Microtubules and Microfilaments in Stimulated Rana pipiens Pars Intermedia Secretory Cells

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There is much speculation concerning the role of microtubules and microfilaments in the process of secretion. Although they have been shown in electron micrographs of pituitary cells (1-3), only a preliminary note (4) has indicated any correlation between microtubules and secretion in the adenohypophysis. Using cytochalasin B to disrupt microfilaments, Schofield (5) suggested that contractile microfilaments in the anterior pituitary might be involved in translocation of granules of growth hormone to the membrane to replace granules of an initial release or in their fusion with the cell membrane.

It has been postulated that microtubules and/or microfilaments are part of a contractile system which could move granules to the cell surface or could be involved in the release mechanism of hormones from other endocrine tissues such as the pancreas (6-9), the adrenal medulla (10, 11), and the thyroid gland (12-14). Because of this support for a microfilament-microtubule hypothesis in these tissues, it is intriguing to question their presence in secreting versus nonsecreting melanophore-stimulating hormone (MSH) secretory cells of the pars intermedia. Background adaptation of amphibians has produced MSH secretory cells demonstrating secreting and nonsecreting characteristics which have been described in detail (15-17).

MATERIALS AND METHODS

Eighteen adult Rana pipiens, obtained from Southwestern Scientific Supply, Tucson, Arizona, were adapted to either white or black backgrounds by maintaining them for 14 days in white or black plastic pans placed under a fluorescent light source. To determine the response to adaptation, the melanophore index (M.I.) of the melanophores in the web of the foot was determined by the Hogben

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Fig. 1. Portion of a typical pars intermedia secretory cell from a 14 day white-adapted frog. The cell is filled with cytoplasmic granules (G). ×13,255.

Fig. 2. Microtubules in a pars intermedia secretory cell from a 14 day black-adapted frog. Microtubules (arrows) are scattered in the cytoplasm with no particular orientation to the granules (G). ×28,048.

and Slome (18) index. For fixation of the pituitaries, the animals were decapitated, the parasphenoid bone removed, and the tissue quickly flooded with 1.5% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.0–7.2). The neurointermediate lobes were dissected free and were fixed at room temperature for 3–4 hr in fresh glutaraldehyde. Postfixation was carried out for 1–1.5 hr in cold 1% osmium tetra-
oxide in 0.10 M phosphate buffer (pH 7.1–7.3), which was composed of 0.10 M sodium orthophosphate and 0.10 M potassium orthophosphate in the ratio of 9.5 to 0.5. The specimens were then rinsed in phosphate buffer, dehydrated in ethanol, and embedded in Epon 812 (19). Light gold to silver thin tissue sections, cut on a Porter-Blum MT-2 microtome with a diamond knife, were mounted on uncoated copper grids and stained for 10–15 min in 4% uranyl acetate in absolute
Fig. 5. Microfilaments adjacent to the nucleus (N) of an active secretory cell. MF and arrow indicate the microfilaments and the microtubule, respectively. Note the random microfilaments in addition to those aggregated into bundles. ×19,759.

Fig. 6. Portions of secreting cells from a 14 day black-adapted frog. Bundles of microfilaments (MF) are oriented parallel to the cell membrane, but none appear to be immediately beneath the cell membrane (arrow). ×14,444.
ethanol (20) followed by 15–30 sec staining in 0.1% lead citrate (21). The grids were examined in a Philips EM 200 electron microscope operating at 60 KV.

RESULTS

The nine black (M.I. 4.5–5.0) and nine white (M.I. 1.0–1.5) adapted frogs provided pars intermedia showing the typical ultrastructural characteristics of secreting and nonsecreting MSH cells, respectively. The most obvious difference in these cells is the presence of cytoplasmic granules (17). In the white-adapted animal the MSH cells, which are nonsecreting, are filled with cytoplasmic granules (Fig. 1) while the secreting cells of the black-adapted frog show few granules (Figs. 2–6).

In addition to other characteristic organelles present, both microtubules and microfilaments are seen in the MSH secretory cell type. Although microtubules are seen in the cells which appear not to be secreting granules, they appear much more frequently in those cells which have all the characteristics of a cell synthesizing and releasing MSH. The microtubules, about 200 Å in diameter, are not restricted to a particular area of the cell, but are scattered throughout the cytoplasm (Fig. 2). Figure 3 shows two secretory granules apparently in contact with the cell membrane and a cross section of two microtubules adjacent to the granules. Also, single microtubules were seen parallel to the cell membrane (Fig. 4), but in none of the secretory cells examined was a microtubular structure seen connecting the cell membrane and a secretory granule. Since this microtubular structure–granule relationship has been described for the pancreas (6, 22), it was reasonable to expect this phenomenon to occur in the pars intermedia.

As with the microtubules, microfilaments appear more common in the cells which are synthesizing and releasing granules than in those which are inactive. The filaments, about 50 Å in diameter, are aggregated in clusters, although they can also be found in groups of two or three filaments. The microfilaments are present in these active cells, but they appear to have no preferential location. In Figs. 3 and 5 they are concentrated adjacent to the cell nucleus, but they are also seen extending into cellular processes away from the nucleus. Although a microfilamentous web, located at the cell periphery, has been described in the beta cells of the pancreas (9), no network of fine filaments was seen immediately beneath the cell membrane in the MSH secretory cells (Fig. 6). Overall, the microfilaments appear as bundles but with no specific location or orientation. Thus, microtubules and microfilaments are present, but they are not seen in the specific location or relationship as previously described for them in the beta cells of the pancreas.

DISCUSSION

The results of the present investigation indicated that stimulated secretory cells of the pars intermedia of Rana pipiens seem to have an increase in their content of microtubules and microfilaments. Because of the recent interest in the role of these organelles in the process of secretion, this observation of these organelles in the secreting versus nonsecreting MSH cells is an intriguing one. Only Pelletier's unpublished data, appearing in Pelletier and Bornstein (4), has previously indicated a difference in the microtubule content of stimulated cells of the adenohypophysis. No electron microscopical study has discussed the presence of microfilaments in stimulated pituitary secretory cells.
Pelletier and Bornstein (4), studying the effect of colchicine in the rat anterior pituitary, demonstrated an accumulation of secretory granules associated with the disappearance of microtubules. Coates (personal communication), in an examination of the rat pars distalis, observed the presence of these organelles in the secretory and stellate cells but not in overwhelming numbers. She did not look in a quantitative manner for a relationship between the presence of microtubules and secretory granules in the pituitary secretory cells, but there did not appear to be the kind of microtubular–microfilamentous granule relationship described by Lacy et al. (6) or the filamentous web as described by Orci et al. (9). Pelletier and Bornstein (4) agreed that microtubules were not abundant in the pituitary and were never seen linking secretory granules to one another or to the plasma membrane. The absence of a microtubular–microfilamentous granule relationship in the pituitary is supported by the present investigation. But, more important is the report of a direct correlation between the presence of microtubules and the state of secretory activity of the pars intermedia cells. In those cells not under hypothalamic control, those in an active secretory state, there seem to be more microtubules present. The observation is also true for microfilaments. Since microtubules and microfilaments have been implicated in the secretory process in other endocrine tissues and now in the anterior pituitary (4), the present report of a difference in these organelles in secreting versus nonsecreting MSH cells is important.

Further work could perhaps use the Vinca alkaloids and cytochalasin B to disrupt the MSH secretory cell microtubules and microfilaments, respectively, and to determine not only by means of bioassay but also by electron microscopy the secretory activity of these cells.

SUMMARY

Because of the support for a microtubule–microfilament hypothesis in the secretory process in other endocrine tissues, it is interesting to question their presence in secreting versus nonsecreting MSH cells of the pars intermedia. The nine black- and nine white-adapted frogs provided pars intermedia showing the typical ultrastructural characteristics of secreting and nonsecreting MSH cells, respectively.

Both microtubules and microfilaments were seen in the MSH cells. Although microtubules were seen in the cells which appeared not to be secreting granules, they appeared much more frequently in cells which had all the characteristics of a cell synthesizing and releasing MSH. The microtubules, about 200 A in diameter, were scattered through the cytoplasm. In none of the secreting cells examined was a microtubular structure seen connecting the cell membrane and a secretory granule. Also, microfilaments were more common in the cells which were synthesizing and releasing granules than in those which were inactive. There was no specific location for the microfilaments; they were not only concentrated adjacent to the nucleus but also extended away from the nucleus.

Thus, this investigation suggests that stimulated secretory cells of the pars intermedia have an increase in their content of these two organelles. Although other studies have indicated the presence of these structures in pituitary secretory cells, this investigation demonstrates a direct correlation between the presence of the organelles and the state of secretory activity. Since microtubular–microfilamentous structures were not seen connecting either the secretory granules with one another or the granules with the cell membrane, the study suggests an absence of this relationship in the pars intermedia.
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