IMMUNOHISTOCHEMICAL STUDY OF POSTNATAL DEVELOPMENT OF PROLACTIN-PRODUCING CELLS IN C57BL MICE

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Sexual dimorphic changes in postnatal development of prolactin (PRL)-producing cells in the anterior pituitary gland in C57BL mice were immunohistochemically investigated. When examined at birth, PRL cells were already present in both male and female mice. With advancement of age, PRL cells increased in number. The shape of PRL cells at birth was oval, but on day 7 irregular-shaped PRL cells appeared. The shape of immunoreactive secretory granules in PRL cells were spherical at birth and also at day 7. However, on day 14 polymorphic PRL granules began to appear in type III PRL cells in both male and female mice. The ratios among type I, II, and III PRL cells and also the total number of all types of PRL cells were not different between male and female mice until 4 weeks of age. Meanwhile, type II PRL cells were predominant in both sexes. From 5 weeks of age, type III PRL cells began to increase in number in female mice only, thus sexual dimorphism in PRL cell types became noticeable during pubertal changes. The present results suggest that postnatal morphological, particularly sexual dimorphic, changes observed in secretory granules as well as cell shapes of PRL cells are mainly due to the reflection of endocrine functions in pubertal female mice.

Various kinds of heterogeneity and sexual dimorphism in prolactin (PRL) have been reported and this subject was recently reviewed (10). Moreover, by a high performance liquid chromatography applied to the extract of the pituitary gland of adult C57BL mice, male and female PRL variants were purified and were found to be different from each other in their amino acid compositions (11).

Concerning PRL cells in mice, the prenatal or postnatal development had been investigated by conventional morphological methods (16, 23, 25), however, no light and electron microscopic immunohistochemical studies have been reported.

The present study was undertaken to examine the timing and process of the appearance of sexual dimorphism in PRL cells during postnatal periods in male and female mice by immunohistochemical procedures.

MATERIALS AND METHODS

Male and female mice of the C57BL strain were used in the present study. Mice were killed at birth (day 0 of age) and at 1, 2, 3, 4, 5, 6 and 24 weeks of age by

cervical dislocation. Immediately thereafter, the pituitary glands were removed, and fixed in a picric acid formaldehyde solution (21) for 2 hr at 4°C. These tissues were postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 1 hr and dehydrated through a graded series of ethanol solution and embedded in Epon 812. The PRL cells were identified by light and electron microscopic indirect immunohistochemical method using anti-mouse PRL rabbit serum (9).

In all electron micrographs examined, the total numbers of nuclei of PRL cells in each cell type and all the anterior pituitary cells including PRL cells were counted. Any cells revealing only parts of their cytoplasm were not counted. Electron micrographs used for cellular counting were taken from ultrathin sections located in the areas of 10 grids of a #200 square mesh. For the cell count, three to four mice were used in each group.

Classification criteria of PRL cells:

PRL cells were classified into types I, II and III according to the criteria previously established for adult mouse PRL cells in our laboratory (9): the criteria being based mainly upon sizes and shapes of immunoreactive PRL secretory granules as follows: The type I PRL cell is oval or polygonal in cell shape, and contains spherical PRL secretory granules, about 100 nm in diameter, and poorly developed endoplasmic reticulum and Golgi apparatus. The type I PRL cell is generally scarce in the pituitary gland of adult mice of both sexes. The type II PRL cell is most frequently seen in the pituitary gland of adult male mice. The type II PRL cell shows cup-shaped or triangular cellular configuration and possesses elongated cytoplasmic processes by which it embraces oval-shaped neighboring cells containing non-PRL-immunoreactive secretory granules. The type II PRL cell contains spherical PRL secretory granules, 150–200 nm in diameter, and moderately developed cytoplasmic organelles. The type III PRL cell is oval or polygonal in its cell shape and is found predominantly in the pituitary gland of adult female mice. The type III PRL cell contains variably-sized polymorphic secretory granules, 300 nm in maximal diameter, and well developed cytoplasmic organelles.

RESULTS

Immunoreactive PRL secretory granules were already found in some cells in the anterior pituitary gland at day 0 of age in both male and female mice, although the number of PRL cells was still very scarce. The percentage of PRL cells among all the anterior pituitary cell increased as ages became advanced (Fig. 1). The shape of all PRL cells was oval at day 0 of age (Fig. 2a). Some irregular-shaped PRL cells began to appear at 1 week of age (Fig. 2b), and were thereafter generally observed (Figs. 2c–f).

In both sexes, PRL secretory granules at days 0 and 7 of age were two types of spherical ones resembling those of type I and type II PRL cells, respectively (Figs. 3a, b). Polymorphic PRL secretory granules similar to those of type III PRL cells appeared at 14 days of age, and then three types of PRL secretory granule-containing cells became observable (Figs. 3c–e, Fig. 4).

In both male and female mice, type II PRL cells were predominant and the ratios between all types of PRL cells and each of three types of PRL cells were not different between the two sexes until 4 weeks of age. However, after 5 weeks of age,

FIG. 1. The comparison of the frequency of postnatal appearance of PRL cells in the anterior pituitary gland between male (M) and female (F) mice.

type III PRL cells appreciably increased in number in the pituitary gland of female mice, thus sex dimorphism of PRL cells became evident (Fig. 4).

DISCUSSION

The first appearance of PRL cells judged by conventional morphological criteria (3, 15, 24) has been reported to be at 5 days after birth in male mice (23), or at 18 days of age in female mice and at 21 days of age in the male mice (25). In the present immunohistochemical study, PRL cells resembling type I PRL cells were already identifiable at birth both in male and female neonatal mice. However, immunoreactive PRL cells resembling type III cells containing polymorphic secretory granules, which are supposed to be the same as the classical PRL cell identified by conventional morphological criteria, began to appear at 14 days of age in both sexes. It might be possible that early stages of PRL cells are already present in the developing pituitary gland of a mouse fetus. PRL secreted by fetal mouse pituitaries in organ culture was reported to have caused milk secretion in mammary glands *in vitro* (12). PRL cells have been identified in fetal pituitary glands by immunohistochemical observations in rats (4, 6, 20), hamster (22), pig (5), sheep (14) and also in human (1, 2).

Immunoreactive PRL cells observed in the pituitary gland of immature mice were found in the present study to increase in number and also to change the shape and size of their secretory granules as ages advanced. Until 4 weeks of age, type II PRL cells were predominant in both sexes, and the total number of all types of PRL cells and the ratios among type I, II, and III PRL cells were not different between male and female mice. Only in female mice, however, type III PRL cells began to increase in number from 5 weeks of age, and sexual dimorphism became apparent from early pubertal period. Before puberty, the serum levels of PRL were reported to be low (13). Immunoreactive PRL secretory granules were found to alter their

FIGS. 2a-f. Immunohistochemical photomicrographs of the pituitary gland. $\times 1800$

a. 0 day-old male mouse. b. 7 day-old female mouse. c. 3 week-old male mouse. d. 3 week-old female mouse. e. 24 week-old male mouse. f. 24 week-old female mouse.

FIGS. 3a-e. Immunohistochemical electron micrographs of the pituitary gland.

- a. Type I PRL cell in 0 day-old female mouse. $\times 10,000$
Arrows indicate type II PRL cells which were omitted from the count of PRL cells, because the nuclei of these cells did not appear in this electron micrograph.
- b. Type II PRL cell in 7 day-old female mouse. $\times 10,000$
- c. Type III PRL cell in 14 day-old male mouse. $\times 10,000$
- d. Type III PRL cell in 3 week-old female mouse. $\times 10,000$
- e. Type III PRL cell in 3 week-old male mouse. $\times 8,000$

morphological features by hyperprolactinemia caused by pituitary isografts in adult female mice and these changes were reversed when hyperprolactinemia was suppressed by treatments with 2-brom- α -ergocriptine (8). Sasaki and Sano (17-19) reported biological effects of the ovary on sexual dimorphic differentiation of PRL cells in mice. Estrogen administered exogenously was reported to be responsible for feminization of PRL cell types in treated adult male mice (7).

The present study suggests that sexual dimorphic changes occurring in PRL cells, particularly in morphological features of immunoreactive PRL secretory granules, may be closely related to the developmental changes in endogenous endocrine activities in female mice during puberty.

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FIG. 4. The ratios between the total number of PRL cells and the number of each of type I, II, or III PRL cells are compared between male and female mice.

REFERENCES

1. Baker, B. L. and Jaffe, R. B.: The genesis of cell types in the adenohypophysis of the human fetus as observed with immunohistochemistry. *Am. J. Anat.* 143; 137-162, 1975.
2. Baker, B. L. and Yu, Y.-Y.: An immunocytochemical study of human pituitary mammotropes from fetal life to old age. *Am. J. Anat.* 148; 217-240, 1977.
3. Barnes, B. G.: Electron microscopic studies on the secretory cytology of the mouse anterior pituitary. *Endocrinology* 71; 618-628, 1962.
4. Chatelain, A., Dupouy, J. P. and Dubois, M. P.: Ontogenesis of cells producing polypeptide hormones (ACTH, MSH, LPH, GH, prolactin) in the fetal hypophysis of the rat: influence of the hypothalamus. *Cell Tiss. Res.* 196; 406-427, 1979.
5. Dacheux, F.: Differentiation of cells producing polypeptide hormones (ACTH, MSH, α - and β -endorphin, GH, and PRL) in the fetal porcine anterior pituitary. *Cell Tiss. Res.* 235; 615-621, 1984.
6. Daikoku, S.: Development of hypothalamic-hypophysial axis in rats. *Abstracts Int. Symp. on Pituitary Gland*, 52, 1984.
7. Harigaya, T., Hashimoto, H. and Hoshino, K.: Effects of estrogen on prolactin cells in male mice. *Proc. Int. Symp. on the Pituitary Gland*. Excerpta Medica, Amsterdam-Oxford, (in press)
8. Harigaya, T. and Hoshino, K.: Immunohistochemical identification of prolactin-producing cells in pituitary isografts in CBA mice. *Anatomischer Anzeiger*, (in press)
9. Harigaya, T., Kohmoto, K. and Hoshino, K.: Immunohistochemical identification of prolactin-

- producing cells in the mouse adenohypophysis. *Acta histochem. cytochem.* 16; 51–58, 1983.
10. Hoshino, K.: Sexual dimorphism in prolactin. In *Endocrine Correlates of Reproduction*, ed. by K. Ochiai et al., Japan Sci. Soc. Press, Tokyo/Springer-Verlag, Berlin, 145–159, 1984.
 11. Hoshino, K.: Sexual dimorphism and heterogeneity of prolactin cells. *Proc. Int. Symp. on the Pituitary Gland. Excerpta Medica, Amsterdam-Oxford* (in press)
 12. Kohmoto, K. and Bern, H. A.: Occurrence and secretion of prolactin in fetal mouse pituitaries. *Proc. Soc. Exp. Biol. Med.* 137; 807–809, 1971.
 13. Michael, S. D., Kaplan, S. B. and Macmillan, B. T.: Peripheral plasma concentrations of LH, FSH, prolactin and GH from birth to puberty in male and female mice. *J. Reprod. Fert.* 59; 217–222, 1980.
 14. Parry, D. M., McMillen, I. C., Robinson, J. S. and Torburn, G. D.: Immunocytochemical localisation of prolactin and growth hormone in the perinatal sheep pituitary. A morphological and quantitative study. *Cell Tiss. Res.* 197; 501–514, 1979.
 15. Sano, M.: Further studies on the theta cell of the mouse anterior pituitary as revealed by electron microscopy, with special reference to the mode of secretion. *J. Cell Biol.* 15; 85–97, 1962.
 16. Sano, M. and Sasaki, F.: Embryonic development of the mouse anterior pituitary studied by light and electron microscopy. *Z. Anat. Entwickel-Gesch.* 129; 195–222, 1969.
 17. Sasaki, F. and Sano, M.: Role of the ovary in the sexual differentiation of prolactin and growth hormone cells in the mouse adenohypophysis during postnatal development: a stereological morphometric study by electron microscopy. *J. Endocr.* 85; 283–289, 1980.
 18. Sasaki, F. and Sano, M.: Role of the ovary in the sexual differentiation of prolactin and growth hormone cells in the mouse adenohypophysis: a stereological morphometric study by electron microscopy. *J. Endocr.* 93; 117–121, 1982.
 19. Sasaki, F. and Sano, M.: Role of the ovary in sexual differentiation of lactotrophs and somatotrophs in the mouse adenohypophysis: a stereological morphometric study by electron microscopy. *J. Endocr.* 99; 355–360, 1983.
 20. Setalo, G. and Nakane, P. K.: Functional differentiation of the fetal anterior pituitary cells in the rat. *Endocrinol. Exp.* 10; 155–166, 1976.
 21. Stefanini, M., De Martino, C. and Zamboni, L.: Fixation of ejaculated spermatozoa for electron microscopy. *Nature* 216; 173–174, 1967.
 22. Thompson, S. A. and Trimble, J. J., III: Immunohistochemical localization of prolactin cells of the pars distalis in the fetal and neonatal hamster. A light and electron microscopy study. *Cell Tiss. Res.* 168; 161–175, 1976.
 23. Wilson, D. B. and Christensen, E.: Postnatal development of somatotrophs and mammotrophs in the pars distalis of the C57BL mouse. *Cell Tiss. Res.* 211; 441–448, 1980.
 24. Yamada, K. and Yamashita, K.: An electron microscopic study on the possible site of production of ACTH in the anterior pituitary of mice. *Z. Zellforsch.* 80, 29–43, 1967.
 25. Yamashita, K.: Electron microscopic observations on the postnatal development of the anterior pituitary of the mouse. *Gunma Sym. Endocrinol.* 6; 177–196, 1969.