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THE EFFECT OF LONG-TERM ETHANOL MATERNAL INGESTION AND WITHDRAWAL ON BRAIN REGIONAL MONOAMINE AND AMINO ACID PRECURSORS IN 15-DAY-OLD RATS

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Abstract—1. The effects of alcohol on brain monoamines were studied in 15-day-old offspring of rats given ethanol until the 21st day of gestation or the 15th day post partum.

2. Increased noradrenaline concentrations were found in limbic system, hemispheres, diencephalon and brain stem of pups from mothers under alcohol treatment, and in hemispheres, diencephalon and brain stem of pups from deprived mothers.

3. Serotonin and 5-hydroxyindol acetic acid were augmented in limbic system, diencephalon and brain stem of pups from mothers under alcohol whereas tyrosine was decreased in all brain regions studied in pups from alcoholic and deprived mothers.

4. Consequently, chronic ethanol ingestion by pregnant rats has deleterious effects on CNS development of 15-day-old offspring which persists 15 days after alcohol withdrawal.

INTRODUCTION

Maternal alcohol ingestion produces adverse effects on central nervous system development (Branchey and Friedhoff, 1973; Rawat, 1975a; Rossett, 1979; Barnes and Walker, 1981; Borges and Lewis, 1981) as well as on offspring behavior (Vincent, 1958; Abel, 1978; Bond, 1981; Leonard, 1981; Peeke *et al.*, 1981). It has been shown that ethanol also affects several aspects of monoamine metabolism in fetal and neonatal brains (Branchey and Friedhoff, 1973; Thadani, *et al.*, 1977; Detering *et al.*, 1980a,b; Miller and Friedhoff, 1980) and we recently reported that chronic ethanol ingestion during pregnancy in rats produced alterations in brain monoamine concentration in offspring during the perinatal phase (Mena *et al.*, 1982). In the present work we studied monoamine concentrations in brain regions in 15-day-old offspring from alcoholic rats to determine whether observed changes persisted when the mothers were deprived of alcohol from the 21st day of gestation.

MATERIALS AND METHODS

Female Sprague–Dawley rats from our colony weighing 169 ± 5 g were maintained under automatically controlled temperature (25 ± 1°C) and 12 hr light–dark cycles (light from 0900 to 2100 hr). They were divided into three groups that received the following treatment.

(1) *Alcoholics*

These were receiving purina chow diet *ad libitum* and 10% (w/v) ethanol added to the drinking water for the first week, 15% for the second week, 25% for the third week, and 30% for the fourth week after which animals were mated and maintained on 30% ethanol until the end of the experiment (15th day postpartum).

(2) *Alcohol-deprived*

Rats receiving the same treatment as the alcoholics until the 21st day of gestation when ethanol solution was replaced by plain tapwater for drinking.

(3) *Controls*

These rats were receiving purina chow diet and water *ad libitum* during the entire experiment.

At parturition, the offspring number was reduced to 8 per litter. All animals (mothers and their offspring) were sacrificed by decapitation between 1000 and 1200 hr on the 15th day postpartum. Offspring brains were immediately dissected according to Carlsson and Lindquist (1973). After dissection, brain parts from 4 pups were pooled, frozen in dry ice, weighed, and stored at −80°C until processing. Monoamine and amino acid determinations were performed by fluorimetric analysis and with the aid of an automatic amino acid analyzer as described elsewhere (Mena *et al.*, 1982). Statistical evaluation of the data was done by the Student *t*-test.

RESULTS

Monoamine and 5-hydroxyindol acetic acid (5HIAA) concentrations in brain regions of 15-day-old rat pups are shown in Table 1. Noradrenaline (NA) concentrations were higher in all regions in pups from alcohol-treated mothers, except for striatum in pups from mothers maintained on alcohol until day 15 (alcoholics) and for limbic system and striatum in pups from alcohol-deprived mothers (deprived) whereas regional dopamine (DA) concentrations were not affected (Table 1). Serotonin (5HT) and 5 HIAA concentrations were higher in the limbic system, diencephalon and brain stem of pups from alcohol vs control mothers while, except for a reduction in 5 HIAA in the brain stem, these effects

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Table 1. Endogenous monoamine concentrations in brain regions of 15-day-old pups

	NA (ng/g)			DA (ng/g)		
	Control mothers	Alcohol mothers	Alcohol-deprived mothers from day 21 of gestation	Control mothers	Alcohol mothers	Alcohol deprived mothers from day 21 of gestation
Limbic	177.0 ± 5.8	221.2 ± 10.9**	183.4 ± 18.0	1173.0 ± 35.6	1207.4 ± 66.4	1234.7 ± 37.5
Striatum	87.5 ± 7.9	106.7 ± 18.8	88.5 ± 2.7	1553.4 ± 41.9	1629.5 ± 67.7	1498.3 ± 116.0
Hemispheres	58.4 ± 1.6	75.8 ± 5.1**	74.6 ± 4.0**	64.3 ± 16.0	94.2 ± 18.0	96.8 ± 12.0
Diencephalon	271.1 ± 7.2	336.3 ± 14.3**	330.1 ± 12.4**	263.4 ± 20.1	292.8 ± 10.6	234.2 ± 17.5
Brain stem	370.3 ± 9.6	448.2 ± 10.4***	408.2 ± 11.3*	29.3 ± 10.0	49.9 ± 7.3	38.0 ± 10.0
	5HT (ng/g)			5HIAA (ng/g)		
Limbic	460.0 ± 19.6	540.6 ± 18.7*	485.0 ± 32.8	169.0 ± 14.5	218.0 ± 14.9*	164.1 ± 17.0
Striatum	294.4 ± 11.7	325.4 ± 23.9	267.8 ± 21.4	120.7 ± 17.9	124.5 ± 14.6	137.7 ± 11.6
Hemispheres	121.8 ± 17.0	131.9 ± 18.0	123.0 ± 6.8	45.4 ± 11.0	58.6 ± 6.8	43.8 ± 2.5
Diencephalon	692.0 ± 11.3	904.7 ± 50.6**	687.4 ± 35.5	414.4 ± 32.2	849.3 ± 85.0**	352.9 ± 40.9
Brain stem	649.0 ± 48.4	987.8 ± 63.5**	770.0 ± 29.0	780.0 ± 61.5	1188.0 ± 125.0*	517.8 ± 31.5**

Results are means ± SEM of 6 determinations per group. Statistical comparison vs pups from control mothers is shown by asterisks: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Table 2. Tyrosine and tryptophan levels in brain regions of 15-day-old pups

	Tyr (nmol/g)			Trp (nmol/g)		
	Control mothers	Alcohol mothers	Alcohol deprived mothers from day 21 of gestation	Control mothers	Alcohol mothers	Alcohol deprived mothers from day 21 of gestation
Limbic	185.8 ± 14.7 (6)	96.7 ± 11.4 (6)**	159.0 ± 9.4 (6)	≤13.6 (6)	55.6 ± 1.9 (3)***	≤14.6 (6)
Striatum	168.3 ± 4.7 (3)	76.2 ± 10.1 (5)***	104.9 ± 15.7 (4)*	19.6 ± 2.4 (3)	36.3 ± 5.6 (6)	17.2 ± 2.6 (4)
Hemispheres	194.0 ± 10.5 (5)	115.9 ± 17.6 (6)**	149.7 ± 4.1 (6)**	7.98 ± 0.7 (6)	23.7 ± 2.7 (6)***	≤5.6 (6)
Diencephalon	182.5 ± 8.6 (5)	111.2 ± 6.8 (5)***	145.3 ± 5.2 (4)*	15.5 ± 0.4 (4)	30.9 ± 0.5 (3)***	≤13.2 (5)
Brain stem	154.2 ± 13.7 (6)	78.8 ± 16.0 (6)**	74.3 ± 14.06 (6)**	≤19.9 (6)	36.3 ± 4.6 (5)**	≤25.0 (6)

Results are means ± SEM of (n) determinations per group. Statistical comparison vs pups from control mothers are shown by asterisks: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Table 3. Catecholamine and tyrosine concentrations in whole brain of mothers 1 hr after i.p. injection of α-methyl *para*-tyrosine (250 mg/kg)

	NA (ng/g)	DA (ng/g)	Tyr (nmol/g)
Controls	299.0 ± 4.5	817.3 ± 44.6	23.9 ± 3.8
Alcohol	348.8 ± 14.3***	814.8 ± 17.8	27.7 ± 2.3
Alcohol-deprived from 21st day of gestation	299.4 ± 13.9	773.9 ± 32.4	23.6 ± 3.7

Animals were sacrificed at 15th day post-partum. Results are means ± SEM of 6 animals. Statistical comparison vs controls are shown by asterisks: ***$P < 0.001$.

were not observed in pups from deprived mothers (Table 1). Tyrosine (Tyr) and tryptophan (Trp) brain region concentrations were also measured as representatives of monoamine amino acid precursors, and values in 15-day-old pups are summarized in Table 2. Tyr concentrations were lower in all brain regions studied in pups from alcoholic vs control mothers. Tyr content was lower in the striatum, hemispheres, diencephalon and brain stem of pups from deprived mothers (Table 2). Trp concentrations were higher in the limbic system, hemispheres, diencephalon and brain stem in pups of alcoholic vs control mothers, but there were no significant changes in Trp levels in pups of deprived mothers (Table 2).

Experimental conditions used in the present study were as in a previous study (Ludeña *et al.*, 1983), where pup growth and metabolic parameters, and maternal nutritional conditions were described. To determine whether maternal alcohol ingestion or withdrawal affected brain monoamine metabolism in the mothers, they were i.p. injected with 250 mg/kg of α-methyl *para*-tyrosine to inhibit catecholamine synthesis and killed 1 hr later. As shown in Table 3, brain NA concentration was significantly higher in mothers under alcohol treatment and unchanged in alcohol-deprived mothers from 21st day of gestation as compared with controls. No differences in brain DA and Tyr concentrations were found in the three experimental groups (Table 3).

DISCUSSION

Alterations in rat brain monoamines during pup development in this model of the fetal alcohol syndrome coincide with previously reported offspring growth retardation (Ludeña *et al.*, 1983), and were probably due to specific effects of maternal ethanol treatment rather than to malnutrition. This hypothesis is supported by the prolonged effect on certain brain monoamine concentrations in 15-day-old pups of mothers alcohol-deprived from day 21 of gestation, when maternal food intake and offspring body weight were completely normalized (Ludeña *et al.*, 1983). It has been shown that maternal alcohol intake affected neonatal brain neurotransmitter concentrations while most differences disappeared in 4-day-old neonates of alcohol-treated mothers (Mena *et al.*, 1982) and that chronic alcohol ingestion in adult rats produced changes in monoamine content in specific brain regions (Mena and Herrera, 1980). Present results indicated that maternal alcohol ingestion affects offspring monoamine concentrations in some but not all brain regions, differences that would be undetectable in whole brain preparations. Maternal alcohol ingestion affected the neonatal noradrenaline and serotonin systems, but not the dopamine system, in agreement with the reported increase in NA brain content in neonates of mothers receiving ethanol either in drinking water (Mena, 1981; Mena *et al.*, 1982) or in liquid diet (Rawat, 1975b). Reported decreases in NA and DA brain levels in pups of mothers under alcohol treatment (Detering *et al.*, 1980a,b, 1981) could be due to differences in the amount and duration of ethanol administration. These factors affect not only the degree but also the direction of effects on brain monoamines because chronic ethanol enhances brain monoamine concentrations in adult rats (Mena and Herrera, 1980) whereas acute treatments significantly decrease DA, 5HT and 5HIAA concentrations (unpublished results).

The mechanism of maternal ethanol action and/or metabolic or behavioural consequences on specific brain regions in 15-day-old rats is still unclear and should await further experimental contributions, but our findings may explain some of the CNS disturbances reported in offspring of alcoholic mothers. Effects in pups of mothers receiving alcohol could be secondary consequences of the alcohol induced decrease in maternal milk secretion (Cobo, 1973) as well as the ethanol content in the milk but these factors cannot explain effects in pups of alcohol-deprived mothers. The disappearance of alcohol effects retarding brain NA depletion following tyrosine hydroxylase inhibition in alcohol-deprived mothers together with the rapid recuperation of maternal and offspring body weights (Ludeña *et al.*, 1983) suggest that most effects of alcohol in the mothers have disappeared 15 days after its withdrawal. Thus observed changes in the brain monoamines of offspring of these deprived mothers are an index of a more permanent alteration in CNS development produced by the ethanol reaching the fetus during its intrauterine life.

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