Effects of Ovariectomy and Administration of Sex Hormones on Prolactin Cell Types in Mice

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Light and electron microscopic immunohistochemistry of prolactin (PRL) cells in the anterior pituitary gland in mice has been investigated and PRL cells in the pituitary gland of both male and female adult mice were classified into three types mainly based upon sizes and shapes of immunoreactive PRL secretory granules¹⁾. The type I PRL cell is scarce in the pituitary gland of both sexes. The type II PRL cell is frequently seen in adult male mice. The type III PRL cell is predominant in adult female mice¹⁾. These sexual dimorphic features of PRL cells became apparent during puberty²⁾. SASAKI and SANO⁶⁻⁸⁾ have previously reported that the important role of ovary for sex differences of PRL cells in the anterior pituitary gland, although their investigations were based on the convenient electron microscopic observation. In the present study, an attempt was made to clarify whether sex hormones would influence the phenotypic differentiation of PRL cell types in mice.

Materials and Methods

Adult male and female mice of the C57BL strain (13-18 weeks old) were used in the present study. They were maintained at $25 \pm 2^\circ\text{C}$ with 12 h light : 12 h darkness, and food (Oriental Mouse Chow, Tokyo) and water were available *ad libitum*.

Ovariectomy was performed in female mice under pentobarbital sodium (Nembutal, Abbott) anaesthesia. Immediately thereafter, ovariectomized mice were implanted subcutaneously with a silastic tube (5 mm long, o.d. 2 mm, plain style 1 mm, Dow Corning) containing estradiol-17 β or testosterone crystal (Sigma), or with an empty silastic tube as controls. Male mice were implanted subcutaneously with a silastic tube containing estradiol-17 β or with an empty silastic tube as controls.

One week after the treatment, mice were killed by heart puncture and the pituitary glands were removed, and fixed in a picric acid formaldehyde solution⁹⁾ for 2 h at 4°C . These tissues were postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 1 h and

マウスプロラクチン細胞分化に対する卵巣除去および性ホルモン投与の影響：針谷敏夫（京都大学医学部，現明治大学農学部，川崎市多摩区 214）

dehydrated through a graded series of ethanol solution and embedded in Epon 812.

The PRL cells in the anterior pituitary gland were identified by electron microscopic indirect immunohistochemical method using anti-mouse PRL rabbit serum provided by Dr. K. KOHMOTO previously described¹⁾. 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, four mice were used and the number of each type of PRL cells was pooled in each group.

PRL cells were classified into types I, II and III according to the criteria established for mouse PRL cells previously¹⁾: 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 II PRL cell usually 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 contains variably-sized polymorphic secretory granules, 300 nm in maximal diameter, and well developed cytoplasmic organelles.

Results and Discussion

Table 1 shows the percentage of total PRL cells among all the anterior pituitary cells and that of each type of PRL cells in the all PRL cells. When ovariectomized female mice were treated with estradiol-17 β , total number of PRL cells in the anterior pituitary gland increased slightly compared with that of the ovariectomized control mice. The ratio of the number of type III PRL cells to the total number of all PRL cells increased, while the ratio of either type I or II PRL cells to the total decreased. Then, type III PRL cells became predominant in mice with estradiol-implants. On the other hand, in testosterone treated mice, the ratio of either type I, II, or III PRL cells to the total number of all PRL cells did not change compared with that in the control, although the percentage of total PRL cells in all the pituitary cells decreased.

In male mice, estradiol-implants also increased the proportion of type III PRL cells and decreased that of type I and II PRL cells. Although the percentage of total PRL cells among all the anterior pituitary cells is slightly increased by the estradiol treatment in

Table 1. Effects of estradiol or testosterone in ovariectomized mice and of estradiol in male mice on the ratio of PRL cells in the total number of anterior pituitary gland cells and the relative proportions of three types of PRL cells.

Experiment		PRL cell	type I	type II	type III
female	ovx control (269 ^a)	45.3%	3.3%	41.5%	55.2%
	ovx + E ₂ (261)	54.8	1.4	3.5	95.1
	ovx + T (397)	36.9	5.1	47.1	47.7
male	control (358)	24.0	12.8	44.8	42.4
	E ₂ (247)	29.2	2.5	10.4	87.1

ovx, ovariectomy; E₂, estradiol-17 β ; T, testosterone. ^a: total number of counted pituitary cells.

male mice, but it is still lower than that of female mice.

In the present experiments, the implantation of estradiol-17 β crystal in adult ovariectomized female mice and non-orchidectomized male mice yielded the increase of type III PRL cells at the expense of types I and II PRL cells. On the other hand, the implantation of testosterone in adult ovariectomized female mice caused no change in the ratio of the number of either type I, II or III PRL cells to total number of PRL cells. In the previous study³⁾, neonatal administration of estrogen in male mice had no effect on the proportion of three types of PRL cells when they were killed after puberty. The existence of ovary during puberty seems to be essential for the induction of sex differences in PRL cells in mice.

SASAKI and SANO⁶⁻⁸⁾ have previously reported sex differences in the morphology of PRL cells in mice and indicated that the presence of ovary after puberty is essential for the female phenotypic differentiation of the anterior pituitary gland. The present study indicates that estrogen is related to the sex-linked phenotypic differentiation of PRL cells, particularly to the appearance of type III PRL cells. The type III PRL cell in female mice was previously found to increase in number during puberty, followed by sex-linked differences in types of PRL cells in mature mice²⁾. The type III PRL cell also increased in grafted pituitary glands in mice and this increase was prevented by the administration of bromocriptine⁴⁾. These data suggest that the differentiation into type III represents the attainment of the high secreted activity of PRL cells. The increase in the number of type III PRL cells in the anterior pituitary gland during puberty seems to correspond to the increase of the serum level of PRL towards puberty in mice⁵⁾.

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