Pharmacology Biochemistry & Behavior, Vol. 11, pp. 605–609. Printed in the U.S.A.

Prenatal Amphetamine Exposure: Ovulation, Sexual Behavior and Hypothalamic Monoamine Content in Rats

OSCAR A. RAMIREZ, HUGO F. CARRER AND ANTONIA G. NASELLO

Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Estafeta 32, 5000 Córdoba, Argentina

Received 30 May 1979

RAMIREZ, O. A., H. F. CARRER AND A. G. NASELLO. *Prenatal amphetamine exposure: ovulation, sexual behavior and hypothalamic monoamine content in rats.* PHARMAC. BIOCHEM. BEHAV. 11(6) 605–609, 1979.—Previous observations have pointed out that amphetamine treatment during pregnancy produces behavioral and neurochemical changes in the male offspring. The present study was undertaken in order to determine whether those findings could be extended to sexual behavior, reproductive function and hypothalamic monoamine metabolism in female rats. It was found that offspring of amphetamine treated rats have greater sensitivity to estrogen and estrogen-progesterone for the induction of sexual receptivity. 5-HT content in medial hypothalamus of estrogen-progesterone treated rats was decreased with respect to controls. On the other hand the content of 5-HIAA was not different; noradrenaline levels in hypothalamus were also normal. Sex cycle duration and ovulatory phenomena were not affected.

Behavioral teratogenesis Prenatal amphetamine Brain monoamine metabolism Sexual behavior

THE use of amphetamine as an anorexic agent during pregnancy has become quite common, often disregarding its psychoactive effects [19]. Since amphetamine can induce teratogenic changes [1,19] it probably crosses the placental barrier, affecting the brain of the fetus. Several reports have indicated that amphetamine administration during pregnancy brings about important modifications in behavioral [10,16] and neurochemical [18] parameters of offspring. Previous work from this laboratory has shown that 0.5 mg of dl-amphetamine given daily during pregnancy induces in the offspring increased conditioned avoidance responses and greater exploratory activity in the open field [18]. These animals were shown to have greater tyrosine hydroxylase (TH) activity and increased catecholamine turnover in whole brain without significant changes in catecholamine levels [18].

Since neuraminergic systems are clearly involved in neuroendocrine as well as behavioral aspects of reproductive function the present experiments were performed to investigate the effects of amphetamine treatment during pregnancy on these facets of reproductive physiology in female offspring, and to correlate the eventual changes with variations in regional levels of brain monoamines.

METHOD

Female offspring of amphetamine treated rats were used. Parents were selected on the basis of their performance in an active avoidance test as previously described [16].

This criterion was used in order to make results comparable with previous data from this laboratory. Females were injected daily during pregnancy with 0.5 mg/kg of dl-amphetamine subcutaneously from the first day (identified by the presence of spermatozoa in the vaginal smear examination made every morning) until delivery. Control groups received saline only. At birth litters were adjusted to 4 females and 4 males, offspring being reared by their natural mother.

Beginning at 3 months of age vaginal smears were taken 5 days per week during at least 4 cycles. On the last day of estrus animals were ovariectomized, the tuba were dissected and the ova were counted under a microscope.

From this time on animals were kept under reversed lighting conditions (lights on from 2200 to 1000 hr). At least 3 weeks after castration rats were injected at the beginning of the dark period with estradiol benzoate 10 μg per kg of body weight. In these and subsequent treatments hormones were dissolved in peanut oil and subcutaneously injected in a volume of 0.1 ml/100 g body weight. Forty eight hours later animals received 2 mg of progesterone per kg of body weight and after 6–8 hr they were brought to an observation cage housing a male. Ten mounts were allowed and the number of lordotic responses were recorded. With these data the lordosis quotient ($\frac{\text{no. of lordosis}}{\text{no. of mounts}} \times 100$) was calculated.

Sexual receptivity induced by estrogen alone was also studied. With at least 3 week intervals between treatments, animals were injected with 25, 50, 100, and 200 μg estradiol benzoate per kg body weight in a random fashion. Estrogen injections were given around 1000 hr and receptivity tests were carried out 54–56 hr later. Approximately the same

TABLE 1

SEXUAL CYCLE AND OVULATION IN OFFSPRING OF RATS TREATED DAILY DURING PREGNANCY WITH AMPHETAMINE 0.5 mg/kg

Group	N	Cycle length			Ovulation	
		4 days	5 days	Irregular	Frequency*	Number of ova/rat (mean ± S.E.)
Control	30	16	5	9	20/21	9.4 ± 0.7
Experimental	20	16	1	3	17/17	10.9 ± 0.7

*Number of rats ovulating/number of rats. Only animals with regular 4 or 5 day cycles were included.

number of experimental and control animals was tested in any single day; animals were tested only once under the same estrogen dose.

Monoamine Assays

At least 4 weeks after behavioral studies were completed animals received either 100 μg of estradiol benzoate or 10 μg of estradiol benzoate plus 2 mg of progesterone. Schedule of hormone administration was the same as outlined above. With the same intervals with respect to hormone injections and at the same time of day that behavioral studies had been performed, rats were killed by decapitation. Brains were immediately removed and placed on a chilled glass plate. The hypothalamus was separated by a frontal section at the level of the anterior border of the optic chiasma, a caudal section at the level of the anterior aspect of the mammillary bodies and lateral sections at the hypothalamic fissures. A horizontal section, at the level of the fornix, separated the hypothalamus from the rest of the brain. The hypothalamus was further divided into anterior and medial portions by a frontal section at the level of the posterior border of the optic chiasma. The anterior portion which included the preoptic area and part of the anterior hypothalamus weighed 13.31 ± 0.5 mg (mean ± SE). The medial portion which included the ventromedial, arcuate, and dorsal hypothalamic nuclei weighed 10.8 ± 0.6 mg. Dopamine (DA), noradrenaline (NA), 5-hydroxytryptamine (5-HT) and 5-hydroxy-indol-acetic acid (5-HIAA) were measured in these fractions according to a method devised in this laboratory (Orsingher *et al.*, to be published) based on the procedure described by Chang [3]. Briefly, tissue was homogenized in acid n-butanol and extracted with solvents. 5-HIAA was extracted from the organic phase with $NaCO_3H$, and after obtaining the o-phthaldialdehyde (O-PT) reaction and heating, fluorophores were measured using an activating wavelength of 360 nm and an emitting wavelength of 470 nm [5]. 5-HT was extracted from the alumina supernatant and purified through DOWEX-50 W X4 [13]. NA and DA were adsorbed in alumina, oxidized with I_2 and heated for the development of the different fluorophores which were then determined fluorometrically.

Concentration of catecholamine data and behavioral data normalized by arc sine transformation were analyzed using Student's t test. Data on ovulation frequency and cycle length were tested by Fisher's exact probability.

RESULTS

Sexual Cycles and Ovulation

Relative frequency of 4 or 5 day-cycles in the offspring of amphetamine treated rats did not differ from controls. Number of rats ovulating at proestrus or number of ova shed per rat were also normal (Table 1).

Sexual Behavior

As can be seen in Fig. 1 offspring of amphetamine treated rats were significantly more receptive than controls after treatment with estrogen and progesterone. To test the hypothesis that this difference in sexual behavior may be due to different sensitivity to estrogen, a dose response curve was obtained. Figure 2 shows the results of these experiments. At all the doses studied, lordosis quotients for the experimental group were higher than for the control group. Significant differences were observed at the doses of 100 and 200 μg of estradiol benzoate per kg b.w. Both points fall in the linear portion of the dose-response curve. It was evident for the 2 observers recording the results throughout the test that animals in the experimental group showed elements of soliciting behavior (darting, hopping) more frequently and intensely than control animals.

Monoamine and 5-HIAA Assay

The results of the NA, 5-HT and 5-HIAA assay are shown in Tables 2 and 3. Results of DA assay are not included since its concentration in both hypothalamic fractions was below the sensitivity of the method. In the offspring of amphetamine treated rats after estrogen-progesterone treatment the concentration of 5-HT in the medial hypothalamic fraction was significantly ($p<0.05$) lower than in control animals. However, the concentration of 5-HIAA was similar in both groups. No significant difference was found in the concentration of 5-HT in medial hypothalamus between experimental and control animals treated with estrogen alone or in the anterior hypothalamic fraction, irrespective of the treatment used. No differences were found in NA levels of both fractions after either hormone administration.

DISCUSSION

Our results show that amphetamine treatment during pregnancy has long lasting effects on the sexual behavior of

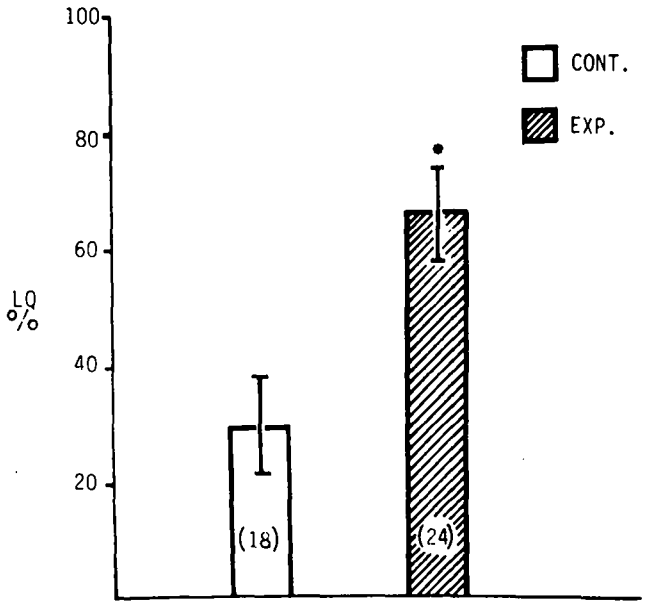

FIG. 1. Sexual receptivity induced by estrogen and progesterone in offspring of amphetamine treated rats. Bars represent mean of lordosis quotient. Vertical lines indicate SE. Number of animals are in parenthesis. *$p<0.001$.

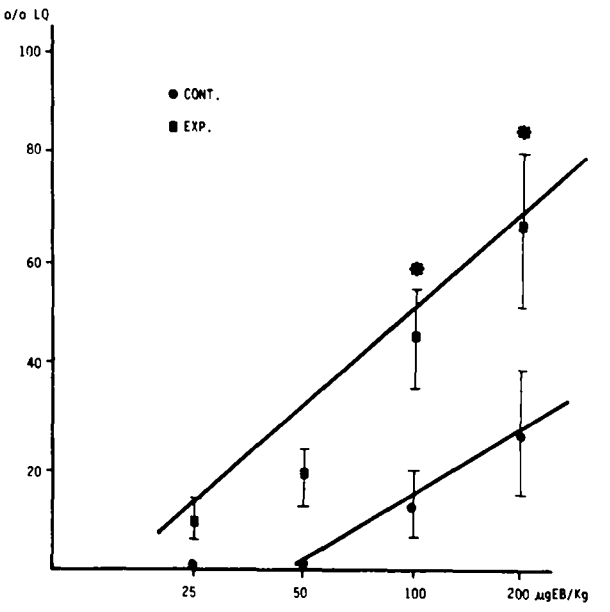

FIG. 2. Sexual receptivity induced by different doses of estrogen in offspring of amphetamine treated rats. Data are mean ± SE. Numbers of animals varies from 10 to 17. *$p<0.01$.Estradiol benzoate dose in log scale.

TABLE 2

HYPOTHALAMIC NA, 5-HT and 5-HIAA CONTENT AFTER ESTROGEN-PROGESTERONE PRIMING IN OFFSPRING OF AMPHETAMINE TREATED RATS (µg/g wet weight; mean ± SE).

	Ant. hypothalamus			Medial hypothalamus		
	NA	5-HT	5-HIAA	NA	5-HT	5-HIAA
Control	1.85 ± 0.06 (7)	1.48 ± 0.19 (7)	1.53 ± 0.07 (8)	2.04 ± 0.19 (7)	2.02 ± 0.27 (7)	1.73 ± 0.15 (8)
Experimental	1.89 ± 0.25 (6)	1.58 ± 0.31 (7)	1.14 ± 0.16 (7)	1.82 ± 0.20 (6)	1.36 ± 0.07* (7)	1.78 ± 0.23 (7)

*Experimental vs control $p<0.05$.
Number of animals are in parentheses.

TABLE 3

HYPOTHALAMIC NA, 5-HT AND 5-HIAA CONTENT AFTER PRIMING WITH 100 µg ESTRADIOL BENZOATE PER kg BODY WEIGHT IN OFFSPRING OF AMPHETAMINE TREATED RATS (µg/g wet weight; mean ± SE)

	Ant. hypothalamus			Medial hypothalamus		
	NA	5-HT	5-HIAA	NA	5-HT	5-HIAA
Control	1.68 ± 0.18 (8)	1.32 ± 0.13 (8)	1.62 ± 0.20 (7)	2.17 ± 0.24 (6)	1.61 ± 0.21 (8)	1.85 ± 0.16 (7)
Experimental	1.92 ± 0.16 (8)	1.49 ± 0.21 (9)	1.77 ± 0.19 (9)	2.32 ± 0.15 (9)	1.75 ± 0.19 (9)	1.79 ± 0.14 (8)

Number of animals are in parentheses.

adult female offspring. It must be noted that no drug treatment was used after birth, which indicates that whatever changes are produced as consequence of the treatment in utero persist at least through the first 10–12 months of life. Offspring of amphetamine treated rats were more receptive after an estrogen-progesterone treatment that mimics the ovarian steroids secretion during the sexual cycle of normal rats [20]. This increased sensitivity of the experimental animals to estrogen-progesterone priming is due at least in part to a greater sensitivity to estradiol benzoate, since the dose response curve for sexual behavioral responses after estrogen alone was clearly displaced to the left (Fig. 2). This fact does not rule out the possibility that in offspring of amphetamine treated rats response to progesterone may also be modified (see below). Different sexual behavioral responses of ovariectomized rats to estrogen or estrogen-progesterone have frequently been causally related with changes in cerebral monoamine content and their metabolism [6,14]. Most evidence favors the existence of a noradrenergic system exerting facilitatory influences on sexual receptivity [6,14]. The increased activity of such a system may be evoked to explain our observation that animals in the experimental group were more sensitive to estrogen or estrogen-progesterone treatment. As a matter of fact, offspring of amphetamine treated rats show other behavioral changes which become evident in active avoidance and exploratory activity tests, equally related to variations in brain catecholamine metabolism [17,18].

Male offspring of rats receiving identical amphetamine treatment as used in these experiments show increased tyrosine hydroxylase activity and greater catecholamine turnover in whole brain [18] and in hypothalamus (O. Ramirez - unpublished results), although no differences were found in catecholamine levels in either case [18].

Also in the present experiments, no difference was found in catecholamine content between control and experimental groups in anterior or posterior hypothalamic fractions of ovariectomized animals primed with estrogen or estrogen-progesterone at doses that produced clearly different behavioral responses. The possibility must be kept in mind that these behavioral differences may, nonetheless, be related with different catecholamine turnover rates. The situation is different for serotonin since in the medial hypothalamic fraction of ovariectomized rats primed with estrogen and progesterone, offspring of amphetamine treated animals had smaller 5-HT content than controls. Since 5-HIAA levels were not different in these animals, suggesting a normal catabolic rate, a decrease in 5-HT content would indicate that synthesis is proceeding at a slower pace. This result coincides with repeated observations that suppression of 5-HT synthesis facilitates sexual behavioral performance in females [6,14] as well as males [11], observations which have been interpreted as proof of the existence of a serotonergic system tonically inhibiting sexual receptivity. Indeed, it has been proposed that progesterone triggers the appearance of behavioral heat by suppressing this putative serotonin dependent inhibitory system [15]. Again our observation agrees with this proposition since the decrease in 5-HT content was evident only in animals receiving progesterone. This fact further substantiates the possibility that a permanent modification of the serotonergic cell's response to progesterone is responsible for the observed difference in sexual behavior induced by amphetamine administration during gestation. Coincidently, the difference in 5-HT content was found only in the medial hypothalamic fraction which included the ventromedial nucleus, where neurons containing 5-HT have recently been found [4]. It has been clearly demonstrated that the ventromedial nucleus plays a key role in the control of sexual behavior in the female rat [2,12]. The suggestion of a decreased activity in the serotonergic pathways in the brain of amphetamine offspring is not contradictory with our finding of normal 5-HIAA levels, since catabolism of serotonin is not necessarily correlated with the activity of serotonin cells [7, 8, 9] and moreover an unknown fraction of brain 5-HIAA originates from intracellular 5-HT catabolism independent of synaptic release [9].

In view of the above considerations it appears most likely that behavioral changes are dependent on changes in neurotransmitter metabolism induced by prenatal amphetamine exposure although other possibilities such as changes in steroid receptors, pharmacokinetics of injected hormones, differences in sensory thresholds, etc cannot be excluded. In spite of the differences observed in sexual behavior, neuroendocrine parameters measured in this study were normal, emphasizing the duality of the structures controlling sexual behavior and ovulatory phenomena.

It can be concluded that amphetamine treatment during pregnancy exerts long lasting effects on monoaminergic metabolism and sexual behavioral responses to ovarian steroids.

REFERENCES

1. Aboul-Enein, H. Y. Amphetamine: a pharmacological profile. *Amer. J. Pharmac.* **143:** 161–164, 1971
2. Carrer, H. F., G. Asch and C. Aron. New factor concerning the role played by the ventromedial nucleus in the control of estrous cycle duration and sexual receptivity in the rat. *Neuroendocrinology.* **13:** 129–139, 1973
3. Chang, C. C. A sensitive method for spectrophotofluorometric assay of catecholamines. *Int. J. Neuropharmac.* **3:** 643–649, 1964
4. Chan-Palay, V. Indoleamine neurons and their processes in the normal rat brain and in chronic diet-induced thiamine deficiency demonstrated by uptake of ^{3}H-serotonin. *J. comp. Neurol.* **176:** 467–494, 1977.
5. Cox, R. H. and J. L. Perhach. A sensitive, rapid and simple method for the simultaneous spectrophotofluorometric determinations of norepinephrine, dopamine, 5-hydroxytryptamine and 5-hydroxyindolacetic acid in discrete areas of brain. *J. Neurochem.* **20:** 1777–1780, 1973.
6. Everitt, B. J., K. Fuxe, T. Hökfelt and G. Jonsson. Role of monoamines in the control by hormones of sexual receptivity in the female rat. *J. comp. physiol. Psychol.* **89:** 556–572, 1975.
7. Gessa, G. L. and R. Gessa. Significato dei metaboliti delle monoamine cerebrali in rapporto alla funzione neuronale. *Acta Neurologica.* **31:** 539–548, 1976.
8. Grahame-Smith, D. G. Studies in vivo on the relationship between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with a monoamine oxidase inhibitor and l-tryptophan. *J. Neurochem.* **18:** 1053–1066, 1971.
9. Green, A. R. and D. G. Grahame-Smith. Effects of drugs on the processes regulating the functional activity of brain 5-hydroxytryptamine. *Nature.* **260:** 487–491, 1976.
10. Hitzemann, B. A., R. J. Hitzemann, D. A. Brase and H. H. Loh. Influence of prenatal d-amphetamine administration on development and behavior of rats. *Life Sci.* **18:** 605–612, 1976.

11. Malmnäs, C. O. Monoaminergic influences on testosterone activated copulatory behaviour in castrated male rat. *Acta Physiol. scand. Suppl.* **395:** 1–128, 1973.
12. Mathews, D. and D. A. Edwards. The ventromedial nucleus of the hypothalamus and the hormonal arousal of sexual behaviors in the female rat. *Hormones Behav.* **8:** 40–51, 1977.
13. Metcalf, G. A rapid method for the simultaneous determination of noradrenaline, dopamine and 5-HT in small sample of brain tissue. *Anal. Biochem.* **57:** 316–320, 1974.
14. Meyerson, B. J., M. Eliasson, L. Lindström, A. Michanek and A. C. Söderlund. Monoamines and sexual behavior. In: *Psychopharmacology, Sexual Disorders and Drug Abuse.* edited by T. A. Ban, J. R. Boissier and J. Gessa. Amsterdam: North Holland, 1973.
15. Meyerson, B. J., H. Carrer and M. Eliasson. 5-hydroxytryptamine and sexual behavior in the female rat. *Adv. Biochem. Psychopharmac.* **11:** 229–242, 1974.
16. Nasello, A. G., C. A. Astrada and O. A. Ramirez. Effects on the acquisition of conditioned avoidance responses and seizure threshold in the offspring of amphetamine treated gravid rats. *Psychopharmacologia.* **40:** 25–31, 1974.
17. Nasello, A. G. and O. A. Ramirez. Openfield and Lashley III maze behaviour of the offspring of amphetamine-treated rats. *Psychopharmacology.* **58:** 171–173, 1978.
18. Nasello, A. G. and O. A. Ramirez. Brain catecholamines metabolism in offspring of amphetamine treated rats. *Pharmac. Biochem. Behav.* **9:** 17–20, 1978.
19. Nelson, M. M. and J. O. Forfar. Association between drugs administered during pregnancy and congenital abnormalities of the fetus. *Brit. med. J.* **1:** 523–527, 1971.
20. Shaikh, A. A. and S. A. Shaikh, Adrenal and ovarian steroid secretion in the rat estrous cycle temporally related to gonadotropins and steroid levels found in peripheral plasma. *Endocrinology.* **96:** 37–44, 1975.