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CEREBRAL ORNITHINE DECARBOXYLASE LEVELS FOLLOWING GESTATIONAL EXPOSURE TO COCAINE

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Abstract—The pre- and postnatal developmental course of cerebral ornithine decarboxylase (ODC) has been studied in infant rats after treatment of pregnant dams with cocaine. Levels of cocaine attained in brains and serum of embryos were not initially increased over corresponding maternal values, but were more persistent. However, cocaine was no longer detectable in these tissues 4 days after administration. The cerebral ODC level of treated pups was initially depressed and subsequently elevated relative to control values. These changes were apparent at times when cocaine was not detected in the developing brain. Results indicate that a transient exposure to cocaine *in utero* may lead to prolonged developmental abnormality.

Key words: ornithine decarboxylase, cocaine, fetal brain, gestational exposure.

The prevalence of illicit cocaine use is sufficiently widespread so as to include a significant population of pregnant women. This results in prenatal exposure of embryos to this drug, which may have adverse effects on infant development.^{8,11} Babies born to cocaine-using mothers have been reported to have significantly reduced birth weight and head circumference, in addition to intellectual deficits.^{6,15}

The purpose of this study was to evaluate cerebral development in gestationally exposed rats, by means of assay of ornithine decarboxylase (ODC), an enzyme with a distinctive developmental profile. The cerebral ODC level is high during maturational cell proliferation, but declines greatly with the cessation of major mitotic activity.³ Several neurotoxic agents have been shown to modulate the ontogenic expression of this enzyme, including methyl-mercury^{4,5} and the prevalent pharmacological agents methadone^{17,19} and nicotine.^{20,22} In view of the rapid responsiveness of ODC to environmental conditions, this enzyme has been proposed as a marker in the detection of developmental deficits.¹⁸

EXPERIMENTAL PROCEDURES

Treatment of rats

Pregnant Sprague–Dawley CD rats (235–280 g) were maintained in groups of four with free access to food and water. At various stages of gestation, rats were subcutaneously injected with cocaine in distilled water or with vehicle alone. The dosage used on each injection day was 5 mg/kg body wt. Dosing was on gestational days 9, 10, 11 (group I); 12, 13, 14 (group II); 15, 16, 17 (group III) and 18, 19, 20 (group IV). Some newborn rats were maintained postnatally by the original mothers, without cross-fostering. At several time points, mothers and young were decapitated, and serum was collected from clotted blood. Brains were rapidly removed, weighed and frozen at -70°C .

Ornithine decarboxylase assay

ODC was assayed by the measurement of evolved $^{14}\text{CO}_2$ from carboxyl- ^{14}C ornithine (64.1 mCi/nmol, New England Nuclear, Boston, MA).¹⁴ Frozen brain tissues were homogenized in 19 vol. 0.04 M Tris–HCl (pH 7.4), adrenals were homogenized in 1.5 ml Tris–HCl and the homogenates were centrifuged (26,000 g, 10 min). 0.9 ml of various tissue preparations was added

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to 50 μ l pyridoxal phosphate solution (1 mM) and 50 μ l [14 C]ornithine in 0.045 dithiothreitol and 0.01 M Tris-HCl, pH 7.2. The final ornithine concentration was 2.5 μ M.

Incubation was carried out for 45 min at 37°C in a sealed tube in a shaking water bath. Labeled CO₂ was trapped on a paper wick containing hyamine which was suspended above the reaction mixture. The reaction was stopped and CO₂ was released by the injection of 0.5 ml 10% trichloroacetic acid into the mixture. The decarboxylation reaction was linear with time under these conditions. Background decarboxylation, not attributable to ODC, was determined by running a parallel incubation in the presence of 5 mM α -difluoromethyl ornithine, a specific inhibitor of ODC,¹² obtained through the courtesy of Dr P. McCann (Merrell Research Center, Cincinnati, OH).

Cocaine determination

The concentration of cocaine in brain and serum was determined by solid phase ¹²⁵I radio-immune assay using a commercially manufactured antibody (diagnostic Product Corp., Los Angeles, CA). Standard amounts of cocaine were added to serum or brain homogenates and concentration curves were developed. The limit of sensitivity of this method corresponded to a concentration of 10 ng cocaine/ml. Cocaine levels have been found to be very stable in the brain after extended storage in the frozen or refrigerated state.²³

The metabolic conversion of cocaine to pharmacologically inactive metabolite benzoylegonine is rapid in blood, but levels of this metabolite do not exceed those of cocaine by more than three-fold in any tissue.²³ We found the immunological cross-reactivity of benzoylegonine with the cocaine antibody to be less than 2.5%. Thus, the values reported here largely represent cocaine rather than its metabolite.

Statistics

Results were analysed using Fisher's Least Significant Difference Test after one-way analysis of variance. Throughout the results, the symbol (*) means $P < 0.05$ or lower using a two-tailed t distribution.

RESULTS

Pregnant rats were injected with cocaine for three consecutive days (5 mg/kg daily). The treatment had no major adverse effect on fetal development as judged by brain and body weight and litter size (Table 1). Furthermore, no physical abnormality was found in any of the embryos from all the treatment groups. Levels of cocaine in serum and brain were determined in maternal and fetal tissues on gestational day 21. Cocaine was only detectable in the most recently injected group; group IV (Table 2). Fetal serum and brain levels were significantly higher than the corresponding maternal tissues. Cerebral ODC levels were depressed relative to controls in experimental groups I, II, and III while only the group (IV) showing detectable cocaine residues showed no such change (Table 2).

In view of the relatively rapid disappearance of detectable cocaine, cerebral and serum levels of this drug were examined shortly after administration of a single dose (5 mg/kg body wt) to rats at the 20th day after conception (Fig. 1).

Table 1. Characteristics of embryos after administration of cocaine to pregnant rats

	Pups/litter	Body wt (g)	Brain wt (mg)
Control	11.4 \pm 0.5	4.11 \pm 0.16	172 \pm 5
Group I	11.8 \pm 0.9	3.91 \pm 0.9	154 \pm 5
Group II	11.0 \pm 0.9	3.95 \pm 0.10	165 \pm 3
Group III	10.7 \pm 1.4	4.01 \pm 0.07	169 \pm 5
Group IV	10.7 \pm 1.2	4.24 \pm 0.16	167 \pm 1

Each data point derived from means from six individual litters \pm S.E.

Table 2. Cocaine levels in maternal and fetal brain at gestational day 21, and ornithine decarboxylase content of fetal brain, following maternal cocaine administration

Dosing group	Cocaine content ($\mu\text{g/g}$ tissue)				Neonatal brain ODC ($\text{pmol CO}_2/45 \text{ g tissue}$)
	Maternal		Fetal		
	Brain	Blood	Brain	Blood	
Control	0	0	0	0	402 ± 33
Group I	0	0	0	0	$285 \pm 12^*$
Group II	0	0	0	0	$301 \pm 2^*$
Group III	0	0	0	0	$265 \pm 19^*$
Group IV	5 ± 2	11 ± 4	14 ± 4	70 ± 8	356 ± 16

Each point derived from a mean of 5–6 individual values \pm S.E.

* Significantly different from control value ($P < 0.05$).

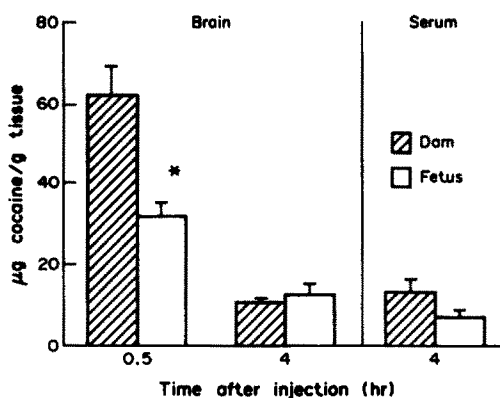


Fig. 1. Cocaine level in maternal and fetal brain and serum after administration to pregnant rats. Rats at the 20th gestational day were subcutaneously injected with 5 mg cocaine/kg body weight and tissues collected for analysis, 0.5 and 4 hr later. *Differs significantly from the corresponding maternal value ($P < 0.05$).

Four hours after injection, cocaine levels in fetal tissues did not differ significantly from the corresponding maternal values. Both levels were much lower than the liver content of cocaine ($3.98 \pm 0.41 \mu\text{g/g}$ tissue 4 hr after drug administration). Thirty minutes after cocaine injection, cerebral levels were higher in the dams than in the fetus (Fig. 1). A relatively long-lasting depression of fetal brain ODC content was apparent after a 3-day drug treatment, despite the rapid disappearance of detectable cocaine.

For this reason, the normal developmental profile of cerebral ODC was compared to that from rat pups of mothers dosed on days 12–14 (group II). This study included both pre- and postnatal time points. The normal developmental course of cerebral ODC showed a marked postnatal decline as previously described²⁹ (Fig. 2). Transient but pronounced elevation of cerebral ODC occurs for a few hours after birth,² but no comparative study of ODC levels was performed at this time. Treated animals expressed a biphasic response to drug administration: an initial prenatal depression of ODC followed by a significant elevation of this enzyme after birth (Fig. 3). These changes were superimposed on the normal developmental decline in enzymic activity.

DISCUSSION

In common with the data reported here, at short times after injection, cocaine was also found by other workers to be more concentrated in maternal, rather than fetal brains of mice.¹⁶ These authors proposed that the drug is actively transported into the mature nerve system. There is also other evidence that supports this concept.¹³ The pharmacokinetics of cocaine may vary considerably between species. In the pregnant mouse, cocaine is barely detectable in brain 6 hr after a single injection of 10 mg/kg body wt.¹⁶ We have found that cocaine levels in the fetal brain, while initially lower than in corresponding maternal brain, appear to be more persistent at longer times after drug administration (Table 2).

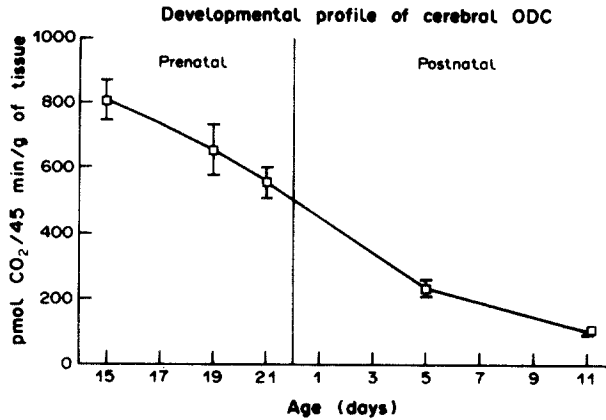


Fig. 2. Developmental profile of cerebral ornithine decarboxylase in perinatal rats. Each point represents the mean \pm S.E. from six pups derived from six individual mothers.

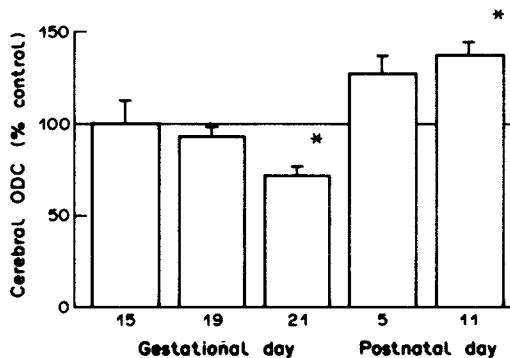


Fig. 3. Effect of gestational exposure to cocaine upon pre- and postnatal developmental profile of ODC. Three consecutive injections of cocaine (5 mg/kg body wt) were given on gestational days 12, 13 and 14. Each point \pm S.E. was derived from 5-6 pups from individual mothers. *Differs significantly from that of corresponding control (saline injected), ($P < 0.05$, two-tailed *t*-test).

In contrast to another drug of abuse, phencyclidine, PCP,¹ cocaine is neither strongly accumulated by fetal brain nor is it retained at a high concentration in that tissue. However, transient gestational exposure to cocaine is capable of causing prolonged significant changes in the developmental profile of an enzyme associated with cell division and growth, first in a downward and then in an upward direction. These modulations are consistent with a developmental delay of mitotic and tissue accretion processes despite the detection of any gross deficit in body or brain weight. In this study we have avoided use of large doses of cocaine which are known to cause reductions in maternal and fetal weights.⁹ In view of the fact that the temporal development of brain regions is not synchronous, it would be desirable to carry out regional studies of the changes described here. However, the relatively small size of the fetal brain makes such a study difficult.

The use of pups that have not been cross-fostered raises the possibility of abnormal maternal behavior as influencing the outcome of this study. Although brain and body weights did not differ significantly between any treated group and the parallel control, this hazard cannot be completely excluded since deprivation of neonatal rats from normal maternal behavior can influence cerebral ODC levels.⁷ However, such impaired development results in a severe depression of, rather than an increase of, pup ODC levels.

In addition to being a useful marker for ontogenic abnormality, rates of polyamine synthesis may directly influence developmental outcome. Specific inhibition of ODC in the perinatal animal can result in later changes in behavioral responses.¹⁰

Postnatal ODC elevations found after gestational exposure to methadone have been taken to represent a prolongation of the time required for the completion of developmental processes consequent to abnormal body or organ growth rates.¹⁹ An initial depression in the maturational pattern of ODC followed by a subsequent elevation can reflect a delayed rate of maturation of CNS neurons.³ This has been reported for several agents including methylmercuric chloride⁴ and ethanol.²⁴ In the case of methyl mercury, an abnormal developmental profile of cerebral ODC occurs at doses that are low enough to be regarded as almost exclusively affecting behavioral rather than biochemical parameters. These doses, as low as 0.05 mg/kg body wt, produce clear-cut abnormalities in open-field and swimming behaviors, but are associated with no other known biochemical or morphological teratologic alterations.²¹

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