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BRAIN CONCENTRATIONS OF BIOGENIC AMINE METABOLITES IN ACUTELY TREATED AND ETHANOL-DEPENDENT RATS

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1 Mass fragmentography was used to measure whole brain concentrations of some of the major metabolites of tyramine, octopamine, dopamine and noradrenaline in acutely treated and in ethanol-dependent rats.

2 Treatments with ethanol, either acutely or chronically, failed to alter significantly brain concentration of *p*-hydroxyphenylacetic and *p*-hydroxymandelic acid (metabolites derived from tyramine and octopamine respectively). The effect on catecholamine metabolites was marked and therefore suggests that ethanol is selective in its effect on central metabolism of biogenic amines.

3 Acute ethanol treatment significantly increased brain concentration of homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylglycol (MHPG). Vanilmandelic acid (VMA) was not affected. All four metabolites (HVA, DOPAC, MHPG and VMA) were increased in the brains of rats rendered dependent on ethanol while still intoxicated (blood ethanol levels above 200 mg/dl). In ethanol-dependent rats undergoing ethanol withdrawal syndrome (no ethanol present in blood), the brain concentrations of HVA and DOPAC were normal while those of MHPG and VMA continued to be elevated.

4 From the decline in the concentrations of HVA and DOPAC after 50 mg pargyline/kg in control rats and rats acutely treated with ethanol, it was concluded that ethanol has no effect on the transport of phenolic acids across the blood brain barrier.

5 No reversal in the metabolism of catecholamines from an oxidative to a reductive pathway, analogous to that produced by ethanol in the periphery, could be established in the brain.

6 The increase in catecholamine metabolite concentrations after ethanol treatment, either acute or chronic, were interpreted as manifestations of increases catecholamine turnover.

Introduction

The involvement of biogenic amines in the mediation of central and peripheral effects of ethanol has been postulated by several authors (for a review see Feldstein, 1971; Truitt & Walsh, 1971; Lahti, 1975). Most studies on the effect of ethanol on the metabolism of biogenic amines have been confined to the peripheral sympathetic nervous system where a reversal from an oxidative to a reductive pathway of several amines is well documented (Smith & Gitlow, 1967; Davis, Brown, Huff & Cashaw, 1967a,b; Tacker, Creaven & McIssac, 1970; Bonham Carter, Karoum, Sandler & Youdim, 1970; Ogata, Mendelson, Mello & Majchrowicz, 1971). Attempts to demonstrate changes in biogenic amine metabolites in the central nervous system following the administration of ethanol have been limited to 5-hydroxytryptamine (von Wartburg, 1962; Lahti & Majchrowicz, 1967, 1969; Feldstein & Sidel, 1969; Tyce, Flock, Taylor & Owen, 1970; Tabakoff, Bulat & Anderson, 1975; Tabakoff, Ritzman & Boggan, 1975). In this paper we describe a study on the brain levels of metabolites derived from tyramine, octopamine, dopamine and noradrenaline in rats either treated acutely with ethanol or rendered physically dependent upon ethanol.

Methods

Animals

Male Sprague-Dawley rats weighing between 200 and 300 g were used. After delivery, the animals were

allowed to acclimatize to animal house conditions for two to four days. The animals had unlimited access to food and water throughout the entire experimental period.

Acute experiments

In two sets of experiments, ethanol was administered intragastrically to 12 rats in a dose of 5 g/kg as a 20% (w/v) aqueous solution. Control animals were given 5 ml 0.9% w/v NaCl solution (saline). Three hours later the rats were decapitated and their brains removed and frozen on dry ice. In one set of experiments, the brains were used for the assay of the acidic metabolites (*p*-hydroxyphenylacetic (PHPA), *p*-hydroxymandelic (PHMA), dihydroxyphenylacetic (DOPAC), homovanillic (HVA) and vanilmandelic (VMA) acids) and the other for the assay of 3-methoxy-4-hydroxyphenylglycol (MHPG).

In another set of experiments designed to study the effect of ethanol on the transport mechanism of HVA and DOPAC across the blood brain barrier, 25 rats were given ethanol as described above. One hour later, pargyline (50 mg/kg), a monoamine oxidase (MAO) inhibitor, was given intraperitoneally. Groups of five rats were decapitated immediately and after intervals of 30, 60, 120 and 180 minutes. Control rats were given saline and treated with pargyline exactly as described for the ethanol-treated rats.

Ethanol-dependent rats

Physical dependence upon ethanol was induced in 20 rats by intragastric administration of a 20% (w/v) ethanol solution at 9–15 g/kg daily in three to five fractional doses over four days. Ten to 15 h after the last dose of ethanol, the rats were separated into two groups depending upon the development of the withdrawal syndrome. The rats in the first group showed signs of varying severity during the prodromal detoxication phase of withdrawal. These rats had blood ethanol levels higher than 200 mg/dl. The second group of rats showed overt signs and responses of an ethanol withdrawal syndrome (Majchrowicz, 1973; 1975). During this phase, little or no ethanol was found in the blood. The ethanol withdrawal phase was characterized by the onset of the withdrawal signs and responses reminiscent of the tremulous, spastic and convulsive stages of ethanol-dependence observed in man (Victor & Adams, 1953; Mendelson & LaDou, 1964). In two sets of experiments five rats from each of the above two groups of ethanol-dependent as well as control rats were decapitated and their brains frozen on dry ice. One set of animals was used for the assay of the acidic metabolites and the other for the assay of MHPG.

Equipment

Combined gas chromatography-mass spectrometry was carried out on a Finnigan Model 3000D

Table 1 Atomic mass units of fragments employed for mass fragmentography of biogenic amine metabolites

Metabolite	*Derivative*	*m/e used for mass fragmentography (origin of fragment)*	*Relative retention time**
p-Hydroxyphenylacetic acid (PHPA)	Methyl ester/pentafluoropropionyl	253 (M^+–$COOCH_3$)/312 (M^+)	1.0
p-Hydroxymandelic acid (PHMA)	Methyl ester/pentafluoropropionyl	387 (M^+–$COOCH_3$–CO)/415 (M^+–$COOCH_3$)	1.0
Homovanillic acid (HVA)	Methyl ester/pentafluoropropionyl	283 (M^+–$COOCH_3$)/342 (M^+)	1.78
Dihydroxyphenylacetic acid (DOPAC)	Methyl ester/pentafluoropropionyl	387 (M^+–$COOCH_3$–CO)/415 (M^+–$COOCH_3$)	1.38
Vanilmandelic acid (VMA)	Methyl ester/pentafluoropropionyl	445 (M^+–$COOCH_3$)/417 (M^+–CH_3OH)	1.50
3-Methoxy-4-hydroxyphenylglycol (MHPG)	Pentafluoropropionyl	*311 (M^+–$OCOHC_2F_5$–COC_2F_5)/458 (M^+–$OCOHC_2F_5$)†	1.22

* Retention time relative to PHPA. † This ion is produced by the transfer of a β hydrogen onto the keto oxygen of the acyl group through a two-step hydrogen rearrangement reaction.

Quadrupole gas chromatograph mass spectrometer employing an 8 ft, $\frac{1}{8}$ inch i.d. 3% SE-54 steel column. The oven temperature was maintained at 200°C or 195°C depending on the type of analysis. The fragments selected for mass fragmentography are summarized in Table 1.

Reagents

Pentafluoropropionic anhydride and pentafluoropropionic imidazole were obtained from Pierce Chemical Company, Rockford, Illinois; SE-54 (GP91C silicon Gum rubber) was obtained from Analab, North Haven, Connecticut. 'Lipopure' methanol was purchased from Applied Science Laboratories, Inc., State College, Pennsylvania. Deuterated reagents used for the synthesis of deuterated compounds came from Merck, Sharpe & Dohme, Canada, Ltd., Montreal, Quebec. All other reagents and compounds used were of the highest purity available. Procedure for the synthesis of the different deuterated isomers are published elsewhere (Karoum, Gillin & Wyatt, 1975a, Karoum, Gillin, Wyatt & Coster, 1975b).

Derivatization

Acidic metabolites were first methylated with 100 µl 20% HCl in methanol (prepared by adding 1 ml acetylchloride dropwise to 4 ml 'Lipopure' methanol; acetylchloride quantitatively reacts with methanol to form HCl and methyl acetate.) After 5 min at room temperature, the mixture was dried under a gentle stream of N_2. The methyl ester was next acylated with 50 µl 10% pentafluoropropionyl imidazole in ethyl acetate by heating at 70°C for 10 minutes. One µl of the derivative was injected.

Three-methoxy-4-hydroxyphenylglycol was derivatized in a mixture of 100 µl ethyl acetate and 100 µl pentafluoropropionic anhydride as previously described (Karoum, LeFevre, Bigelow & Costa, 1973) and the pentafluoropropionyl derivative reconstituted in 50 µl ethyl acetate.

The use of pentafluoropropionyl imidazole in the preparation of the methyl ester/pentafluoropropionyl derivatives of the acidic metabolites consistently gave a higher yield of products than did the pentafluoropropionic anhydride. The opposite was true for MHPG.

Processing of brain tissue

The brains were weighed, individually homogenized in 10 ml 1 N HCl, centrifuged at 20,000 *g* for 15 min, and the clear supernatant used for the analysis of the acidic metabolites.

For the alcoholic metabolites, brains were homogenized individually in 6 ml 0.2 M $ZnSO_4$, to which 6 ml 0.2 M $Ba(OH)_2$ was added. The mixture was further homogenized for 1 min and centrifuged at 20,000 *g* for 15 minutes. The clear supernatants were used for analysis.

Assay of acidic metabolites: vanilmandelic acid, homovanillic acid, p-*hydroxyphenylacetic acid, dihydroxyphenylacetic acid and* p-*hydroxymandelic acid*

Into two 45 ml round-bottom glass centrifuge tubes, 2 ml of the clear supernatant obtained from the brain homogenate was introduced followed by the addition of 100 µl of a mixture of appropriate deuterated isomers (5–20 ng) (VMA-d_3, HVA-d_2, PHPA-d_4, DOPAC-d_5, PHMA-d_2). Internal standards (5–50 ng) of the non-deuterated metabolites were also added to one duplicate. The amount of internal standard used was about the same as that expected in the sample analyzed. The mixture in each tube was saturated with anhydrous Na_2SO_4 and then extracted twice with 10 ml ethyl acetate. Aliquots (8 and 10 ml) of the ethyl acetate extracts were respectively transferred, combined and evaporated under vacuum. The dried residue thus obtained was reconstituted in 0.3 ml ethyl acetate; 0.2 ml transferred into 1 ml microflex tubes (Kontes Glass Company, Evanston, Illinois); the 0.2 ml aliquot dried under N_2 and the metabolites in the dried residue converted to their methyl ester/pentafluoropropionyl derivatives. The deuterated isomers were used to correct for any variance that may have been introduced during the extraction and preparation of the non-deuterated metabolites as will be described later.

Assay of unconjugated 3-methoxy-4-hydroxyphenylglycol (MHPG)

Two ml of the clear supernatant obtained from the brain homogenate was mixed in duplicate with 1 ml 1M acetate buffer pH 6.2. To one duplicate, 10 ng of MHPG from a freshly prepared diluted solution was added followed by the addition of 10 ng of deuterated MHPG (MHPG-d_3) to both tubes. The duplicates were then saturated with anhydrous Na_2SO_4, extracted and processed as described for the acidic metabolites, except that the pentafluoropropionyl derivative was prepared.

Assay of total 3-methoxy-4-hydroxyphenylglycol (MHPG)

One ml of the clear supernatant from the brain homogenate was mixed in duplicate with 1 ml 1 M acetate buffer pH 6.2. To one duplicate, 100 µl of diluted MHPG-sulphate solution (corresponding to 20 ng MHPG) was added and the duplicates incubated for 20 h at 40°C with 0.2 ml of a high molecular fraction of glusulase (Karoum *et al.*, 1973). After hydrolysis, MHPG-d_3 (10 ng) was added to all tubes and the samples were processed exactly as described

for the free metabolites. A sample blank containing 2 ml 1 M acetate buffer was included and used to correct for the presence of MHPG (usually about 2 ng/ml) in the glusulase.

Quantification procedure

As described earlier, each brain was analyzed in duplicate (samples A and B) by assaying equal volumes of the clear supernatant obtained from the brain homogenate. To these duplicates (A and B), equal amounts of a deuterated standard were added. An internal non-deuterated standard was also added to one duplicate (A). After processing the samples, the mass fragmentograms of the duplicate samples (A and B), the peak heights of the non-dueterated metabolite and of the corresponding deuterated standard were measured. Metabolite peak heights were expressed as a proportion of the peak height of the corresponding deuterated metabolite. Endogenous metabolite levels were calculated from the change in this ratio due to the addition of the non-deuterated standard to the duplicate (A).

Assay of blood ethanol

Ethanol levels were determined in mixed arterio-venous blood taken immediately after decapitation using an automated adaptation (Majchrowicz, 1971) of the gas chromatographic method of Roach & Creaven (1968).

Results

The concentrations of the various metabolites and blood ethanol levels in the acutely treated rats are shown in Table 2, and those for the ethanol-dependent rats in Table 3. With the exception of one rat that had a blood ethanol concentration of 78 mg/dl, there was no ethanol present in the blood of rats undergoing an ethanol withdrawal syndrome.

The concentrations of PHPA and PHMA, which are metabolites of tyramine (Ewins & Laidlaw, 1910; Schayer, 1953; Armstrong, Shaw & Wall, 1956; Spector, Melmon, Lovenberg & Sjoerdsma, 1963) and octopamine (Armstrong *et al.*, 1956; Pisano, Creveling & Udenfriend, 1960) respectively, were not significantly affected by ethanol in any of the treated rats. However, the concentrations of DOPAC and HVA were significantly increased both in the acutely treated and in the ethanol-dependent rats while still intoxicated. After the clearance of ethanol from the blood, both DOPAC and HVA reverted to normal concentrations in the ethanol-dependent rats. In contrast, MHPG was elevated in the acutely treated as well as in the ethanol-dependent rats irrespective of whether they were intoxicated or undergoing a

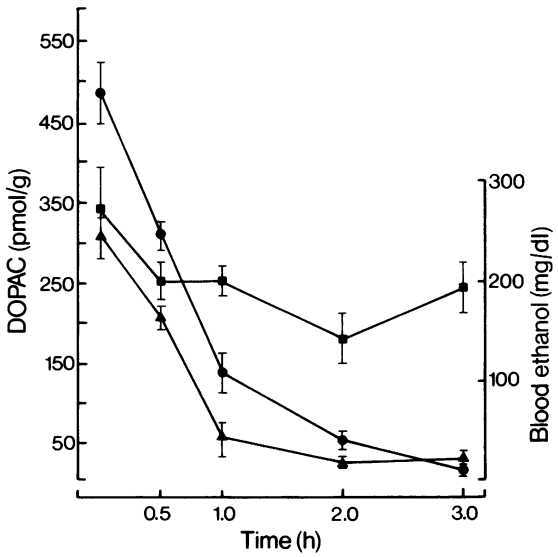

Figure 1 Brain concentrations (pmol/g) of dihydroxyphenylacetic acid (DOPAC) after pargyline. Ethanol (5 g/kg) was given intragastrically 1 h before pargyline (50 mg/kg). Control (▲); intoxicated rats (●); blood ethanol (■). All means for the ethanol-treated rats were significantly higher than those for the controls ($P < 0.05$).

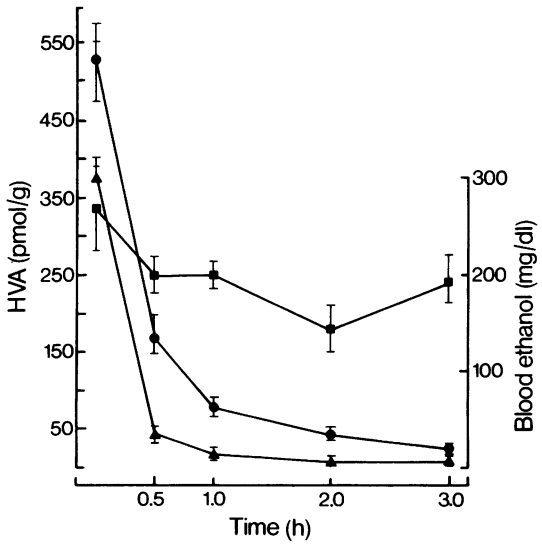

Figure 2 Brain concentrations (pmol/g) of homovanillic acid (HVA) after pargyline (50 mg/kg). Ethanol was given as described in Figure 1. Control (▲); intoxicated rats (●); blood ethanol (■). All means of the ethanol-treated rats were significantly higher than those of the control.

Table 2 Whole rat brain amine metabolites in acute ethanol-treated and control rats*

Description	*No. of rats*	*PHPA*	*PHMA*	*VMA*	*Free MHPG*	*Total MHPG*	*Total MHPG/VMA*	*HVA*	*DOPAC*	*Blood ethanol*
Controls	6	—	18.4 ± 1.6	10.1 ± 2.5	69.6 ± 3.2	479 ± 49	46.2 ± 12	445 ± 24	369 ± 20	0
Treated†	12	—	19.0 ± 2.1	6.5 ± 1.0	99.9 ± 12.5	679 ± 32	103.7 ± 49	857 ± 33.5	556 ± 20	193.4 ± 24
P value	—	—	NS	NS		$P<0.05$	$P<0.005$	$P<0.005$	$P<0.001$	

PHPA = *p*-hydroxyphenylacetic acid; PHMA = *p*-hydroxymandelic acid; VMA = 3-methoxy-4-hydroxyvanilmandelic acid; MHPG = 3-methoxy-4-hydroxyphenylglycol; HVA = homovanillic acid; DOPAC = dihydroxyphenylacetic acid. * Results are expressed in pmol/g tissue as their mean ± s.e. mean. † Rats were given intragastrically 5 g of ethanol in 20% (w.v.) solution per/kg of body weight and decapitated 3 h later.

Table 3 Whole rat brain amine metabolites in ethanol-dependent and control rats*

Description	*No. of rats*	*PHPA*	*PHMA*	*Total MHPG*	*Total MHPG/VMA*	*VMA*	*HVA*	*DOPAC*	*Blood ethanol*
A. Controls	5	164 ± 26	15.2 ± 1.6	414 ± 49	20.8 ± 2	18.6 ± 2	510 ± 39	407 ± 32	—
B. Intoxicated†	5	230 ± 26	22.8 ± 3.8	597 ± 61	13.4 ± 3.9	52.5 ± 11.1	659 ± 42.8	630 ± 67	230 ± 21
C. Withdrawn‡	5	158 ± 5.9	15.2 ± 2.1	804 ± 68	30 ± 12	52.5 ± 12	516 ± 21	429 ± 27	§
P value, A/B		NS	NS	$P<0.05$	NS	$P<0.02$	$P<0.025$	$P<0.02$	—
P value, A/C		NS	NS	$P<0.001$	NS	$P<0.005$	NS	NS	—
P value, B/C		NS	NS	$P<0.05$	NS	NS	$P<0.02$	$P<0.025$	—

Abbreviations as in Table 2. * Results are expressed in pmol/g tissue as their mean ± s.e. mean. † Rats were given intragastrically 20% (w/v) ethanol at 9 to 15 g/kg in three to five fractional doses daily for four days and were decapitated during the prodromal detoxication phase of the withdrawal period while intoxicated. ‡ Rats were treated as described for the intoxicated group (†) but were decapitated while showing overt withdrawal signs (see text). § One rat among this group had a blood ethanol of 78 mg/dl.

withdrawal syndrome. The concentration of VMA was elevated only in the ethanol-dependent rats. It is worth mentioning that 3-methoxy-4-hydroxyphenylethanol (MHPE) (the alcoholic counterpart of HVA) was semiquantitatively evaluated in some of the acutely and chronically treated rats and compared with controls. It was found that brain concentration of this metabolite was not markedly affected by ethanol ingestion. Semiquantitative evaluation of MHPE was carried out because the gas chromatographic column employed here does not allow accurate measurement of this metabolite (Karoum, Gillin, Wyatt & Coster, 1975b).

When the concentrations of HVA plus DOPAC and MHPG plus VMA were compared in the control and acutely treated rats, the percentage increase was found to be 53.5 and 58.8, respectively. The percentage increases between the control and ethanol-dependent rats while still intoxicated were 40.8 and 50.4, respectively.

The changes in the concentration of brain HVA and DOPAC in the control and acutely treated rats following pargyline (50 mg/kg) are summarized in Figures 1 and 2, respectively. The curves for each metabolite in the control and in the treated rats were almost parallel and therefore do not suggest a blockade of the metabolites' transport across the blood brain barrier following the administration of ethanol. If the transport of the metabolites were blocked by ethanol then the slopes at any point on the curves for the treated rats will be smaller than those of the controls.

Discussion

The results reported indicate that the effect of ethanol on biogenic amine metabolism is somewhat selective, involving the catecholamines but not the phenolic amines. Thus, while the concentrations of the phenolic amine metabolites (PHPA and PHMA) were not significantly changed by ethanol, those derived from catecholamines (HVA, DOPAC, VMA and MHPG) were significantly increased (see Tables 2 and 3).

The reversal from an oxidative to a reductive pathway prevalent in the metabolism of peripheral biogenic amines was not found to occur in the rat brain for dopamine or noradrenaline. These findings are opposite to observations from human experiments where ethanol reduced the urinary excretion of 5-hydroxyindoleacetic acid (Olson, Gursey & Vester, 1960; Rosenfeld, 1960; Davis *et al.*, 1967a) and VMA (Davis *et al.*, 1967b; Bonham Carter *et al.*, 1970; Ogata *et al.*, 1971) with a concomitant increase of the corresponding alcoholic metabolites, 5-hydroxytryptophol and MHPG. A similar phenomenon was reported in the rat for metabolites of tyramine (Tacker *et al.*, 1970). The above inability of ethanol to produce a reversal in the metabolism of biogenic amines in the brain is supported by reports on the effect of ethanol on 5-hydroxytryptamine metabolism in the whole rat brain (Tyce *et al.*, 1970; Carlsson & Lindqvist, 1973) and in brain slices (Eccleston, Reading & Ritchie, 1969).

The brain metabolism of β-hydroxylated biogenic amines (noradrenaline and octopamine) have been reported by several workers (Rutledge & Jonason, 1967; Breese, Chase & Kopin, 1969a,b; Eccleston *et al.*, 1969) and predicted by a computer simulation approach to produce mostly the alcoholic metabolites (Turner, Illingworth & Tipton, 1974). Biogenic amines with no β-hydroxyl groups, on the other hand, were reported to form mostly the acidic metabolites. The results described here on brain catecholamine metabolites are in agreement with the above observations. Thus, MHPG was found to be the major metabolite of noradrenaline while HVA and DOPAC were found to be the predominant metabolites of dopamine. Furthermore, these metabolites were found to continue to predominate after acute and chronic administration of ethanol (see Table 2 and 3). In this connection, it is interesting to note that although acute ethanol treatment significantly increased the ratio of total MHPG to VMA, chronic ethanol ingestion had no effect on this ratio (see Table 2 and 3).

The observed increase in the concentration of brain catecholamine metabolites following ethanol treatment might result from the blockade of transport or an increased rate of formation; the latter could be due to blockade of catecholamine uptake or to an increased amine release and/or turnover. In order to explain how ethanol influences brain catecholamine metabolism, each of these possibilities warrants consideration.

Inhibition of the transport of dopamine metabolites across the blood brain barrier by ethanol can be excluded on the basis of direct evidence. The data presented here (Figures 1 and 2) illustrate that the rates of depletion of HVA and DOPAC after inhibition of monoamine oxidase by pargyline are not affected by ethanol treatment. However, in a recent report Tabakoff *et al.*, 1975a, claimed that ethanol inhibits 5-hydroxyindoleacetic acid transport from human spinal cord and from the mouse brain (Tabakoff *et al.*, 1975b). If this blockade could be confirmed in the rat brain employing a similar approach to that described here for HVA and DOPAC (see Figures 1 and 2), then these findings will establish for the first time a profound difference in the effect of ethanol on the metabolism of cerebral dopamine and 5-hydroxytryptamine. The failure of ethanol to alter significantly the concentrations of PHPA and PHMA indicates that the transport of these two metabolites is also not affected by ethanol.

Concentrations of ethanol and acetaldehyde within the physiological ranges (Majchrowicz & Mendelson, 1970) were reported to have no effect on the uptake of

noradrenaline by synaptic vesicles (Roach, Davis, Pennington & Mordyke, 1973; Lahti & Majchrowicz, 1974). The concentration of ethanol (0.22 M) reported by Israel, Carmichael & MacDonald, 1973, to decrease noradrenaline uptake by synaptic vesicles, is physiologically unattainable. Therefore, at the relatively moderate concentrations of blood ethanol observed in the acutely treated and ethanol-dependent rats, interference with catecholamine uptake cannot be expected to play a very important role in the explanation of the observed increase in brain catecholamine metabolites.

The confused picture on the effect of acute ethanol treatment on brain steady-state levels of catecholamines suggests a complex underlying mechanism that may or may not involve depletion. For example, ethanol was reported by some workers to decrease brain concentration of noradrenaline (Gursey *et al.*, 1959; Gursey & Olson, 1960; Carlsson, Magnusson, Svenson & Waldeck, 1973) while others could not confirm such findings (Murphy, Guze & King, 1962; Effron & Gessa, 1963; Rudas & Vaca, 1964). As for dopamine, the general consensus is that ethanol minimally affects brain dopamine (Haggendal & Lindqvist, 1961; Carlsson *et al.*, 1973; Hunt & Majchrowicz, 1974). No clear opinion, therefore, can be formulated on the possible releasing effect of ethanol *in vivo*. However, *in vitro* evidence suggests that ethanol can release dopamine (Seeman & Lee, 1974; Darden & Hunt, 1975). Whether this release is responsible in whole or part for the observed increase in the catecholamine metabolites following ethanol treatments cannot be ascertained from the present data, and therefore, further experiments are required.

Finally, increased turnover rates of dopamine and noradrenaline are the processes most likely to explain satisfactorily the observed increase (over 50%) in the molar concentrations of their metabolites. Whether the increase in the turnover rates was secondary to catecholamine release or to other phenomena is yet to be resolved. Increased turnover rates of several amines were shown to be associated with increased concentrations of their metabolites (Meek & Neff, 1973; Sharman, 1973). Therefore, the results summarized in Tables 2 and 3, indicate that during the time when ethanol is present in the body, whether after acute or chronic treatments, dopamine and noradrenaline turnover rates are increased. During withdrawal when no ethanol is present in the blood, the dopamine turnover rate either decreases or reverts to normal, while that of noradrenaline remains elevated. The increased turnover of noradrenaline suggested here after ethanol administration is partly supported by reports from several other workers (Corrodi, Fuxe & Hökfelt, 1966; Carlsson *et al.*, 1973; Hunt & Majchrowicz, 1974; Pohorecky, 1974; Pohorecky, Jaffe & Berkeley, 1974). Corrodi *et al.* (1966) could not detect any changes in the turnover of dopamine 15 min after ethanol, while Hunt & Majchrowicz (1974) observed a decrease 2 h after ethanol. Evidence for increased dopamine turnover produced by ethanol was found in two indirect studies. In the first (Carlsson *et al.*, 1973), ethanol was found to increase the production of both radioactive noradrenaline and dopamine from tritiated tyrosine. In the second, moderate doses of ethanol were found to increase the elevation in dihydroxyphenylalanine in both dopamine and noradrenaline dominated areas of the brain following inhibition of aromatic amino acid decarboxylase (Carlsson & Lindqvist, 1973). An increased turnover rate of dopamine following ethanol treatment is indicated from our results after monoamine oxidase inhibition (see Figures 1 and 2). It appears, therefore, that our data are at variance with the reports of Corrodi *et al.* (1966) and Hunt & Majchrowicz (1974) on the effects of ethanol on dopamine turnover. However, we are of the opinion that the turnover rate of dopamine as well as its steady-state levels are dynamically changing after ethanol ingestion. For these reasons, unless stringent precautions are taken to maintain a constant steady-state level of ethanol in the blood as well as sampling the concentration of dopamine at different periods during the course of the experiment, the turnover rates determined may misrepresent what is actually happening. Thus, it is possible that the finding of Corrodi *et al.* (1966) represents the average turnover rate from 15 min after ethanol treatment up to the time the experiment was concluded and that of Hunt & Majchrowicz (1974), represents the average turnover rate 2 h after ethanol administration until the end of the experiment when the blood ethanol levels were falling.

The results reported here allowed us to gain some insight into the mechanism associated with the signs and responses of ethanol withdrawal in the rat. The significant drop in the concentrations of HVA and DOPAC during withdrawal as compared with the intoxicated ethanol-dependent rats (see Table 3) together with the continuous elevation of brain VMA and MHPG clearly indicates that during withdrawal the response of the noradrenergic neurones is different from that of the dopaminergic neurones. The drop in HVA and DOPAC concentrations in the brain indicates that during the withdrawal syndrome, dopamine turnover is lower than in the intoxicated rats, suggesting a decline in dopamine turnover rate following ethanol elimination from the blood. Hunt & Majchrowicz (1974) and Darden & Hunt (1975) observed a reduction in dopamine turnover rate and release during withdrawal. More specific experiments are required to determine whether withdrawal signs and responses are associated with either decreased dopamine and/or increased noradrenaline turnover. These experiments suggest that any stress effects during the withdrawal period are distinct from other

stresses (electric shock, cold exposure) which have been shown to elevate dopamine turnover (Bliss & Ailion, 1971).

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