An Electron Microscopical Study on Effects of Synthesized Estrogen on the Anterior Pituitary of Male Mice

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Abstract. Effects of natural estrogen and synthetic nonsteroidal estrogens on the fine structure of the anterior pituitary cells of the male mouse were studied. As estrogenic compounds, estradiol-17β, hexestrol, and two hexestrol difatty acid esters (hexestrol dicaprylate and hexestrol dicaprylate) were used. Seven days after injection with a single dose of 100 μg per adult male mouse, the hexestrol difatty acid esters induced striking morphological alterations in the gonadotrophic cells, including LTH cells. LTH cells were hypertrophied drastically and LH cells relatively, while FSH cells exhibited an atrophic appearance.

In order to confirm the suppressive effect of the estrogens on FSH cells, male mice were orchidectomized and then injected with the same dose of each estrogen. This treatment completely suppressed postcastration changes in FSH cells. This also resulted in an exaggeration of estrogen-induced LTH cell hypertrophy. Alterations induced by estradiol-17β were similar to but milder than those in the case of the hexestrol difatty acid esters, although hexestrol exerted no morphological effects.

The difatty acid ester of hexestrol, hexestrol dicaprylate, was prepared by Hamada (unpublished data) for the first time. Since then it has been under investigation for usefulness in the animal production and veterinary practice in many laboratories. In the present study, hexestrol difatty acid esters were used to clarify the nature of effects of estrogenic compounds on the anterior pituitary cytology of the male mouse. It has been found that the synthetic estrogenic compound has stronger effects on the gonadotrophs, including LTH cells of the pituitary gland, than natural estrogen, i.e., estradiol-17β.

Materials and Methods

Two experiments were conducted in this study.

The initial experiment, adult male mice of the C3Bl strain were injected subcutaneously with a single dose of 100 μg of estradiol-17β, hexestrol, hexestrol dicaprylate, or hexestrol dicarboxate. Estradiol-17β and hexestrol were suspended in 5% gum arabic in distilled water, and the two hexestrol difatty acid esters were diluted with cotton seed oil. The dose of injection was always 0.1 ml.

In the subsequent experiment, adult mice were orchidectomized and injected with the same dose of each of the estrogens within one hour following the operation. Untreated intact and orchidectomized animals were used as controls. All the experimental animals were killed seven days after treatment.

The anterior pituitary tissues were taken out from them just after autopsy and processed routinely for electron microscopy (Yamashita [21]). Thick sections about one μ in thickness were obtained from Epon embedded materials and stained with toluidine blue for the survey of the tissues by light microscopy.
Results

Since the fine structure of the anterior pituitary of normal mice has been described in detail by many authors (Barnes [1], Sano [15], Yamada and Yamashita [20]), the normal fine structure of LTH, FSH, and LH cells is reviewed herein only briefly as a basis for comparison with that of experimentally altered cells of these types which are the authors' principal concern in the present study.

LTH cells are small and irregular in outline, and contain characteristic granules of variable size and shape. The number of cells of this type is not so small in male mice, in which many small profiles of the cells are observed (Figs. 1 and 3).

FSH cells are large and round or oval in shape, containing round, homogeneous granules of moderate electron density (Fig. 8).

LH cells are smaller than FSH cells and contain dense secretory granules, which are comparable in size with the latter cells.

FSH cells were markedly hypertrophied following orchidectomy (Fig. 11). They were enlarged due to an increase in amount, as well as vacuolar dilatation, of rough endoplasmic reticulum and the development of Golgi complex. Granules contained in them were reduced in both size and number; though the formation of granules was facilitated in the Golgi apparatus. LH cells did not show so drastic alterations as those of FSH cells after castration, but tended to exhibit the apparently enhanced function of the cytoplasm. No LTH cells appeared to undergo any conspicuous change after castration.

1. Effect of estrogen administration on LTH, FSH and LH cells

Administration of two hexestrol difatty and acid esters, i.e., hexestrol dicaprylate and hexestrol dicaprylate, caused hyperplasia and marked hypertrophy of LTH cells (Fig. 4). The nuclei of enhanced LTH cells were large and rather polymorphous, containing large, conspicuous, occasionally annular nucleoli of high electron density. The cytoplasm of these cells was also hypertrophied markedly (Fig. 4). Mitochondria were numerous and usually rod-shaped. Rough endoplasmic reticulum was highly developed and arranged in parallel arrays, occupying a large part of the cytoplasm. It was very common for rough endoplasmic reticulum to have concentric whorls (Nebenkern) (Fig. 6). At the center of these whorls, there were frequently smooth-surfaced vesicles. The Golgi complex was extremely well developed and contained many newly formed secretory granules.

Many small immature granules were observed around the Golgi complex, but mature granules were reduced in number and scattered in the periphery of the cytoplasm. At the vascular poles of LTH cells, images of discharging granules were rather frequently observed. Abundant free ribosomes were distributed throughout the cytoplasm.

These remarkable changes in LTH cells, especially the enlargement of nuclei and rough endoplasmic reticulum, were so prominent that the light microscopy of thick Epon sections made it possible to identify LTH cells readily and assess the functional state of these cells. By the aid of the light microscope, it was shown that LTH cells increased in number following administration with the hexestrol difatty acid esters. This was also well evidenced by the frequent occurrence of LTH cells at mitosis and the concomitant increase in the number of mitotic endothelial cells. The enlargement of the anterior pituitary of the
treated animals was attributable to this hyperplasia of LTH cells.

FSH cells were reduced in size and number following administration with the hexestrol difatty acid esters (Fig. 9). They were irregular in outline due to compression by neighboring cells. Accordingly, they appeared partially in a single ultrathin section and could be detected only by careful electron microscopy. The reduction in cellular size was chiefly because the cytoplasm contained a small amount of rough endoplasmic reticulum and the poorly developed Golgi complex. Secretory granules were rather decreased in number, although they occupied a large part of the reduced cytoplasm.

LH cells were enlarged in size and observed rather frequently (Figs. 5 and 9). They contained well developed organelles. Rough endoplasmic reticulum was often arranged in several parallel arrays. The Golgi complex was large, showing an active formation of granules. Secretory granules were small in number and exhibited a preferential orientation along the cell membrane. Many figures showing discharge of granules were present at the vascular pole of the cell (Fig. 5). Less prominent changes in the anterior pituitary cells were observed in mice treated with estradiol-17β than in those treated with the two hexestrol difatty acid esters, while no morphological changes in these cells were detected from mice administered with hexestrol.

2. Effects of estrogens on LTH, FSH and LH cells of castrated mice

In order to confirm the above-mentioned findings on the effects of estrogen treatment, some mice were orchidectomized and then injected with a single dose of each estrogen. As a result, they presented a drastic augmentation of hyperplasia and hypertrophy of LTH cells and a marked inhibition of possible postcastration hypertrophy of FSH cells (Figs. 2 and 10). In the estrogen-treated castrates, hypertrophic LTH cells occupied a large part of the anterior pituitary tissue (Fig. 2). Mitotic figures were frequently seen among LTH cells. It is also interesting to note that annulate lamellae were detected in a few of the LTH cells in the animals treated with hexestrol difatty acid esters (Fig. 7). In the castrates, hexestrol difatty acid esters exhibited the highest effect on LTH cells, estradiol-17β a moderate effect, and hexestrol no effect at all.

FSH cells showed no remarkable cytological changes in the groups treated with hexestrol difatty acid esters (Figs. 2 and 9). In the estradiol-treated castrates, postcastration hypertrophy of FSH cells was inhibited incompletely. Hexestrol had no ability to inhibit the response of any animal to orchidectomy.

There were no significant differences in any change of LH cells among the experimental groups treated with different sorts of hexestrol difatty acid esters.

Discussion

There have been many electron microscopic studies indicating that estrogens exert an effect to enhance the function of LTH cells and to induce frequently hyperplasia or formation of tumors of this type of cells [6, 10, 13, 16, 18]. The authors' morphological findings on the effects of estradiol-17β and the two hexestrol difatty acid esters on the LTH cells of male mice coincide with the descriptions made from those studies.

The response of LTH cells to the above-mentioned effective estrogens was augmented by combined orchidectomy. This result confirms well the above-mentioned
finding on the estrogen actions, and may be explained by the elimination of an antagonistic action of endogenous androgen. In male mice treated with hexestrol difatty acid esters which induced an especially drastic augmentation of the LTH cell response, it is interesting to note that annulate lamellae were observed in a few LTH cells. These lamellae were reported to have been detected in certain undifferentiated cells or tumor cells [8]. The occurrence of this type of organelle in the anterior pituitary was first described by Watari and Tsukagoshi [18] in the LTH cells of an estrogen-induced tumor in rats. Thus, the finding of this type of organelle in LTH cells suggests that these cells may have been in a highly stimulated state.

In their disc electrophoretic analysis of the anterior pituitary, Jones et al. [7] observed that the administration of estradiol-17β to normal or orchidectomized male rats had resulted in an increase in staining intensity of the prolactin (LTH) band, while untreated orchidectomized rats showed no alteration in the band. Their finding lends support to the authors' interpretation of the morphological findings on LTH cells obtained from the present investigation under similar experimental conditions.

In the literature cited above, there is no information on the FSH or LH cells of estrogen-treated animals.

It was found in the present electron microscopical study that estrogens had exerted an inhibitory influence on the function of FSH cells and an enhancing effect on the function of LH cells. The suppressive action of estrogens on FSH cells was confirmed further since a definite prevention of postcastration hypertrophy of FSH cells had occurred to estrogen-treated castrates. Paesi et al. [12] reported that estradiol benzoate had diminished the content of FSH in intact and castrated male rats. Recently, Gay and Dever [4] also mention that a postorchidectomy rise in serum FSH had been inhibited in the rat by daily subcutaneous injections with estradiol benzoate. These findings coincide with the results of the present experiment.

In the literature cited above, there are some contradictory opinions on the effects of estrogens on the synthesis, storage, and release of LH in the pituitary. It has generally been believed that estrogens suppress the pituitary gonadotropic potency. Alternatively, a stimulative action of estrogens on the LH secretion has been proposed by a number of workers. It is well known that administration of estrogens induces a release of LH in immature female animals [5, 11, 14], ovariectomized animals [2, 17] and pregnant animals [3]. Recently, Weik et al. [19] reported that estrogen had exerted both a positive and a negative effect on the synthesis and release of LH in the pituitary gland, depending on the dosage used, and that its high dose had suppressed both synthesis and release of LH.

In view of these data in the literature, it is considered that the effect of estrogen on the synthesis and release of LH may greatly be influenced by age, sex, physiological and experimental conditions, and varying time-dose relationships in the respective animal.

Accordingly, under these circumstances, it is somewhat difficult to compare the morphological conclusions drawn on the LH cell in the present investigation with the data given above. The present observation on the LH cells of estrogen-treated male mice suggests an enhancement of the release and production of secretory granules in these cells. Namely, the stimulative
influence of estrogen on these cells was manifested by such decrease in secretory granules as attributable to the enhanced release and by a development of the protein-producing apparatus in the cytoplasm. These findings may probably be supported by the evidence that the administration of estrogens induces an acute release of LH, as cited above. It should also be noted that the present experimental data were obtained from materials collected within seven days after the treatment, or during a period when a high secretion of LH granules was expected to continue. Leavitt [9] reported that estradiol-17β increased the number of gonadotrophs in the anterior pituitary of ovariectomized mice, although he did not divide the gonadotrophs into two types, i.e., FSH and LH cells. In the present experiment with estradiol-17β, the postcastration hypertrophy of FSH cells was inhibited incompletely.

The present experimental design may not be qualified as a physiological model for studying the LH cell function, since the large doses of estrogens and male mice were employed. The results of the present investigation that LH cells appeared after estrogen treatment may raise a problem on the functional morphology of these cells. Further detailed observation will be required to determine whether LH cells are hypertrophied following the estrogen treatment or not.

References
[16] Schelin, U., Lundin, P. M., and Bartholdson,


**Explanation of Figures**

Fig. 1. Portion of an anterior pituitary from a control male mouse. LTH cells with moderately developed cell organelles are seen among STH cells. ×1,900.

Fig. 2. Portion of an anterior pituitary from a castrated mouse treated with hexestrol dicaproate. LTH cells with well developed endoplasmic reticulum occupy the greater part of this figure. Note a small FSH cell in the upper right corner of the figure. ×1,900.

Fig. 3. Portion of an anterior pituitary from a control male mouse. ×3,200.

Fig. 4. LTH cells of a male mouse treated with hexestrol dicaproate. Note well developed cell organelles and large secretory granules in the cytoplasm. ×4,900.

Fig. 5. LH cell of a male mouse treated with hexestrol dicaprylate. Note the well developed cytoplasm and images of discharge of secretory granules (arrows). ×4,900.

Fig. 6. Portion of an anterior pituitary from a hexestrol dicaprylate-treated male mouse. An LTH cell contains a typical “Nebenkern” in the cytoplasm. ×4,900.

Fig. 7. LTH cells of a castrated mouse treated with hexestrol dicaproate. Note annulate lamellae in an LTH cell. ×4,900.

Fig. 8. Portion of an anterior pituitary from a control male mouse. An FSH cell possesses a large area of cytoplasm containing a relatively small number of secretory granules. ×3,200.

Fig. 9. Portion of an anterior pituitary from a hexestrol dicaproate-treated male mouse. A small FSH cell and well developed LH cells are noted. ×3,200.

Fig. 10. Portion of an anterior pituitary from a castrated mouse treated with hexestrol dicaproate. Small FSH cells with poorly developed cytoplasm are seen. Compare them with an FSH cell stimulated by orchidectomy in the next figure. ×3,200.

Fig. 11. Portion of an anterior pituitary from an orchidectomized mouse. An FSH cell with well developed cell organelles, especially a large Golgi area, dilated endoplasmic reticulum, and a few, small scattered secretory granules. ×3,200.

**Abbreviations**

| A .......... | ACTH cell |
| C .......... | Capillary |
| f .......... | Follicular cell |
| N .......... | Nebenkern |
| S .......... | STH cell |
| AN .......... | Annulate lamellae |
| F .......... | FSH cell |
| L .......... | LH cell |
| P .......... | LTH cell |