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Effect of ethanol on the hydroxylation of tyrosine and tryptophan in rat brain *in vivo*

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Various doses of ethanol were injected intraperitoneally into rats. After 20 min, 3-hydroxybenzylhydrazine (NSD 1015), an inhibitor of aromatic amino-acid decarboxylase was injected and 30 min later the animals were killed. The amount of dopa accumulating in brain as a consequence of decarboxylase inhibition was significantly increased by moderate doses of ethanol (up to 4 g kg^{-1}). There was no corresponding effect on 5-hydroxytryptophan. The effect on dopa was found both in a dopamine- and a noradrenaline-dominated area of the brain, suggesting that catecholamine synthesis is stimulated by ethanol in both types of neuron. The data lend support to the hypothesis of Carlsson, Engel & Svensson (1972) that cerebral catecholamines are involved in the central stimulation induced by ethanol.

We previously reported that a large dose of ethanol, given orally to mice, increased the net yield of [^{3}H]dopamine and [^{3}H]noradrenaline, formed in the brain from intravenously injected [^{3}H]tyrosine (Carlsson, Magnusson & others, 1973). Also the ratio of [^{3}H]dopamine to [^{3}H]noradrenaline was increased by this treatment. Since ethanol was found to decrease brain catecholamine levels and enhance their metabolism (cf. Corrodi, Fuxe & Hökfelt, 1966), the data suggested a stimulating action of this agent on the synthesis of catecholamines in the brain.

Studies in mice and rats showed that the well-known stimulating action of moderate doses of ethanol (1–2 g kg^{-1}) on motility could be prevented by α-methyl-*p*-tyrosine, an inhibitor of tyrosine hydroxylase, given in doses which caused no significant decrease in motility (Carlsson, Engel & Svensson, 1972). These observations have been supported by experiments in man (Ahlenius, Carlsson & others, 1973) and suggest that some central actions of ethanol may depend at least in part on intact catecholamine synthesis.

In the above biochemical experiments, large doses of ethanol, causing severe intoxication, were required to demonstrate an increased yield of [^{3}H]catecholamines. It seemed questionable whether an increased catecholamine synthesis might play a causative role in the central stimulation induced by moderate doses of this agent.

In this laboratory a direct *in vivo* method for investigating the probable rate-limiting step of catecholamine synthesis, *i.e.* the hydroxylation of tyrosine, has been developed (Carlsson, Davis & others, 1972). This method measures the accumulation of 3,4-dihydroxyphenylalanine (dopa) after the administration of an inhibitor of aromatic amino-acid decarboxylase. Unpublished observations in this laboratory suggest that, at least under certain conditions, this method is more sensitive than previous more indirect techniques for measuring catecholamine synthesis.

In the present investigation we have examined the effect of moderate doses of ethanol on catecholamine synthesis, using the new method referred to above.

METHODS

Male Sprague-Dawley rats, 210–300 g, were fed on commercially available pellets (Anticimex, Stockholm, Sweden). Ethanol, 20% (w/v), was injected intraperitoneally in various doses, followed after 20 min by the aromatic amino-acid decarboxylase inhibitor NSD 1015 (3-hydroxybenzylhydrazine HCl, Smith & Nephew Research Ltd., Gilston Park, Harlow, England), 100 mg kg^{-1}. The animals were decapitated 30 min after the injection of NSD 1015.

The whole brain (without the olfactory lobes) was dissected and placed on a glass plate over ice. In one series of experiments the brain was dissected as follows. The corpus callosum was sectioned in the midline and the lateral ventricles opened. The dopamine-rich limbic areas containing *e.g.* the olfactory tubercles and nucleus accumbens (medial part) were dissected by exposing the anterior commissure in its entire course. A cut was made from the anterior commissure to the lateral border of the olfactory tubercle. Thus, a brain part was obtained located rostrally and medially to the anterior commissure, rostrally to the optic chiasm and including the olfactory tubercles. Thereafter, the corpora striata were removed and then the rest of the hemispheres (including hippocampus), and the cerebellum. The remaining brain stem was divided by a section from the rostral border of the corpora quadrigemina to the rostral border of the pons. Thus, the following brain parts were obtained: (1) the limbic dopamine-rich part ,(2) the corpora striata, (3) the remainder of the hemispheres (referred to as hemispheres), (4) diencephalon, (5) lower brain stem, and (6) cerebellum.

Immediately after dissection the brain parts were frozen on dry ice. The parts of 3 rats were pooled and weighed.

The pooled brain parts, or in another series of experiments, single whole brains were homogenized in 10 ml 0·4 N perchloric acid containing 5 mg $Na_2S_2O_5$ and 20 mg EDTA. The extracts were purified on a strong cation exchange column (Dowex 50) (Atack & Magnusson, 1970; Kehr, Carlsson & Lindqvist, 1972). The following spectrophotofluorimetric analyses were made: tyrosine (Waalkes & Udenfriend, 1957), dopa (Kehr & others, 1972), dopamine (Carlsson & Waldeck, 1958, modified by Atack, 1973), noradrenaline (Bertler, Carlsson & Rosengren, 1958), tryptophan and 5-hydroxytryptophan (5-HTP) (see Bédard, Carlsson & Lindqvist, 1972).

RESULTS

Various doses of ethanol were injected intraperitoneally and the decarboxylase inhibitor NSD 1015 was injected 20 min afterwards. After another 30 min the rats were killed. Fig. 1 shows the effect of ethanol pretreatment on the amount of dopa accumulating in the whole brain during the 30 min following decarboxylase inhibition. A dose-dependent increase approaching 50% after the highest dose (4 g kg^{-1}) is seen. There was no concomitant change in tyrosine, 5-HTP or tryptophan levels (Table 1).

Ethanol enhanced the accumulation of dopa both in the striatum, i.e. a dopamine-rich area, and in the hemispheres, i.e. an area where noradrenaline predominates (Table 2). However, the effect appeared to be more pronounced in the striatum than in the hemispheres. In other brain parts a positive but not significant correlation between the dopa level and the dose of ethanol was found.

Ethanol did not cause any significant change in the level of dopamine or noradrenaline in any of the brain regions examined.

Table 1. *Amino-acids in rat brain after treatment with various doses of ethanol, followed by an inhibitor of aromatic amino-acid decarboxylase.* Ethanol was injected i.p. 50 min and NSD 1015 (100 mg kg^{-1}) 30 min before death. Control rats received NSD 1015 only. Single whole brains were analysed. The means ± standard errors of the means are shown. Figures within brackets indicate number of experiments (rats).

	Dopa ng g^{-1}	Tyrosine $\mu g\ g^{-1}$	5-HTP ng g^{-1}	Tryptophan $\mu g\ g^{-1}$
Control	154 ± 4 (12)	17·1 ± 1·10 (8)	133 ± 17 (8)	5·4 ± 0·21 (8)
Ethanol 1 g kg^{-1}	191 ± 8* (8)	15·1 ± 0·60 (4)	144 ± 20 (8)	5·7 ± 0·30 (8)
Ethanol 2 g kg^{-1}	181 ± 10† (8)	17·1 ± 0·54 (4)	131 ± 11 (8)	4·9 ± 0·28 (8)
Ethanol 4 g kg^{-1}	227 ± 20‡ (4)	15·9 ± 0·39 (4)	—	—

* Differs from control ($P < 0.005$) and from 4 g kg^{-1} ($P < 0.025$).
† Differs from control ($P < 0.025$) and from 4 g kg^{-1} ($P < 0.005$).
‡ Differs from control ($P < 0.001$). (Analysis of variance + *t*-test.)

Table 2. *Effect of various doses of ethanol on the accumulation of dopa in rat brain regions induced by an inhibitor of the aromatic amino-acid decarboxylase.* Ethanol was injected i.p. 50 min and NSD 1015 (100 mg kg^{-1}) 30 min before death. Control rats received NSD 1015 only. The means ± standard errors of the means are shown. Each value comprises pooled parts of 3 rat brains.

	Dopa, ng g^{-1}			
	Striatum	Hemisphere	Lower brain stem	No. of experiments
Control	372 ± 19	71 ± 4	96 ± 11	3
Ethanol 0·5 g kg^{-1}	455 ± 27	78 ± 5	122 ± 5	2
Ethanol 1 g kg^{-1}	575 ± 36	76 ± 6	114 ± 13	3
Ethanol 2 g kg^{-1}	492 ± 36	89 ± 3	122 ± 11	3
Ethanol 4 g kg^{-1}	690	84	139	1
Correlation coefficient	0·72	0·58	0·56	
P	<0·01	<0·05	<0·05	

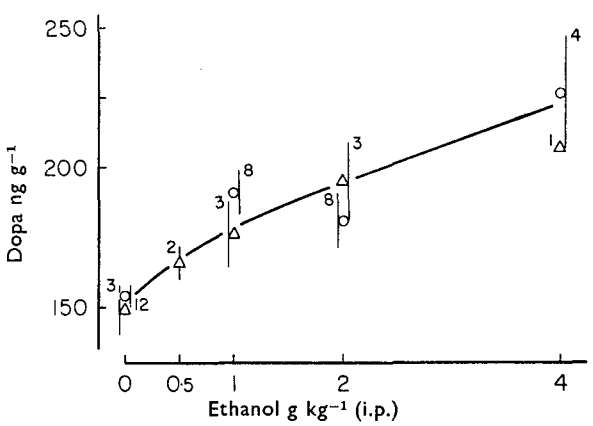

FIG. 1. Dopa in whole rat brain after various doses of ethanol, followed by an inhibitor of the aromatic amino-acid decarboxylase. Ethanol was injected i.p. 50 min and NSD 1015 (100 mg kg^{-1}) 30 min before death. The means ± s.e. and the number of experiments are shown. ○ Individual whole brains analysed. △ Pooled brain parts from three animals analysed (see Methods); whole brain data calculated from brain part data.

Three rats were given ethanol 4 g kg^{-1} (i.p.) and killed 50 min later. Their whole brains were analysed for dopa and 5-HTP. No dopa and small, probably insignificant amounts of 5-HTP (about 10 ng g^{-1}) were detected. Thus the accumulation of dopa observed in brain slices exposed to ethanol *in vitro* (Kaniike & Yoshida, 1963) did not take place under the present *in vivo* conditions.

DISCUSSION

In the present investigation a dose-dependent effect of ethanol on the amount of dopa accumulating in the brain after decarboxylase inhibition has been found. This may indicate that ethanol in moderate dosage enhances the rate of tyrosine hydroxylation in rat brain. The effect was found in areas rich in both dopamine and noradrenaline and thus the effect is probably exerted on both types of catecholamine-carrying fibre systems. However, the effect on dopamine synthesis appears to be more pronounced. These observations are in excellent agreement with our earlier observations on mice (see Introduction). However, the isotope method used in that study may be less sensitive, in that larger doses of ethanol were required to obtain a detectable effect.

The accumulation of 5-HTP, induced by decarboxylase inhibition, was not influenced by ethanol, indicating that we are dealing with a selective action on catecholamine synthesis. This is further supported by the absence of a change in tyrosine or tryptophan levels. The reason for this accumulation of dopa remains obscure. Perhaps ethanol stimulates the impulse flow in catecholamine-carrying nerve fibres. However, other possibilities cannot be excluded.

In any event the present observations lend additional support to the hypothesis that the central action of ethanol is at least partly mediated by an effect on the brain catecholamines (see introduction).

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