

MINI-REVIEW**Hormone-induced granular convoluted tubule-like cells in mouse parotid gland**Shingo Kurabuchi¹, Takanori Matsuoka¹, and Kazuo Hosoi²¹*Department of Histology, The Nippon Dental University School of Life Dentistry, Tokyo, Japan ; and*²*Department of Molecular Oral Physiology, Institute of Health Biosciences, the University of Tokushima Graduate School, Tokushima, Japan*

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

Keywords : granular convoluted tubule (GCT) cell, androgen, thyroid hormone, parotid gland (PAG), mouse

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.

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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

5 α -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

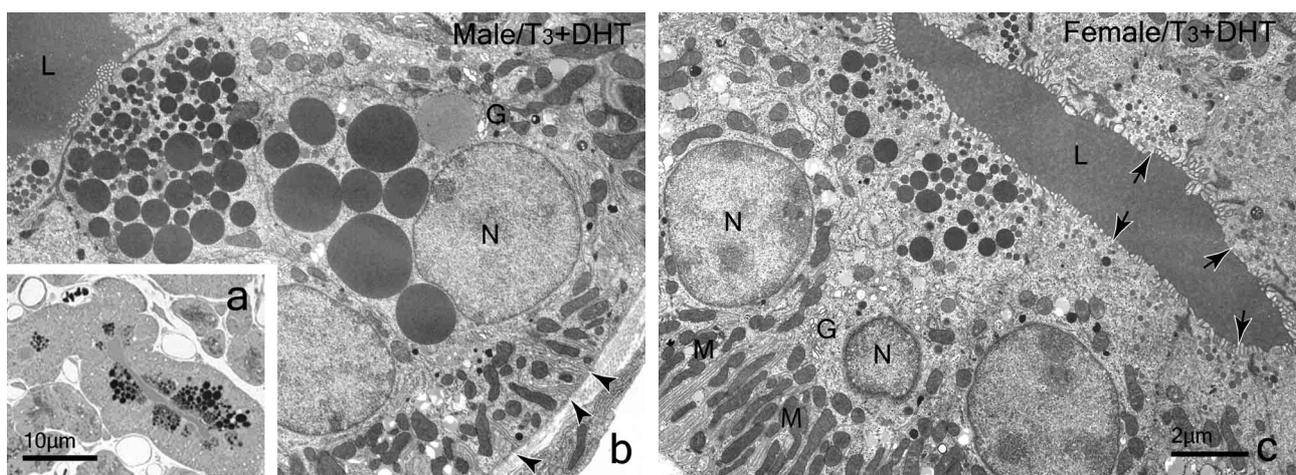


Fig. 1. LM (a: Heidenhain's iron hematoxylin staining) and TEM (b, c) micrographs of hormone-modulated SD cells in PAGs from male (a, b) and female (c) mice (Reference 9). Secretory granules in the full-fledged granular cells and acinar cells were darkly stained using this staining method, and the granules in the former cells show remarkable intercellular variations in their size and density (a). Remarkably larger granules (sometimes beyond 2.5 μ m in diameter) are packed into the apical two-thirds of large-type full-fledged granular cells (a, b). Rather large granules (within 1 μ m in diameter) sparsely occupy the apical one-third of small-type full-fledged granular cells (a, c). A few small electron-dense secretory granules (approximately 0.1~0.3 μ m in diameter) are scattered throughout the subluminal cytoplasm of the SD cells in intact and hormone-injected mice (arrows in c), but these granules are not detected using LM (a). The arrowheads indicate basal membrane infoldings. G, Golgi apparatus; L, lumen; M, mitochondria; N, nucleus of full-fledged granular cells.

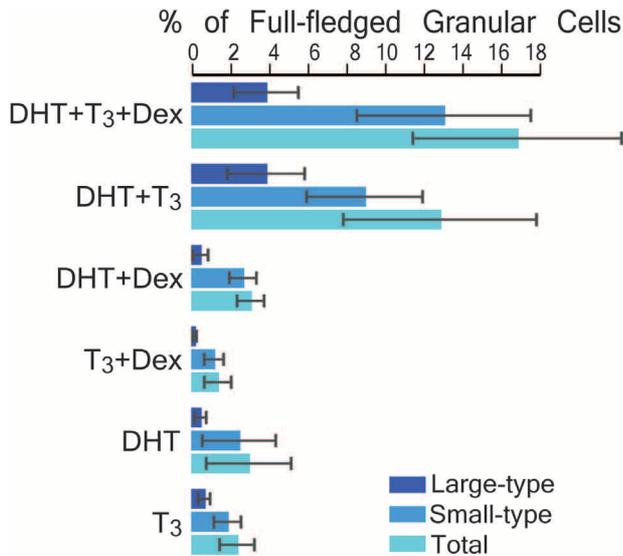


Fig. 2. Bar graph, showing the percentages of full-fledged granular cells in the interlobular SD segments of the PAGs of male mice injected with T_3 , DHT, and Dex, either alone or in combination (Reference 9). As no granular cells were detected in the intact or Dex-alone group, these two groups were 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 \pm SD for 4 animals. $^{\S}P < 0.001$, compared with T_3 -alone, DHT-alone, T_3 +Dex, or DHT+Dex, using a Student *t*-test.

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 PAG in male mice were significantly high, accounting for approximately 12% of the cells in the T_3 +DHT group and 18% of the cells in the three-hormone group, and large-type GCT-like cells were prominent in these two groups (Fig. 2). Dex alone did not induce full-fledged granular cells in any duct segments. T_3 or DHT administered alone, or concomitantly with Dex, induced only a few GCT-like cells, accounting for less than 3% of the population of SD cells in each group, many of which were relatively small. Furthermore, numerous SD cells in each hormone-injected mice group retained their original form, exhibiting

only a few small secretory granules (Fig. 1c), despite the supraphysiological hormone conditions.

GCT-like cells were also hormonally induced in the PAGs of female mice, but the percentage of these cells was remarkably low. All the GCT-like cells in the female PAG were classified as a small-type: their secretory granules were relatively small and sparse and well-developed basal infoldings were observed as usual (Fig. 1c). The SD cells in female glands may be intrinsically less responsive to hormones, since the nuclear distribution of androgen receptors in mouse SMG is much greater in males than in females (11), although no information concerning other hormone receptors in salivary glands is available.

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.

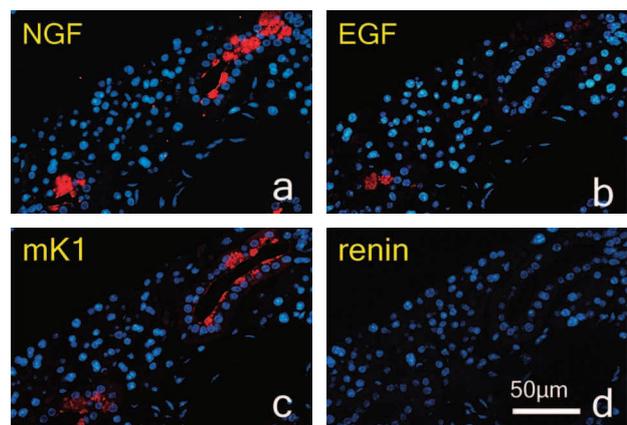


Fig. 3. Immunofluorescence micrographs (Cy3, red) for NGF (a), EGF (b), mK1 (c), and renin (d) in semi-serial sections of PAGs from male mice, injected with T_3 +DHT (Reference 12). Labels for NGF and mK1 are present on the secretory granules of full-fledged granular cells and the subluminal edge of many SD cells (a, c). Labels for EGF (b) are found only in a few full-fledged granular cells, corresponding to large-type granular cells. 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 α -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 α -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 α -adrenergic and cholinergic activation, similar to the situation in SMG GCT cells (17-20), but differed from that in SLG GCT-like cells.

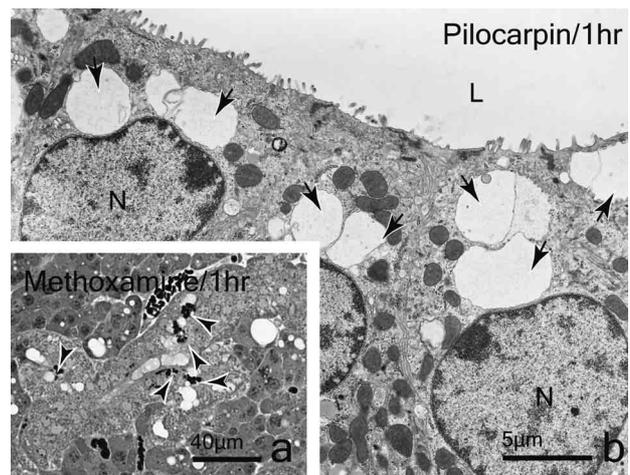


Fig. 4. LM (a; Heidenhain's iron hematoxylin staining) and TEM (b) micrographs of hormone-induced GCT-like cells in the PAGs from male mice at 1 hr after the injection of methoxamine (a) or pilocarpine (b). The GCT-like cells in the SD segment and acinar serous cells have been considerably depleted of secretory granules, but a few rare secretory granules are visible near the vacuoles (arrowheads in a) in the SD segments. Large vacuoles (arrows in b), which appear to be signs of the discharge secretory products from the GCT-like cells, are dotted in some cells in SD segments. L, lumen; N, nucleus of the cells, containing large vacuoles.

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

1. Mori M, Takai Y, Kunikata M : Biologically active peptides in the submandibular gland-Role of the granular convoluted tubule. *Acta Histochem Cytochem* 25 : 325-341, 1992
2. Gresik EW : The Granular Convoluted tubule (GCT) cell of rodent submandibular gland. *Microsc Res Techn* 27 : 1-24, 1994
3. Kurabuchi S, Hosoi K : Sexual dimorphism and hormonal regulation of granular convoluted tubule (GCT) cells of mouse submandibular gland-immunocytochemical analysis. (in Japanese) *J Masticat Health Soc* 10 : 61-70, 2001
4. Kurabuchi S, Gresik EW : Ultrastructural study of hormonally responsive striated duct cells in the mouse sublingual gland. *Odontology* 89 : 34-40, 2001
5. Gresik EW, Barka T : Epidermal growth factor, renin, and protease in hormonally responsive duct cells of the mouse sublingual gland. *Anat Rec* 205 : 169-175, 1983
6. Hazen-Martin DJ, Landreth G, Simson AV : Immunocytochemical localization of nerve growth factor in mouse salivary glands. *Histochem J* 19 : 210-216, 1987
7. Kurabuchi S, Gresik EW, Yao C, Hosoi K : Hypophysectomy and hormonal therapy modulate mK1-immunopositive duct cells in the mice sublingual glands. *J Mol Hist* 39 : 499-507, 2008
8. Kurabuchi S, Hosoi K : Immunocytochemical localization of mK1, a true tissue kallikrein, in the mouse parotid gland : sexual dimorphism and effects of castration and hypophysectomy. *Odontology* 92 : 73-76, 2004
9. Kurabuchi S : Repeated androgen and thyroid hormone injections modulates the morphology of hormone-responsive duct cells in the mouse parotid gland. *Odontology* 94 : 29-37, 2006
10. Tandler B, Gresik EW, Nagato T, Phillips CJ : Secretion by striated ducts of mammalian major salivary glands : review from an ultrastructural, functional, and evolutionary perspective. *Anat Rec* 264 : 121-145, 2001
11. Kyakumoto S, Kurokawa R, Ohara-Nemoto Y, Ota M : Sex difference in the cytosolic and nuclear distribution on androgen receptor in mouse submandibular gland. *J Endocrinol* 108 : 267-273, 1986
12. Kurabuchi S, Hosoi K : Immunocytochemical study of granular duct cells with a hormonally enhanced granular cell phenotype in the mouse parotid gland. *Odontology* 97 : 57-61, 2009
13. Kurabuchi S, Hosoi K, Gresik EW : Developmental and androgenic regulation of the immunocytochemical distribution of mK1, a true tissue kallikrein, in the granular convoluted tubule of the mouse submandibular gland. *J Histochem Cytochem* 50 : 135-145, 2002
14. Gresik EW, Barka T : Immunocytochemical localization of epidermal growth factor during the postnatal development of the submandibular gland of the mouse. *Am J Anat* 151 : 1-10, 1978
15. Chabot J-G, Walker P, Pelletier G : Thyroxine accelerates the differentiation of granular convoluted tubule cells and the appearance of epidermal growth factor in the submandibular gland of the neonatal mouse. A fine structural immunocyto-chemical study. *Cell Tissue Res* 248 : 351-358, 1987
16. Gresik EW, Michelakis A, Barka T : Immunocytochemical localization of renin in the submandibular gland of the mouse during postnatal development. *Am J Anat* 153 : 443-450,

- 1978
17. Hosoi K, Aoyama K, Ueha T : Effect of autonomic agents on the amount of androgen-dependent granules in convoluted tubular cells of the mouse submandibular gland, Canadian J Physiol Pharmacol 56 : 634-641, 1978
 18. Hirata Y, Orth DN : Secretion of epidermal growth factor, nerve growth factor, and renin-like enzymes by dispersed male mouse submandibular gland cells *in vitro*. Endocrinology 107 : 92-97, 1982
 19. Johnson DA, Cortez JE : Chronic treatment with beta adrenergic agonists and antagonists alters the composition of proteins in rat parotid saliva. J Dent Res 67 : 1103-1108, 1988
 20. Bedi GS : The effect of adrenergic agonists and antagonists on the expression of proteins in rat submandibular and parotid glands. Critic Rev Oral Biol Med 4 : 565-571, 1993