Biphasic Effect of Acute Ethanol Administration on Rat Liver Tyrosine–2-Oxoglutarate Aminotransferase Activity

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1. Acute ethanol administration causes a biphasic change in rat liver tyrosine aminotransferase activity. 2. The initial decrease is significant with a 200 mg/kg dose of ethanol, is prevented by adrenoceptor-blocking agents and by reserpine, but not by inhibitors of ethanol metabolism, and exhibits many of the characteristics of the inhibition caused by noradrenaline. 3. The subsequent enhancement of the enzyme activity by ethanol is not associated with stabilization of the enzyme, but is sensitive to actinomycin D and cycloheximide. 4. It is suggested that the initial decrease in aminotransferase activity is caused by the release of catecholamines, whereas the subsequent enhancement may be related to the release of glucocorticoids.

Mood disorders are well-known clinical features of acute and chronic alcoholism and alcohol-withdrawal states. The possible involvement of 5-hydroxytryptamine and catecholamines in mood regulation has therefore led workers to examine the effects of ethanol on the metabolism of these biogenic amines in animal brains and on factors influencing it. One of the peripheral factors influencing brain 5-hydroxytryptamine synthesis by altering the availability of circulating tryptophan to the brain (for a review, see Badawy, 1977) is the activity of liver tryptophan pyrrolase, which controls the quantitatively important (kynurenine) pathway of tryptophan degradation in man, rat and certain other animal species (see Badawy & Evans, 1976a), and we have presented evidence suggesting that changes in rat liver tryptophan pyrrolase are involved in the acute and chronic effects of ethanol on brain 5-hydroxytryptamine synthesis (Badawy & Evans, 1976b; Badawy *et al.*, 1979).

Whereas evidence for an important role for brain tryptophan concentration in 5-hydroxytryptamine synthesis is considerable (see Curzon & Knott, 1974, and references cited therein), that for a similar role for brain tyrosine concentration in catecholamine synthesis is increasing (Carlsson & Lindqvist, 1978; Gibson & Wurtman, 1978, and references cited therein). It follows therefore that peripheral factors influencing brain tyrosine concentration may play an important role in central catechol-

amine synthesis. A strong candidate for such role is the activity of liver tyrosine–2-oxoglutarate aminotransferase (EC 2.6.1.5), which controls the quantitatively most important route of tyrosine degradation. Very little work has, however, been done on the possible effects of ethanol on this hepatic enzyme. Kenney *et al.* (1965) reported briefly that acute ethanol administration to rats causes some enhancement, whereas the enzyme in the isolated perfused rat liver has been reported to be either unaltered (Mørland *et al.*, 1972) or stabilized (Mørland, 1977) by the addition of ethanol to the perfusion medium. We have therefore performed a detailed study of the effects of acute ethanol administration *in vivo* on rat liver tyrosine aminotransferase activity, the results of which form the subject of the present paper.

Materials and Methods

Chemicals

The following compounds were gifts from the sources indicated in parentheses: actinomycin D (Merck, Sharp and Dohme, Hoddesdon, Herts., U.K.), the α -adrenoceptor-blocking agents dibenamine [*N*-(2-chloroethyl)dibenzylamine] and dibenylne (phenoxybenzamine) (Smith, Kline and French Laboratories, Welwyn Garden City, Herts., U.K.), the aldehyde dehydrogenase inhibitor disulfiram (tetraethylthiuram disulphide) (Burk Pharmaceuticals, Shalford, Surrey, U.K.), the β -adrenoceptor-blocking agent propranolol [1-(isopropylamino)-3-(naphthylloxy)propan-2-ol]

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(Imperial Chemical Industries, Alderley Edge, Macclesfield, Cheshire, U.K.).

The alcohol dehydrogenase inhibitor pyrazole was purchased from Koch-Light Laboratories, Colnbrook, Bucks., U.K., whereas all other chemicals were obtained from BDH Chemicals and Sigma (London) Chemical Co. (both of Poole, Dorset, U.K.) and were of the purest commercially available grades.

Animals and treatments

Locally bred male Wistar rats (150–210 g) were maintained on cube diet 41B (Oxoid, Basingstoke, Hants., U.K.) and water and were killed by stunning and cervical dislocation between 12:45 and 15:15 h.

All chemicals were administered intraperitoneally at various times before death and in the doses given with the individual results. Ethanol was mixed with 0.9% (w/v) NaCl and was injected in a volume of 20 ml/kg body wt. A mixture of dimethylformamide and 0.9% NaCl (1:4, v/v) was used to dissolve ergotamine tartrate (an α -adrenoceptor-blocking agent), which was injected in a volume of 2 ml/kg, whereas dimethylformamide alone (1 ml/kg) was used to dissolve disulfiram and reserpine. All other compounds were dissolved in 0.9% NaCl (with, if necessary, adjustment of pH to 7.3) and were injected in volumes of 1–2 ml/kg or occasionally in larger volumes. Control rats received equal volumes of the appropriate solvent(s).

Determination of liver tyrosine aminotransferase activity

This was performed by a modification of the method of Diamondstone (1966).

(a) *Preparation of homogenates.* Fresh liver was homogenized in 0.14 M-KCl/0.0025 M-NaOH (at 0–2°C) for 1 min at 1100 rev./min by using a glass homogenizer with a loose-fitting Teflon pestle. Normally a 10% (w/v) homogenate was used, but, when the enzyme activity was expected to be high, a 5% (w/v) homogenate was prepared.

(b) *Incubation mixture.* The 6.4 ml mixture contained the following components in the volumes and strengths of solutions and final concentrations indicated: tyrosine, 5 ml of 7.68 mM (6 mM); sodium diethyldithiocarbamate, 0.2 ml of 0.12 M (3.75 mM); 2-oxoglutaric acid, 0.2 ml of 0.3 M (9.375 mM); liver homogenate, 0.1 ml of 5 or 10% (w/v); sufficient 0.2 M-potassium phosphate buffer (pH 7.3) and/or other compounds to make up the total volume. The holoenzyme activity was determined in the absence, whereas the total enzyme activity was obtained in the presence, of added (40 μ M) pyridoxal 5'-phosphate in a volume of 0.2 ml. Other concentrations of the cofactor were also used in some experiments. All the above compounds (except the homogenate) were made in the above phosphate buffer, as were ethanol,

acetaldehyde and sodium acetate when added *in vitro* (10 mM each). The tyrosine solution was prepared by dissolving 69.58 mg in about 0.9 ml of 2 M-KOH and gradual addition of the 0.2 M-potassium phosphate buffer (pH 7.3) to make up 50 ml. Only small changes in pH (0.1) were observed. The tyrosine solution must be prepared immediately before use to prevent precipitation on storage.

(c) *Assay.* The ice-cold mixture (6.4 ml) already in 25 ml conical flasks was incubated at 37°C under air with shaking (120 oscillations/min) for 5 or 20 min depending on enzyme activity, which was found in separate experiments to be linear with time for at least 1 h. The reaction was stopped by the addition of 1.2 ml of 30% (w/v) trichloroacetic acid. After shaking for a further 2 min, the contents of flasks were filtered on Whatman no. 1 filter paper. To 3.8 ml of the filtrate was added 0.2 ml of 10 M-NaOH, and the mixture was immediately stirred mechanically and left for 30 min at room temperature ($22 \pm 1^\circ\text{C}$). The A_{331} was then determined against a water blank. Each rat assay was performed in quadruplicate and was accompanied by three tissue blanks that were similarly treated, except that the trichloroacetic acid was added before the liver homogenate. Tyrosine aminotransferase activity was then calculated from an absorption coefficient of $24900 \text{ M}^{-1} \cdot \text{cm}^{-1}$ after correction for the 80% maximum conversion of 4-hydroxyphenylpyruvate into 4-hydroxybenzaldehyde (Diamondstone, 1966).

In the original method of the above author, 10 M-NaOH was used both to stop the reaction and to produce 4-hydroxybenzaldehyde. This treatment caused a turbidity that could not be removed by filtration or centrifugation even when the amount of liver was decreased to only 1 mg (A. A.-B. Badawy, unpublished work). This turbidity was also not caused by the presence of diethyldithiocarbamate, but was overcome by the use of trichloroacetic acid, which has been reported by Diamondstone (1966) to cause a turbidity.

Determination of the half-life of tyrosine aminotransferase

The enzyme activity was determined at 4 h after administration of ethanol (5 ml/kg) or an equal volume of 0.9% NaCl. Cycloheximide (10 mg/kg) was then injected (i.e. at 4 h after the above treatments) and the activity was again determined 1 and 2 h later. Aminotransferase half-life was calculated from the rate of decline in enzyme activity at 0, 1 and 2 h after cycloheximide administration, as plotted semilogarithmically.

Statistical analysis of results

This was performed by using Student's *t* test.

Results and Discussion

Effects of acute ethanol administration on rat liver tyrosine aminotransferase activity

The time-course of the above effects of a 4 g/kg dose of ethanol is shown in Fig. 1. Ethanol exerted a biphasic effect on aminotransferase activity. The initial decrease in both holoenzyme and total enzyme activities was maximum (62 and 34% respectively; $P = 0.005-0.001$) at 1 h, whereas the subsequent enhancement was highest (11.2- and 8.6-fold respectively; $P < 0.001$) at 6 h. Activities then gradually returned to near-basal values at 16–20 h after ethanol administration.

The effects of various doses of ethanol were also

examined (Fig. 2). At 1 h (Fig. 2a), the maximum decrease in holoenzyme and total enzyme activities (55 and 40% respectively; $P = 0.005-0.001$) was achieved by a 1.6 g/kg dose. It is remarkable that significant ($P < 0.025$) decreases in enzyme activities (of 20 and 14% respectively) were observed at 1 h after administration of a dose of ethanol (0.2 g/kg) equivalent to 0.5–1 pint of commercial beer consumed by a 70 kg human. By contrast, the ethanol-induced enhancement of the enzyme activity observed at 6 h (Fig. 2b) was not produced by the 0.8 g/kg dose of ethanol, but was dose-dependent in the dose range of 1.6–4.0 g/kg body wt.

The above biphasic effect of ethanol is not produced by a direct action of ethanol or its metabolites acetaldehyde or acetate, because these agents do

Fig. 1. Time-course of the effects of acute ethanol administration on rat liver tyrosine aminotransferase activity

Ethanol (4 g/kg) was administered intraperitoneally at zero time. The enzyme activity was determined, at various times, as described in the Materials and Methods section in either the absence (holoenzyme activity; ●) or the presence (total enzyme activity; ○) of added (40 μM) pyridoxal 5'-phosphate. Values are means $\pm$ S.E.M. for each group of four rats.

Fig. 2. Effects of various doses of ethanol on rat liver tyrosine aminotransferase activity

Rats received various doses of ethanol in volumes of 20 ml/kg each and were killed at 1 h (a) or at 6 h (b) after injection. Control rats received an equal volume of 0.9% NaCl (zero-dose values). The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity; ●) or the presence (total enzyme activity; ○) of added (40 μM) pyridoxal 5'-phosphate. Values are means $\pm$ S.E.M. for each group of four rats.

not affect the basal enzyme activities *in vitro* when tested at 10 mM concentrations. The holoenzyme and total enzyme activities (in μmol of 4-hydroxyphenylpyruvate formed/h per g wet wt. of liver, $\pm$ s.e.m. for each group of four rats) were as follows: control (27.1 ± 2.5 and 54.1 ± 4.4); ethanol (26.7 ± 2.7 and 57.0 ± 5.4); acetaldehyde (23.6 ± 3.8 and 42.1 ± 6.8); sodium acetate (26.7 ± 3.2 and 57.8 ± 6.5). The failure of ethanol and acetaldehyde to act *in vitro* confirms previous findings (Mørland, 1977). NADH was not tested *in vitro* in view of the close proximity of its absorption maximum to that of 4-hydroxybenzaldehyde.

Mechanism of the early ethanol-induced decrease in tyrosine aminotransferase activity

The initial decrease in enzyme activity observed at 1 h after ethanol administration is also not caused by the ethanol metabolites acetaldehyde and acetate, because it is not prevented by pretreatment of rats with the inhibitors of alcohol dehydrogenase and aldehyde dehydrogenase (pyrazole and disulfiram respectively) (Table 1). These two inhibitors cause no significant ($P > 0.10$) changes in the basal enzyme activities.

Acute ethanol administration stimulates the release from the adrenal medulla and from other

sources (see Majchrowicz, 1973) of catecholamines, which are known to inhibit liver tyrosine aminotransferase activity both *in vitro* (Black & Axelrod, 1969) and after administration (Black & Axelrod, 1970). The possibility was therefore considered that catecholamines (and in particular noradrenaline as the most potent aminotransferase inhibitor of this group) may mediate the early ethanol-induced decrease in enzyme activity. Evidence supporting this possibility is provided by the finding (Table 1) that the inhibitory effect of ethanol is prevented by pretreatment of rats with the adrenoceptor-blocking agents dibenamine, dibenylamine, ergotamine and propranolol, and by the depletor of catecholamine stores reserpine. In control rats, dibenamine, propranolol and, more effectively, reserpine increased the holoenzyme activity, thus increasing the saturation of the apoenzyme with its pyridoxal 5'-phosphate cofactor. A possible explanation of this effect is that catecholamine-modifying agents interfere with the binding of pyridoxal 5'-phosphate to noradrenaline (see Black & Axelrod, 1969) by either depleting the latter or blocking its actions. This suggestion also provides support for the hypothesis (Black, 1970) that liver tyrosine aminotransferase activity may be regulated by a mechanism involving the interaction of nor-

Table 1. *Effects of pretreatment of rats with various agents on the 1h-ethanol-induced decrease in liver tyrosine aminotransferase activity*

Rats were killed at 1 h after administration of ethanol (4 g/kg) or an equal volume of 0.9% (w/v) NaCl (20 ml/kg). Reserpine (20 mg/kg) or dimethylformamide was injected 24 h before, whereas all other compounds were given 0.5 h before, ethanol or 0.9% NaCl in the following doses: pyrazole (272 mg/kg); ergotamine tartrate (2.5 mg/kg); dibenamine (50 mg/kg); dibenylamine (10 mg/kg); propranolol (10 mg/kg); disulfiram (100 mg/kg). The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added ($40 \mu\text{M}$) pyridoxal 5'-phosphate. Values are means $\pm$ s.e.m. for each group of four rats. Values in column (3) are compared with those in column (1), and those in column (4) are compared with those in column (2) and the significance of differences is indicated as follows: * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; † $P < 0.005$; †† $P < 0.001$.

Tyrosine aminotransferase activity (μmol of 4-hydroxyphenylpyruvate formed/h per g wet wt. of liver)

Pretreatment	0.9% NaCl-treated rats		Ethanol-treated rats	
	Holoenzyme (1)	Total enzyme (2)	Holoenzyme (3)	Total enzyme (4)
0.9% NaCl	30.8 ± 2.0	61.1 ± 0.2	$20.3 \pm 1.2^\dagger$	$40.0 \pm 1.6^{\dagger\dagger}$
Pyrazole	30.5 ± 3.2	54.9 ± 5.0	$19.6 \pm 1.3^{**}$	$40.1 \pm 1.1^*$
Ergotamine	30.1 ± 0.4	60.9 ± 0.2	30.2 ± 2.5	61.8 ± 1.8
0.9% NaCl	30.3 ± 1.8	65.0 ± 5.4	$17.7 \pm 1.7^{\dagger\dagger}$	$37.5 \pm 4.4^{***}$
Dibenamine	41.0 ± 2.9	79.0 ± 5.0	41.5 ± 3.1	86.2 ± 7.5
Dibenylamine	32.0 ± 2.1	61.0 ± 1.5	34.7 ± 2.9	64.0 ± 6.6
Propranolol	38.7 ± 2.4	77.5 ± 5.2	34.0 ± 2.5	69.0 ± 7.8
Dimethylformamide	30.6 ± 3.6	69.4 ± 6.3	$15.4 \pm 1.5^{***}$	$41.1 \pm 3.0^{***}$
Disulfiram	33.0 ± 1.8	69.8 ± 3.4	$16.6 \pm 2.7^\dagger$	$39.3 \pm 5.7^\dagger$
Dimethylformamide (24 h)	52.3 ± 1.7	95.0 ± 7.7	$40.0 \pm 1.9^\dagger$	$76.0 \pm 5.1^*$
Reserpine	101.3 ± 9.1	113.7 ± 4.2	108.7 ± 9.5	125.7 ± 4.3

adrenaline with the above cofactor. Further work with catecholamine-modifying drugs may therefore be of interest in this context.

Further evidence for the involvement of catecholamines in the early ethanol-induced decrease in tyrosine aminotransferase activity is provided by a number of similarities of actions of ethanol and noradrenaline on the enzyme. These are illustrated by experiments involving the time-course of the noradrenaline effect, combined injections of ethanol plus noradrenaline and the response of the enzyme from rats treated with either agent towards pyridoxal 5'-phosphate *in vitro*.

The time-course of the effects of noradrenaline administration on the enzyme activity is shown in Fig. 3. Noradrenaline exerted a biphasic effect; an initial decrease in holoenzyme and total aminotransferase activities of 39–43% ($P < 0.005$) at 1 h and a subsequent enhancement exhibiting a maximum (86 and 39% respectively; $P = 0.005–0.001$) at 2.5 h. Activities then returned to the basal values at 4–5 h. The early noradrenaline-induced decrease resembles in magnitude that caused by ethanol (see Fig. 1).

The effects of administration of ethanol, noradrenaline or both on the enzyme activity were compared at 1 h (Table 2). Ethanol alone decreased the holoenzyme and total enzyme activities by 40 and 35% respectively ($P = 0.01–0.005$), whereas the corresponding decreases caused by noradrenaline were 49 and 43% respectively ($P < 0.005$). Combined administration of ethanol and noradrenaline did not result in an additive decrease in enzyme activities when the effects were compared with the corresponding separate ones. This suggests that ethanol and noradrenaline do not act by different mechanisms.

The effects of pyridoxal 5'-phosphate added *in vitro* were examined in control rats and in those

treated with ethanol or noradrenaline 1 h previously (Fig. 4). Maximum activation of basal aminotransferase was produced by a 10 μM concentration of the cofactor. The ethanol- or noradrenaline-induced decrease in activity was not greatly influenced by a large excess of added pyridoxal 5'-phosphate.

The noradrenaline effect on aminotransferase

Fig. 3. Time-course of the effects of noradrenaline on rat liver tyrosine aminotransferase activity
Noradrenaline (1 mg/kg) was administered intraperitoneally at zero time. The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity; ○) or the presence (total enzyme activity; ●) of added (40 μM) pyridoxal 5'-phosphate. Values are means $\pm$ S.E.M. for each group of four rats.

Table 2. Effects of ethanol, noradrenaline or both on rat liver tyrosine aminotransferase activity at 1 h after administration

Rats received two injections 5 min apart and were killed 1 h after the second injection. The first injection was of either noradrenaline (1 mg/kg) or an equal volume (2 ml/kg) of 0.9% NaCl, whereas the second injection consisted of ethanol (4 g/kg) or an equal volume (20 ml/kg) of 0.9% NaCl. The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added (40 μM) pyridoxal 5'-phosphate. Values are means $\pm$ S.E.M. for each group of four rats. The holoenzyme and total enzyme values of test rats are compared with those of control (0.9% NaCl only) animals and the significance of differences is indicated as follows: *** $P < 0.01$; † $P < 0.005$; †† $P < 0.001$.

Injections		Tyrosine aminotransferase activity (μmol of 4-hydroxyphenylpyruvate formed/h per g wet wt. of liver)	
First	Second	Holoenzyme activity	Total enzyme activity
0.9% NaCl	0.9% NaCl	38.7 ± 2.6	75.2 ± 4.9
0.9% NaCl	Ethanol	23.2 ± 1.8†	48.7 ± 3.8***
Noradrenaline	0.9% NaCl	23.7 ± 2.0†	42.6 ± 2.7†
Noradrenaline	Ethanol	20.4 ± 2.3†	39.7 ± 3.3††

activity reported in the present work (Fig. 3) differs from that reported by Black & Axelrod (1970) in female adrenalectomized Sprague-Dawley rats in exhibiting a reversed order of the two opposite elements of the biphasic change. Further work is required to explain this difference.

Mechanism of the later ethanol-induced enhancement of tyrosine aminotransferase activity

The almost 6-fold enhancement of the enzyme activity observed at 5 h after ethanol administration

Fig. 4. Effects of pyridoxal 5'-phosphate *in vitro* on liver tyrosine aminotransferase activity from control, ethanol-treated and noradrenaline-treated rats

Rats were killed at 1 h after administration of 0.9% NaCl, ethanol (4 g/kg) or noradrenaline (1 mg/kg) and the enzyme activity was then determined as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence of various concentrations of pyridoxal 5'-phosphate. Values are means $\pm$ S.E.M. for each group of four rats. Symbols: $\circ$, control rats; $\bullet$, ethanol-treated rats; $\blacktriangle$, noradrenaline-treated rats.

is not much altered by pretreatment of rats with ergotamine (Table 3), which is capable of preventing the early ethanol-induced decrease (see Table 1). A number of suggestions can be made from these observations: (1) the enhancement is not related to, or caused by, the initial decrease in enzyme activity. This is further supported by the finding (Fig. 2) that, whereas a 1.6 g/kg dose of ethanol causes maximum inhibition at 1 h, the later enhancement is dose-dependent in the dose range of 1.6–4.0 g/kg; (2) the ethanol-induced enhancement is not mediated by tyrosine that could have accumulated in the liver as a result of the initial decrease in its catabolism; (3) the ethanol enhancement is also unlikely to be catecholamine-mediated, particularly since the noradrenaline-induced stimulation of the enzyme activity is only moderate (Fig. 3); (4) the ethanol enhancement is unlikely to be tryptophan-mediated. This is because, although a catecholamine-dependent increase in the availability to the liver of circulating free tryptophan is observed early after acute ethanol administration, ergotamine is also capable of preventing this increased availability (Badawy & Evans, 1975, 1976b).

The ethanol enhancement of the enzyme activity is also not caused by an effect on enzyme degradation. Thus, when the half-life of tyrosine aminotransferase was determined by administration of cycloheximide to rats pretreated 4 h previously with either ethanol (4 g/kg) or an equal volume of 0.9% NaCl, it was found that the holoenzyme and total enzyme from control rats exhibited half-lives of 2.03 and 2.09 h respectively, whereas the corresponding values in ethanol-treated rats were 2.02 and 2.01 h respectively.

The exclusion of the above factors as mediators of the ethanol enhancement of tyrosine aminotransferase activity leaves us with the possible involve-

Table 3. Effects of pretreatment of rats with various agents on the 5 h-ethanol-induced enhancement of liver tyrosine aminotransferase activity

Rats were killed at 5 h after administration of ethanol (4 g/kg) or an equal volume (20 ml/kg) of 0.9% NaCl. The following compounds were administered 0.5 h before ethanol or 0.9% NaCl: 0.9% NaCl (2 ml/kg); ergotamine tartrate (2.5 mg/kg); actinomycin D (0.7 mg/kg); cycloheximide (10 mg/kg). The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added (40 μ M) pyridoxal 5'-phosphate. Values are means $\pm$ S.E.M. for each group of four rats. The values in columns (3) and (4) are compared with those in columns (1) and (2) respectively and the significance of differences is indicated as follows: * $P < 0.05$; † $P < 0.005$; †† $P < 0.001$.

Pretreatment	Tyrosine aminotransferase activity (μ mol of 4-hydroxyphenylpyruvate formed/h per g wet wt. of liver)			
	0.9% NaCl-treated rats		Ethanol-treated rats	
	Holoenzyme activity (1)	Total enzyme activity (2)	Holoenzyme activity (3)	Total enzyme activity (4)
0.9% NaCl	40 $\pm$ 3	72 $\pm$ 3	239 $\pm$ 13††	417 $\pm$ 19††
Ergotamine	77 $\pm$ 3	109 $\pm$ 1	235 $\pm$ 9††	443 $\pm$ 9††
Actinomycin D	37 $\pm$ 2	73 $\pm$ 3	53 $\pm$ 3†	94 $\pm$ 7*
Cycloheximide	61 $\pm$ 5	99 $\pm$ 4	63 $\pm$ 3	110 $\pm$ 6

ment of hormones and/or pyridoxal 5'-phosphate. Aminotransferase enhancement by insulin is insensitive to actinomycin D (see Walker, 1977), whereas that by administered pyridoxine is only partially sensitive to this agent (see Badawy, 1972). The finding (Table 3) that the ethanol enhancement is completely inhibited by actinomycin D therefore excludes the possible involvement of insulin and the cofactor. Moreover, the ability of actinomycin D and also cycloheximide to prevent the ethanol effect (Table 3) suggests that the latter requires the synthesis of both protein and mRNA. Increased synthesis of apo-(tyrosine aminotransferase) is the mechanism by which glucocorticoids (and certain other hormones) enhance the enzyme activity (for a review, see Walker, 1977), and this involves an increased rate of synthesis (not degradation) of tyrosine aminotransferase-specific mRNA (Nickol *et al.*, 1978). The fact that acute ethanol administration enhances the release of adrenal glucocorticoids (see Majchrowicz, 1973) is therefore consistent with the possible involvement of these hormones in the ethanol effect.

Concluding remarks

The present results and those reported previously (Badawy & Evans, 1975, 1976b) suggest that the release of adrenal components plays a major role in the acute effects of ethanol on the hepatic metabolism of tryptophan and tyrosine and on brain 5-hydroxytryptamine synthesis. It remains to be seen whether the effects of ethanol on liver tyrosine metabolism reported here are involved in its actions on brain catecholamine synthesis.

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