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LH-Immunoreactivity and Ultrastructural Changes in the Pituitary Gland of Post-castrated Rats: Quantitative and Morphologic Studies

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Shang-Ming Yu, Sung-Ling Yu, Mei-Miao Chiu and Kwan-Hwa Lin (1997) LH-Immunoreactivity and ultrastructural changes in the pituitary gland of post-castrated rats: quantitative and morphologic studies. *Zoological Studies* **36**(3): 230-239. At 1-d postcastration of 2-mo-old rats, LH-immunoreactive (IR) cells were not hypertrophied in the female pituitary gland but a few LH-IR cells were hypertrophied in that of males. At 2-d postcastration, a few LH-IR cells were hypertrophied in the female pituitary gland and contained small cytoplasmic vacuoles in males. At 4-d postcastration, a few hypertrophied LH-IR cells contained a few small cytoplasmic vacuoles in the female pituitary gland and contained coalesced vacuoles in males. At 7-d postcastration, numerous hypertrophied LH-IR cells contained a few small cytoplasmic vacuoles in the female pituitary gland and contained coalesced vacuoles in males. At 14-d postcastration, a large number of hypertrophied LH-IR cells contained numerous cytoplasmic vacuoles in the female pituitary gland and had become arranged in irregular, interconnecting cords in males. At the electron microscopic level, cisternae of the rough endoplasmic reticulum (RER) were slightly dilated in non-hypertrophied gonadotrophs of the female pituitary gland but moderately dilated in hypertrophied gonadotrophs of males at 1-d postcastration. At 2-d postcastration, cisternae of the RER were slightly dilated in hypertrophied gonadotrophs of the female pituitary gland and moderately dilated in those of males. At 4-d postcastration, cisternae of the RER were moderately dilated in hypertrophied gonadotrophs of the female pituitary gland and had coalesced in those of males. At 7-d postcastration, cisternae of the RER remained moderately dilated in hypertrophied gonadotrophs of the female pituitary gland and had coalesced and markedly dilated in those of males. At 14-d postcastration, cisternae of the RER were similar to those in hypertrophied gonadotrophs of female and male pituitary glands at 7-d postcastration. Most of the secretory granules were small in size but a few large ones were also present in the cytoplasm of female and male hypertrophied gonadotrophs.

Key words: Castration, Immunocytochemistry, LH (luteinizing hormone), Pituitary, Morphometry.

It is known that the anterior pituitary gland produces several different hormones including glycoproteins and polypeptides. The cell types in the anterior pituitary are characterized on the basis of the shape and size of their secretory granules (Farquhar and Rinehart 1954, Kurosumi and Oota 1968). However, at least 3 types of gonadotrophs have been found in the rat pituitary gland (Moriarty 1976, Childs et al. 1980, Kurosumi et al. 1991). Different cell types have been demonstrated to correlate with various physiologic states (Yang et al. 1980). Castration can increase LH (luteinizing hormone) in the pituitary (Piacsek and Meites 1966, McNeilly et al. 1980) and in plasma (Swerdloff et al. 1971, Ojeda and Ramirez 1972, Goomer et al. 1977, Grandison et al. 1977) of male and female rats. An early investigation demonstrated that castration increases hypothalamic content of luteinizing hormone releasing factor (LHRH) (Piacsek and Meites 1966). Conversely, castration results

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in a reduction in the hypothalamic content of LHRH but causes no change in the pituitary content of FSH (McNeilly et al. 1980). The effect of castration on hypertrophic gonadotrophs in the pituitary is markedly suppressed after estradiol injection (Shiino and Yamauchi 1983). The rise in serum LH in castrated rats can be depressed by prolactin, mediated via the hypothalamus (Grandison et al. 1977). A single injection of LHRH increases the amount of serum LH and FSH in castrated rats (Morris and Azmatullah 1982). Little information is available on the systematic cytologic changes in the pituitary gonadotrophs after castration. The purpose of this study was to investigate the effect of castration on male and female pituitary glands in rats in the short-term after castration. The experiments were designed to correlate morphologic events in the pituitary at the ultrastructural level with immunocytochemical staining changes at 1- to 14-d postcastration.

MATERIALS AND METHODS

Sprague-Dawley female rats were maintained under controlled temperatures (20-25 °C) and a light:dark schedule of 14h:10h daily. Gonads of the animals ($n1$ = 15, $n2$ = 28, sham = 7) were removed at 2-month-old and sacrificed at 1-day ($n1$ = 6, $n2$ = 3), 2-d ($n1$ = 7, $n2$ = 3), 4-d ($n1$ = 3, $n2$ = 3), 7-d ($n1$ = 6, $n2$ = 3), and 14-d ($n1$ = 6, $n2$ = 3) postcastration. The numbers within the parentheses indicate the number of female ($n1$) and male ($n2$) animals in that age group. Pituitary glands were removed from the skull, fixed in paraformaldehyde-lysine-periodate fixative (McLean and Nakane 1974), postfixed with osmium tetroxide (Yu 1993), dehydrated in a graded series of ethanol, immersed in propylene oxide, infiltrated in an Epon-Araldite/propylene oxide mixture (1:1), and cured in freshly prepared embedding media. Semithin sections (1 μm thickness) were cut on an ultramicrotome, placed on the glass slide, and then immunolabeled with anti-LH antiserum for light microscopy. Ultrathin sections (60-90 nm thickness) were cut on the ultramicrotome, placed on copper or nickel grids, stained with lead citrate and uranyl acetate, and examined with a JEOL electron microscope.

The immunocytochemical staining method was the avidin-biotin-peroxidase complex procedure of Hsu et al. (1981) with the following steps: (1) incubation in rabbit antisera to rat beta luteinizing hormone (LH) (diluted 1:1000 to 1:2000, NIDDK, Baltimore, MD, USA) for 14-16 h at room temperature; (2) incubation in goat anti-rabbit biotinylated gamma-globulin (Vectastain ABC kit, Vector Laboraties, Burlingame, CA, USA) for 1-2 h; (3) incubation in ABC reagent (Vectastain ABC kit) for 1-2 h; (4) treatment with 3,3'-diaminobenzidine tetrahydrocholride (DAB, 0.3 mg/ml, Sigma Chemical Co., St. Louis, MO, USA) in 0.05 M Tris buffer containing 0.002% hydrogen peroxide at pH 7.6, until immunoreactive sites were visible. To demonstrate cellular specificity of rabbit antiserum to rat beta LH, negative and positive controls were processed separately: for negative control staining, normal rabbit serum or phosphate buffered saline was used to replace rabbit antiserum; for positive control staining, rabbit antiserum to rat prolactin was used to replace rabbit antiserum.

Quantitative measurements were made using the Image-Pro Plus Data Analysis Program (Media Cybernetics, Silver Spring, MD, USA). In measuring LH-IR cells, only the number and the cross-sectioned area through the middle of the immunolabeled cell were included. No corrections were made for shrinkage between fresh tissue and structures measured through the WinFast T230 TV video system in the plastic sections. All the data were stored in the computer and analyzed by SPSS for Windows. The statistical comparisons of cross-sectional area in multiple groups used one-way analysis of variance (one-way ANOVA). The post hoc analyses for significance between any 2 groups were conducted by the Student-Newman-Kuels test.

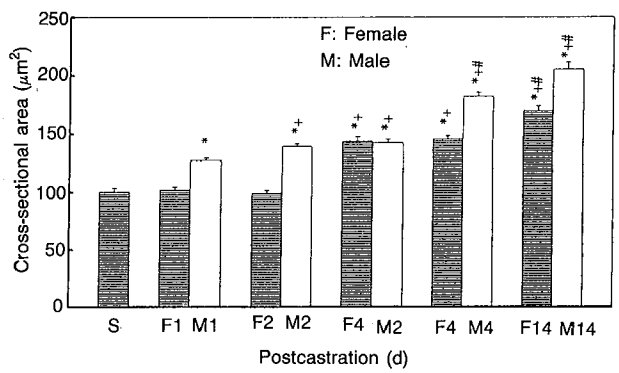

Fig. 1. Mean cross-sectional area with standard error for LH-IR cells of female and male pituitary glands. S: sham-castrated; F1, F2, F4, F7, F14: postcastration day 1, 2, 4, 7, and 14 of female rats, respectively; M1, M2, M4, M7, M14: postcastration day 1, 2, 4, 7, and 14 of male rats, respectively; * denotes $p < 0.05$, for data compared with S, (or F1, F2) group; + denotes $p < 0.05$, for data compared with M1 group; # denotes $p < 0.05$, for data compared with M2 (or M4, F4, or F7) group.

RESULTS

Quantitative analysis

As shown in Fig. 1, there were no significant differences in the mean cross-sectional areas of LH-IR cells in female pituitary glands between 1-d postcastration (101.98 ± 2.50 μm^2) or sham-operation (100.14 ± 3.55 μm^2). Cross-sectional area (99.10 ± 1.75 μm^2) maintained no significance difference through 2-d postcastration, but the area was significantly larger at 4-d postcastration (143.80 ± 3.99 μm^2) and at 7-d postcastration (145.62 ± 2.78 μm^2) than at 2-d postcastration in females. Furthermore, cross-sectional area was also significantly ($p < 0.05$) larger at 14-d postcastration (169.55 ± 4.33 μm^2) than at 7-d postcastration.

The cross-sectional area of LH-IR cells was significantly larger at 1-d postcastration in the male pituitary gland (127.36 ± 1.95 μm^2) than in that of females (101.98 ± 2.50 μm^2). In male rats, cross-sectional area was also significantly larger at 2-d

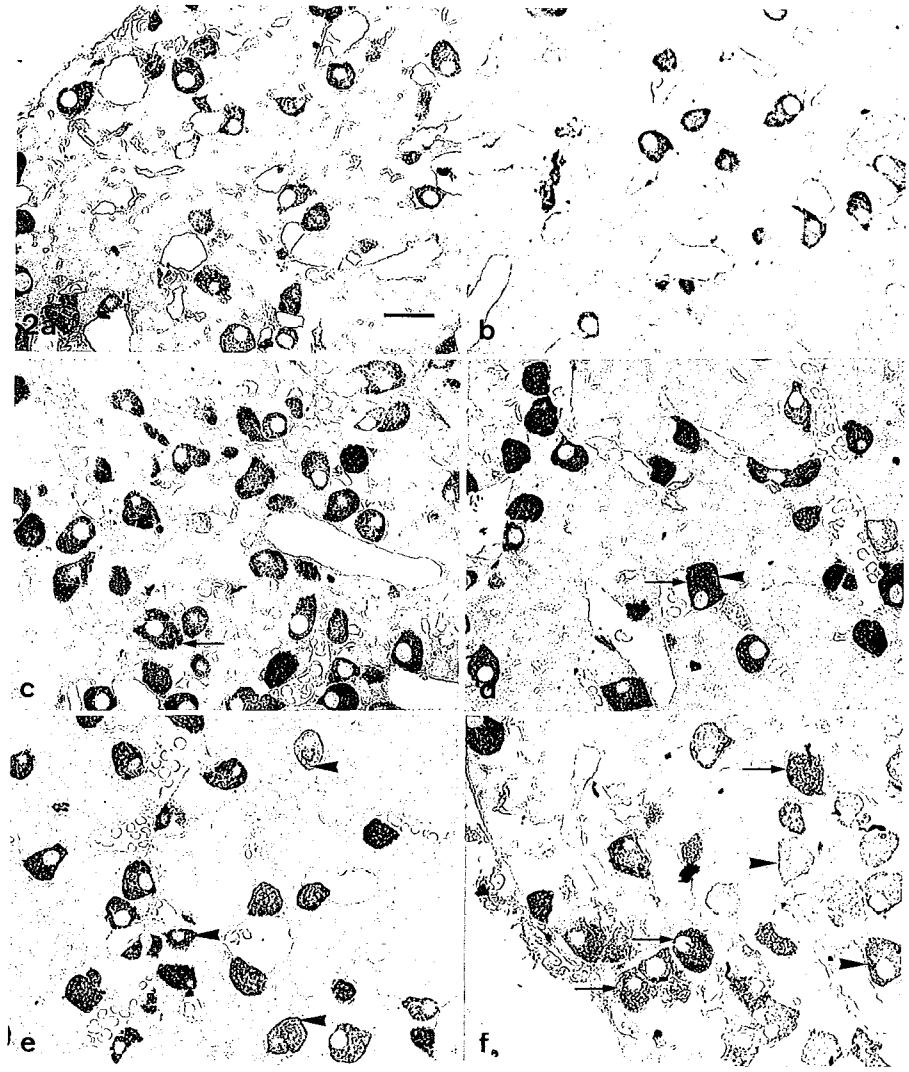

Fig. 2. Light micrographs of LH-immunoreactivity in the female pituitary gland. (a) Sham-castration. LH-immunoreactive (IR) product is found in non-hypertrophied gonadotrophs. (b) One day postcastration. Intense LH-IR product remains similar to that in sham-castrated female rats. (c) Two days postcastration. A few LH-IR cells have become hypertrophied (arrow). (d) Four days postcastration. A few hypertrophied LH-IR cells (arrow) contain a few small cytoplasmic vacuoles (arrowhead). (e) Seven days postcastration. Numerous hypertrophied LH-IR cells contain a few small cytoplasmic vacuoles (arrowhead). (f) Fourteen days postcastration. A large number of hypertrophied LH-IR cells (arrows) contain numerous cytoplasmic vacuoles. Note the hypertrophied LH-IR cells (arrowheads) containing no cytoplasmic vacuoles. Scale bar = 25 μm.

postcastration (139.42 ± 2.31 μm^2) and at 4-d postcastration (142.44 ± 3.19 μm^2) than at 1-d postcastration. Cross-sectional area was significantly larger at 7-d postcastration (182.00 ± 3.66 μm^2) than at 4-d postcastration. Cross-sectional area at 14-d postcastration (205.30 ± 6.09 μm^2) was significantly larger than at 7-d postcastration in males.

Light microscopy

LH-immunoreactivity in the pituitary gland of the female rat

In negative control staining, no specific immunoreactive product was found in male or female rat pituitary glands. In positive control staining, immunoreactive product was localized in mammotrophs which contained large irregular secretory granules which differed from the immunolabeled LH cells.

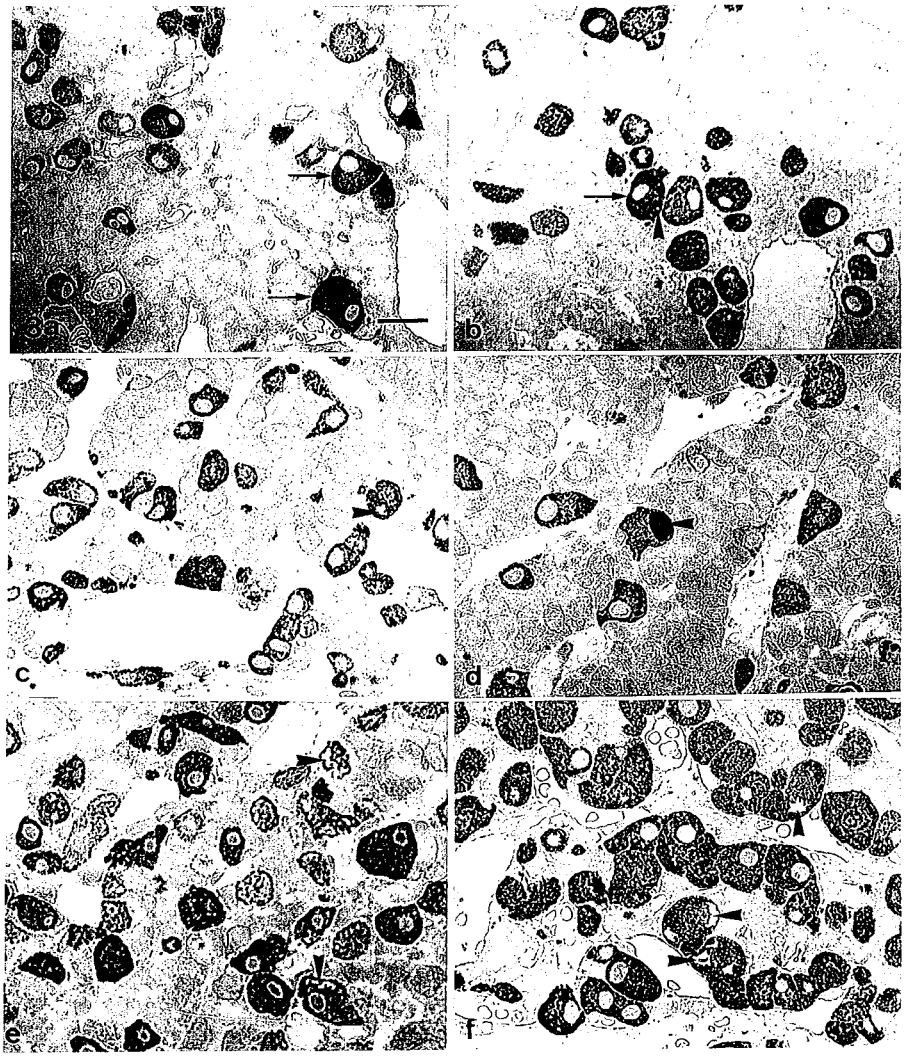

Fig. 3. Light micrographs of LH-immunoreactivity in the male pituitary gland. (a) One day postcastration. A few hypertrophied LH-IR cells (arrows) are found in the pituitary gland. (b) Two days postcastration. A few hypertrophied LH-IR cells (arrow) contain a few small cytoplasmic vacules (arrowhead). (c) Four days postcastration. A few hypertrophied LH-IR cells contain coalesced vacuoles (arrowhead). (d) Four days postcastration. Immunolabeled dense bodies (arrowhead) are also frequently found in the male pituitary gland. (e) Seven days postcastration. Numerous hypertrophied LH-IR cells contain coalesced vacuoles (arrowheads). (f) Fourteen days postcastration. A large number of hypertrophied LH-IR cells are arranged in irregular, interconnecting cords. Some hypertrophied LH-IR cells contain coalesced vacuoles (arrowheads) and numerous cytoplasmic vacuoles in the cytoplasm. Scale bar = 25 μm.

In sham-castrated female rats, LH-immunoreactive (IR) product was found in non-hypertrophied gonadotrophs, and no cytoplasmic vacuoles were found in the LH-IR cells of the pituitary gland (Fig. 2a). At 1-d postcastration (Fig. 2b), LH-IR cells were found to be similar to those in sham-castrated females. At 2-d postcastration, a few LH-IR cells had become hypertrophied (Fig. 2c). At this age, the cross-sectional area of LH-IR cells showed no significant difference compared with that at 1-d

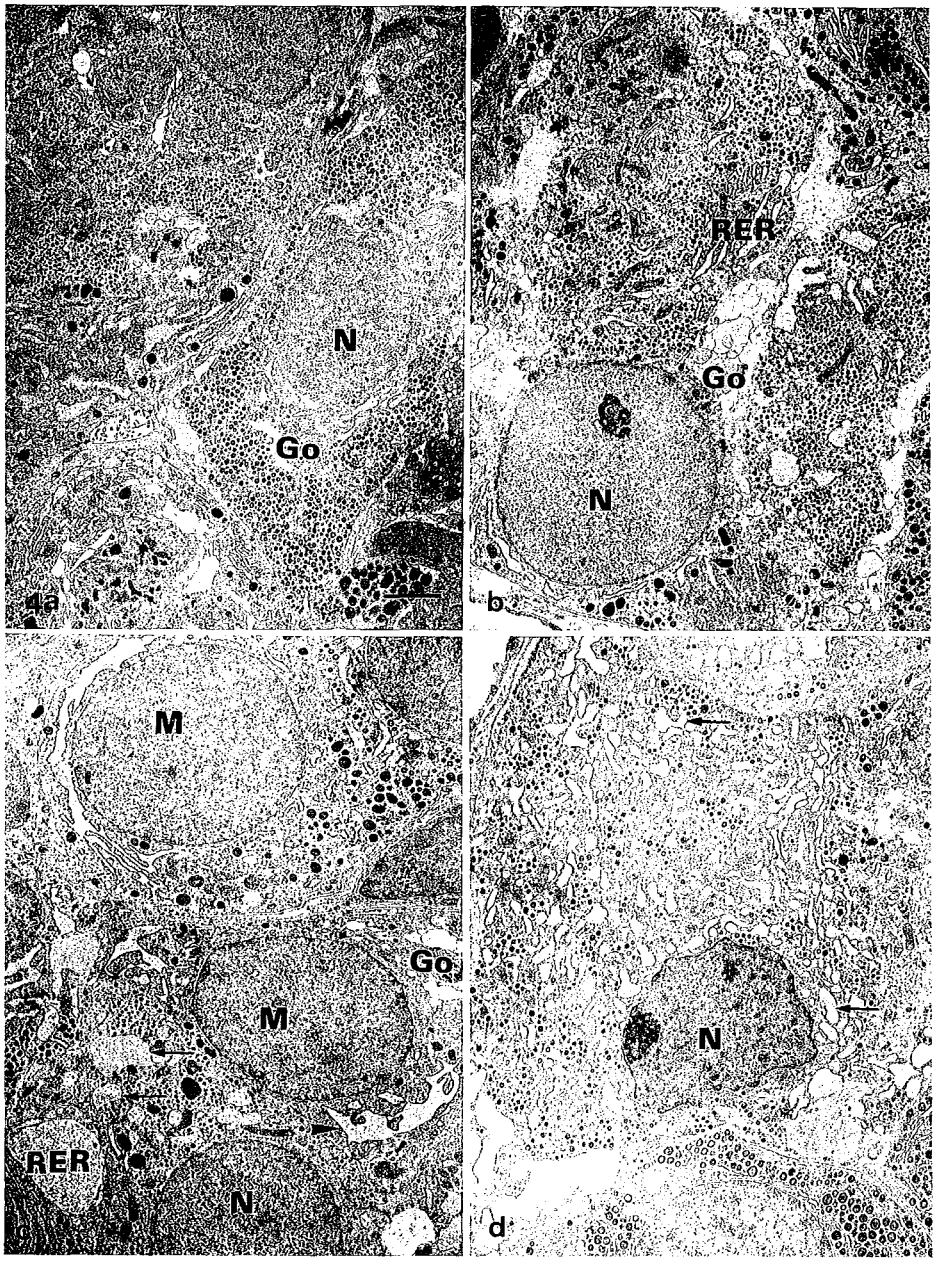

Fig. 4. Electron micrographs of gonadotrophs in the female pituitary gland. (a) One day postcastration. A gonadotroph (N) contains well-developed cisternae of the Golgi complex (Go) and a slightly dilated cisternae of the rough endoplasmic reticulum. (b) Two days postcastration. A gonadotroph (N) contains moderately dilated cisternae of the Golgi complex (Go) and slightly dilated cisternae of the rough endoplasmic reticulum (RER). (c) Four days postcastration. A gonadotroph (N) contains moderately dilated cisternae of the rough endoplasmic reticulum (arrows). Occasionally a markedly dilated cisterna (RER) is present. Note the adjacent mammotrophs (M) bearing large secretory granules and containing coalesced and moderately dilated cisternae of the rough endoplasmic reticulum (arrowhead). (d) Seven days postcastration. A gonadotroph (N) contains much moderately dilated cisternae of the rough endoplasmic reticulum (arrows). Scale bar = 2 μm.

postcastration. At 4-d postcastration, an increase in number of hypertrophied LH-IR cells was significant, and a small number of these hypertrophied LH-IR cells contained a few small cytoplasmic vacuoles (Fig. 2d). At 7-d postcastration, numerous hypertrophied LH-IR cells contained a few small cytoplasmic vacuoles (Fig. 2e). At 14-d postcastration, a large number of hypertrophied LH-IR cells contained numerous cytoplasmic vacuoles (Fig. 2f).

LH-immunoreactivity in the pituitary gland of the male rat

At 1-d postcastration, a few hypertrophied LH-IR cells were found in the male rat pituitary gland (Fig. 3a). At 2-d postcastration, a small number of hypertrophied LH-IR cells contained a few small cytoplasmic vacuoles (Fig. 3b). At 4-d postcastration, a few hypertrophied LH-IR cells contained

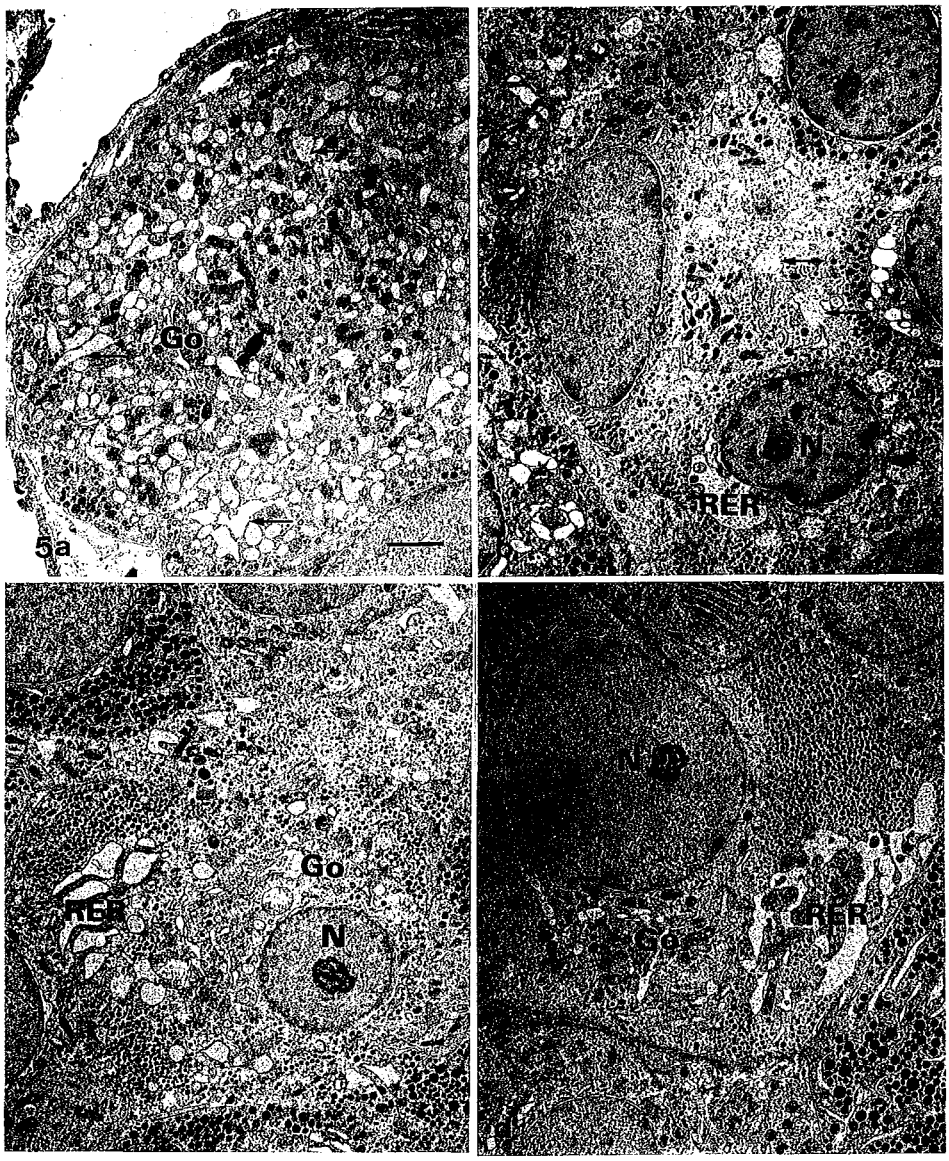

Fig. 5. Electron micrographs of gonadotrophs in the female (a) and male (b-d) pituitary gland. (a) Fourteen days postcastration. A gonadotroph contains many moderately dilated cisternae of the rough endoplasmic reticulum (arrows). (b) One day postcastration. A gonadotroph (N) contains well-developed cisternae of the Golgi complex (arrows) and a few moderately dilated cisternae of the rough endoplasmic reticulum (RER). (c) Two days postcastration. A gonadotroph (N) contains some moderately dilated cisternae of the rough endoplasmic reticulum (RER). Note that the cisternae of the Golgi complex (Go) are rather poorly developed. (d) Four days postcastration. A gonadotroph (N) contains coalesced and moderately dilated cisternae of the rough endoplasmic reticulum (RER). Scale bar = 2 μm.

coalesced vacuoles (Fig. 3c) or immunolabeled dense bodies (Fig. 3d). At 7-d postcastration, numerous hypertrophied LH-IR cells contained coalesced vacuoles (Fig. 3e). At 14-d postcastration, a large number of hypertrophied LH-IR cells was found in the pituitary gland, and some of them contained numerous cytoplasmic vacuoles and large coalesced vacuoles (Fig. 3f). Most of the immunolabeled LH-IR cells were arranged in irregular, interconnecting cords.

Electron microscopy

Gonadotrophs in the pituitary gland of the female rat

At 1-d postcastration, gonadotrophs in the female rat pituitary gland contained well-developed cisternae of the Golgi complex and slightly dilated cisternae of the rough endoplasmic reticulum (Fig. 4a). Small secretory granules were numerous in the cytoplasm. At 2-d postcastration, hypertrophied gonadotrophs contained moderately dilated cisternae of the Golgi complex and slightly dilated cisternae of the rough endoplasmic reticulum (Fig. 4b). Small secretory granules had accumulated in the cytoplasm. At 4-d postcastration, hypertrophied gonadotrophs contained some moderately dilated cisternae of the rough endoplasmic reticulum (Fig. 4c). Most of the secretory granules were small but a few large ones were also present in the cytoplasm. At 7-d postcastration, hypertrophied gonadotrophs contained many moderately dilated cisternae of the rough endoplasmic reticulum (Fig. 4d). Most secretory granules were small and scattered in the cytoplasm. At 14-d postcastration, hypertrophied gonadotrophs were similar to those found in the female pituitary gland at 7-d postcastration (Fig. 5a).

Gonadotrophs in the pituitary gland of the male rat

At 1-d postcastration in male rats, hypertrophied gonadotrophs contained well-developed cisternae of the Golgi complex and a few moderately dilated cisternae of the rough endoplasmic reticulum (Fig. 5b). Most secretory granules were small in size and accumulated at 1 pole of the cytoplasm. At 2-d postcastration, hypertrophied gonadotrophs contained some moderately dilated cisternae of the rough endoplasmic reticulum (Fig. 5c). Small secretory granules had accumulated at 1 pole of the cytoplasm. At 4-d postcastration, hypertrophied gonadotrophs contained coalesced and moderately dilated cisternae of the rough endoplasmic reticulum (Fig. 5d). Small secretory granules and coalesced, dilated cisternae of the rough endoplasmic reticulum had accumulated at 1 pole of the cytoplasm. At 7-d postcastration, hypertrophied gonadotrophs contained coalesced and markedly dilated cisternae of the rough endoplasmic reticulum (Fig. 6a). Small secretory granules were scattered among the coalesced, dilated cisternae of the rough endoplasmic reticulum. In addition, many moderately dilated cisternae of the rough endoplasmic reticulum were located in the hypertrophied gonadotrophs in patterns similar to those found in the female pituitary gland at 14-d postcastration. At 14-d postcastration, hypertrophied

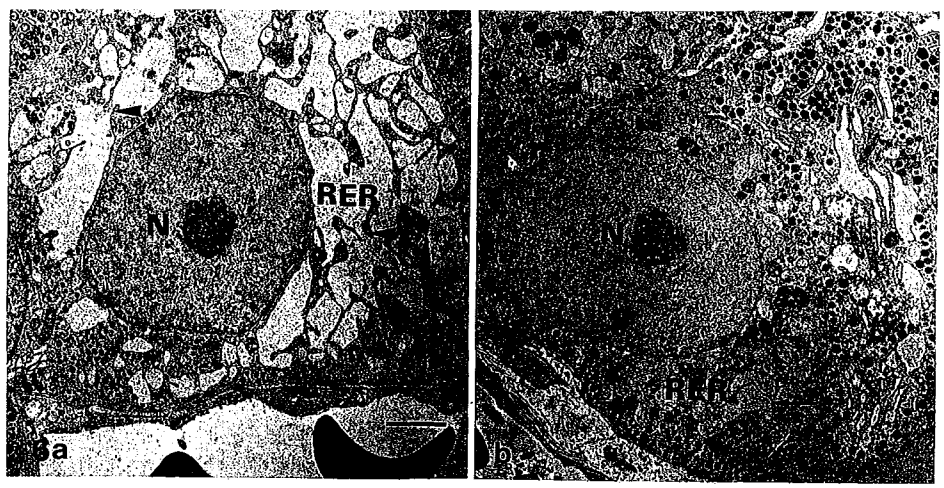

Fig. 6. Electron micrographs of gonadotrophs in the male pituitary gland. (a) Seven days postcastration. A gonadotroph (N) contains coalesced and markedly dilated cisternae of the rough endoplasmic reticulum (RER). The arrowheads indicate the junctions of the adjacent coalesced cisternae of the rough endoplasmic reticulum. (b) Fourteen days postcastration. A gonadotroph (N) contains numerous moderately dilated cisternae (arrows) of the rough endoplasmic reticulum (RER). Scale bar = 2 μm.

gonadotrophs were similar to those in the male pituitary gland at 7-d postcastration (Fig. 6b).

DISCUSSION

The gonads are under the control of the pituitary gland. The pituitary secretions are in turn regulated by the gonadal steroids which act on adenohypophysial luteinizing hormone (LH) and hypothalamic LH releasing hormone (LHRH) to reduce their secretion (Piacsek and Meites 1966). Castration results in the removal of the negative feedback of gonadal steroid inhibition and causes increases in hypothalamic LHRH content and serum LH level (Shin and Howitt 1975), LH mRNA (Perheentupa et al. 1993), pituitary LH content (McNeilly et al. 1980), TRH-like immunoreativity (Akinsanya et al. 1995), neurokinin A (Debeijek et al. 1992), and nitric oxide synthase protein (Ceccatelli et al. 1993). The pituitary LH level falls gradually in 2-5 d after castration, then increases to the control level by day 8 after castration and reaches twice the control level by day 13 (Shin and Howitt 1975). By using immunocytochemical staining, the present study demonstrated that LH-immunoreactivity dramatically increases in amount after castration both in the female and male pituitary gland, especially at 14-d postcastration in males. Our findings provide immunocytochemical morphologic data to support previous reports. Several factors have been considered for this pituitary LH increment. Mitosis is presumably a major factor for proliferation of gonadotrophs in the anterior pituitary after castration (Sakai et al. 1988), and differentiation from immature cells into gonadotrophs is another possibility (Inoue and Kurosumi 1981).

Gonadotrophs are of 2 different cell types: one secreting FSH and the other secreting LH. FSH cells possess large and small secretory granules, while LH cells contain only the small type of secretory granules (Kurosumi and Oota 1968). Immunocytochemical staining indicates that LH and FSH may be contained within the same cell (Nakane 1970). LH-immunoreactivity is predominant in small secretory granules, while large ones are occasionally positive but mostly react negatively (Kurosumi et al. 1991). Our present study indicates that hypertrophied LH-IR cells contain coalesced cytoplasmic vacuoles after castration. Such vacuoles correspond to the fusion of the dilated cisternae of the rough endoplasmic reticulum in the gonadotrophs. Our findings are similar to previous work by Somer et al. (1988) utilizing alcohol-treated rats. They demonstrated by ultrastructural analysis that the vacuole originates from, and anastomoses with, dilated cisternae of the rough endoplasmic reticulum.

It is of interest, however, to note that the occurrence of coalesced, dilated cisternae of the rough endoplasmic reticulum in gonadotrophs is predominantly restricted to the cell type containing small secretory granules. This finding agrees with that of the castration results which show no change in the pituitary content of FSH (McNeilly et al. 1980). In a previous study, castration resulted in a striking sex difference in plasma LH levels in adult rats; by 2-d postcastration, serum LH was 5- to 10-fold higher in males than in females (Elskus et al. 1995). The present work demonstrates a similar finding: that the appearance of cytoplasmic vacuoles is slightly earlier in males by 2-d postcastration than in females by 4-d postcastration. A likely explanation for our results is that the hypertrophy of the gonadotroph indicates highly active synthesis of gonadotropic hormones. Furthermore, this gonadotropin synthesis is revealed by the cytoplasmic vacuoles at the light microscopic level or by the dilation of the rough endoplasmic reticulum and the Golgi complex in the gonadotrophs at the electron microscopic level. The coalesced vacuoles appear to result from the fusion of elements of the rough endoplasmic reticulum. In summary, this study shows that a sex difference in responsiveness of the gonadotrophs to castration closely parallels changes in the hypertrophy and coalesced vacuoles in the female and male pituitary. Detailed correlation of LH-IR, FSH-IR, and simultaneously colocalized LH-FSH-IR cells to ultrastructural changes will be investigated in the near future by using a double immunocytochemical labeling technique.

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大白鼠去勢後腦下垂體之黃體促素免疫染色反應與超微結構變化：形態計量與形態學之研究

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在去勢後第一天，雌性大白鼠的腦下垂體的黃體促素免疫染色反應細胞並不膨大且與對照組相同，而在雄性大白鼠的腦下垂體卻具有一些膨大的黃體促素免疫染色反應細胞，即具有一些更加膨脹的粗糙內質網小池。在去勢後第二天，在雌性大白鼠的腦下垂體內可發現一些膨大的黃體促素免疫染色反應細胞。此時在雄性大白鼠的腦下垂體內膨大的黃體促素免疫染色反應細胞含有空泡存在，亦即含有一些更加膨脹的粗糙內質網小池。在去勢後第四天，在雌性大白鼠的腦下垂體內膨大的黃體促素免疫染色反應細胞也含有空泡存在。但在雄性大白鼠的腦下垂體內膨大的黃體促素免疫染色反應細胞含有融合性空泡，亦即含有一些更加膨脹融合的粗糙內質網小池。在去勢後第七天，在雌性大白鼠的腦下垂體內有許多膨大的黃體促素免疫染色反應細胞也含有空泡存在。但在雄性大白鼠的腦下垂體內許多膨大的黃體促素免疫染色反應細胞卻含有融合性空泡，亦即含有一些更加膨脹更顯著融合的粗糙內質網小池。在去勢後第十四天，在雌性大白鼠的腦下垂體內有更多膨大的黃體促素免疫染色反應細胞含有空泡。但在雄性大白鼠的腦下垂體內有更多膨大的黃體促素免疫染色反應細胞相互排列並連接成不規則的索狀構造，並且一些膨大的黃體促素免疫染色反應細胞含有融合性空泡。在雌性和雄性大白鼠兩者的腦下垂體內，大部份的膨大的性腺細胞含有較小的分泌顆粒，僅有一小部份的膨大的性腺細胞含有較大的分泌顆粒。

關鍵詞：去勢，免疫細胞化學，黃體促素，腦下垂體，形態計量。

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