ORIGINAL ARTICLE ISSN: 2455-5274

Demonstration of Solitary Chemosensory Cells in the Nasopalatine and Vomeronasal Ducts of Rat by α-gustducin and GAP-43 Immunohistochemistry.

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Date of Submission: ***01-03-2018***
Date of Acceptance: ***01-04-2018***
Date of Publishing: ***21-05-2018***

ABSTRACT

Background: In the present study, we used immunohistochemistry for α -gustducin and Growth Associated Protein-43 (GAP-43) to examine the spatial distribution of the solitary chemosensory cells primarily in the nasopalatine ducts of rat at the time of weaning, which is lack in the literature. **Methods:** We found abundant solitary cells labeled with α-gustducin in the nasopalatine duct and vomeronasal organ of rats. In the nasopalatine duct, these cells were more frequent in the medial wall epithelium; meanwhile appreciable number of α-gustducin labeled cells were localized only in the neuroepithelium portion of the vomeronasal organ. We found the number of these cells increased toward the entries of the nasopalatine and vomeronasal ducts into the nasal cavity. We also found GAP-43 heavily expressed in the core of nasopalatine duct, close to the basement membrane and around the blood vessels and cavernous spaces of the vomeronasal organ. **Results:** GAP-43 labeled axons apposed the solitary chemosensory cells closely, either coursing along or wrapping the solitary chemosensory cells. Individual cells were apposed by one or a few intraepithelial nerve fibers and a single fiber sometimes contacted a few solitary chemosensory cells. Intraepithelial GAP-43 labeled fibers were more frequent toward the nasal cavity and the entry of nasopalatine and vomeronasal ducts in close association with the solitary chemosensory cells. **Conclusion:** We conclude that α -gustducin-expressing cells alongside the GAP-43 intraepithelial nerves in the nasopalatine and vomeronasal ducts suggests that they share the same transduction mechanisms.

Keywords: *Chemosensory cells, olfactory, palate, smell, taste.*

INTRODUCTION

The olfactory system of mammals consists of several subsystems, each of which may serve distinct functions by using different signal transduction pathways and projecting to different brain areas (Ma et al. 2003; Kociánová et al. 2006). The vomeronasal complex is one of these subsystems, composed primarily of the vomeronasal organ (VNO), which lies along both sides of the ventrorostral aspect of the nasal septum. In rodents and bats, the VNO opens directly into the nasal cavity close by the nasopalatine (NPD), which extends through the incisive canal, from the incisive papilla in the mouth to the floor of the nasal cavity (Estes 1972). The presence of NPD is required to detect the odors of ingested food in most mammals. Thus, the two NPD contribute to the vomeronasal system, which also includes neural projections from the VNO to the vomeronasal (accessory olfactory) bulb (Døving and Trotier 1998). Descriptions in the literature have largely

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focused upon the vomeronasal epithelium, which consists of a sensory and a non-sensory epithelium lining the medial and lateral side of the cavity, respectively. Solitary chemosensory cells (SCCs) are distributed in the respiratory tree (Merigo et al. 2005; Tizzano et al. 2011) and the VNO (Zancanaro et al. 1999; Ogura et al. 2010) of rodents. These SCCs have neurogenic nature as they expressed GAP-43 and some neuronal markers (Dennis et al. 2003; Ma et al. 2003; Weiler and Benali 2005). In this report, we aim to demonstrate the distribution of SCCs and the associated nerves primarily in the NPD using α - gustducin and GAP-43 immunohistochemistry in the rat, which is still lacking in the available literature.GAP-43, is a neuronal membrane phosphoprotein, which is well known to be expressed in large amounts by neurons during development, and exhibited excellent validity for labeling the gustatory nerves in the embryonic tongue and the subsequent innervation of adult taste buds in sheep (Mistretta and Haus 1996), rat (Mbiene and Mistretta 1997; Wakisaka et al. 1998; El Sharaby et al. 2004), and mouse (Ringstedt et al. 1999). In addition, we used antibody against α-gustducin, G-protein specifically expressed by mature light (type 2) taste bud cells (Tabata et al. 1995; Yang et al. 2000; El Sharaby et al. 2004).

MATERIALS ANDMETHODS

The nasopalatine papilla (NP) and nasopalatine duct (NPD) and adjacent parts of the nasal septum were collected from 10 healthy Sprague-Dawley rats of both genders at 1-2 months of age. Dissected specimens were fixed in 4% formaldehyde for 3-4 days, decalcified with 7.5% EDTA for 2-3 weeks at 4°C, and then soaked in 20% sucrose/ PBS at 4°C overnight for cryoprotection. Tissue blocks were oriented and transverse 15 µm thick sections were cut with a cryostat at -25 °C, thaw-mounted onto poly-L-lysine-coated glass slides, and air dried for 90 min prior to staining. For double immunofluorescence, stained sections with polyclonal α-gustducin were labeled with FITC conjugated anti67 rabbit for 60 min, rinsed with PBS and subsequently incubated with monoclonal GAP-43 (1:3000; Chemicon International, CA) for 60 min. Then, they were incubated with Cy3- conjugated anti-mouse IgG diluted 1:500 in PBS (Molecular Probes, OR, USA) for 90 min at room temperature. Sections were cover-slipped with Vectashield and viewed with a fluorescence microscope (Axioskop 2 plus, Carl Zeiss) using the appropriate exciting filter. The specificity of the primary antibodies against α -gustducin and GAP-43 have been reported in a previous literature (El Sharaby et al. 2004). As controls for double immunofluorescence, sections were incubated with normal serum instead of the primary antibody, and these sections resulted in no specific reactions.

RESULTS

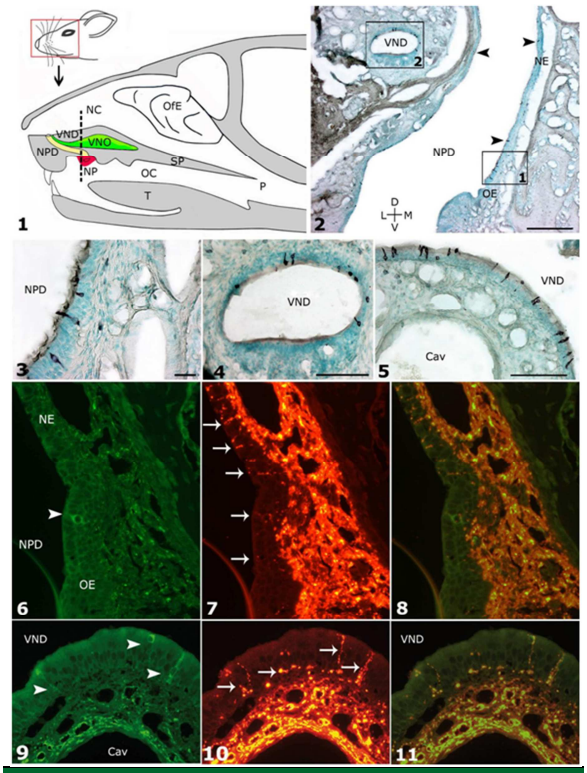

Figure 1: Schematic drawing of a parasagittal view through a rat's head showing the position of nasopalatine duct (NPD) and vomeronasal organ (VNO). NC - nasal cavity; OC – oral cavity; OfE - olfactory epithelium; P - pharynx; SP - soft palate; T - tongue.

Figure 2: Section at the cutline in A showing α-gustducin labeled solitary cells (SCCs) (arrowheads) in the medial and lateral wall epithelium of the NPD. Note: transition of the medial wall epithelium from the taste bud region (OE) to the respiratory epithelium (NE). Bar: 200 µm.

Figure 3: Magnification of the right box in B showing bipolar α-gustducin labeled SCCs in the medial wall epithelium of the NPD. Bar: 10 µm.

Figure 4: Magnification of the left box in B showing bipolar α-gustducin labeled SCCs exclusively in the sensory epithelium (dorsal) of the entry passageway of vomeronasal duct (VND). Bar: 50 µm.

Figure 5: Appreciable number of bipolar α-gustducin labeled SCCs only in the neuroepithelium portion with one process reaching the surface epithelium contacting the lumen of NPD while the second process extended toward the base of the epithelium where abundant blood vessels and cavernous spaces were found (Cav). Bar: 50 µm.

Figure 6-8: Nasopalatine duct of rat labeled with α-gustducin (6) and GAP-43 (7) and merged immunofluorescence (8). In Fig. 6, arrowheads show typically bipolar α-gustducin

labelled SCCs. In Fig. 7, heavily GAP-43 expression was evident in the core of NPD close to the basement membrane. Arrows show heavily labeled fibers in the papillary epithelium especially toward the nasal cavity and in close association with the SCCs (× 40 magnification)
Figure 9-11: Vomeronasal organ of rat labeled with α-gustducin (9), GAP-43 (10) and merged immunofluorescence (11). In Fig. 9, arrowheads show typically bipolar α-gustducin labelled SCCs extending along the whole neuroepithelium portion from the surface epithelium contacting the lumen of the VND to the base of epithelium. In Fig. 10, extensive GAP-43 expression was evident close to the basement membrane, around the blood vessels and near the cavernous spaces (Cav) of the VNO. Arrows show heavily labeled fibers either coursing along or wrapping the SCCs (× 40 magnification).

The oral opening of the NPD extended vertically upwards on each side of the NP and then obliquely rostromedially through the incisive canal to the nasal cavity [Figure 1]. The epithelial lining of the duct was thinner toward its entry in the floor of the nasal vestibule [Figure 2]. The VNO coursed rostrally on each side of the nasal septum, lateral to the NPD, where the sensory epithelium became dorsal and the non-sensory epithelium ventral. The duct of VNO coursed rostrally, until it opened close by the entry of NPD. We found heavily labeled cells with α-gustducin along the entire length of the NPD and the VNO [Figure 2-5]. In the NPD, these SCCs were longitudinally oriented with a slender apical process directed toward the lumen and the other toward the basal membrane. They were substantially abundant in the medial wall epithelium as well as the entry of the duct into the nasal cavity, while fewer cells were found in the lateral wall epithelium of the duct [Figure 3]. In the VNO, appreciable number of SCCs labeled with α-gustducin were localized in the neuroepithelium portion, with one process reaching the lumen surface epithelium while the second process extended toward the base of the epithelium where abundant blood vessels and cavernous spaces were found [Figure 4 and 5]. In the present study, the double immunofluorescence of GAP-43 with α-gustducin was helpful to examine the relationship of SCCs with the surrounding nerves in the NPD and VNO [Figure 6-11]. Extensive expression of GAP-43 was remarkable in the core of the NPD especially close to the basement membrane, and the labeled nerves entered the papillary epithelium of the duct passing toward the nasal cavity in close association with the SCCs [Figure 8-9]. In the VNO, extensive expression of GAP-43 was also evident close to the basement membrane, around the blood vessels and cavernous spaces [Figure 10-11]. GAP-43 labeled axons apposed the SCCs labeled with α-gustducin closely, either coursing along or wrapping the SCCs. Interestingly, individual SCCs that found only in the neuroepithelium portion of the VNO were opposed by one or a few GAP-43 labeled intraepithelial nerve fibers, and a single fiber sometimes contacted a few SCCs.

DISCUSSION

In this report, we found appreciable number of solitary cells expressing α-gustducin and associated with nerves heavily labelled with GAP-43 along the entire length of the NPD and in the neuroepithelium portion of the VNO of rat. Individual SCCs were remarkably opposed by one or a few intraepithelial nerve fibers and a single fiber sometimes contacted a few SCCs. Intraepithelial GAP-43 labeled fibers were more frequent toward the nasal cavity and the entry of NPD and VND in close association with the α-gustducin labelled SCCs. These cells are morphologically identical to the SCCs distributed in the respiratory tree (Merigo et al. 2005; Tizzano et al. 2011) and VNO of mouse (Zancanaro et al. 1999; Ogura et al. 2010). These SCCs were longitudinally oriented and more frequent in the medial wall epithelium. This result, which is not reported before is consistent with the demonstration of α -gustducin heavy labeled cells in the taste buds that found only in the medial wall epithelium of the NPD of rat (El Sharaby et al. 2004). We found appreciable number of SCCs labeled with α-gustducin in the neuroepithelium portion of VNO.
However, Zancanaro et al. (1999) found α -gustducin positive cells exclusively in the neighboring non-receptor epithelium in the VNO of mouse. In accordance to Ogura et al. (2010), most of the intraepithelial fibers, at this region, are necessary to innervate the SCCs, which relay sensory information onto the trigeminal fibers. Our findings support that the chemoreceptors in the NPD and VNO could operate as detectors of irritating stimuli (Finger et al. 2003). Further studies are required to investigate a possible phylogenetic contribution between these cells in both the NPD and VND as stated by Hoon et al. (l999). In conclusion, the finding that α -gustducin-expressing cells alongside the GAP-43 intraepithelial nerves that we found in the NPD and VND suggests that they share the same transduction mechanisms.

CONCLUSION

Acknowledgments
The author wishes to thank Prof. Satoshi Wakisaka, Department of Oral Anatomy and Developmental Biology, Osaka University Graduate School of Dentistry, Osaka, Japan for providing the materials and

revising the manuscript. This study was funded by a research grant in aid from the STDF, Ministry of Higher Education and Scientific Research, Egypt and JSPS Core-to-Core Program, B. Asia-Africa Science Platforms.

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How to cite this article: El Sharaby AA, Nomir AG, Elgarhy AK. Demonstration of Solitary Chemosensory Cells in the Nasopalatine and Vomeronasal Ducts of Rat by α-gustducin and GAP-43 Immunohistochemistry. Acad. Anat. Int. 2018;4(1):31-34.

Source of Support: Nil, **Conflict of Interest:** None declared.