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Dopamine causes ultrastructural changes in prolactin cells of tilapia (*Oreochromis niloticus*)

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Abstract. This study was undertaken to examine ultrastructural changes induced by dopamine in fish prolactin cells. Tilapia adenohypophyses were incubated with dopamine and evaluated by electron microscopy. The quantities of rough endoplasmic reticulum (RER) in prolactin cells increased and the number of secretory granules were decreased by dopamine (10^{-6} mol/l) treatment. Another set of adenohypophysial tissues was placed back into control medium for 10 min following a 3 h incubation period with dopamine (10^{-6} mol/l) (RE10 min group). This group had significantly less RER than the 3 h dopamine-treated tissue, and the shape of many granules in the RE10 min group changed from spherical to rod-like. In addition, some of the granule content appeared to diffuse out of granules since some were not fully surrounded by membrane. It was therefore hypothesized that the rod-shaped granules might be the result of prolactin secretion by diffusion.
Key words. Tilapia; prolactin; rough endoplasmic reticulum; secretory granules; dopamine; electron microscopy; in vitro.

Euryhaline fish are able to tolerate wide ranges of environmental salinity while stenohaline species cannot. To tolerate freshwater habitats, euryhaline species secrete a large amount of prolactin, a major hormone that helps to maintain the physiological content of sodium ions in the body.

The major role for prolactin in teleostean fish, first identified by Pickford and Phillips [1], is related to osmotic regulation in freshwater [2]. Most hypophysectomized euryhaline fish will die if they are kept in freshwater but will live if they are placed in seawater; loss of sodium ions is the major factor responsible for the death following hypophysectomy [3]. On the other hand, hypophysectomized fish can survive in freshwater if they are injected with prolactin. Therefore prolactin is necessary for survival in freshwater, but it is not required for survival in seawater.

Like other protein hormones, prolactin is synthesized by the rough endoplasmic reticulum (RER) and then packaged into immature granules by the Golgi apparatus. These immature granules coalesce into mature granules before their storage, destruction by lysosomal enzymes, or exocytotic release from the plasma membrane into the perivascular space [4–6].

The hypothalamus in teleosts regulates prolactin synthesis and release of these processes. The prolactin release inhibiting factor (PIF) is dopamine [7]. In mammals, dopamine acts as a hormone, released from the hypothalamic neurons into the hypophyseal portal system and carried to the pituitary. Teleosts, however, do not have this portal system and their pars distalis is directly innervated by neurons in the ventral region of the hypothalamus [8, 9]. Dopamine acts as a neurotransmitter in teleosts and therefore, steady-state physiological levels of dopamine cannot be determined. Dopamine activates dopamine D_2-receptors in prolactin cells, which causes a reduction in adenylyl cyclase activity and thus inhibits cyclic AMP production. The reduced amounts of cyclic AMP induce inhibition of prolactin secretion. This is a well-accepted model for dopamine action on prolactin release [7]. However, although the inhibitory action on adenylyl cyclase activity is a major mechanism of dopamine inhibition, it is not the only one. Cyclic AMP stimulates prolactin secretion, and reduced amounts of cyclic AMP induce a lesser amount, but dopamine is also able to inhibit the cyclic AMP-induced prolactin secretion [10]. The inhibitory action of cyclic AMP-induced prolactin release indicates that dopamine acts on a site(s) after the cyclic AMP event in the cascade reaction from D_2-receptor to prolactin secretion. Dopamine may also act on sites other than those involved in the adenylyl cyclase system [11, 12].

We have chosen tilapia for this attempt to establish a foundation for future studies on prolactin secretion for the following reasons: (1) being a euryhaline species, the effect of changing salinity can be studied under conditions that simulate migration between fresh water and

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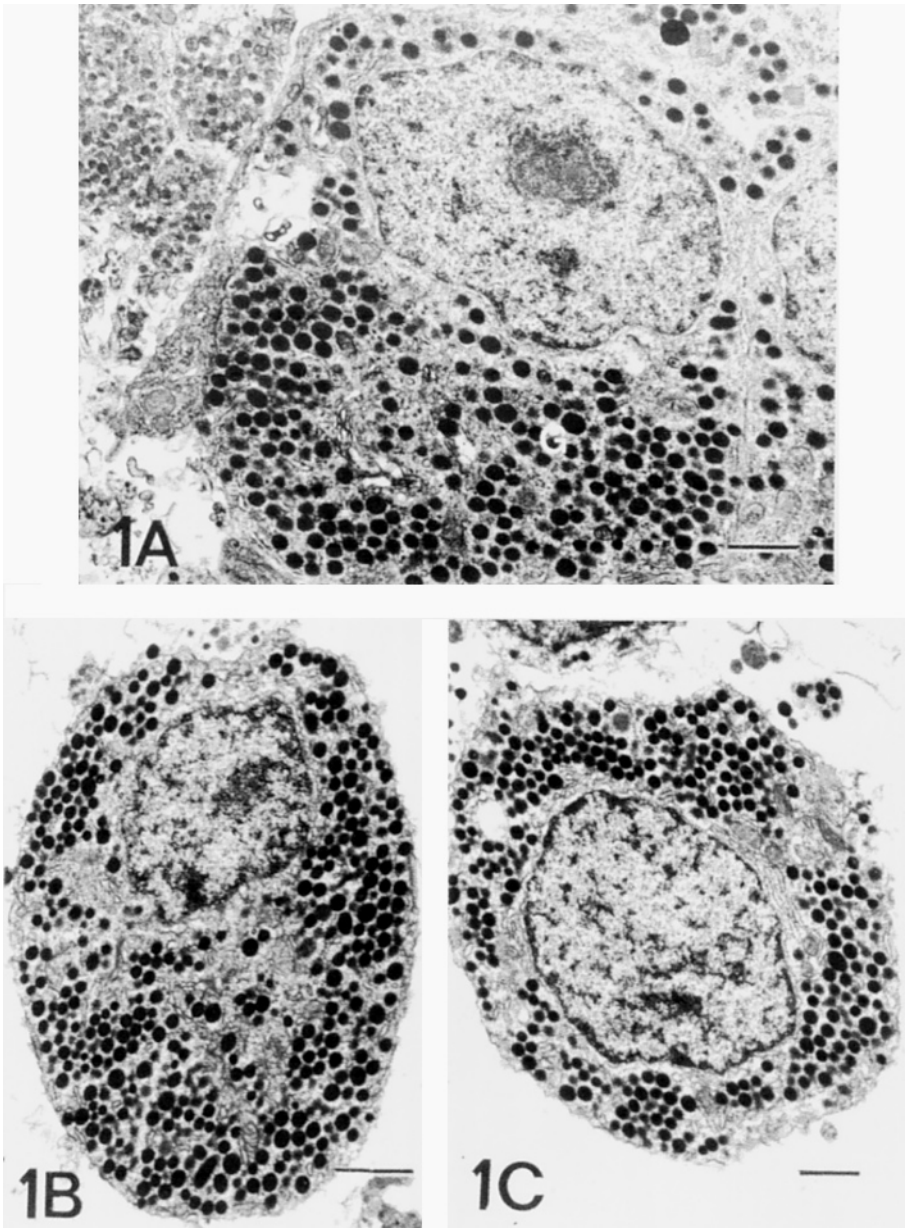

Figure 1. (*A*) A representative prolactin cell incubated in medium (control) for 3 h following a preincubation period. Prolactin cells contain a large number of granules (G); bar = 1 μm. (*B*) Prolactin cells incubated in dopamine (10^{-6} mol/l)-containing medium for 5 min; bar = 1 μm. (*C*) Prolactin cells incubated in dopamine (10^{-6} mol/l)-containing medium for 10 min; bar = 1 μm.

salt water, (2) the body weights (100–300 g) are practical for handling in a small laboratory setting, and (3) the prolactin cells are clustered together in the rostral part of the pituitary, so that homogeneous populations of prolactin cells can be harvested by microdissecting the rostral pituitary [13].

Previous studies with mammalian pituitary cells have shown that ultrastructural changes induced by dopamine administration are correlated with inhibition of prolactin secretion [14, 15]. We have examined the changes in tilapia prolactin cells after dopamine treatment to establish the effect of dopamine on ultrastructures.

Materials and methods

Animals. Live male tilapia (*Oreochromis niloticus*), African teleosts, weighing between 138 and 222 grams, were purchased from Northern Tilapia Inc. (Lindsay, Ontario, Canada). They were raised in freshwater at 22–25 °C under 13–15 h light. Usage of the tilapia was approved by the Animal Care Committee at Queen's University.

Incubation. Dulbecco's modified Eagle's Medium (Gibco Lab., Burlington, Ontario) supplemented with 1 g/l bovine serum albumin (Sigma Chemical Co., St. Louis, MO., U.S.A.) and 10^{-4} mol/l ascorbic acid

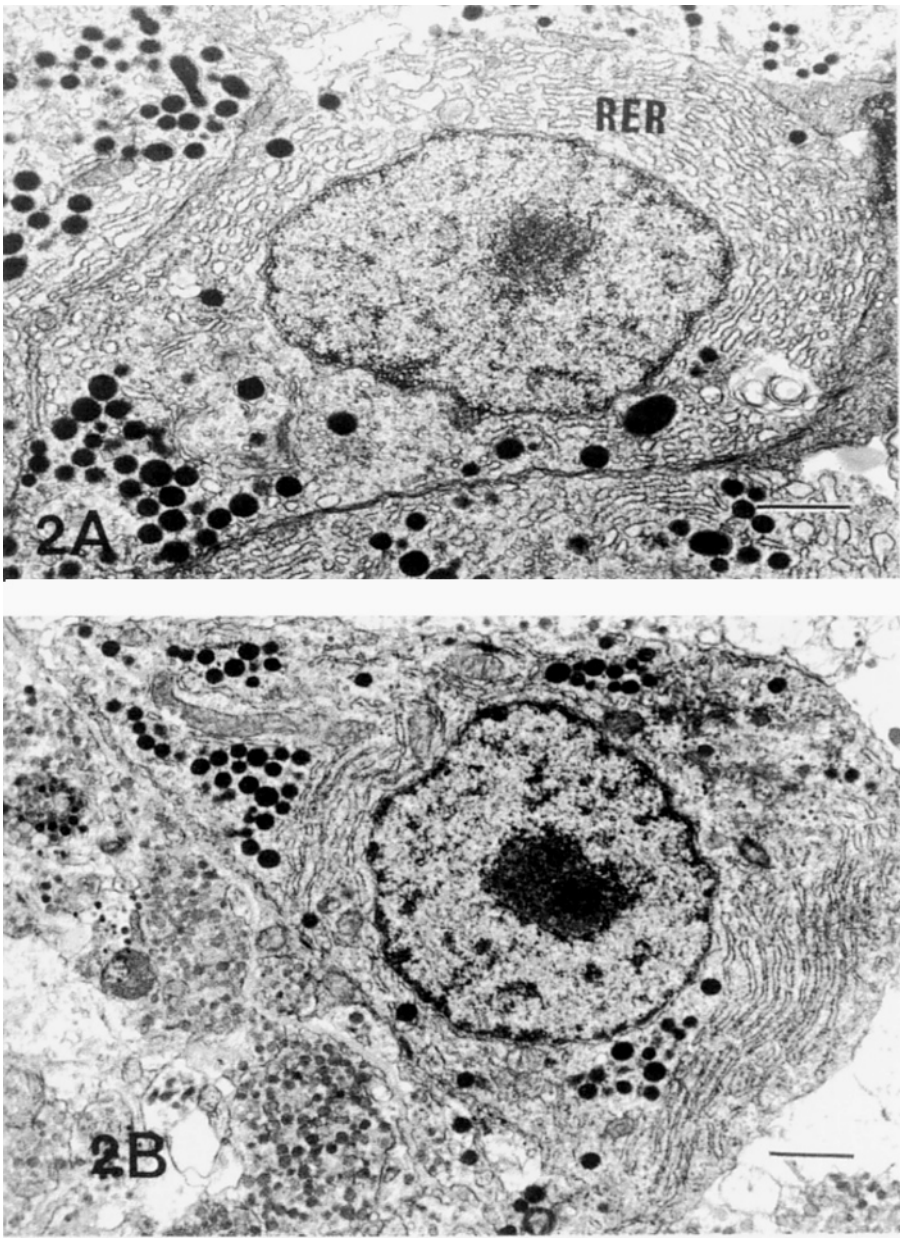

Figure 2. (*A*) A representative prolactin cell incubated in dopamine (10^{-6} mol/l)-containing medium for 30 min (*A*). The percentage of rough endoplasmic (RER) significantly increased, whereas that number of granules significantly decreased in comparison to the control prolactin cells (fig. 1*A*); bar = 1 μm. (*B*) Prolactin cells incubated in dopamine (10^{-6} mol/l)-containing medium for 3 h (*B*); bar = 1 μm.

(Sigma) (DMEM-BSA) was used. Dopamine (Sigma) solution (10^{-6} mol/l dopamine in DMEM-BSA) was prepared immediately before the experiment.

Tilapia were decapitated and their pituitaries rapidly harvested. The pituitary tissue was cut with a scalpel blade into small fragments (<1 mm^3), three pituitaries were pooled and placed in Eppendorf centrifuge tubes containing 1 ml of DMEM-BSA, where they were preincubated for 1 h before the experimental medium was introduced. At the end of the preincubation period, the medium was removed and replaced with fresh DMEM-BSA medium for 3 h or newly made dopamine solution for periods of 5 min, 10 min, 30 min or 3 h. In addition, tissues incubated in dopamine for 3 h were placed back in the control medium for 10 min (RE10 min group). The incubation experiments were performed at 25 °C.

Morphology and stereological analysis. Pituitary tissues were immediately immersed in fixative containing 2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M cocodylate buffer, pH 7.2 [16]. Tissues were post-fixed in 1% osmium tetroxide in the same buffer, stained en bloc with saturated uranyl acetate in 50% ethanol, dehydrated in graded series of ethanol and embedded in

Table 1. Relative volumes of subcellular components of prolactin cells.

Incubation period	N	Granule volume (%)		RER volume (%)		Nucleus volume (%)	
Control	8	33.13 ± 1.52		2.07 ± 0.74		29.63 ± 2.61	
5 min	5	32.96 ± 1.84	NS	5.13 ± 1.00	NS	24.44 ± 2.14	NS
10 min	5	27.06 ± 2.50	NS	12.01 ± 1.54	NS	26.43 ± 5.14	NS
30 min	12	13.85 ± 2.99	*	27.36 ± 4.08	**	27.74 ± 1.85	NS
3 h	5	16.12 ± 4.75	*	28.90 ± 2.77	**	26.03 ± 6.81	NS
RE 10	7	24.43 ± 1.64	NS, n	13.78 ± 2.06	NS, s	28.46 ± 2.10	NS

N = number of randomly selected cells. NS = not significant, *significant, **highly significant from control. s = significant from 3 h group, n = not significant from 3 h group. RE 10 = 10 min incubation in control medium after 3 h incubation with 10^{-6} mol/l dopamine.

araldite (J.B.E.M., Montreal). Ultrathin sections were collected onto 300-mesh grids, stained en face with alkaline lead citrate and examined under Hitachi H-7000 electron microscope.

Stereological analysis was perfomed on a randomly selected pituitary block. The grid was scanned to locate a square which was completely covered by sections; no structural details could be observed in this mode, thus visual bias was minimized. Five micrographs, one from or near to the upper right corner of each square, were photographed at a set magnification of $\times 4000$ and contact-printed. Only cells with visibly large nuclei and intact cell membranes were used for analysis. Morphological changes in prolactin cells were analyzed in order to evaluate dopamine effect on the ultrastrucure. Prolactin cells were recognized by morphological characteristics [15]. Volume percent of secretory granules, RER and nucleus were estimated from the prints by a 100-point sampling grid modified from Weibel, Kistle and Scherle [17]. The sum of the points from the five micrographs, expressed as percentages, was considered to be the sample value for that specimen. The mean of estimates in each group was calculated and compared with those obtained from other treatment groups. Statistical significance was assessed with a one-way analysis of variance followed by Bonferroni's modified t test (Instat, Graphpad, San Diego, CA, U.S.A.). A p value less than 0.05 or 0.01 was considered to be significant or highly significant, respectively. Data are expressed as means $\pm$ standard error.

Results

Prolactin cells have characteristic shapes and sizes; homogeneously dense and very dark-staining, the largest of any adenohypophysial cell type with diameters between 170 and 350 nm, dumbbell shaped during maturation and round or ovoid in the mature state. They are easily identified by their ultrastructure.

In control prolactin cells, a large number of secretory granules were present. They were mainly circular or ovoid and were relatively evenly distributed throughout the cell (fig. 1a).

Following incubation with dopamine (10^{-6} mol/l), a tendency towards polarization (uneven distribution) of granules within the cell appeared as early as 10 min later (fig. 1b). After 30-min and 3-h incubation periods, the majority of granules were found concentrated in specific areas (figs. 2a and b). During incubation with dopamine, the mean granule percentage of cell volume decreased with time (figs. 1 and 2, Table 1). After a 5-or 10-min incubation with dopamine, the mean percentage cell volume occupied by granules was not significantly different from that of the control group. However, after longer incubation periods with dopamine the mean granule percentages significantly decreased from the control value of $33.13 \pm 1.52\%$ (control) to $13.85 \pm 2.99\%$ after 30 min and $16.12 \pm 4.75\%$ after 3 h (figs. 1 and 2, Table 1).

During the incubation with dopamine a new ultrastructure, RER, appeared (figs. 2a and b). The percentage of cell volume occupied by RER increased progressively, as the incubation period with dopamine increased, from $2.07 \pm 0.74\%$ (control) to $5.13 \pm 1.00\%$ at 5 min, to $12.01 \pm 1.54\%$ at 10 min, to $27.36 \pm 4.08\%$ at 30 min, to $28.90 \pm 2.77\%$ at 3 h. However, the increase with respect to the control levels did not become significant until after the 30 min incubation period (Table 1). No significant changes were observed in the percentage of cell volume occupied by the nuclei during any of the experimental periods (Table 1).

When the adenohypophyseal tissues were incubated for 10 min in the control medium after 3 h incubation with the 10^{-6} mol/l dopamine solution (RE10 group), the mean cell volume percentage occupied by granules increased from $16.12 \pm 4.75\%$ (3 h group) to $24.43 \pm 1.64\%$ (RE10 group); however, the increase was not significant. In addition, the percentage of RER significantly decreased from $28.90 \pm 2.77\%$ (3 h group) to $13.78 \pm 2.06\%$ in the RE10 min group.

Another distinguishable change during the 10 min recovery period (RE10 min group) was in the shape of the granules. A large number of polymorphous rod-shaped granules appeared during the recovery period. These rod-shaped granules, many of which were only partially covered by membrane, were rarely observed in

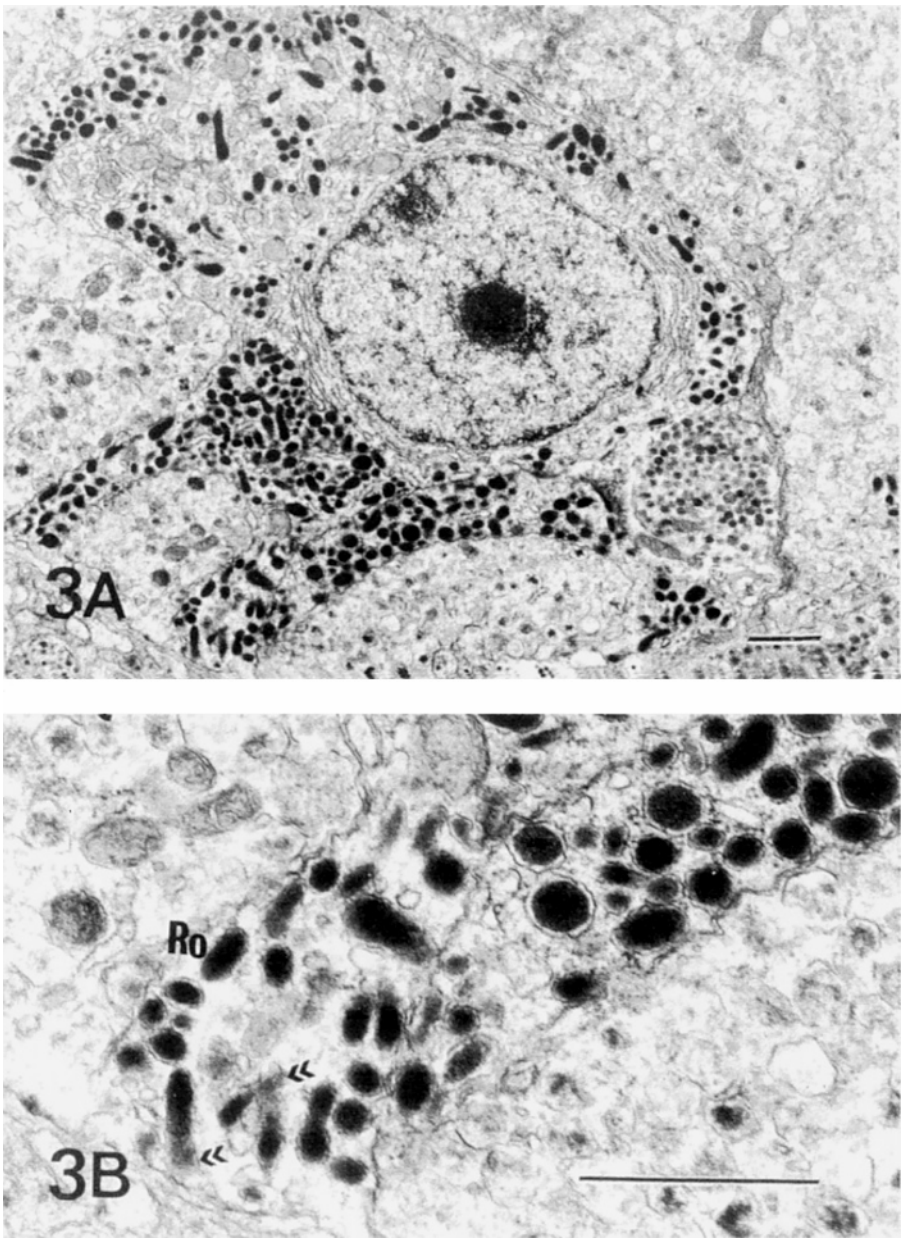

Figure 3. (*A*) A representative *eta* cell incubated in control medium for 10 min following 3 h incubation with dopamine (10^{-6} mol/l) (*A*). The quantity of RER significantly decreased in comparison to that of the control cells (fig. 1*A*); bar = 1 μm. (*B*) Higher magnification of fig. 3*A*, displaying rod-shaped granules (Ro) (*B*). Granule content is dense within area covered by membrane, but density decreases (arrows) toward uncovered end of granule; bar = 1 μm.

the other experimental groups. The granule content was dense within the area covered by membrane, but decreased in density towards the uncovered end of the granule (fig. 3).

Discussion

Tilapia prolactin cells are localized in the rostral pars distalis, in contrast to mammals whose prolactin cells are not heavily concentrated in any region of the pars distalis. The localization to a particular site could help the hypothalamus to innervate prolactin cells and regulate their functions. While dopamine receptors in prolactin cells inhibit prolactin release in both mammals and teleosts [7, 18], teleostean hypothalamic prolactin releasing factor (PRF) plays a more prominent role than that of mammals [19]. Dopamine not only acts on D_2-receptors in prolactin cells, but also stimulates GH secretion through D_1-like-receptors [20] and modulates GTH secretion in teleosts [21, 22]. Tilapia secretes two different prolactins, prolactin-I and prolactin-II. Prolactin-I has more effective Na^+-retaining action than

prolactin-II [23], but it has not been clarified whether both types of prolactin are stored in the same granule or each has its own store.

We originally began a project to demonstrate omega (Ω)-shaped exocytotic processes on membrane two minutes after pimozide injection into rats, when a maximum rate of prolactin release occurs. Dopamine was used as a reference control for the project. Ultrastructure of the pimozide-treated group was not different from that of the control group. However, to our surprise, there was a massive increase of RER in the dopamine-treated prolactin cells [15]. The change did not occur in any other types of adenohypophysial cell. The observation, therefore, suggests that the dopamine action is not a nonspecific general phenomenon but specific to prolactin cells, probably through D_2-receptors. However, more direct evidence is required to define receptor involvement. Block of dopamine action with specific antagonists such as pimozide would provide further evidence that dopamine acts on D_2-receptors. We have examined the tilapia prolactin cells further to elucidate the relationship between action of dopamine and ultrastructural changes.

The morphology of prolactin cells in rat and tilapia appears to be the same [14, 15, 24]; the cells are angular in shape and the granules are polymorphous. These characteristics are unique among pituitary cells and thus it is relatively easy to identify prolactin cells by their morphology. It is interesting to note that the RER volume in prolactin cells is also increased when tilapia is under acid stress [24]. According to morphological characteristics, the newly formed structures which appear in large quantity following dopamine treatment are RER. However, they are only temporary structures; they disintegrate quickly (within a few minutes) after dopamine is withdrawn. These facts imply that the structures play a short term physiological role and may not be involved in protein synthesis, the major function of RER. The exact physiological function of the RER structures is not known. It could be related to a mechanism by which dopamine inhibits prolactin secretion, since the volume of RER increases while under the inhibitory influence of dopamine and returns to normal levels following removal of dopamine from the incubation medium (RE10 group). Dopamine has been shown to inhibit not only prolactin release, but also prolactin synthesis [25].

Our previous experiments with rats suggested that RER provided a physical barrier to prolactin secretion by relocating around the periphery of the cell [15]. However, this was not found to be the case with tilapia because the RER did not have any tendency to sequester granules within the centre of the cell. In fact, the RER was often seen encircling the nucleus, thereby advancing the granules toward the periphery. The reason for this is not yet known.

Prolactin granules are 'tough' structures that can withstand strong physical shearing forces [26] and thus do not change shape simply as a result of some experimental artefacts. However, many granules became rod- or dumbbell-shaped after a ten-minute recovery period (RE10 min group), suggesting that the morphological changes of granules are a biological phenomenon. The rod-shaped granules appeared within ten-minutes of incubation in the control medium following removal of dopamine inhibition. This ten-minute period could be too short for the de novo synthesis of prolactin, which forms 'dumbbell-shaped' granules by merging immature granules. However, it is possible that the newly shaped granules are derived from a reformation of tightly packed, high density mature granules. It is interesting to see that many of these granules are not completely covered by membrane (fig. 3b). The lack of membrane in part of the newly shaped granules suggests that the granule may be disintegrating, with resulting diffusion of its prolactin content out of the cell. However, we cannot exclude the possibility that slanted cuts of the rod-shaped granules could cause them to appear less dense on one side, which could then result in a thinner and therefore less visible membrane. It is well established that a large amount of prolactin is secreted when the inhibitory action of dopamine is removed [11], commonly called 'rebound release'. It is possible that the prolactin is released by diffusing out of the cell after dopamine inhibition is removed.

We have observed that dopamine decreased the number of prolactin granules and increased the quantity of RER in spite of its inhibitory action on prolactin secretion and synthesis. The majority of spherical granules became rod- or dumbbell-shaped during a period of inhibitory dopamine withdrawal. This change in granule morphology could be related to 'rebound release'.

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