**MINI-REVIEW**

**Hormone-induced granular convoluted tubule-like cells in mouse parotid gland**

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Abstract: Most striated duct (SD) cells in the adult mouse parotid gland (PAG) have a few small secretory granules. These granules, however, are usually too small and sparse to be detected using light microscopy. Our serial studies have suggested that these PAG SD cells belong to a group of hormone-responsive granular duct cells, similar to the granular convoluted tubule (GCT) cells found in the submandibular gland. These studies also indicate that and some PAG SD cells may be capable of developing a granular cell phenotype under supraphysiological conditions of androgenic and thyroid hormones, leading to more abundant, and more kinds of GCT-specific secretory polypeptides. Here, the cytology of hormone-modulated SD cells, the immunocytochemistry of their secretory products, and their secretory responses to some autonomic agents are reviewed. Finally, the close similarity of the duct systems of the three major salivary glands in mice is critically emphasized. J. Med. Invest. 56 Suppl.: 290-295, December, 2009

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**INTRODUCTION**

The granular convoluted tubule (GCT) is a specialized segment of the striated duct (SD) in the rodent submandibular (SMG) duct system, and GCT cells are known to have abundant large secretory granules. These cells are up-regulated by pituitary-dependent hormones and are sexually dimorphic, being larger in males than in females. GCT cells synthesize and externally secrete a large number of biologically active polypeptides, such as epidermal growth factor (EGF), nerve growth factor (NGF), renin, and members of the kallikrein gene family (for reviews, see 1-3). A few morphologically similar granular duct cells have also been detected in the SD of the mouse sublingual gland (SLG), usually in males, and rarely in females (4). Such GCT-like cells in the SLG express GCT-specific secretory products (5-7) and are also up-regulated by pituitary-dependent hormones (7). GCT-like cells were induced in the SD of the SLG in female mice treated with androgen (5). However, GCT-like cells have not been detected in the duct segments of the parotid gland (PAG) in any rodent species. In the adult mouse PAG, which is examined in this mini review, many intra-lobular SD cells and excretory duct cells contain a few small secretory granules (8, 9). Likewise, the presence of small secretory granules in the SD cells of the major salivary glands in many mammalian species has been reported, suggesting that SD cells are involved in the secretion of organic products (10). However, the composition of these secretory granules remains largely unknown.
SEXUAL DIMORPHISM AND DEPENDENCY OF PITUITARY-DEPENDENT HORMONES

Light microscopy (LM), and semithin sections stained using routine histological methods indicate that the SD cells in mouse PAG of either sex appear to be devoid of secretory granules, have a round nucleus at their center, and have well-developed striations at their bases. Meanwhile, transmission electron microscopy (TEM) reveals that many SD cells have poor Golgi apparatus, sparse rough endoplasmic reticulum (RER), and well-developed basal membrane infoldings associated with elongated mitochondria in addition to containing electron-dense secretory granules. These secretory granules are very small and sparse, scattered throughout the subluminal edges, and rather larger and more abundant in males than in females, exhibiting moderate sexual dimorphism. Castration in male mice decreased the number and size of the secretory granules in the SD cells, resulting in an appearance similar to that of female mice. Hypophysectomy in male mice resulted in either the complete disappearance or a significant reduction in the number of secretory granules. Taken together, these results indicate that many SD cells in the PAG correspond to a hormone-responsive granular duct cell type, being dependent on pituitary-dependent hormones and exhibiting androgen-regulated sexual dimorphism (8).

HORMONE-INDUCED GCT-LIKE CELLS IN MOUSE PAG

5α-Dehydrotestosterone (DHT, 20 mg); dexamethasone (Dex, 10 mg); and T3 (1 mg per kg body weight), either singly or in combination, were subcutaneously injected into male and female mice every other day for two weeks. The concomitant injection of T3+DHT or all three hormones enhanced granular cell phenotype in some SD cells in the PAG, converting them into full-fledged granular cells (9). The secretory granules in these cells were large enough to be visible using LM, but inter- and intra-cellular variations in their size and density were observed (Fig. 1a), and the full-fledged granular cells were roughly classified into small and large cell types (see Figs. 1 and 2). TEM confirmed that well-developed full-fledged granular cells, corresponding to the large cell type, had numerous large electron-dense secretory granules in their apical two-thirds and exhibited distended RER cisternae and modest larger Golgi apparatus in their perinuclear cytoplasm, a few short membrane striations at their bases (Fig. 1b). The secretory granules in such full-fledged granular cells were remarkably...
larger than those in non-modulated SD cells with low hormone sensitivity (Fig. 1a-c). However, the secretory granules even in the large, full-fledged granular cells were still smaller, than the secretory granules of the SMG GCT cells in the same mice. Therefore, the hormone-induced full-fledged granular cells exhibited cytological features corresponding to the SMG GCT cells of adult female mice or young male mice, since their secretory granules were relatively small and they retained their basal membrane striations.

The percentages of such hormone-induced GCT-like cells in the SD segments of the PAGs of male mice injected with T3, DHT, and Dex, either alone or in combination (Reference 9). As no granular cells were detected in the intact or Dex-alone group, these two groups were excluded from this graph. The full-fledged granular cells were classified into small-type and large-type cells, the former containing comparatively smaller secretory granules, and the latter containing larger secretory granules as viewed using LM (see Fig. 1). All the values in each group are the means± SD for 4 animals. \(*p<0.001\), compared with T3-alone, DHT-alone, T3+Dex, or DHT+Dex, using a Student t-test.

**CHARACTERIZATION OF HORMONE-INDUCED GCT-LIKE CELLS**

The expression and localization of SMG GCT-specific secretory products were examined in hormone-modulated PAG SD cells using an immunofluorescent method. Many SD cells in intact mice and hormone-injected mice were immunoreactive for NGF and mK1, and both labels were visible throughout the subluminal edges, which contained only a few small secretory granules (12). The secretory granules of the GCT-like cells were also labeled for NGF, EGF, and mK1 (Fig. 3a-c). EGF labeling was limited to a small population of GCT-like cells (Fig. 3a). No labeling for renin was detected in any of the duct cells (d). The nuclei are counterstained with DAPI (blue).
3b), many of which were large-type GCT-like cells. No granular cells immunoreactive for renin were detected in the PAG duct system (Fig. 3d). Immunoelectron microscopy confirmed that gold particles, suggesting the presence of these polypeptides, were restrictedly localized on the secretory granules (8).

During the postnatal development of GCT cells in mice SMGs, mK1 first appeared approximately 1 to 2 weeks of age (13), while EGF and NGF appeared at about 3 weeks of age (2, 14, 15); the expression of renin was detected at 5 weeks of age (16). All immature GCT cells expressed mK1, but the inhibition of mK1 was observed at 4 weeks of age, corresponding to the onset of puberty. Nearly all the SMG GCT cells in adult male mice expressed EGF, NGF and renin, while less than 1% of the population of GCT cells expressed mK1 (13). Therefore, mK1 appears to be a neonatal type of secretory product that is down-regulated by pituitary-dependent hormones; this unusual characteristic differs from that of other GCT-specific secretory products. In contrast, mK1 synthesis in the PAG was not inhibited in all hormone-induced GCT-like cells, and no GCT-like cells were positively labeled for renin. These results suggest that the hormone-induced GCT-like cells in the PAG correspond to immature SMG GCT cells at a stage just prior to the onset of puberty.

SECRETION IN RESPONSE TO METHOXAMINE AND PILOCARPINE

The secretory responses to the \( \alpha \)-adrenergic agent, methoxamine (20 mg/kg body weight), and the cholinergic agent, pilocarpine (10 mg/kg body weight) were examined in hormone-induced PAG GCT-like cells and compared with those in SMG GCT cells and SLG GCT-like cells in the same hormone-injected male mice. In the PAG and SMG, no changes in the duct segments were observed within 15 min after the injection of methoxamine or pilocarpine, despite marked depletion of secretory granules that occurred in approximately half of the acinar cells in both glands. At 30 min to 1 hr after the injection of both secretagogues, the discharge of numerous secretory granules from the PAG GCT-like cells and the SMG GCT cells, as well as from almost all the acinar cells, was observed. The SMG GCT cells sometimes possessed vacuole-like pits, corresponding to traces of secretory product discharge, and a marked decrease in the number of secretory granules. The GCT lumina were highly distended. Nearly all the secretory granules appeared to have been discharged from the PAG GCT-like cells, and only a few rare secretory granules remained near the large vacuoles (Fig. 4a). Several large vacuoles, corresponding to the traces of secretory product discharge from the GCT-like cells, were dotted throughout the SD segments (Fig. 4a, b). In contrast, neither \( \alpha \)-adrenergic nor cholinergic stimulation affected the discharge of the secretory granules from either the GCT-like cells or acinar mucous cells in the SLG. We concluded that the secretion of secretory products into saliva from hormone-induced GCT-like cells in the PAG is mediated predominantly by \( \alpha \)-adrenergic and cholinergic activation, similar to the situation in SMG GCT cells (17-20), but differed from that in SLG GCT-like cells.

CONCLUSION

In mice, the three major salivary glands each exhibit specific secretory acini units and duct systems. The acini are composed of seromucous-type secretory cells in the SMG, serous-type secretory cells in
the PAG, and a combination of mucous and serous cells in the SLG. The duct system of each gland also exhibits unique characteristics in intact mice, as described above. Our serial studies report for the first time that the granular cell phenotype of some PAG SD cells can be altered to resemble that of GCT-like cells under supraphysiological conditions of androgenic and thyroid hormones, emphasizing a much greater similarity among the duct systems of the three major salivary glands in mice than has been previously appreciated. The GCT and GCT-like cells develop from SD precursors, have similar morphological and fine structural features, are sexually dimorphic, are multi-hormonally regulated, and synthesize and secrete similar GCT-specific biological active polypeptides. However, the duct system of each gland in intact mice exhibits unique characteristics. Interestingly, both GCT-like cells and acinar mucous cells in the SLG showed no response to α-adrenergic and cholinergic activation, even though the SMG and the SLG have the same secretomotor innervation from the facial nerves.

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REFERENCES


