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Brain Catecholamines Metabolism in Offspring of Amphetamine Treated Rats

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NASELLO, A. G. AND O. A. RAMIREZ. *Brain catecholamines metabolism in offspring of amphetamine treated rats.* PHARMAC. BIOCHEM. BEHAV. 9(1) 17–20, 1978.—Previous observations have pointed out that treatment with amphetamine during pregnancy produces behavioral modifications in adulthood. In order to elucidate some possible brain biochemical mechanisms that could explain the behavioral changes observed we have determined the endogenous content of brain dopamine and noradrenaline, the in vivo rate of conversion of tyrosine-C^{14} in such amines and the activity of tyrosine-hydroxylase, the rate limiting enzyme in catecholamines biosynthesis. We did not observe modifications in the endogenous content of dopamine and noradrenaline but did observe an increase in the conversion rate of tyrosine-C^{14} in such amines and also in the tyrosine-hydroxylase activity. These results indicate that treatment with amphetamines during fetal age produces catecholamine metabolism modifications that persist throughout adulthood, although the influence of behavioral modifications of the mothers cannot be ruled out. The relationship with behavioral changes is discussed.

Amphetamine Pregnancy Brain catecholamine metabolism

OVER the last years, the use of amphetamines by women during pregnancy has become common. It is used as a psychopharmacological agent and, more frequently, as an appetite supressant [16,18]. For this reason many authors have been interested in studying the effects of prenatal administration of amphetamine on different post-natal parameters in experimental animals ([5, 8, 9, 13, 14, 15, 17, 19] Nasello *et al.*, submitted). Some of them have correlated behavioral modifications with brain catecholamine (CA) levels [8,14] but the results are somewhat contradictory. Thus, a decrease of CA in total brain of rat offspring at 35 days of age has been observed, but this change was apparent only in some brain regions at 84 days of age, when mothers were injected with 3 mg/kg of d-amphetamine, once a day, after the fifth day of pregnancy until delivery. Offspring of both ages had an increased motor activity but this increase was more apparent in the eldest ones when the changes in CA were smaller. They also reported that the dose used by them caused pup mortality until 12 days of age [8]. On the other hand, the mice injected with 5 mg/kg of d-amphetamine, once a day, during the last third of pregnancy, showed an increase in whole brain noradrenaline (NA) and dopamine (DA) at 30 days of age but no differences from control NA and DA brain levels at 75 days of age were observed. These offspring also presented a heightened open-field activity level, which was more prominent at 75 days than at 30 days of age [14].

In previous papers we have reported behavioral changes in rat offspring whose mothers received 0.5 mg/kg of amphetamine, once a day, during the whole pregnancy ([15] Nasello *et al.*, submitted). We proposed that those changes might be due to a modification of brain CA metabolism. Present studies were carried out to corroborate this hypothesis.

METHOD

Prenatal Treatment

Albino rats of our colony (Wistar origin) were selected by their learning ability in a shuttle box as has been previously described [15]. Females were injected daily during pregnancy with 0.5 mg/kg of d,l-amphetamine sulfate (Purest) subcutaneously after the first day (determined by inspection of the vaginal smear each morning) until delivery. After birth, no drug was administered to either the dams or the pups. Control group received saline only. At birth the size of the litters was adjusted to eight (when possible, four females and four males). Offspring were reared by their natural mothers until weaning at one month of age, as is normal in our laboratory. They were not cross-fostered at birth, as a model of human drug use. Animals were maintained in a temperature regulated room (23 ± 2°C) on a 12 hr light–12 hr dark cycle and they had food and water available ad lib. All the following studies were made with 90–120 day old male rats, 250–300 g body weight of at least six different litters distributed at random. As has been stated previously, the treatment does not modify body weight of the offspring of amphetamine treated rats [15].

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Catecholamine Assay

Rats were killed by decapitation. Whole brains of rats born from control and amphetamine treated mothers were immediately removed and homogenized in 0.4 N perchloric acid. NA was assayed spectrofluorometrically after adsorption and elution from alumina according to Euler and Lishajko [7]. DA was assayed from the eluate by the method of Carlsson and Waldeck [4] with modifications as described by Drujan *et al.* [6].

Conversion Rate of Tyrosine-C^{14} to DA and NA in Total Brain

Tyrosine-C^{14} (100 μCi/kg IV) was injected 30 min prior to sacrifice. Total brains were removed and homogenized in 0.4 N perchloric acid and centrifuged. Total homogenated radioactivity was determined in an aliquot of 0.1 ml. The supernatant solutions were treated with alumina to adsorb catecholamines. The alumina columns were then eluted and DA and NA in the effluent were separated by Dowex 50-X8 column [2]. Radioactivity of both fractions was determined [21].

Tyrosine Hydroxylase Assay

Tyrosine hydroxylase (TH) activity was measured by duplicated determinations with the coupled (hog kidney) decarboxylase method [22], with no major modifications, using l-tyrosine-l-C^{14} as substrate. For this assay the whole brains were homogenized in a 0.001 M sodium phosphate buffer (pH 7).

Protein Determination

An aliquot of the supernatant from the enzyme assay was used for protein determination by the method of Lowry *et al.* [10] with bovine serum albumina as standard. Brain dry weights were determined simultaneously.

Statistical Methods

Differences between groups assessed statistically by a two-tailed Student *t* test. They were considered significant when p was equal to or less than 0.05.

RESULTS

A can be seen in Table 1 this treatment does not modify the endogenous content of CA in total brains. Nevertheless, the conversion rate of tyrosine-C^{14} into DA and NA is markedly higher in the treated group. The total homogenated radioactivity was the same in both groups (Table 2). In the experimental group there is also an increase in the activity of TH, the rate limiting enzyme in CA biosynthesis, expressed as nmoles of $^{14}CO_2$ evolved per gram of fresh tissue per hr (Table 3). There were no changes either in the protein or in the water content of the brains (Table 4). Therefore, the increase in the enzyme activity is probably due to an increase of the specific activity.

TABLE 1

BRAIN DA AND NA CONTENT IN OFFSPRING OF RATS TREATED DURING PREGNANCY (μG/G W.W. ± SE). AMPHETAMINE DOSE: 0.5 MG/KG

	Dopamine	Noradrenaline
Saline	0.970 ± 0.082 N=9	0.369 ± 0.024 N=9
Amphetamine	0.871 ± 0.056 N=10	0.327 ± 0.016 N=10

N: number of animals

DISCUSSION

Previous investigations in our laboratory have shown that the treatment used in the present studies resulted in behavioral modifications of adult offspring, such as an increase in learning ability, motor activity and hippocampal seizure susceptibility ([15] Nasello *et al.*, submitted).

These changes might be due to a modification of central catecholamines metabolism. Our present results do not show any variations in the endogenous CA content although treated group values tend to be slightly smaller. Other authors have reported changes of central CA content of offspring when they administered amphetamine during preg-

TABLE 2

TYROSINE-C^{14} CONVERSION INTO CA IN BRAIN OF OFFSPRING OF RATS DURING PREGNANCY (C.P.M./G. W.W. OF THE INDIVIDUAL VALUES AND THEIR RESPECTIVE MEANS ± SE). AMPHETAMINE DOSE: 0.5 MG/KG

	Total homogenated radioactivity		Dopamine-C^{14}		Noradrenaline-C^{14}	
	Saline	Amph.	Saline	Amph.	Saline	Amph.
Exp. 1	—	—	391	548	166	341
Exp. 2	56.560	50.007	415	471	183	203
Exp. 3	54.648	74.223	327	527	65	202
Exp. 4	70.870	57.362	375	587	116	166
Means	60.693	60.531	377†	533*	132¶	228§
± SE	±5.119	±7.168	±19	±24	±27	±39

signficance levels:
* vs. †: $p<0.0005$
§ vs. ¶: $p<0.05$

TABLE 3

TYROSINE-HYDROXYLASE ACTIVITY IN BRAIN OF OFFSPRING OF RATS TREATED DURING PREGNANCY (NMOLES/G W.W/H. ± SE). AMPHETAMINE DOSE: 0.5 MG/KG

Saline	Amphetamine
7.91 ± 0.56 N=10	9.56 ± 0.25 N=10

N: number of animals
Significance level: $p<0.02$

TABLE 4

PROTEIN AND WATER CONTENT IN BRAIN OF OFFSPRING OF RATS TREATED DURING PREGNANCY. AMPHETAMINE DOSE: 0.5 MG/KG

	Protein mg/g w.w ± SE	Water % w.w. ± SE
Saline	113.12 ± 0.97 N=7	81.15 ± 0.10 N=7
Amphetamine	114.12 ± 0.64 N=5	80.98 ± 0.14 N=5

N: number of animals

nancy and they have correlated these modifications with behavioral alterations [8,14] (see Introduction). It is interesting to note that the major behavioral changes are observed when the CA content modifications are no longer apparent or occur only in some brain areas. This is in agreement with our present findings.

When we study a more dynamic aspect of these amines metabolism, we notice that conversion rate of tyrosine-C^{14} into DA and NA is markedly increased. There is also a higher level of tyrosine hydroxylase activity, the rate limiting enzyme in CA biosynthesis, which is probably due to an increase of specific activity since there are no changes either in protein or in water content of the brain. It has also been described by others that a treatment during pregnancy may produce changes in brain TH activity in the offspring that persists throughout adulthood. Some of them have observed an increase when they used reserpine and d,lα-methyl-p-tyrosine in chickens [11,12], although it has been described that, in rats, reserpine produced a decrease of this enzyme activity [1]. When ethanol was used some authors supported an increase of TH activity [3] that was not observed by others [20]. These changes in brain TH activity may or may not be accompanied by changes in the endogenous brain CA levels. The kind of treatment used by the different authors was not the same, and, moreover, it has been suggested that there is a critical period for drug administration during pregnancy to induce brain TH activity modifications [1,11]. Our results show an increase of brain TH activity without modification of endogenous CA content. This may indicate that administration of amphetamine during pregnancy produces a higher brain CA turnover in adult offspring. This may account for the behavioral changes observed ([15] Nasello *et al.*, submitted). However, the influence of behavioral modifications in the mothers which received the drug cannot be ruled out because cross-fostering had not been done.

These animals with permanent modification of brain CA turnover may be used as experimental tools to investigate the participation of catecholaminergic mechanisms in different experimental situations, although regional studies would also be desirable.

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