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EFFECT OF MATERNAL ETHANOL INGESTION ON NEONATAL RAT BRAIN AND HEART ORNITHINE DECARBOXYLASE*

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Abstract—Perinatal exposure of developing rats to ethanol caused an alteration in the developmental pattern of ornithine decarboxylase (ODC) activity in heart and brain which was different for the two tissues. In brain, an initial decrease in ODC activity was followed by normal or supranormal levels before declining again, whereas heart ODC activity initially was increased but then declined to the low levels of activity characteristic of adult heart tissue. Withdrawal from ethanol at birth or at 3 and 5 days postnatally produced alterations in brain and heart ODC activity that were different from those seen in pups exposed to ethanol continuously throughout development, indicating that the biochemical changes are dependent on the duration of exposure as well as the time at which withdrawal is initiated. The altered ODC developmental patterns in the brain and heart are consistent with the hypothesis that maternal ethanol administration exerts significant effect on fetal polyamine metabolism and ultimately upon the growth and development of these tissues.

Ornithine decarboxylase (EC 4.1.1.17) (ODC) catalyzes the conversion of ornithine to putrescine, the first and probable rate-limiting step in polyamine biosynthesis [1, 2]. Stimulation of animal and bacterial RNA polymerase *in vitro* by physiological concentrations of polyamines has been well documented [3–5], and polyamines may play a regulating role in protein synthesis [6, 7]. Polyamine, nucleic acid and protein synthesis have been shown to vary in a parallel fashion in a variety of rapidly growing systems, such as bacteria [8–10], *D. melanogaster* [11, 12], amphibian embryo [13], chick embryo [14–16], regenerating mouse and rat liver [16–19] and developing central nervous system [15, 20]. These and other studies suggest that ODC activity is stimulated in conditions in which growth is induced [21–28].

Brain regions of fetal and neonatal rats have a characteristic developmental pattern of ODC activity which is sensitive to alterations induced by drug or hormonal treatment [29–31], and it has been suggested that perturbations of this pattern may be used as an early index of central nervous system maturation. The present study was undertaken to determine whether chronic ethanol ingestion by pregnant rats affects the pattern of ODC activity in the heart and brain of the offspring.

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METHODS AND MATERIALS

Timed pregnant rats (Zivic-Miller) were housed individually in breeding cages and on day 11 of gestation were started on a nutritionally complete liquid diet (Sustacal). The experimental group was given Sustacal containing 6.8% (v/v) ethanol starting on day 13 of gestation, while controls received Sustacal made isocaloric to the ethanol diet by the addition of sucrose. To insure that both groups ate the same amount, control intake was restricted to that consumed by the experimental group throughout the study; consumption averaged approximately 70 ml/day, and was measured daily. In cross-fostering studies, pups born of ethanol-treated mothers were transferred at 0, 3 and 5 days of age to control mothers until killed. The pups were divided into five groups: (1) control, (2) those exposed to ethanol prenatally plus postnatally, (3) those withdrawn at birth, (4) those withdrawn at 3 days postnatally, and (5) those withdrawn at 5 days postnatally. The pups were weighed and killed by decapitation at intervals of several days from 2 days prenatally to 17 days postnatally, and heart, cerebellum and the rest of the brain were assayed for ODC activity. For cerebellum and heart, tissues from two to three animals were pooled for each determination until 5 days of age.

Tissues were weighed, homogenized in 20 vol. of ice-cold 10 mM Tris–HCl (pH 7.2), and the homogenate was centrifuged at 26,000 *g* for 20 min. An aliquot of the supernatant (approximately 2–5 mg protein) was assayed for ODC activity by generating $^{14}CO_2$ from DL[1-^{14}C]ornithine using a modification of the method of Russell and Snyder [1]. The incubation medium for brain contained final concentrations of 0.5 mM dithiothreitol, 0.5 mM pyridoxal 5′-phosphate, 0.25 μCi DL[1-^{14}C]ornithine and 125 μM unlabeled L-ornithine. In order to increase the sensitivity

of the assay for heart tissue, no unlabeled ornithine was added to the incubation medium. The $^{14}CO_2$ was trapped using hyamine hydroxide and counted by liquid scintillation spectrometry. A number of duplicate assays were run after dialysis or in the presence of saturating concentrations of ornithine and the results were identical.

Data are presented as means and standard errors, and levels of significance calculated by the Student's *t*-test (unpaired).

DL[1-^{14}C]ornithine monohydrochloride (sp. act. 43.1 mCi/m-mole) was obtained from New England Nuclear Corp. L-Ornithine monohydrochloride and pyridoxal 5'-phosphate were obtained from Sigma Chemical Co. and dithiothreitol was from Bachem Feinchemikalien AG.

RESULTS

Effect of ethanol on body and tissue weights. Over the course of postnatal development, control rats increased in body weight from approximately 11 g at 3 days to 28 g at 17 days of age (Table 1). Pups exposed to ethanol continuously throughout development showed a retardation in weight gain starting at 10 days of age. In cross-fostering studies, pups exposed to ethanol and withdrawn at 0, 3 and 5 days of age showed a weight gain similar to that of controls.

No marked changes occurred in weights of brain minus cerebellum in any of the ethanol-treated groups (Fig. 1). However, cerebellum weights in developing rats exposed continuously to ethanol were significantly below control at 13 and 17 days of age (Fig. 2). In contrast, few changes were seen in pups undergoing postnatal withdrawal.

Table 1. Effect of maternal ethanol administration on body weights of developing rats

Treatment	Age in days	Body wt*
Control		10.9 ± 0.27 (28)
Continuous ethanol	3	10.4 ± 0.30 (28)
Withdrawn at day 0		9.6 ± 0.28 (6)
Control		13.7 ± 0.36 (28)
Continuous ethanol		13.0 ± 0.41 (24)
Withdrawn at day 0	5	12.9 ± 0.08 (6)
Withdrawn at day 3		13.5 ± 0.66 (11)
Control		17.2 ± 0.43 (12)
Continuous ethanol		18.2 ± 0.32 (11)
Withdrawn at day 0	8	18.6 ± 0.50 (6)
Withdrawn at day 5		17.3 ± 0.58 (6)
Control		23.2 ± 0.62 (11)
Continuous ethanol	10	19.3 ± 0.89†(14)
Withdrawn at day 5		21.9 ± 0.55 (12)
Control		22.4 ± 0.81 (6)
Continuous ethanol	12	21.9 ± 1.05 (6)
Withdrawn at day 0		23.4 ± 0.50 (6)
Control		23.9 ± 0.51 (12)
Continuous ethanol	13	20.5 ± 0.54†(14)
Withdrawn at day 5		24.6 ± 0.49 (6)
Control		27.7 ± 0.48 (6)
Continuous ethanol	17	21.5 ± 0.40†(6)

* Results are expressed as mean ± standard error of the number of animals in parentheses.

† Denotes significant difference vs control ($P < 0.05$).

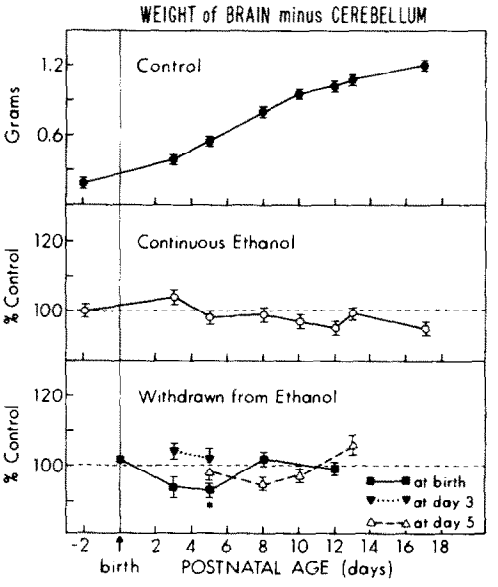

Fig. 1. Weight of brain minus cerebellum in developing rats. The group of rats indicated as continuous ethanol were given ethanol continuously from day 13 of gestation. Other ethanol-exposed pups were transferred to control mothers as indicated on the graph. Points and bars represent means ± standard errors of 6–18 determinations at each age; asterisks denote significant differences (at least $P < 0.05$).

In pups exposed to ethanol throughout development, heart weights were normal until 10 days of age, after which time weights were significantly below control (Fig. 3). In contrast, pups withdrawn at birth showed a marked increase in the rate of heart weight gain to a level of 140 per cent of control by 12 days of age. However, animals withdrawn at 5 days of age showed no difference from controls.

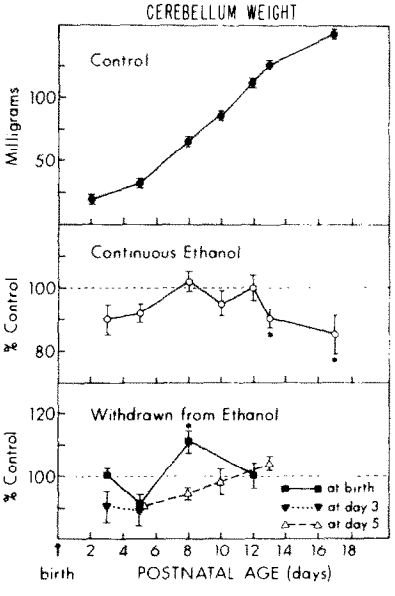

Fig. 2. Weight of cerebellum in developing rats. Description is the same as for Fig. 1.

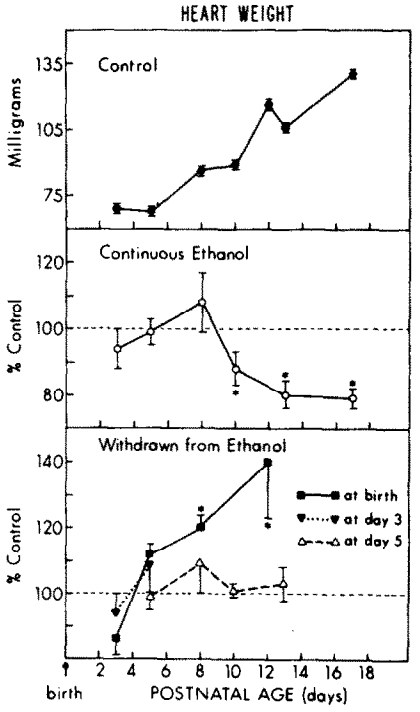

Fig. 3. Weight of heart in developing rats. Description is the same as for Fig. 1.

Effects of ethanol on ornithine decarboxylase activity. In control pups, ODC activity in brain minus cerebellum was high initially and then declined to low values by day 12 (Fig. 4). In pups exposed continuously to ethanol, ODC activity was deficient at

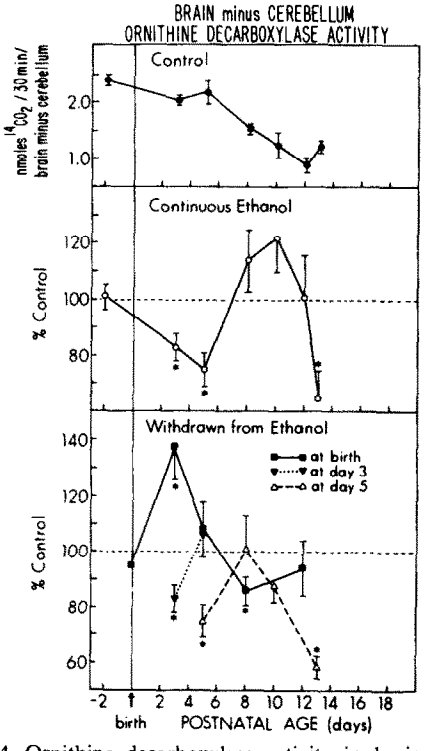

Fig. 4. Ornithine decarboxylase activity in brain minus cerebellum of developing rats. Description is the same as for Fig. 1.

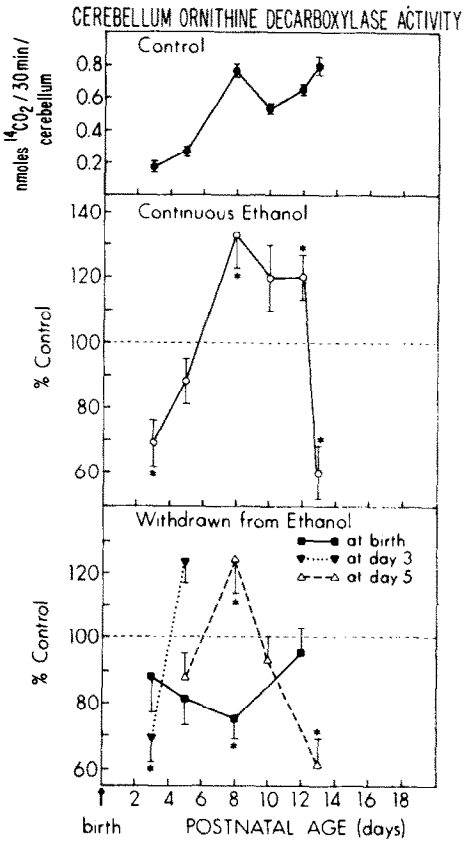

Fig. 5. Ornithine decarboxylase activity in cerebellum of developing rats. Description is the same as for Fig. 1.

3 and 5 days of age, but rebounded to normal or supranormal levels (8–12 days) before declining again. In cross-fostering studies, withdrawal initiated an immediate increase in ODC activity followed by a subsequent decline. The relative changes in ODC activity in the continuous ethanol group vs those withdrawn at 0 or 5 days are similar, but the patterns are displaced in time and initial level of ODC activity.

Cerebellar ODC activity in controls increased with age through day 13 (Fig. 5) and declined subsequently (data not shown). In neonates exposed to ethanol throughout development, an initial decrease in cerebellar ODC activity was followed by a subsequent increase between 8 and 12 days of age; however, by 13 days a significant decrease in activity was again observed. Activity patterns after withdrawal at 3 and 5 days are similar to that observed with continuous ethanol and to those described above for brain minus cerebellum. However, withdrawal at birth resulted in a unique pattern in that no rebound increase in ODC was observed and levels remained below normal.

In controls, ODC activity per heart slowly increased with age (Fig. 6). In pups exposed to ethanol throughout development, heart ODC activity was increased between 3 and 8 days, but then declined to low activity characteristic of adult heart tissue. Withdrawal at 0 or 3 days resulted in an increase in ODC activity relative to controls, and this response was most marked at 12 days of age. However, in pups withdrawn at day 5, heart ODC activity declined steadily.

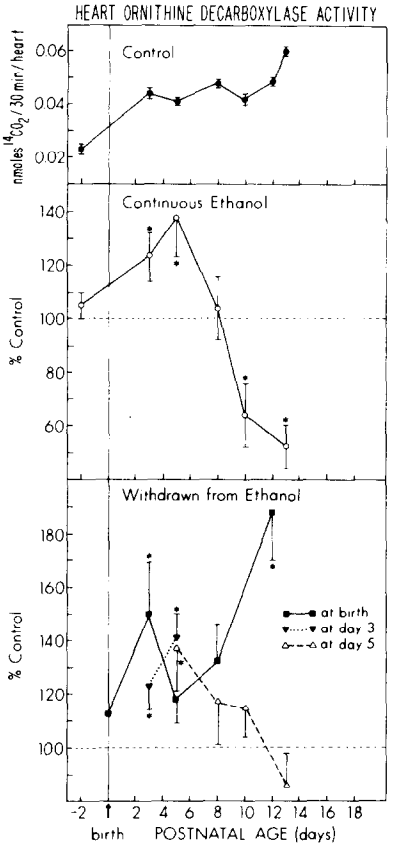

Fig. 6. Ornithine decarboxylase activity in heart of developing rats. Description is the same as for Fig. 1.

DISCUSSION

Ethanol crosses the placental barrier [32, 33] and can affect the developing fetal central nervous system and other tissues. There is clinical evidence to suggest that infants from alcoholic mothers may have abnormal physical growth [34], and recent studies have shown that both acute and chronic ethanol administration to pregnant rats results in decreased ribosomal protein synthesis in fetal brain [35].

In the present study, maternal ethanol administration produced alterations in the developmental pattern of ODC in brain and heart, accompanied by correlative alterations in the growth of these organs. These data are particularly interesting in view of the known involvement of polyamines in nucleic acid and protein synthesis in a variety of proliferating and developing tissues [5–7, 15–20], and the strong evidence that putrescine levels vary directly with alterations of ODC under a number of experimental circumstances [1, 29, 36, 37]. However, the present data do not distinguish between a direct or indirect [30] effect of ethanol on the polyamine system.

Although the immediate postnatal level of ODC activity was below control in brain but above control in heart, the basic pattern of the subsequent alteration in the various tissues was characterized by an initial increase followed after a few days by a marked decrease. However, the pattern was displaced toward earlier ages in heart compared to brain. In the two tissues that are undergoing the most marked development and differentiation in the postnatal period (cerebellum and heart), the altered ODC pattern correlates with a subsequent significant lag in tissue weight.

To determine the extent to which the ethanol-induced abnormal development could be prevented by postnatal termination of ethanol exposure, animals were transferred to control mothers at different ages. The most marked effect of withdrawal in brain was the immediate initiation of the same "up–down" pattern that was observed at a later time with continuous ethanol; the sole exception to this pattern in brain occurred in the cerebellum after withdrawal at birth. In heart, the pattern after withdrawal at 3 or 5 days again coincides with the pattern seen after continuous ethanol. However, in heart as with cerebellum, withdrawal at birth appears to elicit a different response in ODC activity. In this unique case, continuously elevated ODC activity correlated with a consistent increase in heart weight.

In conclusion, these data show that perinatal ethanol exposure significantly alters polyamine metabolism and growth in developing heart and brain and that the magnitude and direction of change depend upon both the period of exposure and the tissue studied.

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