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Monoamine Metabolism in Rat Brain Regions Following Long Term Alcohol Treatment

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Summary

Female Wistar rats (150—200 g) were treated with ethanol (15 % w/v) for 21 days and compared with control rats given water. Ethanol administration produced a reduction of fluid and food consumption and changes in the metabolism of cerebral monoamines. There was an increase in serotonin (5-HT) turnover statistically significant in the striatum, and a decrease in noradrenaline (NA) turnover in ethanol rats as compared to controls.

Endogenous NA levels were significantly increased in the diencephalon and dopamine (DA) levels were increased in the striatum. After inhibition of catecholamine synthesis with α-methyltyrosine (α-MT), NA depletion was significantly retarded but no changes in DA depletion were noted. DOPA accumulation after decarboxylation inhibition showed no significant change in any brain region studied.

Introduction

Although the ability of alcohol to produce tolerance and physical dependence has long been acknowledged, little progress has been made to clarify the mechanisms causing these conditions. Previous studies of the effects of chronic ethanol administration on animal brain monoamine concentration, synthesis or turnover have yielded contradictory results largely because of differences in species and/or strains of animals tested, doses of ethanol, routes of administration, treatment duration, choice of control groups, and methods utilized.

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Different strategies of ethanol administration (see review in *Mello*, 1973) have been successful in producing steady high levels of ethanol in blood, however, all methods used such as induced ethanol drinking (*Ratcliffe*, 1972; *Cicero* and *Smithloff*, 1973); ethanol liquid diet (*Freund*, 1969; *Branchey et al.*, 1971; *Ogata et al.*, 1972); inhalation (*Goldstein* and *Pal*, 1971; *Goldstein*, 1972); schedule-induced polidypsia (*Falk*, 1972); and gastric intubation (*Essing* and *Lam*, 1968; *Wallgren* and *Barry*, 1970; *Majchrowicz*, 1975) have been based on forced administration of ethanol.

No consistent findings have been reported concerning changes in the monoamine metabolism during and after prolonged administration of ethanol (*Corrodi et al.*, 1966; *Post* and *Sun*, 1973; *Carlsson* and *Lindqvist*, 1973; *Carlsson et al.*, 1973; *Frankel et al.*, 1974; *Liljequist et al.*, 1975; *Pohorecky et al.*, 1974; *Hunt* and *Majchrowicz*, 1974 a, b; *Griffiths et al.*, 1974; *Ahtee* and *Svartström-Frazer*, 1975; *Pohorecky et al.*, 1978). We have studied the influence of free ethanol drinking treatment on monoamine synthesis and turnover in rat brain regions, as well as on fluid and food consumption.

Materials and Methods

Forty-eight female, albino Wistar rats (154 ± 6 g at the start of each experiment) were housed four to a cage and maintained on purina chow diet and water "ad libitum" with artificial daylight from 9 a.m. to 9 p.m. Ethanol was administered freely in drinking water (15 % w/v) while control rats drank plain water. Three groups of rats (16 animals in each) were used for the following determinations:

Endogenous monoamine and 5-HIAA levels in rat brain regions.

Regional DOPA and 5-HTP accumulation 30 min after inhibition of L-aromatic amino acid decarboxylase, by NSD 1015 (3-hydroxybenzylhydrazine, HCl; 100 mg/kg i.p.). Tyrosine and tryptophan levels.

Cerebral monoamine depletion 1 hour after tyrosine hydroxylase inhibition with α-methyltyrosine (α-MT, 250 mg/kg i.p.).

Animals were sacrificed by decapitation between 10 a.m. and 12 a.m., and the whole brain was immediately dissected on an ice-cold glass Petri dish according to the method of *Carlsson* and *Lindqvist* (1973) into: (1) dopamine-rich limbic portion containing the olfactory tubercle, nucleus cinguris, and allocortex from the medial basal surface of the temporal lobe; (2) the corpora striata; (3) the rest of the cerebral hemispheres; (4) the diencephalon; and (5) the lower brain stem.

After dissection, brain parts were frozen on dry ice, weighed and stored at -80 °C. The extraction of amines, precursors and metabolites was performed after homogenization in 25 ml plastic tubes containing 10 ml of ice-cold 0.4 N perchloric acid, 0.2 ml of 10 % EDTA and 0.1 ml of 5 %

$Na_2S_2O_5$. The extract was purified on a strongly acidic cation exchange column (Dowex 50W-X4) according to *Atack* and *Magnusson* (1970). Fluorimetric analyses were made for: NA (*Bertler et al.*, 1958); DA (*Atack*, 1973); 5-HT, 5-HIAA and 5-HTP (*Atack* and *Lindqvist*, 1973); DOPA (*Kehr et al.*, 1972); tyrosine (*Waalkes* and *Udenfriend*, 1957); and tryptophan (*Bédard et al.*, 1972).

The recovery of the columns was between 70 and 100 % and uncorrected results were used. Statistics were done by Student's test and values were considered significant when $P < 0.05$.

Results

Fluid and Food Consumption

As seen in Table 1, the group of water control animals had significantly higher fluid and food intake than the ethanol treated animals. In the ethanol group, 25 % of the calory intake corresponded to ethanol and their total daily caloric intake was slightly lower than in controls (Table 1).

Table 1. *Effect of ethanol treatment (15 % w/v) on fluid and food consumption in the rats*

Group	Food intake* g/24 hours	Fluid intake ml/24 hours	Total kcal/ 24 hours
Ethanol (N = 24)	11.48 ± 0.45	12.84 ± 0.64 (9.26 g/kg/day)	45.15
Control (N = 24)	18.31 ± 0.67	33.58 ± 1.24	53.86
P	< 0.001	< 0.001	

Length of treatment: 21 days.
* Mean of all treatment period.
Results are means ± S.E.M.

DOPA and 5-HTP Formation After NSD 1015

No changes in DOPA accumulation were observed in any of the brain regions studied in ethanol treated or control animals. However, there was a statistically significant increase in 5-HTP accumulation in the striatum of ethanol treated rats as compared with their controls (Table 2). Tyrosine levels tended to increase in the alcoholic rat, reaching significance in the brain stem (Table 2). Tryptophan levels in the alcoholic animals increased significantly in the striatum, hemispheres and brain stem regions (Table 2).

Table 2. *Regional DOPA and 5-HTP accumulation, 30 min after decarboxylation inhibition by NSD 1015 (3-hydroxybenzylhydrazine, HCl; 100 mg/kg i.p.)*

	DOPA (ng/g)		5-HTP (ng/g)		Tyr (μg/g)		Trip (μg/g)	
	Control	Alcohol	Control	Alcohol	Control	Alcohol	Control	Alcohol
Limbic	230 ± 36	233 ± 19	172 ± 17	173 ± 8	15.18 ± 0.40	16.28 ± 0.74	5.14 ± 0.19	5.9 ± 0.36
Striatum	408 ± 60	390 ± 26	92 ± 9	121 ± 5*	18.11 ± 1.84	19.71 ± 2.48	5.02 ± 0.18	5.86 ± 0.24*
Hemispheres	70 ± 6	72 ± 3	103 ± 5	98 ± 4	12.80 ± 1.23	13.79 ± 0.28	4.99 ± 0.37	7.79 ± 0.55**
Diencephalon	253 ± 21	238 ± 11	212 ± 29	245 ± 23	14.41 ± 1.03	16.89 ± 0.71	4.51 ± 0.12	5.29 ± 0.54
Brain stem	138 ± 3	127 ± 7	207 ± 12	238 ± 23	13.06 ± 0.23	15.57 ± 0.44**	4.02 ± 0.12	5.42 ± 0.53*
No. of experiments	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)

The means ± S.E.M.

P values of the differences between alcohol and control groups are shown by asterisks: * = $p < 0.05$, ** = $p < 0.01$.

Table 3. *Endogenous monoamine and 5-HIAA levels in rat brain regions after 21 days of ethanol (15 % w/v)*

	NA (ng/g)		DA (ng/g)		5-HT (ng/g)		5-HIAA (ng/g)	
	Control	Alcohol	Control	Alcohol	Control	Alcohol	Control	Alcohol
Limbic	457 ± 41	460 ± 39	2373 ± 151	2310 ± 143	765 ± 74	733 ± 55	172 ± 36	116 ± 23
Striatum	223 ± 22	234 ± 37	4067 ± 211	5290 ± 215*	495 ± 31	656 ± 42*	197 ± 29	209 ± 7
Hemispheres	238 ± 13	263 ± 14	—	—	378 ± 24	410 ± 31	52 ± 11	68 ± 5
Diencephalon	432 ± 21	528 ± 27*	158 ± 66	268 ± 22	1048 ± 120	1028 ± 52	123 ± 10	124 ± 43
Brain stem	368 ± 16	401 ± 23	49 ± 14	23 ± 7	724 ± 60	878 ± 77	90 ± 11	127 ± 10
No. of experiments	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)

Results are means ± S.E.M.

P values of the differences between alcohol and control groups are shown by asterisks: * = $p < 0.05$.

Endogenous Monoamine and 5-HIAA Levels

NA—There was an increase in NA in alcoholic rats, as compared to water control rats, that reached statistical significance in the diencephalon (Table 3).

DA—The level of DA in the striatum of alcoholic rats was higher than in controls. No significant changes in DA were observed in the other regions studied in both groups (Table 3).

5-HT—There was an increase of 5-HT in the striatum of the ethanol treated rat as compared to water control animals, but no changes were found in any other brain regions (Table 3).

5-HIAA—No changes were noted in 5-hydroxyindoleacetic acid content in either group (Table 3).

Monoamine Levels After Catecholamine Synthesis Inhibition

NA level in the ethanol rats after α-MT was significantly increased as compared to controls (Table 4). No changes were found in DA depletion after inhibition of tyrosine hydroxylase (Table 4).

Table 4. *Whole brain monoamine levels 1 hour after tyrosine hydroxylase inhibition with MT (250 mg/kg i.p.)*

NA (ng/g)			DA (ng/g)		
Control	Alcohol	P	Control	Alcohol	P
310 ± 32 (7)	480 ± 25 (8)	< 0.01	522 ± 32 (7)	532 ± 60 (8)	n.s.

Results are means $\pm$ S.E.M.

Discussion

Ethanol treatment did not alter the CA synthesis rate measured as the accumulation of DOPA in any of the dissected brain parts studied, and similar results have been reported by *Liljequist* and *Engel* (1977). However, there was an increased level of DA in the striatum, as reported also by *Post* and *Sun* (1973) and an increase in NA in all dissected regions that reached statistical significance in the diencephalon (increased cerebral NA levels have also been reported by *Post* and *Sun*, 1973; *Littleton et al.*, 1974; *Chopde et al.*, 1977).

After tyrosine hydroxylase inhibition with α-MT, we found a significant retarded depletion in NA in the whole brain and no changes

in DA turnover, in agreement with other reports (*Corrodi et al.*, 1966; *Ahtee* and *Svartström-Frazer*, 1975). These results indicate that chronic ethanol treatment may induce a decrease in the release of NA, mainly in the diencephalon; NA turnover decrease has also been reported by *Thadani* and *Truitt* (1973).

Acetaldehyde, formed in the oxydation of ethanol, may be exerting a sympathomimetic action by liberating catecholamines from peripheral storage sites (*Schneider*, 1971; *Walsh et al.*, 1970), and by increasing plasma norepinephrine (NE) (*Walsh* and *Truitt*, 1968). *Badawy et al.* (1979) reported that chronic ethanol administration affected rat liver tryptophan pyrrolase, decreasing the activity of the haem-free predominant form or apoenzyme by increasing NADH and NADPH concentration in the liver (*Morland*, 1974; *Badawy* and *Evans*, 1975). Liver tryptophan pyrrolase is one of the peripheral factors that affects brain 5-hydroxytryptamine synthesis by altering the availability of circulating tryptophan to the brain (*Badawy et al.*, 1979). We found increased tryptophan levels in the striatum, hemispheres and brain stem and, after NSD 1015, 5-HTP accumulation was also increased in the striatum as were 5-HT endogenous levels. This finding suggests that chronic ethanol treatment induces an increase in 5-HT synthesis in the striatum, and the biochemical changes in our study provide further evidence for the hypothesis that central monoamine mechanisms are influenced by chronic ethanol treatment.

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