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Research Articles

Ultrastructural changes in rat mammotropes following incubation with dopamine

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Abstract. Cultured mammotropes incubated with dopamine for one hour exhibited changes in ultrastructure indicative of actively depressed biosynthetic and secretory activity. Peripheral relocation of rough endoplasmic reticulum appeared to create a barrier to secretory granule release by exocytosis. A decrease in the numbers of secretory granules indicated a decrease in prolactin production and enhanced lysosomal activity.
Key words. Pituitary; adenohypophysis; mammotrope; dopamine; prolactin.

It is well established that prolactin is under the inhibitory influence of the hypothalamus[1,2], and dopamine is generally accepted to be the physiological prolactin inhibiting factor[3,4]. Binding of dopamine to receptors in the plasma membrane of mammotropes inhibits both the synthesis of prolactin and the release of secretory granules[5]. In previous studies[6,7], we reported that after administration of dopamine to estradiol-primed male rats, extensive changes in mammotrope ultrastructure occurred as follows: 1) relocation of rough endoplasmic reticulum to the periphery of the cell, 2) fewer secretory granules adjacent to the cell membrane, 3) fewer exocytoses and 4) increased numbers of intracellular lipid bodies associated with secretory granules (putative granule disposal system). Similar results were obtained using the dopamine agonist, ergocristine[8]. More recently, it was reported that dopamine induced similar ultrastructural changes in mammotropes from pituitary grafts homografted into the anterior chamber of the eye in female albino rats[9]. These investigations indicate that the dopamine-induced ultrastructural changes are part of a mechanism by which prolactin release is inhibited. The present study was undertaken to examine mammotropes of pituitary cell aggregates following incubation with dopamine; by using an in vitro system, mammotropes are isolated from the influence of the hypothalamus and the action of dopamine can be more easily studied.

Materials and methods

Male Sprague-Dawley rats (CD) weighing 275–300 g were purchased from the Canadian Breeding Farms and Laboratories (Charles River, Montreal, Quebec). Following decapitation, adenohypophyses were quickly excised and sliced into small fragments. The tissue was dispersed for 2 h at 37 °C in a Spinner's flask containing Spinner's Minimum Essential Medium (S-MEM) supplemented with trypsin (0.05%) and BSA (0.1%). When dispersion was complete, the solution was centrifuged to collect the cells. The supernatant was discarded while the cells were resuspended in 10 ml S-MEM containing 0.02% lima bean trypsin inhibitor and then recentrifuged. The cells were then placed in a culture medium (pH 7.4) containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 2.5% fetal calf serum, 15% horse serum, 0.14% sodium bicarbonate and 40U/ml penicillin-G. One pituitary equivalent of cell suspension in culture medium was transferred to each of two plastic petri dishes which were not treated for cell culture, so that cells formed aggregates instead of adhering to the bottom of the culture dish. The cells were maintained in culture for 5–7 d and then incubated in either DMEM or $1 \times 10(-6)$ mol/L dopamine in DMEM for 1 h.

Cell clusters were fixed in 0.1 M cacodylate-buffered (pH 7.2-7.4) glutaraldehyde followed by postfixation in 0.1 M cacodylate-buffered osmium tetroxide and embedding in Jembed 812. Ultrathin sections were stained with uranyl acetate and lead citrate[10], and examined and photographed on a Hitachi 500 electron microscope.

To compare quantitative changes between groups, random micrographs of mammotropes were taken; only cells with visible nuclei and intact cell membranes were used. Forty cells from cell cultures incubated with dopamine were compared to 40 cells from control cultures. The amount of peripheral rough endoplasmic reticulum (RER) was classified according to a previous method[8] as 1) class A, less than 25% of the total cell perimeter (average 13%); 2) class B, 25–50% (average 40%); 3) class C, 50–75% (average 60%), and 4) class D, greater than 75% (average 90%). The numbers of granules and intracellular lipid bodies per cell as well as the numbers of granules adjacent to the cell membrane were also detemined. The means from each group were compared using the Student's t-test.

Results

In both control and dopamine-treated cell cultures, mammotropes were the predominant cell type and were identified according to ultrastructural characteristics determined previously[11]: 1) prominent rough endoplasmic reticulum, 2) well-developed Golgi complex and 3) typically large (600–900 nm) and polymorphous secretory granules. Intracellular lipid bodies were observed in both groups, but no significant difference was found when comparing the mean number per cell in both groups. Putative lysosomes and multivesicular bodies were frequently seen in the cytoplasm.

Control mammotropes. Untreated mammotropes showed characteristics of synthetically active cells, viz., well-developed RER arranged in stacks of parallel lamellae, an eccentric nucleus and a large Golgi complex containing numerous immature secretory granules (figs 1 and 2). Mature secretory granules were seen throughout the cytoplasm.

Dopamine-treated mammotropes. Following dopamine treatment, mammotropes displayed more RER at the periphery of the cell (figs 3 and 4). The percent difference of the cell perimeter occupied by concentric RER between the dopamine-treated (85.0 ± 2.0) and control (54.2 ± 3.4) mammotropes was highly significant ($p < 0.01$). In dopamine-treated mammotropes, 85.0% were class D (>75% of the cell perimeter occupied by RER) as compared to 17.5% of controls. Following dopamine treatment, mature secretory granules were fewer in number and most were separated from the cell membrane by parallel rows of RER. The smaller, more immature secretory granules associated with the Golgi complex were also fewer in number in the dopamine-treated mammotrophs (fig. 4). As well as a significant ($p < 0.01$) decrease in the mean number of secretory granules per cell in dopamine-treated (38.1 ± 1.4) as compared to control (62.2 ± 5.3) mammotropes, there were significantly ($p < 0.01$) fewer secretory granules in close proximity to the cell membrane of dopamine-treated (9.2 ± 0.8) as compared to control mammotropes (13.8 ± 1.4).

Discussion

We have demonstrated that cultured mammotropes incubated with dopamine for 1 h exhibited ultrastructural changes which indicate that secretory activity was inhibited.

Although mammotropes of the rat characteristically contain large polymorphous secretory granules, Nogami and Yoshimura[12] demonstrated prolactin cells that contain smaller, spherical secretory granules. Following injection of dopamine into rats with grafted pituitaries, Ishibi and Shiino[9] reported increased numbers of smaller, spherical secretory granules in mammotropes but there was an absence of immunoreactivity for prolactin in this type of granule following use of the protein A-gold procedure. Rennels et al.[13] stated that although mammotropes cannot always be identified solely on the basis of granule size, this is probably the most useful criterion.

Control mammotropes resembled the typical cultured mammotropes described by Tixier-Vidal[14] in that they showed characteristics of synthetically active cells as described by Farquhar[15,16] and which included a well-developed RER in the form of parallel oriented lamellae, an eccentric nucleus and a large Golgi complex containing numerous immature granules occupying a volume approaching that of the nucleus. Mature secretory granules were found throughout the cell, many of which were adjacent to the cell membrane.

When compared to controls, mammotropes exposed to dopamine showed a relocation of RER to the periphery of the cell and fewer secretory granules adjacent to the cell membrane. These parallel stacks of RER adjacent to the cell membrane appear to form a barrier that prevents secretory granules from reaching the cell membrane for release by exocytosis. This observation is consistent with reports from others[17,18] and our previous in vivo studies[6–8] which examined the effect of administration of dopamine and the dopamine agonist, ergocristine, on estradiol-primed male rats. In the latter studies, we concluded that because the peripheral rearrangement of RER was observed within 2 min after dopamine administration, it was unlikely to be the result of de novo synthesis of membrane but rather of rapid movement of membrane within the cell. Such movement could be explained by the microtrabecular lattice described by Porter and Tucker[19] which is a system of filaments that are said to support and move cell organelles.

Previous studies reported an accumulation of mature secretory granules within the cytoplasm of mammotropes exhibiting a dopamine-induced RER barrier[6,18]. In the present study we observed that dopamine-treated mammotropes contained significantly fewer secretory granules per cell (average 38.1 ± 1.4) than untreated mammotropes (62.2 ± 5.3). Because the mammotropes in the present study were incubated with dopamine for 1 h as opposed to receiving a single injection in our previous studies, their biosynthetic activity was decreased by continuous exposure to the inhibitory effects of dopamine[20]. Dopamine is known to induce decreases in intracellular cyclic AMP which in turn contributes to reduction of prolactin release[21,22]. It has also been demonstrated that dopamine induces the stimulation of lysosomal enzymes in mammotropes[23]; in a process known as crinophagy, lysosomes fuse with excess secretory granules which are then degraded[24–26]. Thus, with prolactin biosythetic activity inhibited and lysosomal enzymes stimulated in mammotropes exposed to dopamine, it is not surprising that over time there would be a reduction in the numbers of secretory granules.

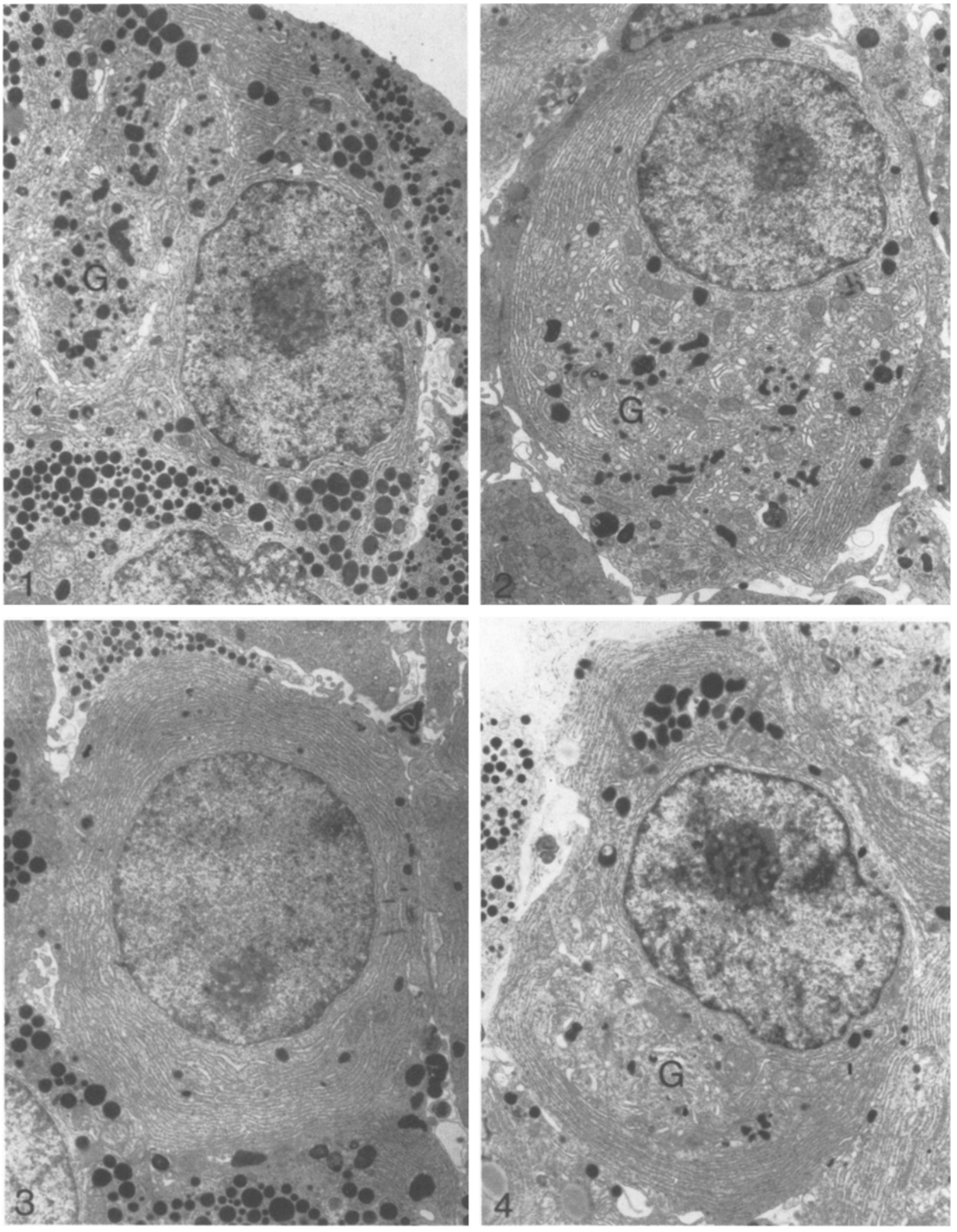

Figure 1. Control mammotrope showing mature secretory granules throughout the cytoplasm and numerous immature secretory granules within the large Golgi complex (G). × 7,150.

Figure 2. Control mammotrope showing well-developed RER and large Golgi complex (G) containing numerous immature secretory granules. × 6,720.

Figure 3. Dopamine-treated mammotrope showing peripheral RER and very few secretory granules. × 6,900.

Figure 4. Dopamine-treated mammotrope showing peripheral RER with most of the relatively few secretory granules located away from the cell membrane. The Golgi complex (G) contains a small number of immature secretory granules. × 7,280.

In our previous studies[6,7], 2 min following dopamine administration to estrogen-primed male rats, we reported an increase in numbers of intracellular lipid bodies closely associated with secretory granules and tentatively suggested that they were part of a putative granule disposal system. In subsequent studies[27,28], we proposed that this lipid accumulation in mammotropes may be degraded through a lysosomal pathway in folliculo-stellate cells. Because there were no changes in numbers of intracellular lipid bodies in the present study, it is possible that during the longer exposure to dopamine, the intracellular lipid body phase of the mammotropes had passed.

We have demonstrated that dopamine causes ultrastructural changes in cultured mammotopes. Peripheral relocation of RER appeared to create a barrier to exocytosis while a decrease in numbers of secretory granules indicated depressed biosynthetic and enhanced lysosmal activity.

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