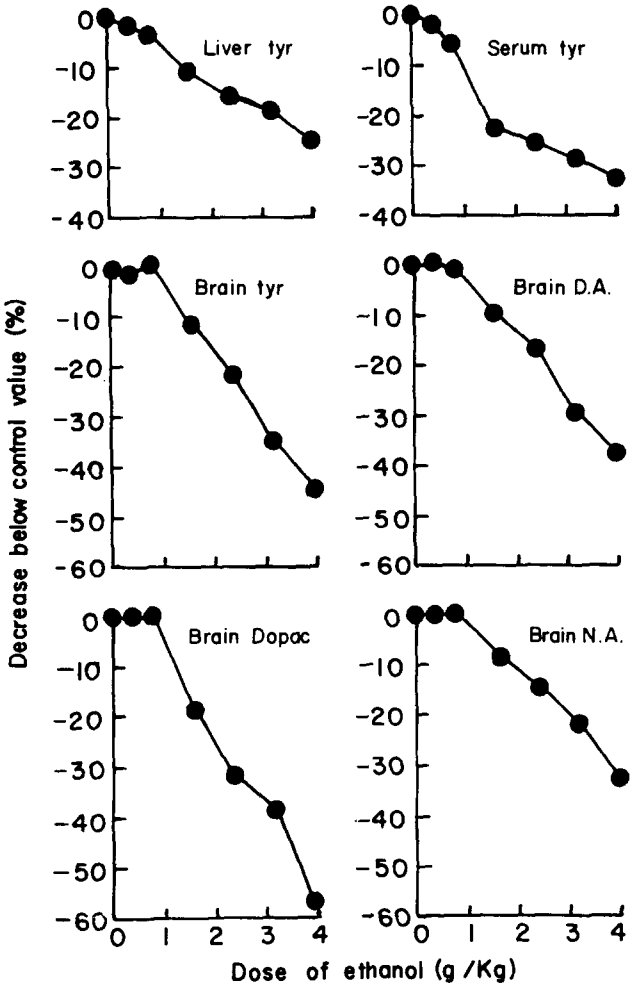

FIG. 4. Effects of various doses of ethanol at 4 hr on rat tyrosine metabolism. Rats received an IP injection of either ethanol (0.4–4 g/kg) or an equal volume (20 ml/kg) of 0.9% NaCl and were killed 4 hr later. The ethanol effects are expressed as the percentage decreases below control values (those in 0.9% NaCl-treated rats) and are based on means of 5 animals per group. The data are the outcome of 3 separate experiments, each of which had its own control group, the values in which (in μg/ml of serum or per g wet wt. of tissue; means±SEM for 5 rats) were as follows: liver tyrosine (19.72±0.67, 17.42±0.28 and 18.77±0.81); serum tyrosine (16.96±0.45, 18.48±0.23 and 15.88±0.10); brain tyrosine (19.00±0.51, 19.32±0.31 and 18.65±0.33); brain dopamine (0.91±0.017, 0.70±0.006 and 0.62±0.011); brain dopac (0.30±0.004, 0.37±0.028 and 0.51±0.015); brain noradrenaline (0.34±0.012, 0.49±0.005 and 0.56±0.010).

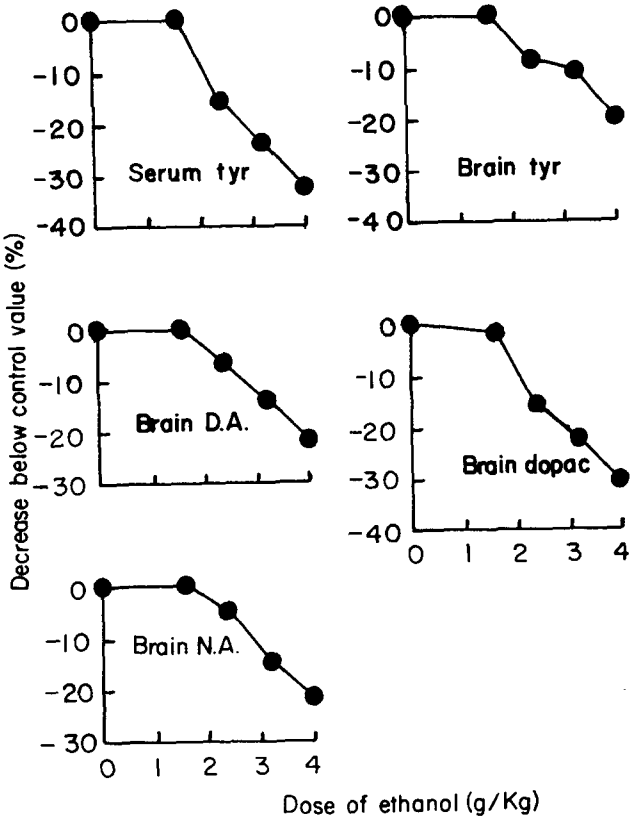

FIG. 5. Effects of various doses of ethanol at 12 hr on rat tyrosine metabolism. Rats received an IP injection of either ethanol (1.6–4 g/kg) or an equal volume (20 ml/kg) of 0.9% NaCl and were killed 12 hr later. The ethanol effects are expressed as the percentage decreases below control values (those in 0.9% NaCl-treated rats) and are based on means of 5 rats per group. The data are the outcome of 2 separate experiments, each of which had its own control group, the values in which (in μg/ml of serum or per g wet wt. of brain; means±SEM of 5 rats) were as follows: serum tyrosine (21.48±0.92 and 22.44±0.86); brain tyrosine (16.74±0.30 and 15.08±0.43); brain dopamine (0.80±0.005 and 1.05±0.007); brain dopac (0.43±0.028 and 0.35±0.031); brain noradrenaline (0.38±0.017 and 0.51±0.005).

creased availability of circulating tyrosine to the brain. This, however, does not exclude an additional mechanism, namely that of enhancement of tyrosine hydroxylase activity, as the results described in the text suggest. Whereas the above hepatic mechanism is suggested here for the first time, enhancement of cerebral catecholamine synthesis at early time points after acute ethanol administration has previously been reported [6, 7, 22, 23, 24]. Only two reports are at variance with these findings: the absence of any changes in catecholamine concentrations at 1 hr after intravenous ethanol administration [11] and the decrease in these pa-

TABLE 2

DECREASE IN CONCENTRATIONS OF COMPETING AMINO ACIDS IN SERUM AT 5 HR AFTER ETHANOL ADMINISTRATION TO RATS

	Treatment	
	Concentration (μM)	
Serum amino acid	0.9% NaCl	Ethanol
Valine	122.0 ± 8.2	82.5 ± 1.9†
Isoleucine	71.2 ± 1.3	51.4 ± 3.1‡
Leucine	107.4 ± 6.6	77.0 ± 3.1†
Tyrosine	72.1 ± 5.1	55.6 ± 5.0*
Phenylalanine	72.2 ± 5.5	57.8 ± 2.8*
Tryptophan	86.1 ± 8.0	65.4 ± 4.0*

Rats received an IP injection of either ethanol (4 g/kg) or an equal volume (20 ml/kg) of 0.9% NaCl and were killed 5 hr later. Values are means ± SEM for each group of 6 rats. The significance of the differences is indicated as follows: *$p<0.05$; †$p<0.005$; ‡$p<0.001$.

rameters at 1 hr following inhalation of the drug [13]. A possible explanation of these two latter findings is that of rapid absorption of ethanol via these two routes altering the time-response relationship (see Fig. 1) such that catecholamine synthesis could have returned to normal, or become inhibited at the time of its measurement.

Ethanol effects have also been examined at 15 min after treatment, and it was found that noradrenaline concentration was unaltered by intraperitoneal administration of a 3 g/kg dose to mice [19], whereas turnover of this amine was enhanced by treatment of rats with a 5 g/kg dose of the drug by stomach tube [14]. We cannot discuss these two opposite findings in relation to the present work, because we have not examined such early effects; the two findings could, however, be explained in terms of differences in doses of ethanol and routes of its administration and possibly also species differences.

The results in Fig. 3 of the present work merit further comment in relation to a previous similar study [7]. Thus, although authors of this previous study demonstrated an enhancement of dopa synthesis at 50 min after administration of doses of ethanol of 0.5–4 g/kg (and at 30 min after treatment with compound NSD-1015), they, unlike us, did not detect any increases in cerebral tyrosine concentration. Similarly, although we have observed increases in catecholamine concentrations at 1 hr after ethanol administration in the absence of compound NSD-1015 (Figs. 1 and 2), the above authors did not [7]. No explanation of this difference between the two studies can as yet be offered.

Mechanism of 2–4 hr-Ethanol-Induced Inhibition of Rat Brain Catecholamine Synthesis

The results in Fig. 1 suggest that the inhibition of catecholamine synthesis which occurred at 2–4 hr after ethanol treatment is caused by a mechanism involving decreased availability of circulating tyrosine to the brain. This decreased availability may be the result of enhanced hepatic tyrosine degradation (as is suggested by the observed decrease in the hepatic concentration of the amino acid, see Fig. 1) secondary to the previously reported [2] glucocorticoid-mediated enhancement of liver tyrosine aminotransferase activity that follows the initial inhibition. It was found in our previous study [2] that this enhancement of enzyme activity reached a maximum at 6 hr after ethanol administration and was dose-dependent in the ethanol dose range of 1.6–4 g/kg. That this enhancement of liver tyrosine aminotransferase activity is the cause of the 2–4 hr-ethanol-induced inhibition of cerebral catecholamine synthesis is further suggested by the findings (Fig. 4) that doses of ethanol below 1.6 g/kg were ineffective in inhibiting catecholamine synthesis, whereas those of 1.6–4 g/kg caused significant and dose-dependent decreases in tissue tyrosine and brain catecholamine levels.

Whereas this hepatic mechanism is suggested here for the first time, inhibition of cerebral catecholamine synthesis has previously been reported in mice and rats at 1.5–5.5 hr after oral or intraperitoneal administration of doses of ethanol of 4–7 g/kg [9, 14, 18]. By contrast, increases in concentrations of brain catecholamine metabolites have been reported to occur at 3 hr only in two studies in which ethanol was given intragastrically to rats [16,20]; additionally, the concentrations of dopamine and noradrenaline have been shown [13] to return to normal values at 2–3 hr following the start of ethanol inhalation by mice. The findings of the first two [16,20] of these latter three reports cannot be explained at present in relation to the present work, whereas that of the third [13] may, as discussed earlier, be due to rapid absorption of the drug after inhalation.

The ability of compound NSD-1015 to overcome the 4 hr-ethanol-induced decreases in serum and brain tyrosine concentrations (see text) may be explained by possible inhibition of liver tyrosine aminotransferase activity by the former agent (see also [4,8]) and/or blockade of the ethanol enhancement of the enzyme activity. These results do, however,suggest that, whereas compound NSD-1015 could be used successfully *in vivo* to monitor the enhancement of dopa synthesis by agents such as tyrosine or ethanol at 1 hr, as well as its inhibition by amino acids competing with tyrosine for cerebral uptake (see [4, 7, 8, 26, 27] and Fig. 3 of the present work), this decarboxylase inhibitor cannot be used to monitor inhibition of dopa synthesis by agents (e.g., ethanol at 4 hr) that decrease tyrosine availability to the brain by enhancing liver tyrosine aminotransferase activity. Such limitation also illustrates the need to develop alternative decarboxylase inhibitors which do not influence peripheral tyrosine (and possibly also other aromatic amino acid) metabolism and disposition.

Mechanism(s) of Return of Rat Brain Catecholamine Synthesis to Normal at 6 hr and of Its Enhancement at 8 hr After Ethanol Administration

The return of cerebral catecholamine synthesis to normal at 6 hr after ethanol administration (Fig. 1) was surprising. We expected the inhibition previously observed at 2–4 hr to continue, in view of the fact that ethanol enhancement of liver tyrosine aminotransferase activity, which appears to cause the above inhibition, is known [2] to reach a maximum at 6 hr. That this latter enhancement was still accelerating hepatic tyrosine degradation is suggested by the observed (Fig. 1) decrease in the concentration of the amino acid in the liver at 6 hr. The return of cerebral catecholamine synthesis to normal at 6 hr can, however, be simply explained by the return to normal of serum (and hence brain) tyrosine concentrations. The return of serum tyrosine concentration to normal at 6 hr cannot be explained at present, except, perhaps, by the possibility that tyrosine release into the circulation is enhanced. Determination of concentrations of circulating amino acids revealed, however, no evidence of a generalized increase in the size of the circulating amino acid pool at 6 hr (see text). The return of serum tyrosine concentration to normal at 6 hr may therefore indicate a specific change in disposition of this amino acid, the nature of which clearly requires investigation.

The return of cerebral catecholamine synthesis to normal at 6 hr after ethanol administration, however, represents a dramatic reversal of the earlier inhibition, and could be considered as evidence of accelerated synthesis. For this to occur, it is possible that factors initiating it, which are most likely related to brain tyrosine concentration, could have begun to intervene at an earlier time interval. The results in Table 2 show that ethanol decreased the concentrations of all competing amino acids. These decreases (and those in other circulating amino acids) may be insulin-mediated, because this hormone is known [25] to enhance amino acid uptake by skeletal muscle. It should be noted here that, in view of the ability of insulin to increase the concentration of total serum tryptophan [10,12], the observed decrease in serum tryptophan (Table 2), which confirms a previous finding [1],

could be explained, as suggested [1], by the enhanced degradation of this amino acid by hepatic tryptophan pyrrolase.

That the enhancement of brain catecholamine synthesis observed at 8 hr after ethanol administration (Fig. 1) is caused by an increased cerebral uptake of tyrosine is suggested by the concomitant increase in brain tyrosine concentration and the associated and quantitatively similar decrease in that of the circulating amino acid (Fig. 1). The mechanism of this increased cerebral uptake is most likely that of decreased competition from neutral amino acids, as similar changes have been reported [10] in insulin-treated rats. It is not clear, however, if this decreased competition involves all competing amino acids, because their concentrations were not decreased at 6 or 8 hr. A more likely mechanism is that of decreased competition from tryptophan itself, because its circulating concentration is known [1] to be decreased by 52% at 7 hr and by 30% at 8 hr.

The 8 hr effects of ethanol on cerebral catecholamine metabolism have previously been examined in only one study [21], which showed that intraperitoneal administration of a 3 g/kg dose of the drug to mice enhanced dopa synthesis, as determined by the use of another decarboxylase inhibitor, compound NSD-1024 (3-hydroxybenzyloxyamine dihydrogen phosphate). Our present findings (Fig. 1) therefore confirm those in the above report [21].

Mechanism of the 12 hr-Ethanol-Induced Inhibition of Rat Brain Catecholamine Synthesis

Inhibition of catecholamine synthesis at 12 hr after ethanol administration has already been reported [5], and was suggested to involve increased degradation of brain tyrosine by the previously reported [3] enhancement of the cerebral transamination of this amino acid. It was also concluded [3] that any hepatic influence on tyrosine availability to the brain that was observed earlier was no longer evident from 6 hr onwards, and that enhancement of transamination of brain tyrosine not only decreases the intracerebral availability of the amino acid for catecholamine synthesis, but can also play a quantitatively important role in influencing the disposition of circulating tyrosine. Similar conclusions could be drawn from results previously obtained [17] at 2 hr after cortisol administration.

Enhancement of transamination of brain tyrosine at 12 hr after ethanol administration was found to be dose-dependent in the dose range of 1.6–4 g/kg [3]. The results in Fig. 5, showing that the inhibition of cerebral catecholamine synthesis was dose-dependent in the dose range of 2.4–4 g/kg, adds further support to the above conclusion; the failure of the 1.6 g/kg dose of ethanol to cause any significant effects on tyrosine and catecholamine concentrations (Fig. 5) is explained by the finding [3] that this dose enhanced brain "tyrosine aminotransferase" activity only moderately (11–16%) and, since the activity of this enzyme is small in general, such enhancement may not be sufficient to influence intracerebral tyrosine levels.

General Conclusions and Comments

The present findings have demonstrated the ability of acute ethanol administration to exert multiple effects on rat brain catecholamine synthesis that are both time- and dose-dependent. Much of the controversy surrounding the literature on this subject can now be explained on the basis of differences in doses of ethanol, and time intervals after its administration, and routes of its administration. Species differences do not appear to be important in this case. The various findings of the present work, particularly those showing that cerebral catecholamine synthesis can be enhanced or inhibited by very moderate changes in brain tyrosine concentration, not only provide considerable support for the concept that brain tyrosine hydroxylase is normally unsaturated by its amino acid substrate, but also establish the existence of an inverse relationship between cerebral catecholamine synthesis and the activities of tyrosine-transaminating enzymes in the liver and brain.

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REFERENCES

1. Badawy, A., A.-B. and M. Evans. 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. *Biochem J* **160:** 315–324, 1976.
2. Badawy, A. A.-B., B. M. Snape and M. Evans. Biphasic effect of acute ethanol administration on rat liver tyrosine-2-oxoglutarate aminotransferase activity. *Biochem J* **186:** 755–761, 1980.
3. Badawy, A. A.-B., B. M. Snape and M. Evans. Biphasic enhancement of rat brain tyrosine aminotransferase activity by acute ethanol administration. *Subst Alcohol Actions Misuse* **1:** 517–526, 1980.
4. Badawy, A. A.-B. and D. L. Williams. Enhancement of rat brain catecholamine synthesis by administration of small doses of tyrosine and evidence for substrate inhibition of tyrosine hydroxylase activity by large doses of the amino acid. *Biochem J* **206:** 165–168, 1982.
5. Badawy, A. A.-B., D. L. Williams, B. M. Snape and M. Evans. Brain tyrosine aminotransferase: identity and possible role in the regulation of catecholamine synthesis. *Biochem J* **204:** 622–624, 1982.
6. Bustos, G. and R. H. Roth. Effect of acute ethanol treatment on transmitter synthesis and metabolism in central dopaminergic neurons. *J Pharm Pharmacol* **28:** 580–582, 1976.
7. Carlsson, A. and M. Lindqvist. Effect of ethanol on the hydroxylation of tyrosine and tryptophan in rat brain *in vivo*. *J Pharm Pharmacol* **25:** 437–440, 1973.
8. Carlsson, A. and M. Lindqvist. Dependence of 5-HT and catecholamine synthesis on concentrations of precursor amino-acids in rat brain. *Naunyn Schmiedebergs Arch Pharmacol* **303:** 157–164, 1978.
9. Carlsson, A., T. Magnusson, T. H. Svensson and B. Waldeck. Effect of ethanol on the metabolism of brain catecholamines. *Psychopharmacology (Berlin)* **30:** 30–36, 1973.

10. De Montis, M. G., M. C. Olianas, B. Haber and A. Tagliamonte. Increase in large neutral amino acid transport into brain by insulin. *J Neurochem* **30:** 121–124, 1978.
11. Efron, D. H. and G. L. Gessa. Failure of ethanol and barbiturates to alter brain monoamine content. *Arch Int Pharmacodyn Ther* **142:** 111–116, 1963.
12. Fernstrom, J. D. and R. J. Wurtman. Elevation of plasma tryptophan by insulin in rat. *Metabolism* **21:** 337–342, 1972.
13. Griffiths, P. J., J. M. Littleton and A. Ortiz. Changes in monoamine concentrations in mouse brain associated with ethanol dependence and withdrawal. *Br J Pharmacol* **50:** 489–498, 1974.
14. Hunt, W. A. and E. Majchrowicz. Alterations in the turnover of brain norepinephrine and dopamine in alcohol-dependent rats. *J Neurochem* **23:** 549–552, 1974.
15. Hunt, W. A. and E. Majchrowicz. Alterations in neurotransmitter function after acute and chronic treatment with ethanol. In: *Biochemistry and Pharmacology of Ethanol,* vol. 2, edited by E. Majchrowicz and E. P. Noble. New York: Plenum Press, 1978, pp. 167–185.
16. Karoum, F., R. J. Wyatt and E. Majchrowicz. Brain concentrations of biogenic amine metabolites in acutely treated and ethanol-dependent rats. *Br J Pharmacol* **56:** 403–411, 1976.
17. Laboritt, H. and F. Thuret. The action of hydrocortisone on the activity of the cerebral tyrosine transaminase and on the level of cerebral norepinephrine and dopamine. Physio-pathologic deductions. *Res Commun Chem Pathol Pharmacol* **17:** 77–85, 1977.
18. Pohorecky, L. A. Effects of ethanol on central and peripheral noradrenergic neurons. *J Pharmacol Exp Ther* **189:** 380–391, 1974.
19. Rawat, A. K. Brain levels and turnover rates of presumptive neurotransmitters as influenced by administration and withdrawal of ethanol in mice. *J Neurochem* **22:** 915–922, 1974.
20. Reggiani, A., M. L. Barbaccia, P. F. Spano and M. Trabucchi. Dopamine metabolism and receptor function after acute and chronic ethanol. *J Neurochem* **35:** 34–37, 1980.
21. Tabakoff, B. and P. L. Hoffman. Alterations in receptors controlling dopamine synthesis after chronic ethanol ingestion. *J Neurochem* **31:** 1223–1229, 1978.
22. Thadani, P. V., B. M. Kulig, F. C. Brown and J. D. Beard. Acute and chronic ethanol-induced alterations in brain norepinephrine metabolites in the rat. *Biochem Pharmacol* **25:** 93–94, 1976.
23. Thadani, P. V. and E. B. Truitt, Jr. Effect of acute ethanol or acetaldehyde administration on the uptake, release, metabolism and turnover of norepinephrine in rat brain. *Biochem Pharmacol* **26:** 1147–1150, 1977.
24. Wajda, I. J., I. Manigault and J. P. Hudick. Dopamine levels in the striatum and the effects of alcohol and reserpine. *Biochem Pharmacol* **26:** 653–655, 1977.
25. Wool, I. G. Relation of effects of insulin on amino acid transport and on protein synthesis. *Fed Proc* **24:** 1060–1070, 1965.
26. Wurtman, R. J. and J. D. Fernstrom. Control of brain monoamine synthesis by diet and plasma amino acids. *Am J Clin Nutr* **28:** 638–647, 1975.
27. Wurtman, R. J. and J. D. Fernstrom. Control of brain neurotransmitter synthesis by precursor availability and nutritional state. *Biochem Pharmacol* **25:** 1691–1696, 1976.
28. Yamauchi, T. and H. Fujisawa. A simple and sensitive fluorometric assay for tyrosine hydroxylase. *Anal Biochem* **89:** 143–150, 1978.