Effects of Progesterone on Concentrations of Monoamines in Hypothalamic Areas and Plasma Prolactin Levels in Rats (43136)

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Abstract. **The role of progesterone to increase prolactin (PRL) secretion on the first estrous day in pubertal rats was compared with its role in adult cyclic rats. The first estrus was induced by the administration of pregnant mare serum gonadotrophin (5 IU) at 28 days of age. A subcutaneous administration of 2.5 or 7.5 mg of progesterone/100 g body wt significantly increased the concentration of plasma PRL in pubertal rats within 4 hr. The PRL level obtained after progesterone administration was greater than that in similarly treated adult rats. The concentration of dopamine in the arcuate nucleus-median eminence (ARC-ME) in pubertal rats significantly decreased after a lower dosage of progesterone was administered, but no change was found in the preoptic area concentration. In adult estrous rats, the concentration of dopamine in the ARC-ME showed a tendency to decrease after the administration of a larger dose of progesterone (7.5 mg/100 g body wt). No change was observed in the concentrations of indoleamines in the preoptic area and ARC-ME after the administration of progesterone in both pubertal and adult rats. The concentrations of dopamine in the preoptic area and ARC-ME were lower in pubertal rats than in adults. The concentration of 5-hydroxyindole acetic acid and the ratio of 5-hydroxyindole acetic acid to 5-hydroxytryptamine in the ARC-ME were higher in pubertal rats than in adults. These results indicate that progesterone causes a greater increase in tonic PRL secretion in pubertal rats than in adult rats and that a lower hypothalamic dopamine activity and a higher serotonin activity in pubertal rats may account for these differences.** [P.S.E.B.M. 1990, Vol 195]

We (1) reported previously that progesterone could induce the nocturnal prolactin (PRL) surge more easily in ovariectomized pubertal rats than in ovariectomized adult rats and suggested that the mechanism regulating PRL secretion was more sensitive to progesterone in peripubertal rats than in adult rats. Since dopamine has been well known as an inhibitor of PRL secretion in adult rats (2) and since administration of a dopamine synthetic enzyme (decarboxylase) inhibitor (NSD-1015) increased plasma PRL levels in ovariectomized pubertal rats with progesterone implants (3), dopamine may be involved in the mechanism of PRL secretion induced by progesterone in peripubertal rats. Moreover, the involvement of serotonin in the hypothalamus in PRL secretion induced by progesterone administration has been reported by several workers (4–7) The aim of this study was to elucidate the participation of hypothalamic dopamine and serotonin in PRL secretion induced by progesterone during the first estrous cycle of pubertal rats with special reference to the results obtained in adult rats.

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Materials and Methods

Animals. Virgin female Wistar-Imamichi strain rats bred in our laboratory were used at the pubertal (80–95 g body wt, 31 days of age) and adult (180–210 g body wt, 60–70 days of age) stages. They were housed in individual cages under controlled conditions of light (14-hr light:10-hr dark, lights on at 05:00 hr) and temperature (24 ± 2°C) with free access to food (CE2; Nihon CLEA, Yokohama, Japan) and water. In pubertal rats, pregnant mare serum gonadotrophin (5 IU/0.1 ml 0.9% NaCl; Sankyo Pharmaceuticals KK, Kawasaki, Japan) was injected between 08:00 and 09:00 hr at 28 days of age. The vaginal opening and cornification of the smear were observed 3 days after pregnant mare serum gonadotrophin injection. Adult rats showing at least two consecutive regular 4-day cycles were used.

Progesterone (2.5 or 7.5 mg/100 g body wt) was injected subcutaneously to pubertal and cyclic adult rats between 07:00 and 08:00 hr on the day of estrus. Animals in the control group were injected with 0.2 ml of sesame oil. They were decapitated 4 hr after progesterone injection.

Some of the animals treated with the lower dose of progesterone or oil at 07:00 hr were intraperitonealy injected with α-methyl-*para*-tyrosine (250 mg/kg body wt; Sigma Chemical Co., St. Louis, MO) at 09:00 hr and were decapitated 2 hr later. The turnover rate of dopamine in the dissected hypothalamic area was calculated from the degree of a decrease in dopamine concentration during 2 hr after α-methyl-*para*-tyrosine injection according to the method of Brodie *et al.* (8).

The animal room was kept quiet for at least 1 hr before the blood or brain samples were taken. Animals were decapitated within 10 sec after being removed from the cage. Trunk blood was collected for plasma PRL radioimmunoassay. The brain was removed within 30 sec after decapitation, placed dorsal side up on dry ice in a cooling box, and frozen as quickly as possible. The frozen brain was stored at −70°C and analyzed for amines within 1 month.

Brain Dissection. Serial frontal sections were cut in a freezing condition in a cooling chamber (−5 to −10°C) by using a cooled stainless razor blade. The thickness was measured to the nearest 1/20 mm with a slide caliper (Hardened Stainless; Kanon, Tokyo, Japan). The area of the brain excised was orientated by the brain atlas of Sherwood and Timiras (9) and Paxinos and Watson (10) for pubertal rats and adult rats, respectively. The preoptic area (POA) was punched out with a cooled stainless tube (i.d., 1.5 mm) from a frontal section covering between Å 7.5 and Å 6.5 of the atlas in pubertal rats and that covering between 9.1 mm and 7.8 mm anterior from the 0 plane of the atlas in adult rats. The arcuate nucleus-median eminence (ARC-ME) was punched out with a cooled stainless tube (i.d., 1 mm) as a half circle from a frontal section covering between Å 5.5 and 4.0 of the atlas in pubertal rats and that covering between 6.9 and 5.2 mm anterior from the 0 plane in adult rats. The center of the tube was placed at the third ventricle to punch out both POA and ARC-ME.

Extraction of Amines. All microdissected tissues were homogenized in 200 μl of 0.2 *N* perchloric acid containing 10^{-4} *M* cysteine and 4 ng of *N*-methyl hydroxytryptophan (NMHT) as an internal standard, and the homogenate was then kept for 30 min under ice-cold conditions. The homogenate was centrifuged at 12,000*g* for 5 min at 4°C. The supernatant was filtered by using cellulose filters of 0.22-μm pore size (Nihon Millipore KK, Tokyo, Japan). The filtrate was used for amine analysis. Protein contents in the precipitate after the homogenate was centrifuged were determined according to the method of Lowry *et al.* (11) using bovine serum albumin as a standard. Protein contents in the POA in pubertal and adult rats were 0.147 ± 0.006 mg (mean ± SE, $n = 21$ rats) and 0.166 ± 0.009 ($n = 16$), respectively. Those in the ARC-ME were 0.131 ± 0.009 mg ($n = 21$) and 0.154 ± 0.009 ($n = 16$) in pubertal and adult rats, respectively.

Analysis of Amines. Contents of amines were measured by reverse-phase high-performance liquid chromatography with electrochemical detection according to the procedure of King *et al.* (7) with a slight modification. Filtrate (40 μl) from tissue extracts prepared by the procedure described below was injected onto a reverse-phase column (250 × 4.6 mm; 7-μm particle size; Chemcosorb 7-ODS-H Chemco KK, Osaka, Japan) and was eluted with a mobile phase consisting of 0.1 *M* citric acid, 0.1 *M* sodium acetate, and 12% (v/v) methanol at pH 3.7. A flow rate of 0.75 ml/min was produced by using a 2150 high-performance liquid chromatography pump (LKB Bromma, Sweden). The sensitivity of an electrochemical detector (LC 4B; Bioanalytical System Inc., West Lafayette, IN) was set at a 1- or 2-nÅ range, and the applied potential was maintained at 0.71 V to Ag/AgCl reference electrode using a glassy carbon electrode. The peak of amines was recorded by a chromatogram processor (7000 AS; System Instruments Co., Tokyo, Japan).

Authentic amines used were: 5-hydroxy-DL-tryptophan, 5-hydroxytryptamine hydrochloride, and 5-hydroxyindole-3-acetic acid for 5-HTP, 5-HT, and 5-HIAA (Sigma), respectively, NMHT (Aldrich, Milwaukee, WI), and dopamine dydrochloride (Katayama KK, Osaka, Japan).

The retention time of the authentic amine was 6.00 min for dopamine, 8.36 min for 5-HTP, 10.42 min for 5-HT, 11.36 min for NMHT, and 27.06 min for 5-HIAA. Chromatogram of the extract from the tissue showed three peaks corresponding to each retention time for dopamine, 5-HT, or 5-HIAA under the condition of analysis. The mean recovery percentage of each amine extracted at a range of 0.5–4 ng was 98.6 ± 1.1 (mean ± SE, 12 samples), 98.0 ± 0.8 (12 samples), 97.6 ± 0.4 (12 samples), 96.9 ± 0.9 (12 samples), and 98.3 ± 0.5% (12 samples) for dopamine, 5-HTP, 5-HT, 5-HIAA, and NMHT, respectively. Standard curves of each amine were linear over a range of 0.1–0.8 ng. In every analysis, a standard sample was prepared and processed in the same way as every unknown sample. The standard sample consisted of a mixture of 4 ng each of 5-HTP, 5-HT, 5-HIAA, dopamine, and NMHT in 200 μl of 0.2 *N* HCl_4. Amounts of each amine in the sample were calculated by the method of the internal standard. All data of amines are expressed as nanograms of amine per milligram of protein.

Measurements of Hormones. Plasma PRL was determined by using the rat PRL radioimmunoassay

kit provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (Bethesda, MD). Each sample (15 μl) was assayed in duplicate. Plasma PRL values were expressed in terms of NIDDK rat PRL-RP-3 preparation. The intraassay coefficient of variation was 7.6% at the level of 236.6 pg/tube. The lowest detectable level was 62.5 pg/tube.

Progesterone was extracted with diethyl ether from 5 μl of plasma. Progesterone in the extracts was assayed by radioimmunoassay with a rabbit antiserum to progesterone-3-carboxymethyloxime-bovine serum albumin (Teikoku Hormone Manufacturing Co., Kawasaki, Japan), progesterone (Sigma) and [1, 2, 6, 7-^{3}H(N)] progesterone (sp act, 96.5 Ci/mmol; Dupont, NEN Research Products, Boston, MA). The intraassay coefficient of variation was 5.4% at the level of 250 pg/tube. The least detectable level of progesterone was 25 pg/tube.

Statistical Analysis. Results were expressed as mean ± SE. Analysis of variance followed by Duncan's multiple range test was used to assess the statistical difference at $P < 0.05$ among group means (more than two).

Results

Plasma concentrations of PRL 4 hr after the injection of progesterone at the dosage level of 2.5 or 7.5 mg/100 g body wt in pubertal rats were significantly higher than those in oil-treated rats ($P < 0.05$, Fig. 1). In adult rats, plasma concentrations of PRL increased significantly after the administration of 7.5 mg, but not 2.5 mg, of progesterone ($P < 0.05$, Fig. 1). The increased level of plasma PRL after the administration in adult rats was significantly lower than that in pubertal rats ($P < 0.05$, Fig. 1).

Concentrations of dopamine in the ARC-ME of progesterone-treated pubertal animals were only about half of those in oil-treated animals, and the difference was significant ($P < 0.05$, Fig. 1). In the POA, concentrations of dopamine in progesterone-treated animals were similar to those in oil-treated animals (Fig. 1). Significantly lower concentrations of dopamine in the POA or the ARC-ME were observed in pubertal rats treated with oil or progesterone compared with those of equivalent groups in adults ($P < 0.05$, Fig. 1).

The dopamine turnover rate in the ARC-ME after treatment with progesterone in pubertal rats declined significantly to 6.80 ± 0.08 (mean ± SE, $n = 7$ rats) from 12.15 ± 1.52 ($n = 8$) in oil-treated pubertal rats ($P < 0.05$). On the other hand, the rate after treatment with progesterone in adult rats rose to 15.12 ± 2.38 ($n = 6$) from 10.94 ± 1.45 ($n = 5$) in oil-treated adult rats. The dopamine turnover rate in the POA was lower in oil-treated pubertal rats (0.83 ± 0.08, $n = 8$) than in oil-treated adult rats (2.42 ± 0.02, $n = 6$) ($P < 0.05$). These values in the POA were not affected by treatment with progesterone.

No change in the concentrations of 5-HT or 5-HIAA and the ratio of 5-HIAA:5-HT in the POA or the ARC-ME was found in pubertal and adult rats after progesterone administration. The concentration of 5-HIAA and the ratio of 5-HIAA:5-HT in ARC-ME were significantly higher in pubertal than in adult rats ($P < 0.05$, Fig. 2).

The plasma progesterone levels 4 hr after 2.5 and 7.5 mg/100 g body wt of progesterone were 253 ± 43 ng/ml of plasma (mean ± SE, $n = 9$ rats) and 447 ± 66 ($n = 6$), respectively, in pubertal rats and 196 ± 36 ($n = 8$) and 433 ± 34 ($n = 6$), respectively, in adult rats.

Discussion

In addition to our previous report (1) indicating that the nocturnal PRL surge in ovariectomized pubertal rats was able to be generated by a smaller amount of progesterone than that in ovariectomized adult rats,

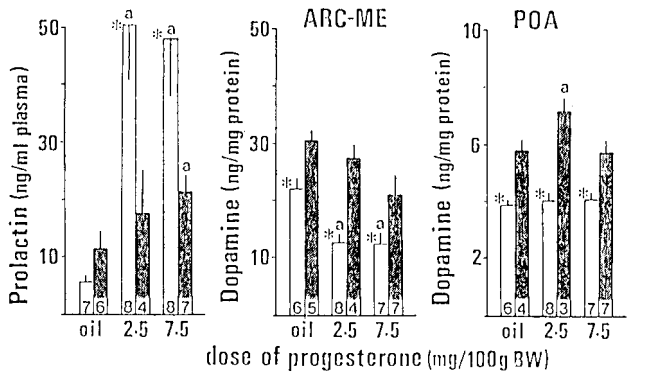

Figure 1. Concentrations of plasma PRL and dopamine in the ARC-ME and the POA 4 hr after progesterone administration at 07:00 hr on estrous day in pubertal (□) and adult (▩) cyclic rats. Values are mean ± SE for four to eight rats and are shown in the bottom of the bars. a, significantly different from the value of the respective control at $P < 0.05$. (Duncan's multiple range test); *, significantly different from the similarly treated adults at $P < 0.05$ (Student's t test). BW, body weight.

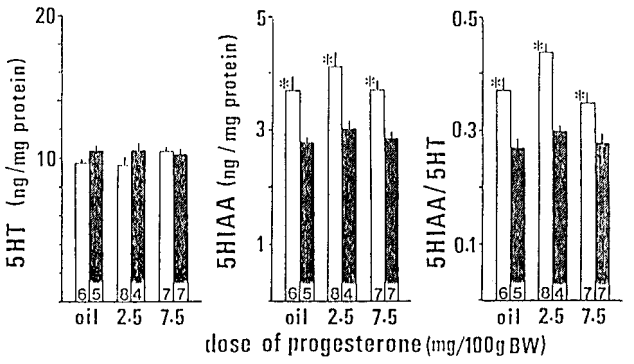

Figure 2. Concentrations of 5-HT and 5-HIAA in the ARC-ME 4 hr after progesterone administration at 07:00 hr estrous day in pubertal (□) and adult (▩) rats. Values are mean ± SE for four to eight rats and are shown in the bottom of the bars. *, significantly different from the similarly treated adults at $P < 0.05$ (Student's t test).

the present result demonstrates that PRL secretion shortly after progesterone administration increased more in pubertal rats than in adults (Fig. 1). Therefore, in pubertal rats, not only the mechanism controlling the daily surge of PRL secretion but also the mechanism controlling the basal secretion of PRL seem to be more sensitive to progesterone.

The present finding that concentrations of dopamine in the POA and the ARC-ME were significantly lower in pubertal rats than in adult rats (Fig. 1) is consistent with our previous results (3) and could be explained by the relatively small amount of tyrosine hydroxylase present in the dopaminergic nerve in pubertal rats. Porter (12) reported that the amount of tyrosine hydroxylase in the median eminence increased from pubertal to adult stages in the rat. The value of dopamine turnover rate in our present study was the same between pubertal and adult rats in the ARC-ME, whereas the rate in the POA was less in pubertal rats than in adult rats. In pubertal rats, dopamine turnover rate in the ARC-ME declined significantly between 2 and 4 hr after progesterone administration at 07:00 hr on estrous day compared with the rate in oil-treated animals. On the other hand, in adult rats, progesterone treatment raised the turnover rate of dopamine in the ARC-ME in comparison to that in oil-treated animals. Therefore, the decrease in the concentration of dopamine in the ARC-ME in pubertal rats treated with progesterone could be ascribed to the direct inhibitory action of progesterone on the activity of tyrosine hydroxylase in dopaminergic neurons in the median eminence. The decline in the activity of this limiting enzyme for dopamine synthesis results in the declining release of dopamine into the portal vessel, which could increase PRL secretion.

Contrary to results seen with pubertal rats, however, the present findings in adult rats treated with the lower dosage of progesterone revealed the increase in dopamine concentration in the POA (Fig. 1). In the previous reports describing the effect of progesterone on the concentration of dopamine in the hypothalamus or the portal vessel, some workers (13, 14) reported an increase in dopamine concentration whereas others did not (15–17).

The finding that the concentration of 5-HT and the ratio of 5-HIAA:5-HT in the ARC-ME were higher in pubertal rats than in adults (Fig. 2) is consistent with that of Watts and Stanley (18). Progesterone treatment at 07:00 hr of estrous day in pubertal or adult rats affected neither the concentrations of 5-HT and 5-HIAA nor the ratio of 5-HIAA:5-HT in the POA and the ARC-ME 4 hr after the treatment (Fig. 2). It has been reported that progesterone stimulates the release of 5-HT from the hypothalamus in the evening and the synthesis in the POA-anterior hypothalamus in the afternoon (6, 7). The difference between these and our findings may be ascribed to the time of sampling in the day.

It has been well known that the dopaminergic neurons in the ARC-ME primarily control a tonic secretion and a surge of PRL secretion in cyclic, pseudopregnant, pregnant, and lactating rats (2, 19–23). The decrease in dopamine concentration in the ARC-ME found after progesterone administration to pubertal rats in our present experiment is explained by a decline in the turnover rate of dopamine after progesterone administration and could result in the increase in plasma PRL concentration. Although many workers have reported that the serotonergic neuron may inhibit dopamine release from the median eminence and/or may stimulate the secretion of PRL releasing factor, which antagonizes the effect of dopamine at the pituitary level (7, 24–27), no change in the concentration of 5-HIAA and the ratio of 5-HIAA:5-HT in the ARC-ME region was obtained in pubertal rats after progesterone treatment in our present experiment. These results suggest that the progesterone-induced increase in the concentration of plasma PRL shortly after the administration in pubertal rats would be mainly due to the decline in the suppressive effects of the dopaminergic neuronal activity in the ARC-ME on PRL release. However, the stimulatory action of the serotonergic neuron on PRL secretion could not completely be excluded under the condition of declining dopaminergic neuronal activity in pubertal rats treated with progesterone, since our present results showed that the concentration of 5-HIAA and the ratio of 5-HIAA:5-HT in the ARC-ME were greater in pubertal rats than in adult females and that these levels remained unchanged after progesterone administration (Fig. 2).

Progesterone causes PRL secretion in pubertal rats more easily than in adult rats on the basis of the difference in the response of dopaminergic neurons in the ARC-ME to progesterone.

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