Hormonally Induced Changes in the Cytoskeleton of Human Thyroid Cells in Culture

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ABSTRACT Serially cultivated thyroid follicular cells are not active in hormone synthesis but retain a thyrotropin-responsive adenylate cyclase. The exposure of such cells to thyrotropin leads to an increase in the concentration of intracellular cAMP and a drastic change in morphology including a total cytoplasmic arborization. The present communication describes these changes at the cytoskeletal level using a cell line derived from a human functioning thyroid adenoma. Phase contrast microscopy showed that the cytoplasmic arborization was preceded by a total disappearance of stress fibers, visible within 20 min of exposure. Small marginal membrane ruffles could also be seen. These morphological changes could also be induced by the addition of dibutyryl cAMP. The action of both thyrotropin and dibutyryl cAMP was potentiated by theophylline. High voltage electron microscopy of whole mounted cells confirmed the loss of stress fibers (microfilament bundles). In addition, thyrotropin treatment led to an uneven redistribution of the cytoplasmic ground substance and to changes in the organization of the microtrabecular lattice. Stereo images demonstrated numerous minute surface ruffles. The thyrotropin-induced arborization was reversible even in the presence of thyrotropin. After 24 h of treatment, cells had flattened and then contained very straight and condensed microfilament bundles.

The results thus demonstrate that thyrotropin induces a disintegration of microfilament bundles in human, partially dedifferentiated, follicular cells and that this effect appears to be caused by cAMP, the second messenger in thyrotropin action. The relation of this event in partially dedifferentiated cells to the effect of thyrotropin in the intact thyroid gland is unclear. The fact that several other cultured hormone-responsive cells round up or become arborized in conjunction with an increase in cAMP levels implies that cAMP may be a major factor in the disassembly of microfilament bundles in these cells.

The principal function of the thyroid gland is to synthesize, store, and release thyroid hormone. The main regulator of these functions is the pituitary hormone thyrotropin (thyroid-stimulating hormone, TSH). It is generally assumed that TSH acts via the activation of adenylate cyclase and that this increases the level of intracellular cAMP. Consequently, dibutyryl cAMP mimics the action of TSH on thyroid cells in vitro and this effect is enhanced by inhibitors of cAMP degradation (1-3).

Isolated thyroid cells in monolayer culture retain some of their functional and morphological characteristics. When confluent primary cultures are exposed to TSH early after explantation, the cells become organized into follicle-like structures (1-5) which maintain the capacity to concentrate iodide and produce thyroid hormone (3, 6). Sparsely seeded or serially cultivated cells lose these functions but retain a TSH-responsive adenylate cyclase. The exposure of such cells to TSH leads to an increase in cyclic AMP content and a profound change in morphology including a complete arborization of the cytoplasm (7-9) indicative of gross changes in the organization of the cytoskeleton and associated cytoplasmic matrix. The present report demonstrates the nature of these changes as revealed by light microscopy and high voltage electron microscopy of whole cells.

MATERIALS AND METHODS

Cell Culture

The human thyroid cell line HTb 31 was initiated from a benign, functioning adenoma (9). These cells are responsive to TSH as shown by a large increase in...
intracellular cAMP after a 60-min exposure to 100 mU/ml of bovine TSH (9). In these studies the cells were routinely grown in 5 cm Corning plastic petri dishes (Corning Glass Works, Corning, NY), using Eagle's minimum essential medium (EMEM) supplemented with 10% fetal calf serum (Flow Laboratories, Rockville, MD) and antibiotics (100 U of penicillin and 50 μg/ml of streptomycin). Subcultivation was done once a week at a 1:2 split ratio using 0.08% trypsin and 0.08% EDTA in phosphate-buffered saline as detaching agents. The thyroid cell line has a finite lifespan and cannot be carried beyond 18 passages (9). For our experiments, only cultures below passage 12 were used. Thyrotropin (TSH; 0.5–1 U/mg) and N6-0'-dibutyryladenosine 3':5'-cyclic monophosphoric acid (dibutyryl cyclic AMP) were purchased from Sigma Chemical Co., St. Louis, MO.

Light Microscopy

The effects on gross morphology of TSH, theophylline and dibutyryl cAMP, respectively, were studied on cover-slip cultures using a Zeiss Ultraphot microscope equipped with phase contrast optics. Photomicrographs were taken on Ektaplan film using ×16 or ×40 Neofluar phase contrast lenses.

High Voltage Electron Microscopy (HVEM)

For HVEM, cells were grown on formvar-coated gold grids (London Finder honeycomb) mounted on cover slips, prepared as previously described (10). The cultures were fixed for 15 min at 37°C using 2% glutaraldehyde in 0.15 M sodium cacodylate, pH 7.3, as fixative. The cells were then rinsed in cacodylate buffer and postfixed in 1% OsO4 in 0.2 M sodium cacodylate for 10 min. The cover slips were rinsed in distilled water and stained with 0.5% uranyl acetate in 0.2 M s-collidine for 15 min, pH 4.5 for 5 min. After rinsing in 0.2 M s-collidine for 15 min and a short rinse in distilled water, the grids were removed from the cover slips and subjected to critical point drying (11). The grids were coated with carbon on both sides and stored in a dessicator. The cells were examined in a JEM 1000 high voltage electron microscope under an accelerating voltage of 1,000 kV.

![Figure 1: Induction of cytoplasmic arborization in human thyroid cells by TSH and dibutyryl cyclic AMP.](image)

(a) Untreated control cells. (b) Cells treated for 6 h with 10 mU of TSH per ml. (c) Cells treated for 6 h with 1 mM theophylline and 10 mU of TSH per ml. (d) Cells treated for 6 h with 1 mM theophylline and 0.5 mM dibutyryl cyclic AMP. Phase contrast microscopy of living cells. Bars, 20 μm. × 330.
FIGURE 2 Disassembly of stress fibers in human thyroid cells treated with TSH and dibutyryl cyclic AMP. (a) Untreated control cells. (b) Cells treated for 20 min with 1 mM theophylline. (c) Cells treated for 20 min with 1 mM theophylline and 10 mU of TSH per ml. (d) Cells treated for 20 min with 1 mM theophylline and 0.5 mM dibutyryl cyclic AMP. Phase contrast microscopy of living cells. Bar, 10 μm. × 825.
RESULTS

Light Microscopy

The human thyroid cells are large, flattened, and epithelial-like with little tendency to overlap (Fig. 1a). After the exposure to 10 mU of bovine TSH per ml for 6 h, ~30% of the cells were arborized (Fig. 1b) with most of the cytoplasm concentrated in the perinuclear area. This effect was strongly potentiated by the addition of theophylline. For example, the addition of 10 mU/ml of TSH in medium supplemented with 1 mM theophylline caused 100% arborization after 6 h (Fig. 1c), whereas the addition of theophylline alone caused no arborization. The effect of TSH on cell morphology was mimicked by the addition of 1 mM dibutyryl cAMP in medium containing 1 mM theophylline (Fig. 1d).

High-power phase contrast microscopy of the untreated thyroid cells showed an abundance of stress fibers, essentially oriented along the long axis of the cells (Fig. 2a). The intra-

![Micrographs of untreated human thyroid cells taken by high voltage electron microscopy.](image)

- (a) Bar, 10 µm. X 850. (b) Part of the central cell in a. Bar, 10 µm. X 3,600. Stress fibers are indicated at sf. (c) A pair of stereo images at yet a higher magnification (X 28,000). Note the relatively dense microtrabecular lattice and its continuity with the linear strands representing the stress fibers (sf). Viewing with a stereo viewer (desk model, Abrams Instrument Company, Lansing, MI) is essential for observing details. Bar, 1 µm.
cellular structures of the completely arborized cells could not, however, be resolved by phase contrast microscopy and the content of stress fibers in these cells could not be determined. We, therefore, analyzed cells that were only briefly treated with 10 mU/ml of TSH and 0.5 mM dibutyryl cAMP, respectively, in medium containing 1 mM theophylline. Cells treated for 20 min were still flattened but showed a somewhat altered structure. Small ruffles were seen along the cell margins and stress fibers had apparently vanished (Fig. 2c). The addition of theophylline alone had no effect on the stress fibers (Fig. 2b) whereas dibutyryl cAMP used briefly, caused the same morphological changes as TSH, i.e., disappearance of stress fibers and induction of marginal ruffling activity (Fig. 2d).

**High Voltage Electron Microscopy (HVEM)**

The untreated thyroid cells were generally thin enough to allow penetration of high energy electrons even in the perinu-
clear area (Fig. 3 a and b). The stress fibers appeared as rather loosely packed bundles that often spanned the entire cell width (Fig. 3 b, g f, and c, f g). In high magnification images, a fairly dense microtrabecular lattice was seen throughout the cytoplasm (Fig. 3 c). The lattice was coextensive with the stress fibers as can readily be seen in stereo pairs (Fig. 3 c).

Treatment for 10 min with 10 mU/ml of TSH had a profound effect on the fine structure of the thyroid cells. Many cells showed a retracting posterior end, a thickening of the perinuclear region and a thin lamellar cytoplasm in the anterior end (Fig. 4 a and b). The thickness of the perinuclear region in this instance made this part of the cell relatively impermeable to the electron beam. In the thinner lamellar regions, the cytoplasmic ground substance was unevenly distributed with dense areas intermingled with very thin areas (Fig. 4 b). No well-defined microfilament bundles were seen but in some places the cytoplasm formed ridges that contained fibrillar structures interpreted as remnants of stress fibers (Stereo Fig. 4 c). Microtubules were visible throughout the cytoplasm (Fig. 4 c, arrows). High magnification showed that the uneven distribution of the cytoplasmic ground substance could be explained in terms of altered organization of the microtrabecular lattice. Thus, areas of lattice condensation were adjacent to areas with scant lattice. In some very thin places the lattice seems to have disappeared entirely (Fig. 4 b, x). A few minute surface ruffles are also evident. Upon prolonged treatment with TSH, arborization became more noticeable (Fig. 5 a). After 20 min the perinuclear condensation of cytoplasm was pronounced and most of the mitochondria were found in this region and generally oriented parallel to the long axis of the cell (Fig. 5 b). In the remaining lamellar regions large areas of thin cytoplasm were intermingled with thicker areas often appearing as ridges (Fig. 5 b).

At this point a multitude of small membrane ruffles projected from the dorsal cell surface. Higher magnifications showed that these ruffles contained a lattice of microtrabeculae (not shown). In the thinner lamellar regions no microfilament bundles remain.

As can be seen in Fig. 5 c and d, dibutyryl cAMP elicited the same change in ultrastructure as TSH. After 20 min of incubation, perinuclear thickening was apparent as well as the disappearance of microfilament bundles and signs of cytoplasmic arborization (Fig. 5 c and d). The addition of 1 mM theophylline along with 0.5 mM dibutyryl cAMP increased the proportion of cells with an altered morphology. However, there were no differences in the fine structure of the responding cells, whether they were exposed to dibutyryl cAMP alone or in combination with theophylline.

Treatment with TSH for several hours led to a complete arborization as shown by light microscopy. The interior of these cells could not be studied in the HVEM due to the thickness of the cytoplasmic branches. After a prolonged incubation, however, most of the cells flattened again, even in the presence of TSH. These were refractory to TSH in the sense that the addition of more TSH did not reinduce arborization.

The "recovered" cells differed in several respects from the untreated control cells. Thus, most of the cytoplasm was still located to the perinuclear region (Fig. 6 a). Stress fibers reappeared but looked very different from those of the untreated cells in that they were often straight and extremely dense (Fig. 6 b and c). In high-magnification stereo images, such condensed stress fibers appeared as hyaline cylinders with no visible internal structure (Fig. 6 c). They were ensheathed by a sleeve of the microtrabecular lattice (Fig. 6 c, m i l).
It is not surprising, then, that the TSH-treated thyroid cells arborize when all stress fibers have disappeared. Arborization of the thyroid cells can be regarded as a pathological response to a normal physiological stimulus, viz. TSH-induced increase in cAMP. In the functioning, cuboidal follicular cell, TSH controls the maintenance of hormone metabolism including apical ruffling and endocytosis of colloid, processes that may be mutually exclusive to the presence of an abundance of stress fibers. In the partially dedifferentiated cells used in this study, TSH treatment leads to an abortive stimulation of ruffling with concomitant disappearance of stress fibers and arborization of the cytoplasm.

**Figure 5** Effects of TSH and dibutyryl cyclic AMP on the fine structure of thyroid cells. The culture was treated for 20 min with 10 mU of TSH per ml (a and b), or 0.5 mM dibutyryl cyclic AMP (c and d). Note the advanced perinuclear thickening and the cytoplasmic arborization (a and c). The stress fibers have disappeared (b and d) and numerous cytoplasmic ruffles project from both the upper and the lower surface, particularly from the thicker areas. The number of microtubules (mt) appears to have increased enormously. (a) Bar, 10 μm × 1,100; (b) Bar, 10 μm × 3,700; (c) Bar, 10 μm × 760; (d) Bar, 10 μm × 3,800.
Arborization in response to hormone treatment is not unique for thyroid cells. A similar event is evoked when adrenocortical cells are treated with adrenocorticotropic hormone (ACTH) (27).

In addition, ovarian granulosa cells treated with follicular stimulating hormone (FSH) (28) and bone cells exposed to parathyroid hormone (29) react in a similar way. All these cell systems have one thing in common, viz. an increase in cAMP induced by the exposure to the specific hormone. The underlying cytoskeletal changes in these other cell systems have not been described, with one exception. Lawrence et al. (28) noticed a significant reduction in microfilament bundles in thin sections of FSH-treated granulosa cells. We would like to suggest that the hormonally induced arborization and cell rounding in the above mentioned cell systems may have a common mechanism, i.e., disintegration of stress fibers that are required for the maintenance of the flattened morphology of the cells. If so,
cAMP may be a major inducer of microfilament bundle disassembly in many types of hormone-responsive cells.

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