OBSERVATION OF FILAMENTS IN THE ADRENAL OF ANDROGEN-TREATED RATS

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Filaments have been reported in the cytoplasm of cells as diverse as columnar epithelial cells (1), epidermal cells (2), neuroglia (3), and neurons (4). In endocrine tissue, concentrations of filaments have been observed in the androgen-producing interstitial cells of the testis (5, 6), whereas the number of these filaments in the cytoplasm of other endocrine tissues is so small that they are only occasionally seen. Increased numbers of filaments have been produced experimentally in neurons (7, 8) and in cultured fibroblasts (9) by treatment with vinblastine or vincristine, inhibitors of mitosis. In the present study, we report the occurrence of similar, conspicuous arrays of filaments in the adrenal gland of androgen-treated rats.

MATERIALS AND METHODS

Female, Sprague-Dawley (Holtzman) rats, 42 days of age, were uninephrectomized and given 1% sodium chloride as drinking solution. Such a regimen has been utilized in our studies of the pathogenesis of experimentally induced hypertension (10). A total of six control rats (group 1) received subcutaneous injections of 0.2 ml of corn oil for 5 days each week. Group 2 consisted of six animals which were treated with 10 mg of methylandrostenediol (MAD, Nutritional Biochemicals, Cleveland, Ohio). Six animals in group 3 were injected with 20 mg of methyltestosterone (MT, Nutritional Biochemicals). Both MAD and MT were dissolved in corn oil, and animals were injected subcutaneously for 5 days each week (11). Animals were sacrificed after 8 wk of injections.

After decapitation, the adrenals were removed, freed of adherent fat and connective tissue, and slices approximately 1 mm in thickness were cut through the entire glandular mass. These slices were fixed for 4 hr in 3% glutaraldehyde buffered to pH 7.2 with 0.1 M phosphate. After fixation, the tissues were washed in ice cold buffer to remove excess glutaraldehyde and stored in cold buffer overnight.

The glands were divided into the three cortical zones by means of a fine scalpel under a dissecting microscope. The zona glomerulosa was isolated by taking thin tangential sections near the surface of the adrenal, the zona fasciculata was identified by the white color imparted by the abundance of lipid, and the zona reticularis was identified by the reddish-brown color due to the absence of lipid.

Tissues were postfixed in 1% osmium tetroxide buffered with 0.1 M phosphate to pH 7.2. After dehydration in a series of chilled ethanol solutions followed by propylene oxide, the tissues were embedded in a mixture of Epon 812 and Araldite (12). The blocks were sectioned with glass knives on a Porter-Blum ultramicrotome. Sections approximately 1 μ in thickness first were cut from each block, stained with toluidine blue, and examined in a light microscope. Before thin sections were cut, the blocks were trimmed to include adrenocortical cells whose zonal position in the adrenal had been identified by light microscopy, insofar as possible. Ultrathin sections were doubly stained with methanolic uranyl acetate (13) and lead citrate (14). Electron micrographs were taken with a Siemens Elmiskop I electron microscope.

RESULTS

Filaments occur in the cytoplasm of zona fasciculata and zona reticularis cells of adrenals from animals treated with the synthetic androgens methylandrostenediol (MAD) (Fig. 1) and methyltestosterone (MT) (Fig. 2). No filaments were observed in the cytoplasm of adrenocortical cells in control animals. The cross-sectional diameter of

* Deceased.

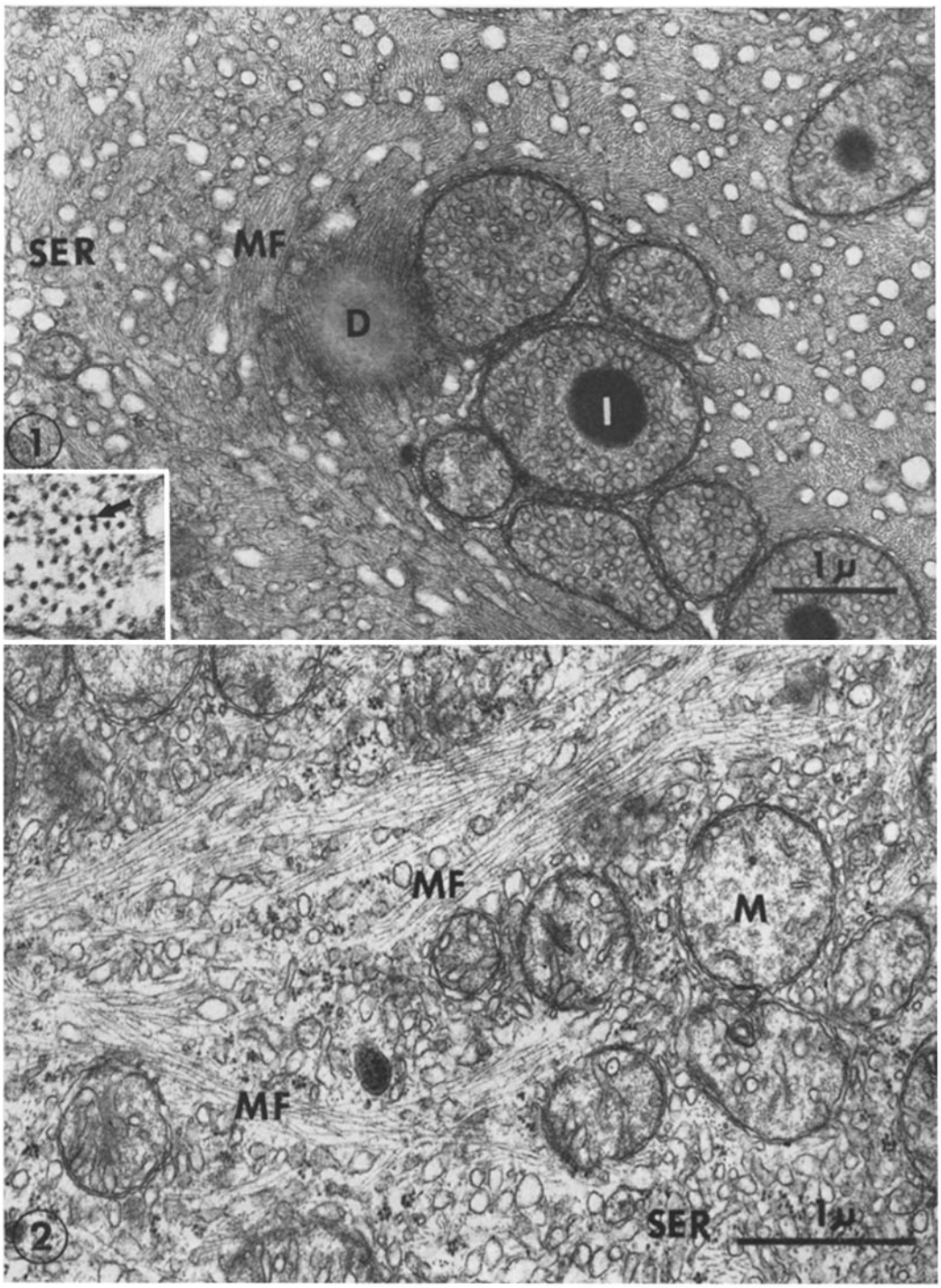

FIGURE 1 Zona fasciculata cell from adrenal gland of MAD-treated animal. The cytoplasm is virtually completely filled with longitudinal and cross-sections of cytoplasmic filaments (*MF*). Filaments can be seen in association with a cytoplasmic droplet (*D*). Dilated tubules of hypertrophic smooth endoplasmic reticulum (*SER*) are scattered throughout the cytoplasm. Mitochondria contain electron-opaque inclusions (*I*). × 18,400. In the *insert*, a high magnification of cross-sections of 70–80 A filaments is seen (arrow). A globular subunit structure can be seen comprising the filaments. × 90,000.

FIGURE 2 Zona fasciculata cell from MT-treated animal. Several bundles of filaments (*MF*) can be seen traversing the cytoplasm. Mitochondria (*M*) contain reduced numbers of cristae. A focal area of hypertrophic smooth endoplasmic reticulum (*SER*) can be seen. × 25,600.

the filaments ranges between 70 and 80 A; subunits comprising the filaments can be discerned which appear to be globular and approximately 30 A in diameter (Fig. 1). When seen in longitudinal section, the filaments often have a beaded appearance.

The filaments were usually found associated together in bundles (Fig. 2). Several bundles could be seen traversing the cytoplasm, sometimes oriented at different angles to each other (Fig. 2). Individual filaments in the bundles were of an indeterminate length and did not branch such that a filament could be seen traversing the cytoplasm for several micra (Fig. 2).

The filaments in adrenals of MAD- and MT-treated animals were similar in morphology and in their distribution throughout the cytoplasm. Whereas a variable number of filaments in individual cells was observed, well over half of zona fasciculata-reticularis cells contained filaments after 8 wk of androgen injections. At this same time, no filaments were identified in cells of the zona glomerulosa. Concentrations of the filaments were frequently seen adjacent to the nucleus. No connections between filaments and cytoplasmic structures could be discerned. Filaments sometimes appeared in close association with cytoplasmic vacuoles (Fig. 1), although filaments were observed adjacent to mitochondria with the same frequency. Occasionally, the cytoplasm of an adrenocortical cell was virtually completely filled with masses of the filaments (Fig. 1), often interspersed among dilated tubules of smooth endoplasmic reticulum.

The adrenal weight in androgen-treated animals was consistently decreased. After 8 wk of treatment with MAD, the adrenal weight in group 2 animals was 51.9 $\pm$ 2.0 mg, whereas that in group 3 animals injected with MT was 50.8 $\pm$ 3.6 mg. The adrenal weight in control animals (group 1) was 60.6 $\pm$ 4.0 mg, a value which was significantly greater than that of either group 1 or group 2 animals.

Other alterations in the ultrastructure of androgen-treated animals were identical to those described previously by Levine and Skelton (15) for MAD-injected animals. Among these changes was hypertrophy of the smooth endoplasmic reticulum, often seen in the form of concentric arrays of lamellar membranes. Sometimes the smooth endoplasmic reticulum was dilated as shown in Fig. 1. Mitochondrial vesicular cristae were generally decreased in number (Fig. 2), in comparison to the numerous vesicular cristae usually found in zona fasciculata cells of control animals (16). In androgen-treated animals, many of these cristae were located peripherally. Furthermore, numerous droplets were scattered throughout the cytoplasm. No microtubules were seen in the cytoplasm of adrenocortical cells of androgen-treated animals, although a single microtubule was sometimes observed in the cytoplasm of adrenocortical cells in control animals.

DISCUSSION

The functional significance of the filaments remains obscure inasmuch as accumulations of filaments have not been reported previously in the adrenal gland. The occurrence of filaments may well reflect an androgen-induced inhibition of mitosis in zona fasciculata-reticularis cells. Direct support for this hypothesis is provided by reports of similar accumulations of filaments in neurons of animals treated with vincristine (7, 8, 17), an inhibitor of mitosis. Although neurons do not divide, vincristine inhibits the formation of microtubules. Mitosis in the adrenal cortex may be inhibited by androgens since the adrenal weight is consistently decreased in the present study as well as in a previous study from our laboratory (11). Furthermore, the proposed inhibition of mitosis and the accumulation of filaments could well be explained by the total absence of microtubules in androgen-treated animals because filaments have been implicated as precursors of microtubules (9). Without microtubules the mitotic apparatus could not be formed, since it is well known that spindle fibers of the mitotic apparatus are composed of microtubules. However, it should be noted that no reports have appeared on mitosis in the adrenal cortex of MAD- or MT-treated animals.

Inhibition of mitosis is also suggested by evidence of cellular degeneration in the same cells containing the filaments. Focal cytoplasmic changes include PAS-positive droplets and vacuolar structures (15). Nevertheless, such cells can synthesize steroids, although the conversion of deoxycorticosterone to corticosterone, the normal major secretory product of the rat adrenal gland (18), is impaired (19). Studies are in progress to determine whether androgen effects are reversible and, therefore, whether the filaments disappear from zona fasciculata-reticularis cells.

Another possible role for the filaments is the

formation of a cytoskeleton for the adrenocortical cells, similar to one of the functions suggested for microtubules by Byers and Porter (20). However, filaments are rarely, if ever, seen in adrenocortical cells of control animals, and it would therefore be difficult to postulate that the filaments produce structural support in the adrenal gland of androgen-treated animals. In addition, filaments are not unique to the adrenal gland of androgen-treated animals since these structures occur in another steroid-secreting cell, the androgen-producing interstitial cell of the testis (5, 6). Furthermore, the observation of morphologically identical structures in neurons (neurofilaments) (17) and in desmosomes (tonofilaments) (2) demonstrates the widespread occurrence of filaments similar to those described in the present study.

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