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The effect of ethanol on the activity of central catecholamine neurones in rat brain

SIR,—There are no apparent changes in the dopamine, noradrenaline or 5-hydroxytryptamine levels in rabbit and rat brain after administration of ethanol (Häggendal & Lindqvist, 1961; Efron & Gessa, 1963). But although the amine levels are unaffected, this does not exclude the possibility that the activity of the central monoamine neurones may be influenced by ethanol. As a result of the development of inhibitors of the rate-limiting step in catecholamine synthesis it has now become possible to examine the activity of the central catecholamine neurones directly at cellular level by the histochemical fluorescence technique (Hillarp, Fuxe & Dahlström, 1966). These experiments showed that the release and synthesis of amine is dependent on neuronal activity (Fuxe & Gunne, 1964; Dahlström, Fuxe, Kernell & Sedvall, 1965; Andén, Corrodi, Dahlström, Fuxe & Hökfelt, 1966; Corrodi & Malmfors, 1966). H 44/68 (DL-α-methyl-tyrosine-methylester) used in this and previous studies (Corrodi, Fuxe & Hökfelt, 1966a) inhibits the biosynthesis of noradrenaline and dopamine without affecting the uptake-storage mechanism of the amine granules (Andén & others, 1966; Corrodi, Fuxe & Hökfelt 1996b; Corrodi & Hanson, 1966).

The present communication affords evidence that changes do occur in the central catecholamine neurones during treatment with ethanol as revealed by both histochemical and biochemical techniques.

Male, Sprague-Dawley rats (150–250 g) were treated with ethanol (2 g/kg as a 5% solution i.p. and H 44/68 (250 mg/kg i.p.). Some animals were given one injection of ethanol and this was followed by H 44/68 15 min later. The rats were then killed 2, 4 or 6 hr later. Other animals were given two doses of ethanol, H 44/68 was administered to these 15 min after the first dose and 4 hr before death; the second dose of ethanol was given 1½ hr before death by which time the animals were asleep without a righting reflex. The whole brains were dissected and analysed separately for dopamine and noradrenaline (Bertler, Carlsson & Rosengren, 1958; Carlsson & Waldeck, 1958; Carlsson & Lindqvist, 1962). Control rats were given either ethanol or H 44/68. The rectal temperature in all animals was found to be normal. After the ethanol the animals showed no signs of peritoneal pain nor did the peritoneal cavity show inflammation.

In the histochemical study the effect of two different doses of ethanol (1 and 2 g/kg) was investigated. Ethanol was administered intraperitoneally once or twice as described above. The animals were killed 4 hr after the i.p. injection of H 44/68 (250 mg/kg) which was given 15 min after the ethanol. Other rats were given ethanol (2 g/kg) by mouth and after H 44/68 treatment as described they

were killed. Control rats were treated with either ethanol or H 44/68. The animals were decapitated under light chloroform anaesthesia; the medulla oblongata, the pons, the mesencephalon, the diencephalon and large parts of the telencephalon were dissected, freeze-dried, treated with formaldehyde gas for 1 hr, embedded in paraffin, sectioned and mounted (Dahlström & Fuxe, 1964; Hamberger, Malmfors & Sachs, 1965).

After treatment with ethanol alone no significant changes were observed in the catecholamine levels of the brain. But, in the animals also treated with H 44/68, there was a greater decrease of noradrenaline but not dopamine in the brain compared with the rats receiving only H 44/68 (Table 1). After two doses of

TABLE 1. NORADRENALINE AND DOPAMINE CONCENTRATIONS IN RAT BRAIN 2, 4 AND 6 HR AFTER ETHANOL (2 G/KG, I.P.) AND H 44/68 (250 MG/KG, I.P.) 15 MIN LATER.
(percent of normal values ± s.e.m.)

Treatment	No. of experiments	Noradrenaline %	Dopamine %
Untreated	30	100·0 ± 2·0	100·0 ± 2·5
2 hr			
Ethanol	4	94·3 ± 3·2	95·0 ± 5·1
H 44/68	4	75·4 ± 1·3	41·9 ± 2·9
Ethanol + H 44/68	4	* 66·7 ± 2·4	47·2 ± 1·8
		* (P < 0·01)	
4 hr			
Ethanol	6	92·1 ± 2·2	88·7 ± 6·2
H 44/68	6	58·7 ± 1·5	26·9 ± 3·0
Ethanol + H 44/68	6	* 44·7 ± 2·4	27·8 ± 2·2
		* (P ~ 0·001)	
6 hr			
Ethanol	4	93·2 ± 3·1	90·0 ± 3·1
H 44/68	4	41·5 ± 1·3	17·4 ± 2·8
Ethanol + H 44/68	4	38·9 ± 1·3	22·2 ± 2·5

TABLE 2. NORADRENALINE AND DOPAMINE CONCENTRATIONS IN RAT BRAIN 4½ AND 1½ HR AFTER ETHANOL (2 G/KG, I.P.) AND 4 HR AFTER H 44/68 (250 MG/KG I.P.).
(Percent of normal values ± s.e.m.)

Treatment	No. of experiments	Noradrenaline %	Dopamine %
Untreated	30	100·0 ± 2·0	100·0 ± 2·5
Ethanol	4	87·4 ± 1·9	98·8 ± 5·0
H 44/68	4	54·0 ± 2·8	29·2 ± 2·7
Ethanol + H 44/68	4	* 33·6 ± 3·1	22·6 ± 1·2
		* (P ~ 0·0005)	

ethanol this decrease of noradrenaline was greater (Table 2). The difference between the test and control animals was most marked 4 hr after H 44/68 injection, somewhat less after 2 hr and not significant after 6 hr. No definite effects could be observed histologically in the dopamine and noradrenaline levels of central monoamine neurones in the parts of the brain 4 hr after ethanol. In the animals given the higher dose of ethanol intraperitoneally twice with H 44/68, the reduction of amine fluorescence in the specific noradrenaline—but not in the dopamine—nerve terminals of the brain was more marked than that observed after treatment with H 44/68 alone. After a single dose of 2 g/kg an accelerated depletion of noradrenaline was observed only in *some* rats, while others showed no definite changes in the rate of fluorescence disappearance compared to controls. Most of the noradrenaline nerve terminal systems of the brain seemed to be affected, e.g. those innervating the nucleus supraopticus,

nucleus paraventricularis, the preoptic area, the nucleus tractus solitarii and nucleus motorius dorsalis n. vagi. The amine levels of the nerve cell-bodies in the various catecholamine cell-groups showed about the same decreases after H 44/68 treatment whether ethanol had been given or not.

The present findings demonstrate that the central noradrenaline neurones are specifically activated, directly or indirectly, 2 to 4 hr but not 6 hr after acutely administered ethanol. This increase in activity would thus result in an increased release and synthesis of noradrenaline which could be revealed after inhibition of synthesis. These findings may explain the inhibitory effects of ethanol on antidiuretic hormone secretion (Hirvonen, Karlsson & Virtanen, 1966) and on oxytocin secretion (Fuchs, 1966), since the noradrenaline nerve terminals surrounding these nuclei probably are inhibitory in function (Fuxe & Hökfelt, 1966). Whether this central effect of ethanol and its known effect on animal and human behaviour to related is not yet known.

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