Compensatory Development of Immature LH Cells after Long-term Gonadectomy: An Immunocytochemical, Electron Microscopic and Cell Count Study

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Abstract

Following long-term castration of male rats (for 3, 6, 12 and 18 months), some populations of five gonadotroph types, i.e., immature cell, types III, III/IV, IV and so-called signet-ring cell, appeared in the pituitary glands. Their ultrastructures were electron microscopically observed after immunohistochemical identification of them using anti-rat LHβ serum. In unoperated control rats, 60 days old, the predominant III/IV type was intermingled with some populations of III and IV types, but no immature LH cells or signet-ring cells were detected among them. In the present observation not all of the gonadotroph types turned into signet-ring cells. Immature small LH cells containing a few small secretory granules began to appear (2.0%) at 3 months, and had increased to a maximum (52.5%) at 18 months. The percentage of the signet-ring cells was high (24.1%) at 3 months, but reduced (2.4%) at 18 months. High populations of small, oval and immature LH cells are assumed to occur as a consequence of mitotic division of the most of immature LH cells. This may compensate for the loss of signet-ring cells in order to maintain the high serum LH and FSH concentrations after long-term castration.

Rat pituitary gonadotrophs increase in number through mitosis during early postnatal life (Matsumura and Daikoku, 1977). The main reason for the increase in gonadotrophs in adult rats may be their active mitotic division (Kurosumi, 1971) because mitotic figures were found in adult gonadotrophs under normal conditions. On the other hand, the mitotic incidence in immunostained gonadotrophs seems very rare in rats at different ages (Shirasawa and Yoshimura, 1982). Yoshimura et al. (1970, 1977a) speculated, on the basis of their cytological studies in postnatal rats, that the prompt increase in pituitary granulated cells was mainly due to the maturation of primordial or undifferentiated cells. The population of gonadotrophs in the adult male rat may remain at a constant level (about 6%), while castration results in a remarkable increase in the population to about 30% (Linkie et al., 1981). Inoue and Kurosumi (1981) and Sakuma et al. (1984) found enhanced mitotic activity of the adult gonadotrophs after castration. However, Yoshimura and Harumiya (1965) postulated that after castration immature basophils may hypertrophy and change into vesiculated castration cells with an abundance of expanded rough endoplasmic reticulum (rER), and eventually turn

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into signet-ring cells, which have been regarded as the destination of castration cells. Yoshimura et al. (1977b) classified male rat pituitary basophils electron microscopically, apart from their functions, into four types, I, II, III and IV including the intermediate types designated as II/III or III/IV. Later, Yoshimura et al. (1981) classified LH/FSH cells, in adult male rats, into four types, II/III, III, III/IV and IV basophils, on the basis of their ultrastructural properties, using the superimposition technique applying immunostaining with anti-rat LHβ serum.

This study deals with the question whether or not all gonadotrophs change into castration cells and also whether or not the high populations of mature gonadotrophs seen after gonadectomy are due only to their own mitotic activity. The purpose of this study is to examine the new development of immature LH cells and their maturation process after gonadectomy through immunohistochemical and cell count procedures.

Materials and Methods

Twenty-six Wistar-Imamichi male rats were orchidectomized at 60 days of age, under sodium pentobarbital anesthesia, and five to seven of them were sacrificed at the operation and 2 weeks, 3, 6, 12 and 18 months after the operation respectively. Blood samples were sucked from the abdominal aorta with syringes and plasma samples were stored at −70°C until radioimmunoassayed. Pituitaries were removed and cut into halves through the sagittal plane. One half was fixed in sublimate formol, dehydrated in ethanol series and embedded in Paraplast. The other half was cut into small pieces with a razor blade and placed in 1% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4. After rinsing with 2% sucrose in the same buffer, the tissues were postfixed in 1% osmic acid solution (Millonig, 1962). The tissues were dehydrated and embedded in Epon-Araldite.

Immunohistochemistry

Ultrathin Epon-Araldite sections and the adjacent semithin sections (1–2 µm thick) were cut with a glass knife. Immunostaining of the semithin section was carried out according to the procedures described in detail by Yoshimura et al. (1981), using anti-rat LHβ serum. The cells corresponding to the immunostained LH cells were observed electron microscopically on the adjacent ultrathin section, applying the superimposition technique (Beauvillain et al., 1975).

The antiserum to rat LHβ was kindly supplied by Dr. F. Yoshimura (Jikei University School of Medicine, Tokyo, Japan). The specificity of the antiserum was already examined in detail by the radioimmunoassay system and the absorption tests (Yoshimura et al., 1981). Paraplast sections were stained with antiserum to human chorionic gonadotropin (hCG, Bioactive Chem. Lab., Tokyo, Japan) by the peroxidase labeled antibody method (Nakane and Pierce, 1967).

Cell count procedure

Five frontal sections through the central part of the pituitary were selected from each rat. The total number of immunostained signet-ring cells was counted under a light microscope. The area of each section was measured with an image analyzer (Mutoh Ind., Tokyo, Japan).

For the precise evaluation of LH cell subpopulations, more than 200 cells from three rats in each group were counted on the electron microscope sections and identified according to the immunostaining for LH cells on the adjacent sections. The cell count was made on the patchwork of the electron micrographs. The gonadotrophs were divided into five types, i.e., immature cell, types III, III/IV, IV and signet-ring cell according to the classification of Yoshimura et al. (1977b).

Radioimmunoassay

The serum concentration of LH and FSH was determined by means of the double antibody radioimmunoassay according to the protocol supplied by NIAMDD, Pituitary Hormones and Antisera Center. The values were expressed in terms of NIAMDD rat-LH-RP-1 or rat-FSH-RP-1.

Findings

Electron microscopy

Three, 6, 12 and 18 months after gonadectomy, four types of mature LH cells still remained in the gland, but elongate...
gonadotrophs identical with castration cells frequently appeared and sometimes contained large colloidal cavities. In my electron microscopical observations, not all of the gonadotrophs (LH cells) turned into castration cells.

Gonadectomy resulted in the appearance of immature LH cells that were small in size, and oval or elongate in shape, sometimes revealing round contours in cross section (Figs. 1, 2 and 7). Ultrastructural features were 1) small cell size, 2) distribution of a few small spherical secretory granules, 100–120 nm in diameter, with rare coexistence of large irregularly shaped granules, some of which resemble lysosomal bodies, 3) moderate development of parallel arrays of rough endoplasmic reticulum (rER), and 4) prominent Golgi apparatus. These immature LH cells seem to be newly developed after long-term castration. The ultrastructurally unaffected mature LH cells were classified into the following categories:

The first category consists of type III basophils of oval or elongated shape containing an abundance of secretory granules 100–150 nm in diameter and several large dense spherical secretory granules 200–400 nm in diameter (Fig. 5). Their parallel arrays of rER are not densely arranged but tend to be dispersed. Some populations of III type cells survived after long-term castration.

The second castegory consists of type III/IV basophils containing a number of secretory granules 150–250 nm in diameter with initial vesiculation of rER (Fig. 1).

The third category consists of IV type (Figs. 6 and 8) filled with dilated cisternae of rER, among which are stored spherical secretory granules 150–250 nm in diameter.

Heavy vesiculation followed by progressive dilation of rER cisternae is the main event in some gonadotrophs changing into castration cells following long-term castration. So-called large castration cells are charaterized by the dense, anastomosing, expanded cisternae within the cells (Fig. 8). They resemble a IV type. When the cisternae fuse into a large cavity, the castration cells change into signet-ring cells (Figs. 3 and 4). The fine structure of the cytoplasm at the surface of the cavity resembles that of IV type. Some aged signet-ring cells with large cavities have a remaining peripheral region which is extremely narrow, showing degenerative or destructive signs (Fig. 4).

Changes in population ratios of five types of LH cells to the total number of LH cells on the electron micrographs

As shown in Fig. 9, in the intact control male rats of 60 days, just before the experimental animals were orchidectomized, LH cells consisted of III (17.1%), III/IV (64.1%) and IV (18.8%) types, among which the cells of III/IV type predominated, but immature LH cells were not detectable. Three months after gonadectomy, immature LH cells began to appear (2%); III/IV type cells decreased in number to 34.3%, while the signet-ring cells reached the maximum number (24.1%). Six months after gonadectomy, IV type cells increased to 30.8% and the immature cells increased to about 5%, while signet-ring cells tended to decrease in number (18.5%). Twelve months after gonadectomy, signet-ring cells were reduced to only 6.8%. Eighteen months after gonadectomy, it was found that immature LH cells represented more than half of the total LH cell population (52.5%). Cells of III type were 13.1% and those of IV type were limited to 4.8% and signet-ring cells were also very scarce (2.4%).

Quantitative changes in immunostained immature LH cells and signet-ring cells at the light microscopical level

The population ratio of all kinds of LH cells increased stepwise being 56.4, 124, 161 and 340 per mm², at intervals of 3, 6, 12 and 18 months after gonadectomy respectively,
Fig. 1. Two types of LH cells from a male mature rat, three months after gonadectomy. They are small oval immature cells (IM) and type III/IV cell with initial vesiculation and a number of small secretory granules. Inset shows the same field on the adjacent thick section immunostained for rat LHβ. X 2600.

Fig. 2. High magnification of an immature LH cell illustrated in Fig 1. This cell is oval in shape, containing parallel arrays of rER and a few minute secretory granules (100-120 nm in diameter). X 8400.
Fig. 3. A signet-ring cell from a male rat at 3 months. This cell contains a large colloidal cavity. The cytoplasm is characterized by numerous secretory granules and cisternae of rER, being sometimes intermingled with a few lysosomal bodies (Ly). X 3200.

Fig. 4. An aged signet-ring cell at 6 months. This cell has the largest colloidal cavity and shows extremely narrow cytoplasm, showing some degenerative or destructive signs. X 2500.
Fig. 5. Type III LH cell, 12 months. Large (450–600 nm) and small (150–350 nm) secretory granules coexist in its cytoplasm, without progressive vesiculation. X 5200.

Fig. 6. Type IV LH cell at 12 months. Its enlarged cytoplasm is heavily vesiculated, containing numerous small secretory granules (160–240 nm in diameter). X 3700.
Fig. 7. A newly developed immature LH cell, at 18 months. These immature LH cells predominate, containing a number of small secretory granules (150–250 nm in diameter) as well as lysosomal bodies (Ly). Flattened rER are arranged in parallel and the Golgi apparatus is prominent. X 6900.

Fig. 8. The remaining Type IV LH cell at 18 months. Its population is greatly reduced, but a few survive. X 3400.
Fig. 9. Changes in the population ratios of five types of gonadotrophs (immature, III, III/IV IV and signet-ring cell) after long-term castration. All types are immunostained with anti-rat LHβ serum and can be identified by the ultrastructural properties on the adjacent ultrathin sections. Total numbers of cells counted were 270, 207, 217, 238 and 705 in control, 3, 6, 12 and 18 months, respectively. IM, immature LH cells; SR, signet-ring cells.

Fig. 10. Changes in the population ratios of total LH cells, immature LH cells and signet-ring cells after long-term castration (3, 6, 12 and 18 months). The randomly selected middle frontal sections (5 sections per rat) were stained with antiserum to hCG. The total numbers of immunostained LH cells and signet-ring cells were counted at the light microscopical level. Population ratios of immature LH cells per mm² mean the values converted from their percentages determined on an electron micrograph (Fig. 9). Columns represent the mean values for 3–5 animals. IM, immature LH cells; SR, signet-ring cells.
and counting areas of each tissue section were 7.8±0.5, 9.0±0.3, 10.3±0.8 and 8.2±0.6 mm² per section at the corresponding intervals.

The population ratios of the immature LH cells per mm² at the light microscopical level were calculated to be 1.2, 6.2, 9.7 and 17.9, at intervals of 2, 3, 6 and 18 months after gonadectomy respectively. They conspicuously increased stepwise in population with time after gonadectomy (Fig. 10). We infrequently found figures of mitotic division mainly in the immature LH cells after long-term castration.

In a chronic state of gonadectomy, not only the cytoplasm but also the colloid substance in the cavity of signet-ring cells was intensely immunostained with anti-hCG serum. The number of immunostained signet-ring cells in five sections in each animal were counted under a light microscope. The incidence of signet-ring cells was highest at 6 months, about twice as high as at the other intervals of 3, 12 and 18 months, during which time they were moderately frequent. The population ratios for signet-ring cells were calculated to be 13.6, 23.0, 11.0 and 8.2 per mm² at intervals of 3, 6, 12 and 18 months after castration, respectively (Fig. 10). During the course of the quantitative analysis by light microscopy, mitotic figures of mature types of gonadotrophs were scarcely observed.

Changes in serum LH and FSH concentrations

The serum LH concentration was very low (20 ng/ml) in the unoperated control male rats (Fig. 11). Two weeks after castration, it rose remarkably and reached a peak at 3 months, measuring 10–12 times the control value, and remained at that level until the 18th month. On the other hand, the serum FSH concentration rose linearly with postoperative time, exhibiting the values 3.5–4 times as great as the control value (400–500 ng/ml) at 18 months.

Discussion

It has been generally accepted that gonadectomy gives rise to an increased population of gonadotrophs, together with a steep rise in the circulating gonadotropin concentration (Schwaltz and Justo, 1977; Lorenzen et al., 1980; Linkie et al., 1981; Childs et al., 1982). Many earlier investigators carried out electron microscopic studies on the changes, mainly focusing on the gonadotrophs following short-term castration (Farquhar and Rinehart, 1954; Kurosumi, 1968, 1971; Kurosumi et al., 1976). Yoshimura and Harumiya (1965) also electron microscopically studied the stepwise changes in gonadotrophs in young male rats (45 days of age) during long-term intervals of from 50 to 300 days after castration. They emphasized that castration might facilitate the transformation of immature gonadotrophs into mature forms which were
destined to turn into the signet-ring cells.

Yoshimura et al. (1977a) classified the basophils, in the normal adult male rats, into four different cell types, including their transitional cells. A recent immunohistochemical study by Yoshimura et al. (1981) showed a range of gonadotrophs which were classified into the II/III, III, III/IV and IV types of basophils through electron microscopic and immunohistochemical observations. However, these different types have not been quantitatively examined. The present immunohistochemical and cell count studies provided data concerning the variation in population ratios of gonadotroph types (Fig. 9): Type III basophils did not show any definite quantitative change after castration. However, type III/IV basophils decreased in number to about a half of the normal value, after castration. The signet-ring cells reached the highest population (24.1%) at 3 months, but thereafter decreased in number, and reached the minimum percentage (2.4%) at 18 months (see Fig. 9). According to cell count data, all the kinds of LH cells stepwise increased in population up to 18 months after castration. However the population density of signet-ring cells reached the maximal level 3 months after castration, and then decreased gradually to 2.4%, 18 months after the operation. This is in contrast with results on changes in the population ratio of IV type cells. This change in the population of the signet-ring cells after castration may depend either upon the transformation of LH cells of IV type into signet-ring cells or upon the subsequent degradation or desquamation of the latter.

The percentage of signet-ring cells determined by EM observation was the highest at 3 months and their population ratio determined by LM observation was the highest at 6 months. This inconsistent result may be because of the remarkable increase in all LH cells types in the gland after castration (Fig. 10). The two results may not really contradict each other because the former is expressed in term of a percentage and the latter in terms of cells population: The population increase of signet-ring cells (1.7 times) was slower than that of total LH cells (2.2 times) during the interval from 3 to 6 months after castration.

On the other hand, the population ratio of the immature LH cells was increased after castration. A particularly sharp increase was seen between 12 and 18 months after castration. This increase may be due either to progressive mitosis of immature LH cells or to the rapid growth of immature LH cells. It is generally known that castration causes a high mitotic rate of gonadotrophs (Pomerat, 1941; Städtler et al., 1970; Inoue and Kurosumi, 1981). Sakuma et al. (1984) reported that mitosis often took place in common pituitary cells involving gonadotrophs in immature rats, and it was found only in the gonadotrophs in mature rats two weeks after castration, although the sectional area and the volume of the pituitary glands did not increase remarkably in those animals. However, the present author could not often find mitotic figures in any type of LH cell during long-term gonadectomy. This conflicting result may be due to the fact that there is a diurnal cycle of mitosis in pituitary cells (Nouët and Kujas, 1975) and that the interval of the metaphase is too short to encounter mitotic division.

It is noteworthy that the immature gonadotrophs do not appear in normal male rats at 60 days of age, but begin to appear 3 months after castration. This may be the first report that abundant small immature LH cells were found to occur after long-term castration. The extraordinary increase in the population of small immature LH cells (more than half of all LH cells) is in striking contrast to the definite loss or desquamation of signet-ring cells at 18 months.

Though the percentages of III, III/IV and IV types and signet-ring cells were less
than 48%, plasma LH and FSH concentrations were 10 and 4 times the normal value, respectively. This suggests that the numerous small and immature LH cells bear the responsibility for the major part of gonadotropin secretion. The present findings strongly suggest that the quantitative increase in small immature LH cells is due to their own mitotic division and that the new development of many immature gonadotrophs seems to be on a morphological basis that perfectly fits the compensation for the loss or desquamation of signet-ring cells in order to maintain the high serum LH and FSH concentrations.

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**References**


Städtler, F., Stöcker, E., Dohm, G. and H. U.


