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THE EFFECT OF MATERNAL PROGESTERONE ON BRAIN MONOAMINE OXIDASE ACTIVITY OF NEONATAL RATS

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SUMMARY

Monoamine oxidase activity was measured in the brains and uteri of neonatal rats exposed to pre- and/or postnatal progesterone. Daily doses of progesterone were injected subcutaneously into pregnant females on gestational days 8–17 and postpartum days 2–6. On postpartum day 1, half of the pups of each litter were cross-fostered to a female in the opposite experimental condition. Pups were sacrificed on postpartum days 1, 3 and 7, and whole brain type A and B MAO activity was determined by a radioisotope assay utilizing ^{14}C-labelled 5-hydroxytryptamine (5-HT) and phenylethylamine (PEA), respectively. Postnatal progesterone elevated both types of MAO activity in the brains of both sexes by day 7. Deamination of both substrates was highest in those animals receiving both pre- and postnatal progesterone. No sex differences in MAO activity were obtained for either control or progesterone-treated rat pups. Uterine MAO activity was not affected by maternal progesterone administration. Progesterone levels measured in blood plasma of 7-day-old animals were significantly higher in those animals receiving postnatal progesterone. These results support the observation that progesterone elevates brain MAO activity in vivo, and that progesterone administered to a lactating female elevates MAO activity in her suckling young. Implications for possible behavioral consequences of MAO alterations are discussed.

INTRODUCTION

The existence of MAO in two forms, A and B, was first described by Johnston[11]. The A form deaminates 5-hydroxytryptamine (5-HT), norepinephrine, dopamine, tyramine, octopamine and tryptamine; the B form metabolizes phenylethylamine (PEA) and benzylamine as well as dopamine, tyramine and tryptamine. Each form

follows a separate time course of development in the brains of rats[3] and mice[12]. Deamination of 5-HT is at 30–40% of adult levels at birth[2,15], whereas, at 6 days of age, type B activity represents only 9% of adult levels, or 3% of total MAO activity[19].

Several lines of evidence indicate that MAO activity may be altered by gonadal hormones. MAO activity varies in accordance with rat estrous[6,8,13,28] and human menstrual[26] cycles, and peaks, when progesterone is known to be at its highest, just prior to diestrus in the rat[8] and soon after day 21 in the human[26]. Exogenous progesterone stimulates MAO activity in rat uterus, ovaries and adrenals[8], although some researchers report finding this effect only in the uterus[4,25]. On the other hand, both estradiol and testosterone reduce MAO activity[16,18]. High levels of gonadal hormones during the second trimester of pregnancy can affect development of the human fetus. Children born to women treated with progesterone in pregnancy were found to progress to higher academic levels than matched controls[5]. Recent work in our laboratory has demonstrated that rats born to progesterone-treated mothers display altered maze-learning ability[10]. The discovery of behavioral changes due to maternal progesterone treatment led us to explore the possibility that changes in MAO activity may in part be mediating the observed effects.

MATERIAL AND METHODS

Preparation of animals

Adult female and male rats of the Long–Evans strain (obtained from Charles River Laboratories) were housed in individual wire cages with ad libitum access to food pellets and water. At least two females were time-mated on any given day to facilitate cross-fostering. On day 8 of pregnancy (counting day 1 as the day when sperm was detected), daily subcutaneous injections of progesterone at a dose of 3.3 mg/kg, or an equivalent amount of the olive oil vehicle, were initiated. Injections were continued through gestational day 17, 5 days prior to birth.

On day 1 following birth, pups were sexed and cross-fostered so as to obtain a similar distribution among each of the 4 experimental conditions: those receiving (1) both prenatal and postnatal progesterone treatment (P-P). (2) prenatal progesterone and postnatal control treatment (P-C), (3) prenatal control and postnatal progesterone (C-P), and (4) prenatal and postnatal control treatment (C-C). Postnatal maternal injections of progesterone or the vehicle were resumed the next day and continued as long as there were pups left in the litter.

Rat pups were sacrificed by decapitation on days 1, 3, 7 and 14 after birth. The brains were rapidly removed and placed in small vials kept in ice for periods of up to 3 h before homogenization. At the same time, the uteri were removed from female pups and frozen at —20 °C, for subsequent MAO activity analysis. The blood of 7-day-old animals was collected in heparinized tubes, centrifuged for 5 min, and the resultant plasma stored at —20 °C. In addition, MAO activity towards 5-HT and PEA was also determined in 4 adult males and 8 females (4 in estrus and 4 in diestrus).

Monoamine oxidase assay

The procedure used was a radioisotope assay using 0.1 m*M* [^{14}C]5-hydroxytryp-

tamine as the substrate for type A MAO and 26 μM [^{14}C]phenylethylamine for type B MAO, essentially as described by Roth[22]. The concentrations of 5-HT and PEA used are similar to their reported K_m values for rat mitochondrial type A and B MAO, respectively[9]. Whole brains were homogenized in 0.1 M potassium phosphate buffer, pH 7.4 and centrifuged twice for 10 min at 600 $\times$ g. The resulting supernatant solutions were diluted to 1/20 of the original concentration and 0.2 ml aliquots were assayed for MAO activity in a total volume of 0.4 ml of 0.05 M potassium phosphate buffer, pH 7.4. Reaction mixtures were incubated for 60 min, at which time 50 μl of 0.4 M hydrochloric acid was added to stop the reaction. Aliquots (0.2 ml) were removed, passed through cation exchange resin columns (Bio-Rex 70) and the effluent collected in scintillation vials. The resin was washed with an additional 2.8 ml of water which was also collected in the vials, and the radioactivity was determined by scintillation spectrometry. Uterine MAO activity was assayed essentially as described above, except that 0.3 ml of the enzyme preparation and 5×10^{-4} M semicarbazide (an inhibitor of plasma amine oxidase) were added to each reaction mixture.

Protein concentrations were determined by the method of Lowry et al.[17]. All values were expressed in nmole of deaminated product formed per 60 min per mg protein.

Progesterone assay

Progesterone levels in 7-day animals were determined with the aid of a radioimmunoassay kit obtained from CIS laboratories, Saluggia, Italy. The frozen plasma was thawed and recentrifuged for another 5 min prior to the assay.

Materials

Radioactively labelled [^{14}C]5-hydroxytryptamine (49.3 mCi/mmole) and phenylethylamine (50.98 mCi/mmole) were obtained from New England Nuclear, Boston, Mass.; unlabelled 5-HT and PEA from Sigma Chemical, St. Louis, Mo.; and pargyline from Abbott Laboratories, Chicago, Ill. Bio-Rex was obtained from Bio Rad Laboratories, Richmond, Calif. All chemicals used were of the purest available from commercial sources.

RESULTS

Brain and body weights

Mean brain and body weights for control and pre- and postnatal progesterone-treated pups were not significantly different on day 1, 3 and 7 after parturition. There was an increase with age in both measures, and the brain/body weight proportion remained constant. In addition, there were no consistent differences in the size of the litters, sex ratio, or length of gestation between progesterone-treated and control females. Cross-fostering was in all cases successful; the females readily accepted pups from other litters.

Sex differences in development of brain MAO

Deamination of PEA and 5-HT in brain was determined in 1, 3, 7 and 14-day-

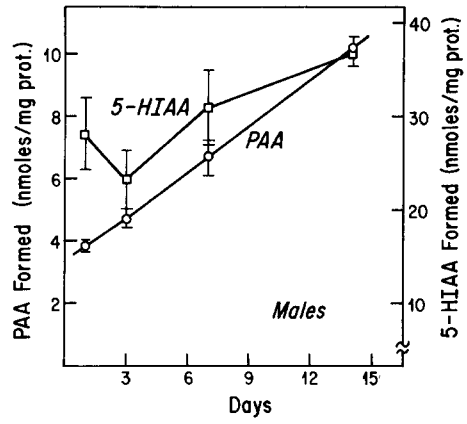

Fig. 1. Type A and B monoamine oxidase (MAO) activity in the developing male rat brain. Reaction mixtures containing 0.1 m*M* [^{14}C]5-hydroxytryptamine or 26 μM [^{14}C]phenylethylamine and brain homogenates (0.050–0.113 mg protein) were incubated for 60 min at 37 °C in a total of 0.4 ml of 0.05 *M* potassium phosphate buffer, pH 7.4. Reactions were terminated by addition of 50 μl of 0.4 *M* HCl and deaminated products formed, 5-hydroxyindoleacetic acid (5-HIAA) and phenylacetic acid (PAA), respectively, were separated by cation exchange chromatography. Data shown are the mean ± S.E. values for 5-HIAA and PAA formed in 1 h. N per group ranges from 7 to 8 rats.

old rat pups whose mothers had received control injections, and untreated adults. Results of these experiments, shown in Figs. 1 and 2, indicate that deamination of both substrates increased with age in both males and females. There were no significant sex differences in MAO activity at any prepubertal age with either substrate. Comparison of Figs. 1 and 2 demonstrates that the increase in type A and B activity in males is similar to that in females. Type B MAO activity appears to be almost identical in both sexes, showing a steady increase during the first two weeks of life. MAO activity towards 5-HT exhibits its sharpest rise between postnatal days 3 and 7, levelling off somewhat thereafter.

Adult values of whole brain MAO activity towards 5-HT and PEA are presented

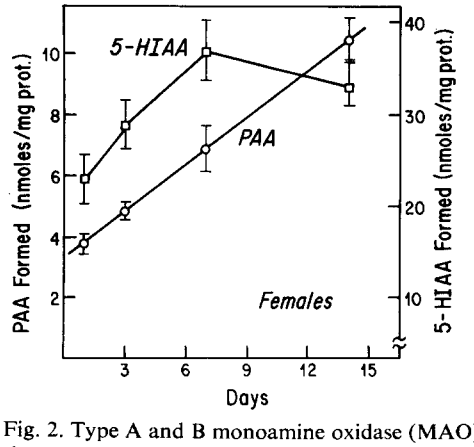

Fig. 2. Type A and B monoamine oxidase (MAO) activity in the developing female rat brain. Points shown are the mean ± S.E. values for metabolites 5-HIAA (5-hydroxyindoleacetic acid) and PAA (phenylacetic acid) formed. N per group ranges from 5 to 8 rats. See Fig. 1 for experimental data.

TABLE I

Type A and B monoamine oxidase (MOA) activity in adult male and female rat brains

Data shown are the mean ± S.E. values for metabolites 5-HIAA (5-hydroxyindoleacetic acid) and PAA (phenylacetic acid) formed. All groups represent the mean of 4 subjects. See Fig. 1 for experimental details.

Sex	*Deaminated product formed (nmoles/mg prot./h ± S.E.)*	
	5-HIAA	*PAA*
Males	31.8 ± 3.4	37.7 ± 3.4
Estrous females	33.6 ± 1.9	37.7 ± 1.0
Diestrous females	50.7 ± 2.1*	37.9 ± 0.9

* Significantly different from males and estrous females ($P < 0.01$), deamination of 5-HT.

TABLE II

Deamination of 5-hydroxytryptamine (5-HT) in whole brain homogenates from neonatal rats exposed to pre- and/or postnatal progesterone

Data shown are the mean ± S.E. values for metabolite 5-HIAA (5-hydroxyindoleacetic acid) formed. See Fig. 1 for experimental details. Numbers in parentheses represent n per group.

Treatment			*5-HIAA formed (nmole/mg prot./h ± S.E.)*		
Prenatal	*Postnatal*	*Sex*	*Day 1*	*Day 3*	*Day 7*
Control	Control	M	28.2 ± 3.9 (5)	23.3 ± 3.2 (7)	31.1 ± 4.0 (6)
		F	23.0 ± 2.6 (7)	28.9 ± 2.8 (7)	36.9 ± 3.2 (5)
Control	Progesterone	M	—	23.8 ± 1.8 (9)	34.5 ± 3.9* (7)
		F	—	25.0 ± 1.6 (7)	38.4 ± 3.9* (6)
Progesterone	Control	M	—	28.1 ± 3.2 (8)	33.4 ± 3.5 (8)
		F	—	24.9 ± 1.7 (9)	30.0 ± 4.1 (7))
Progesterone	Progesterone	M	24.2 ± 2.6 (9)	29.0 ± 2.2 (8)	40.1 ± 4.1* (9)
		F	24.3 ± 2.2 (7)	21.7 ± 2.4 (7)	41.1 ± 4.2* (7)

* With sexes combined, postnatally treated animals (PP + CP) are significantly higher than controls and animals treated only prenatally (PC + CC), $P < 0.01$.

in Table I. Analysis of variance ($F = 16.7$; df = 2,9; $P < 0.01$) followed by Newman–Keuls post hoc tests[27] indicate that diestrous females had higher type A MAO activity than males ($P < 0.01$) and estrous females ($P < 0.01$). MAO activity towards PEA was nearly identical for males and females in both stages of the estrous cycle.

Progesterone effects on brain MAO

The effect of progesterone on 5-HT and PEA deamination in the brains of rat pups is reported in Tables II and III. Analyses of variance[27] of MAO activity reveal no sex differences for either type A or type B MAO. All subsequent analyses were therefore performed with sexes combined. No significant effects of prenatal pro-

TABLE III

Deamination of phenylethylamine (PEA) in whole brain homogenates from neonatal rats exposed to pre- and/or postnatal progesterone

Data shown are the mean ± S.E. values for metabolite PAA (phenylacetic acid) formed. See Fig. 1 for experimental details. Numbers in parentheses represent n per group.

Treatment			*PAA formed (nmole/mg prot./h ± S.E.)*		
Prenatal	*Postnatal*	*Sex*	*Day 1*	*Day 3*	*Day 7*
Control	Control	M	3.89 ± 0.14 (4)	4.89 ± 0.28 (8)	6.76 ± 0.57 (6)
		F	3.81 ± 0.30 (6)	4.93 ± 0.27 (8)	6.89 ± 0.73 (6)
Control	Progesterone	M	—	4.96 ± 0.30 (10)	7.86 ± 0.57* (9)
		F	—	4.59 ± 0.23 (8)	7.35 ± 0.49* (8)
Progesterone	Control	M	—	5.26 ± 0.29 (9)	6.41 ± 0.43** (8)
		F	—	4.62 ± 0.14 (10)	6.26 ± 0.23** (7)
Progesterone	Progesterone	M	3.75 ± 0.17 (10)	5.54 ± 0.33 (9)	8.74 ± 0.62* (9)
		F	3.63 ± 0.22 (8)	4.82 ± 0.21 (8)	8.56 ± 0.76* (7)

* With sexes combined, postnatally treated animals (PP + CP) are significantly higher than controls and animals treated only prenatally (PC + CC), $P < 0.01$.

** With sexes combined, animals treated pre- and postnatally are significantly higher than animals treated only prenatally (PP + PC), $P < 0.01$.

gesterone on type A or type B MAO activity were apparent on postnatal day 1. Significant effects of postnatal progesterone on brain type A MAO activity became evident on postnatal day 7, at which time 5-HT deamination was elevated in animals having received postnatal progesterone (i.e., PP + CP is greater than PC + CC) ($F = 5.94$; df = 1,54; $P < 0.02$). Analysis of the data presented in Table III for PEA deamination by MAO also indicates a significant increase in type B MAO on day 7 due to postnatal progesterone (PP + CP is greater than PC + CC) ($F = 14.13$; df = 1,58; $P < 0.01$). However, due to the smaller variability within groups for PEA deamination, a significant interaction ($F = 7.65$; df = 1,58; $P < 0.05$) between effects of prenatal and postnatal progesterone was detected. Accordingly, animals whose mothers received both pre- and postnatal progesterone (PP) displayed a higher type B MAO activity than those whose mothers received only prenatal progesterone (PC) ($F = 8.29$; df = 1,63; $P < 0.01$). In addition, results presented in Tables II and III also suggest that activity of both forms of the oxidase was higher in animals that received both pre- and postnatal progesterone (PP) than those that received only postnatal progesterone (CP), although this trend did not reach statistical significance.

Uterine MAO activity

Since Southgate[25] and Collins et al.[4] reported that MAO activity increases in the uteri of adult rats treated with progesterone, we were interested in seeing whether we could detect a similar increase in female offspring of progesterone-treated females. Values for deamination of 5-HT and PEA by MAO in neonatal rat uterus are presented in Tables IV and V, respectively. No significant elevations due to pro-

TABLE IV

Deamination of 5-hydroxytryptamine (5-HT) in uterine homogenates from neonatal female rats exposed to pre- and/or postnatal progesterone

Data shown are the mean ± S.E. values for metabolite 5-HIAA (5-hydroxyindoleacetic acid) formed. See Fig. 1 for experimental details. Numbers in parentheses represent n per group.

Treatment		*5-HIAA formed (nmole/mg prot./h ± S.E.)*		
Prenatal	*Postnatal*	*Day 1*	*Day 3*	*Day 7*
Control	Control	19.7 ± 4.5 (4)	18.7 ± 1.6 (6)	24.7 ± 1.6 (6)
Control	Progesterone	—	18.9 ± 2.7 (8)	23.9 ± 3.8 (7)
Progesterone	Control	—	16.0 ± 1.4 (9)	23.5 ± 2.5 (5)
Progesterone	Progesterone	18.7 ± 2.1 (6)	15.9 ± 1.1 (8)	20.5 ± 1.3 (7)

TABLE V

Deamination of phenylethylamine (PEA) in uterine homogenates from neonatal female rats exposed to pre- and/or postnatal progesterone

Data shown are the mean ± S.E. values for metabolite PAA (phenylacetic acid) formed. See Fig. 1 for experimental details. Numbers in parentheses represent n per group.

Treatment		*PAA formed (nmole/mg prot./h ± S.E.)*		
Prenatal	*Postnatal*	*Day 1*	*Day 3*	*Day 7*
Control	Control	5.78 ± 0.96 (4)	7.18 ± 0.51 (6)	8.52 ± 0.70 (6)
Control	Progesterone	—	6.34 ± 0.55 (8)	8.43 ± 0.42 (7)
Progesterone	Control	—	6.05 ± 0.43 (9)	8.30 ± 0.65 (5)
Progesterone	Progesterone	6.35 ± 0.79 (5)	6.60 ± 0.60 (8)	7.98 ± 0.34 (7)

gesterone occurred for either type A or B MAO. Both types of the oxidase were observed to increase with age.

Progesterone analysis

Progesterone was measured in 7-day-old rat pups in order to correlate any differences in MAO activity with levels of circulating progesterone. The resulting data were subjected to an analysis of variance[27] which revealed a significant elevation of plasma progesterone in both groups of pups (CP and PP) whose mothers received progesterone postnatally ($F = 18.0$; df = 1,44; $P < 0.001$). These pups had an average value of 3.99 ± 0.52 ng progesterone per ml plasma, compared to 1.62 ± 0.14 ng/ml in the blood of controls. No significant differences due to sex or prenatal progesterone were obtained.

DISCUSSION

The data reported in this paper demonstrate that the development of type A and B MAO in neonatal rat brain appears to follow different time courses, largely in

agreement with that observed by Jourdikian et al.[12] in mouse brain. They found that type A MAO reaches adult levels by postnatal day 15, whereas type B is only at 50% of adult levels at day 20. Similarly, in rat brain, the B type was found to develop more slowly than the A form of the oxidase, only to reach adult values (32% of total MAO) by postnatal day 25[19]. Our results show a linear increase of the B type of MAO from 13% of total activity at day 1 to 23% of total MAO at day 14. In contrast to the B form, the A form appears to reach a plateau by postnatal day 7. When compared to adult levels obtained in this study, type B increases from 10% of adult levels at birth to 28% by postnatal day 14. On the other hand, type A activity is already at 77% at birth and reaches adult levels within 2 weeks.

Few studies have attempted to find sex differences in the ontogenesis of MAO activity. Hardin[7] reported no differences in MAO activity in the brains of 2-day-old rat pups, using a selective type A substrate, 5-HT. Using a mixed type A and B substrate, tyramine, Kamberi and Kobayashi[13] found no significant differences in MAO activity in whole brain and specific brain regions of male and female adult rats, whereas Skillen et al.[24] reported that adult female rats have higher MAO activity towards 5-HT in brain. Human platelet MAO activity (type B) is generally higher in females[20], prior to, during, and after puberty[23]. Recently, significant differences in adult human brain have also been reported for MAO deamination of benzylamine, with higher activity in females[21]. The present study demonstrates that the development of either type of brain MAO activity in male and female rats does not differ significantly during the first two weeks of life. No sex differences in MAO activity towards PEA or 5-HT are evident until adulthood, at which time diestrous females exhibit greater MAO activity towards 5-HT than that of males or estrous females. In contrast, no sex differences were found with MAO deamination of PEA in adult brain.

The present finding that type A MAO activity is elevated during diestrus is in accord with results of Holzbauer and Youdim[8]. Progesterone levels were found to be highest in the adrenals and ovaries just prior to diestrus, which led these researchers to suggest that this hormone may be enhancing MAO activity at this time. In our study postnatal progesterone treatment is considerably more effective than prenatal treatment in increasing both types of MAO activity in both sexes by day 7, after birth, i.e. C-P $>$ C-C and P-P $>$ P-C. In addition, MAO activity in the C-P and P-P groups represent the two highest values. However, prenatal progesterone does contribute to the enhancing effect of postnatal progesterone, such that P-P animals have higher MAO activity towards both substrates than C-P animals. Thus, progesterone during fetal development may in some way 'prime' some system or systems that result in the elevation of MAO activity, the effect of which is expressed when progesterone is also administered postnatally.

The absence of a corresponding increase in uterine MAO activity due to progesterone in our female rat pups differs with previous findings that uterine MAO activity in adult rats is more susceptible than that of other body organs to progesterone manipulations[4,25]. It can be inferred from the above studies that the increase in uterine MAO activity is primarily localized in the endometrium. In this regard, Karavolas et al.[14] suggest that estrogen priming may be necessary for increased

uterine uptake of progesterone. If this is the case, then levels of estrogen present in the immature rat may be insufficient to induce proliferation of progesterone-sensitive receptors. In the absence of any appreciable progesterone binding in the neonatal uterus, progesterone is rapidly metabolized in the endometrium[1].

Increased MAO activity during development may have behavioral consequences in adulthood. Experiments in our lab reveal that rats treated with progesterone neonatally show altered rates of acquisition of maze learning ability[10]. Similarly, children born to mothers treated with progesterone during pregnancy exhibit above average intelligence[5]. Progesterone during development may accelerate a sequence of maturational events including the enhancement of MAO activity, and this accelerated development may have long-lasting effects. It should be noted here that progesterone exerted no gross maturational effects on infant rats; that is, there were no significant differences on measures of brain and body weights between progesterone-treated and control rats. Thus, the action of progesterone may be due to its influence on specific neurochemical processes during a critical stage of development.

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