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The effect of maternal progesterone injections on fetal development of brain monoamine oxidase of rats

ABIGAIL M. SNYDER, ELAINE M. HULL and JEROME A. ROTH

Dept. of Psychology, State University of New York at Buffalo, Amherst, N.Y. 14226 and (J.A.R.) Dept of Pharmacology and Therapeutics, State University of New York at Buffalo, Buffalo, N.Y. 14214 (U.S.A.)

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The activity of mitochondrial type A and B monoamine oxidase (MAO) in the brain of rats has been shown to increase during the first 3 weeks of life[2]. The type A form of MAO develops more rapidly with adult levels being reached by 14 days of age[3] while the activity of the B-type continues to rise through the third week after birth[7]. Few studies have been undertaken to assay MAO activity in rat fetal brain. Using histochemical methods, Shimizu and Morikawa[8] detected low levels of MAO activity in certain brain areas at 15 days of gestation. Bennett and Giarman[1] assayed MAO activity towards 5-hydroxytryptamine at 20 days of gestation, and found it to be already at 30% of adult levels.

Recently, we have demonstrated an increase in both A and B MAO activity in the brains of 7-day-old rats whose mothers were treated with progesterone before and after parturition[3]. Prenatal progesterone alone had no effect on MAO activity in newborn (1-day-old) rats; however, progesterone was not administered for 5 days prior to birth, so as not to interfere with parturition. Since the newborn rat pups were not exposed to progesterone for 5 days, it cannot be determined what influence progesterone had on brain MAO activity prenatally. Accordingly, the present study was undertaken to determine whether progesterone stimulates one or both types of MAO activity in the brains of fetuses exposed to progesterone via the mother.

Thirty-six female rats of the Long–Evans strain (obtained from Charles River Laboratories) were time-mated and housed in individual plastic cages with ad libitum food pellets and water. Animals were injected daily with either progesterone (3.3 mg/kg) or the olive oil vehicle alone starting day 7 of gestation and continuing until the day of sacrifice.

Fetuses were removed on days 14, 17, 20 and 22 of gestation, following decapitation of the mother. Their brains were rapidly removed, weighed and placed in small vials which were kept on ice for periods of up to 2 h prior to homogenization.

The procedure used was a radioisotope assay using 0.1 mM [^{14}C]5-hydroxytryptamine (5-HT) as the substrate for type A and 0.26 mM [^{14}C]phenylethylamine (PEA) for type B MAO, essentially as described previously[3]. Brains of 14–20-day-old

fetuses were homogenized in 10 vols. of 0.1 M potassium phosphate buffer, pH 7.4, whereas all other brains were homogenized in 5 vols. of buffer. After two centrifugations at 600 × *g* supernatant solutions were diluted as follows: 2.5:1 in the case of 14–20-day-old fetuses and 5:1 for 22-day-old fetuses.

Protein concentrations were determined by the method(s) of Lowry et al.[6]. All values were expressed in nmol of deaminated product formed per 60 min per mg protein.

All data were analyzed with analyses of variance using treatment and days as factors[9].

Radioactively labelled [^{14}C]5-hydroxytryptamine (49.3 mCi/mmol) and phenylethylamine (48.25 mCi/mmol) were obtained from Amersham, Arlington Heights, Ill., and New England Nuclear, Boston, Mass., respectively; unlabelled 5-HT and PEA from Sigma Chemical, St. Louis, Mo.; and pargyline from Abbott Laboratories, Chicago, Ill. Bio-Rex 70 (100–200 mesh) was obtained from Bio Rad Laboratories, Richmond, Calif. All chemicals used were the purest available from commercial sources.

Brain weights of 14, 17, 20 and 22-day-old fetuses are shown in Fig. 1. Progesterone-treated fetuses displayed a nearly linear increase in brain weight from day 14 (0.025 g) to day 22 (0.188 g). The growth rate of the brains of control fetuses was somewhat greater between days 14–17 and 20–22, leveling off slightly between days 17–20. Though this difference in growth rate resulted in a progesterone × day interaction when analyzed with an analysis of variance ($F = 6.78$; df = 1, 3: $P < 0.001$), it is important to point out that no obvious trend toward higher or lower brain weights was apparent for progesterone-treated fetuses.

The effect of progesterone on 5-HT and PEA deamination in the brains of fetuses is reported in Fig. 2A and B, respectively. For both control and progesterone-treated fetuses, type A and B MAO activity in brain increased only slightly between days 14 and 17 of gestation whereas on day 17 a rapid rise in activity of both forms of MAO was noted. An analysis of variance of type A MAO activity revealed a

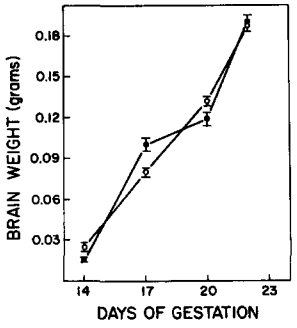

Fig. 1. Brain weights of 14- to 22-day-old fetuses. Statistical differences between progesterone-treated (open circles) and control (closed circles) fetuses are as follows: day 14, progesterone (p) > controls (C), ($F = 5.30$; df = 1; $P < 0.05$); day 17, C > p ($F = 19.37$; df = 1, 36; $P < 0.001$); day 20, p > C ($F = 7.02$; df = 1, 46; $P < 0.02$); day 22, C > p ($F = 4.20$; df = 1, 49; $P < 0.05$). n per group ranges from 8 to 29 animals.

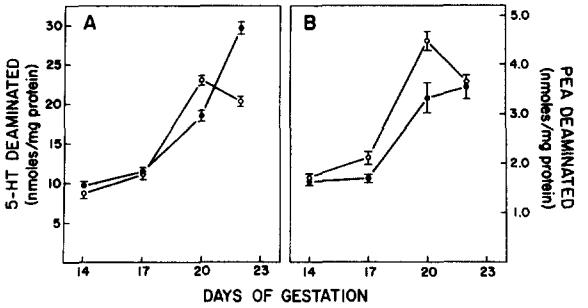

Fig. 2. A: type A MAO activity in the brains of progesterone-treated (open circles) and control (closed circles) fetuses. Reaction mixtures containing 0.1 mM [^{14}C]5-hydroxytryptamine (5-HT) and brain homogenates (0.236–0.320 mg protein) were incubated for 60 min at 37 °C in a total of 0.4 ml of 0.05 M potassium phosphate buffer, pH 7.4. Reactions were terminated by the addition of 50 μl of 0.4 HCl, and the deaminated product was separated by cation exchange chromatography. Points shown represent the mean $\pm$ S.E. value of 5-HT deaminated in one hour. Progesterone-treated animals exhibit higher MAO activity on day 20 ($F = 12.49$; df = 1, 46; $P < 0.002$) and lower activity on day 22 ($F = 51.44$; df = 1, 49; $P < 0.0001$). n per group ranges from 8 to 29 animals. B: Type B MAO activity in the brains of progesterone-treated (open circles) and control (closed circles) fetuses. Experimental details are the same as for Fig. 2A, except 0.26 mM [^{14}C]phenylethylamine (PEA) is used as the substrate. MAO activity is significantly increased in progesterone-treated animals on days 17 ($F = 9.54$; df = 1, 14; $P < 0.01$) and 20 ($F = 11.49$; df = 1, 46; $P < 0.002$). n per group ranges from 6 to 29 animals.

progesterone $\times$ day interaction ($F = 17.92$; df = 3, 147; $P < 0.0001$), which is clearly due to a greater increase in MAO activity in progesterone-treated animals by day 20, with a subsequent decrease in activity, resulting in control animals having higher values at day 22. The overall ANOVA for the activity of the B form of the oxidase in fetal brains also revealed a significant progesterone effect ($F = 5.56$; df = 1, 126, $P < 0.02$). Type B MAO was significantly increased by progesterone on days 17 and 20. After day 20 MAO deamination of PEA in progesterone-treated animals fell to near control values.

The data reported in this paper reveals for the first time the development of the A and B forms of MAO in rat brain during gestation. As early as day 14 of gestation both forms of MAO can be detected. Type A MAO activity of controls rat is almost 20% of the adult levels (48.5 nmol 5-HT deaminated/mg protein) on day 14, and increases most rapidly after gestational day 17, to reach 39% of adult values on day 20 and 61% by day 22, about 36 h prior to birth. Type B MAO activity is at 10% of adult values (15.8 nmol PEA deaminated/mg protein) on day 14, increasing after day 17 to 21% and 23% of adult values by days 20 and 22, respectively.

We also report herein that progesterone chronically administered to pregnant rats stimulates the activity of type B MAO as early as gestational day 17, and elevates A MAO activity by day 20. It is interesting to note that although both forms of the oxidase are present as early as gestational day 14, progesterone treatment for 7 days prior to this time does not yet appear to be able to stimulate MAO activity. This is similar to results reported in this laboratory for progesterone effects on MAO activity in weanling rats whose mothers were treated with an equivalent dose of the hormone[3].

Thus, it would appear that the effects of progesterone on MAO in the developing rat brain require at least one week of continuous administration before oxidase levels are affected.

The decline in MAO activity seen after 20 days of gestation in progesterone-treated fetuses is somewhat puzzling. Progesterone levels in the pregnant rat normally fall off rapidly at this time, which may in part be due to increased metabolism by the fetoplacental unit[4]. If progesterone is being rapidly removed at this time, this may explain the large decrease in the MAO activity at this time. An alternative explanation is that high levels of progesterone are physiologically disruptive at this point of development.

Recent studies in our laboratory demonstrate that progesterone-treated pups perform better on an active avoidance task at 19 days of age (Snyder, unpublished observations). Furthermore, progesterone-treated males exhibited slower performance on a Lashley III maze and less copulatory and aggressive behavior in adulthood[5]. These observations suggest that high levels of progesterone during important periods of development may alter certain aspects of biochemical maturation of the brain. Modification of MAO activity may represent only one change in response to progesterone treatment and it is likely that this steroid also modulates the activity of a variety of other important neurochemical systems during development.

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1 Bennett, D. S. and Giarman, N. J., Schedule of appearance of 5-hydroxytryptamine (serotonin) and associated enzymes in the developing rat brain, *J. Neurochem*, 12 (1965) 911–918.
2 Blatchford, D., Holzbauer, M., Grahame-Smith, D. G. and Youdim, M. B. H., Ontogenesis of enzyme systems deaminating different monoamines, *Brit. J. Pharmacol.*, 57 (1976) 279–293.
3 Franz, J. R., Hull, E. M., Snyder, A. M. and Roth, J. A., The effect of maternal progesterone on brain monoamine oxidase activity of neonatal rats, *Brain Research*, 158 (1978) 397–406.
4 Heap, R. B., Galil, A. K. A., Harrison, F. A., Jenkin, G. and Perry, J. S., Progesterone and oestrogen in pregnancy and parturition: comparative aspects and hierarchical control. In *The Fetus and Birth, CIBA Foundation Symposium*, Elsevier, Amsterdam, 1977, pp. 127–150.
5 Hull, E. M., Franz, J. R., Snyder, A. and Nishita, J. K., Perinatal progesterone treatment and learning, social and reproductive behavior in rats, *Horm. Behav.*, in press.
6 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measurement with the Folin phenol reagent, *J. biol. Chem.*, 193 (1951) 265–275.
7 Mantle, T. J., Garret, N. J. and Tipton, K. F., The development of monoamine oxidase in rat liver and brain, *FEBS Lett.*, 64 (1976) 227–230.
8 Shimizu, N. and Morikawa, N., Histochemical study of monoamine oxidase in the developing rat brain, *Nature (Lond.)*, 184 (1959) 650–651.
9 Winer, B. J., *Statistical Principles in Experimental Design*, McGraw–Hill, New York, 1971.