Alteration of central catecholamine metabolism following acute administration of ethanol

STURE LILJEQUIST*, ARVID CARLSSON, *Department of Pharmacology, University of Göteborg, Fack, S-400 33 Göteborg, Sweden*

Recent studies indicate that the stimulatory properties of small doses of ethanol on behaviour may be dependent on changes in the central catecholamine metabolism (for refs see Engel & Carlsson, 1977; Pohorecky & Newman, 1977). However, there seem to be conflicting results concerning the mode of action by which ethanol exerts its influence on central catecholamine metabolism. Thus it has been reported (1) that ethanol induces a dose-dependent increase in the catecholamine synthesis (measured as the accumulation of dopa following inhibition of central aromatic amino decarboxylase) preferentially in dopamine-rich brain areas in the rat (Carlsson & Lindqvist, 1973), (2) that the catecholamine synthesis remains unchanged after ethanol in both mice and rats (Waldeck, 1974; Bustos & Roth, 1976; Strömbom, Svensson & Carlsson, 1977), and (3) that there is a dose-related decrease of the dopamine synthesis in striatal synaptosomes in rats (Pohorecky & Newman, 1977). In several studies an increased net formation of [^{3}H]dopamine and [^{3}H]noradrenaline from [^{3}H]tyrosine has been reported (Carlsson, Magnusson & others, 1973; Svensson & Waldeck, 1973; Pohorecky & Jaffe, 1975). Furthermore, an ethanol-induced decreased release of dopamine in striatal slices was found by Darden & Hunt (1975), as well as by Gysling, Bustos & others (1976), while increased accumulation of the dopamine metabolite dihydroxyphenylacetic acid (DOPAC) was demonstrated in striatum by Bustos & Roth (1976).

The aim of the present series of experiments was to analyse further the discrepancy between the results concerning the effects of acutely administered ethanol on central catecholamine metabolism. It will be shown that acute administration of ethanol in all probability exerts its influence on catecholamine neurons by interfering with various steps in the catecholamine metabolism.

Female mice (18–20 g) of the N.M.R.I. strain (Anticimex, Sollentuna, Sweden) were used. All drugs were administered by the intraperitoneal route and given a volume of 10 ml kg^{-1} with exception of ethanol 15% v/v) which was given in a volume of 20 ml kg^{-1},3,4-dihydroxyphenylalanine (dopa) was dissolved by adding a few drops of 2M HCl; the final volume thereafter made up by 0·9% NaCl (saline). All other drugs were dissolved in saline. Doses and intervals of administration used are given below. The biochemical analyses were carried out as follows: immediately after decapitation the whole brain was dissected out and placed into ice-cold perchloric acid. Three mouse brains were pooled and weighed. The pooled brains were homogenized in 10 ml 0·4 M perchloric acid containing 5 mg $Na_2S_2O_5$ and 20 mg EDTA. After centrifugation and neutralization the extracts were purified on a strong cation exchange column (Dowex 50 W × 4) (Atack & Magnusson, 1970; Kehr, Carlsson & Lindqvist, 1972). The following spectrophotofluorimetric analyses were carried out: dopa (Kehr & others, 1972), dopamine (Carlsson & Waldeck, 1958; Atack, 1973), noradrenaline (Bertler, Carlsson & Rosengren, 1958), 3-methoxytyramine (Kehr, 1974), and normetanephrine (normetanephrine was eluted together with dopamine, see above. Before the fluorimetric assay 0·1 ml 1M Na_2CO_3 was added to the eluate to destroy the dopamine present. The oxidation of normetanephrine was then performed mainly according to Carlsson & Lindqvist, 1962).

Statistical significances were calculated by Student's *t*-test.

To study the effect of acutely administered ethanol on the formation of 3-methoxytyramine which is assumed to take place after the nerve impulse-mediated dopamine release (Kehr, 1976), the animals were pretreated with ethanol, 2·36 g kg^{-1} (i.p., 90 min before decapitation) i.e. a dose of ethanol which markedly stimulates locomotor activity in mice (for refs see Engel & Carlsson, 1977), or with saline. Thirty min later the monoamine-oxidase inhibitor pargyline 100 mg kg^{-1} (i.p., 60 min before decapitation) was given. The effects of this pretreatment on the accumulation of the *O*-methylated dopamine metabolite 3-methoxytyramine and on the *O*-methylated noradrenaline metabolite normetanephrine, respectively, were studied. As will be seen from Table 1 the administration of a small dose of ethanol markedly retarded the formation of 3-methoxytyramine ($P < 0.005$). No effect of ethanol on the accumulation of normetanephrine could be observed.

Table 1. *Effect of acute ethanol administration on the formation of O-methylated metabolites in whole brain of mice.* Ethanol (2·36 g kg^{-1}, 15% v/v) was given intraperitoneally followed 30 min later by 100 mg kg^{-1} pargyline, 60 min later the animals were decapitated. Concentrations are given in mg g^{-1} ± s.e.m. (n = number of determinations in parentheses). Statistics by *t*-test.

	3-Methoxytyramine	Normetanephrine
Saline	137 ± 20 (9)	105 ± 3 (4)
Ethanol	65 ± 8 (9)	104 ± 5 (4)
	$P < 0.005$	N.S.

* Correspondence.

These results might be interpreted to mean that acute administration of small doses of ethanol inhibit release of dopamine without affecting the release of noradrenaline. To analyse further this possibility, ethanol- and saline-pretreated (i.p.., 90 min before decapitation) animals were injected with 50 mg kg^{-1} benserazid and 100 mg kg^{-1} L-dopa (i.p., 65 min before decapitation) followed 5 min later by 400 mg kg^{-1} α-methyltyrosine (α-MT) and 100 mg kg^{-1} pargyline (i.p., 60 min before decapitation). The effects of dopa loading on the accumulation of dopa, dopamine, noradrenaline, 3-methoxytyramine, and normetanephrine in ethanol- and saline-pretreated animals are shown in Table 2. As will be seen, ethanol significantly ($P < 0.05$) increased the accumulation of dopa in ethanol-pretreated animals following dopa loading. On the other hand, the concentrations of dopamine were significantly ($P < 0.001$) lower in animals treated with ethanol compared with animals pretreated with saline. No effect of the ethanol pretreatment on the concentrations of noradrenaline could be observed. As in the first series of experiments, the formation of the dopamine metabolite 3-methoxytyramine was markedly retarded in ethanol-treated animals ($P < 0.001$). No difference in the accumulation of normetanephrine after dopa loading was observed.

Table 2. *Catechols and O-methylated metabolites in whole brain of mouse after dopa loading: effect of acute ethanol administration.* Ethanol 2·36 g kg^{-1} or saline was given intraperitoneally followed in 25 min by 50 mg kg^{-1} benserazid and 100 mg kg^{-1} L-dopa. After a further period of 5 min 400 mg kg^{-1} α-methyltyrosine and 100 mg kg^{-1} pargyline were given. 60 min later the animals were decapitated. Mean values are ng g^{-1} ± s.e.m. of concentrations from 8 determinations are given. Statistics by *t*-test.

	Dopa	Dopamine	3-Methoxytyramine	Noradrenaline	Normetanephrine
Saline	25986 ±1618	2678 ±40	1527 ±79	846 ±54	95 ±16
Ethanol	30,442 ±1316	1,573 ±124	432 ±32	807 ±12	76 ±17
	$P<0.05$	$P<0.001$	$P<0.001$	N.S.	N.S.

These results indicate that a small dose of ethanol (2·36 g kg^{-1}), which functionally has stimulatory effects on the behavioural activity of mice (see above), appears to influence the metabolism of central catecholamines in a complex manner. The conversion of dopa into dopamine is retarded, possibly due to inhibition of dopa decarboxylase, as indicated by increased concentrations of dopa simultaneously with decreased concentrations of dopamine. Moreover, the formation of 3-methoxytyramine from dopamine is markedly retarded. Whether this retardation is entirely due to lowered substrate availability, or whether the COMT activity is also inhibited, cannot be decided from the present data.

A puzzling phenomenon is the inability of ethanol to inhibit the accumulation of noradrenaline from dopa and its further conversion into normetanephrine, whereas the formation of dopamine and 3-methoxytyramine is markedly inhibited. These observations suggest that we are dealing with a selective action on dopamine neurons leaving the noradrenaline neurons unaffected. Since the dopa decarboxylase and COMT of the two type of neurons are believed to be very similar if not identical, it is at present difficult to envisage the mechanism involved. Theoretically a selective action on the cell membrane of dopaminergic neurons might account for the following sequence of effects, (1) reduced uptake of dopa by the dopaminergic neuron, leading to reduced conversion of dopa to dopamine, (2) reduced release of dopamine from dopaminergic neurons leading to a more marked inhibition of 3-methoxytyramine formation than would be expected from the moderately lowered dopamine concentrations. The dopa concentration of the brain after a loading dose of dopa does not give any information about the dopa concentration in the cytoplasm of dopaminergic neurons. The latter may very well be lowered in spite of an increase of the former.

Taken together, the previous and the present observations indicate that ethanol, even in small doses, may alter the metabolism of central catecholamines by affecting several different mechanisms. In all probability ethanol influences these mechanisms to various degrees depending on prevailing experimental conditions, which perhaps at least partially could explain earlier conflicting results concerning the effects of ethanol on central catecholamine function. Which these mechanisms primarily are is far from clear. However, change in the activity of the various enzymes involved in the metabolism of central catecholamines as well as alteration of different membrane properties, perhaps preferentially related to dopamine neurons, might turn out to be factors of importance when the effects of ethanol on central catecholamine mechanisms will be further elucidated.

An interaction between ethanol and the other drugs used in the present study may have influenced our results.

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Distribution of ampicillin in human whole blood

M. EHRNEBO, *Karolinska Pharmacy, Karolinska Hospital, Fack, S*-104 01, *Stockholm* 60, *Sweden*

Ampicillin is an antibiotic with a low degree of plasma protein binding (Ehrnebo, Agurell & others, 1971). It has previously been shown by several authors that many drugs are bound to the human red blood cells (cf. Ehrnebo, Agurell & others 1974; Hinderling, Bres & Garrett, 1974; Ehrnebo & Odar-Cederlöf, 1977). Suggested mechanisms are binding to intracellular haemoglobin (Wind, Berliner & Stern, 1973), carbonic anhydrase (Beerman, Hellström & others, 1975) and the erythrocyte membrane (Kwant & Seeman, 1969). However, few data are available in the literature on the distribution of ampicillin in human whole blood. In the following study, the equilibrium dialysis technique was used to study the distribution of [^{35}S]ampicillin to the blood cells, plasma proteins and plasma water of human whole blood.

Heparinized whole blood was drawn from five healthy male volunteers, aged 23–28 years. Human plasma, whole blood or a suspension of washed blood cells (500 μl) was introduced on one side of the dialysis membrane (Visking dialysis tubing) in cells of lucite (Ehrnebo & others, 1971, 1974). On the other side was applied [^{35}S]ampicillin (radiochemical purity >98%) in isotonic phosphate buffer pH 7·4. The cell was equilibrated for 5 h at 37°. There was no significant breakdown of ampicillin under these conditions as measured by microbiological assay. Samples (100 μl) were withdrawn from each side and concentration of drug in plasma (C_P), whole blood (C_B), blood cell suspension (C_{BCS}) and buffer solution (C_{BU}) was determined by liquid scintillation counting (Packard Tri-Carb model 3320) by means of the external standard channel ratio method. Samples containing red blood cells were digested with Soluene-100-isoproprenol (1:1) (Packard Instruments) and 30% hydrogen peroxide before adding the scintillation liquid (Instagel, Packard Instruments). The recovery of radioactivity in the digestion procedure was 96·7 s.d. 1·4% (n = 4) and correction for the loss of radioactivity was made before the calculations. The suspension of washed blood cells was prepared by washing the red cell fraction five times with isotonic phosphate buffer and reconstitution to the original haematocrit (McArthur, Dawkins & Smith, 1971). The fraction bound in plasma (f_P) was calculated as

$$f_P = (C_P - C_{BU})/C_P \quad (1)$$

The fraction distributed to the blood cells (λ_{BC}), plasma proteins (λ_{PP}) and plasma water (λ_{PW}) of the whole blood and blood cells (λ_{BCS}) of blood cell suspension was determined according to Ehrnebo & others (1974) as

$$\lambda_{BC} = 1 - C_{PW}(1\text{-}H)/(1\text{-}f_P)C_B \quad (2)$$

$$\lambda_{PP} = C_{PW} f_P (1\text{-}H)/(1\text{-}f_P)C_B \quad (3)$$

$$\lambda_{PW} = C_{PW}(1\text{-}H)/C_B \quad (4)$$