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Regional Differences in Taste Bud Distribution and α-Gustducin Expression Patterns in the Mouse Fungiform Papilla

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Abstract

The regional differences between distribution patterns and α-gustducin expression patterns of the fungiform (FF) taste buds were investigated in the adult mouse, using hematoxylin-eosin staining and immunofluorescence histochemistry on the most anterior region of the tongue (the first millimeter) through the intermediate region of the tongue (the last 1–4 mm). Paraffin sections were prepared from the tip to posterior regions (anterior and intermediate region containing the FF taste buds) of the adult mouse tongue. Results indicate that there were significant regional differences in size and density of taste buds, the cell counts of the single taste bud, and the α-gustducin–immunoreactive taste buds between the 2 regions. The taste bud had a characteristic onion-like appearance, and the α-gustducin–immunoreactive cell was spindle shaped with elongated processes extending from the base to the pore of the taste buds. These results provide a detailed insight to better understand regional descriptions of mouse taste bud density and size and α-gustducin expression with the mouse model.

Key words: α-gustducin, fungiform papillae, mouse, regional difference, taste bud

Introduction

The gustatory system in mammals is apparently important for selective ingestion of nutrients through the oral cavity (Harada et al. 2000), which is dependent on the development of taste buds. There are different subpopulations within the oral cavity of the rat, such as fungiform (FF), foliate (FL), circumvallate (CV), and soft palate (Mistretta 1972; Ganchrow et al. 1986; Hosley and Oakley 1987). The appearance and maturation of taste buds among the subpopulations in the oral cavity in postnatal rat, hamster, and common marmoset (Mistretta 1972; Hosley and Oakley 1987; Belecky and Smith 1990; Harada et al. 2000; Yamaguchi et al. 2001), and the age-related increases in taste bud volume were observed during development primarily a function of cell addition in the rat papilla (Hosley and Oakley 1987; Hendricks et al. 2004). However, the regional differences in the density and size of taste buds within the same subpopulation are not fully understood, such as the FF papillae situated on the anterior two-thirds of the tongue.

Gustducin is a heterotrimeric guanine nucleotide–binding protein (G-protein), which was first reported in rats (McLaughlin et al. 1992) and then confirmed in man (Takami et al. 1994). Gustducin is reported to be associated with bitter or sweet transduction and is expressed in taste cells of the CV, FL, and FF papillae of rat lingual tissue. In rat taste buds, the α-subunit of gustducin has been found in cells with characteristics of Type II (light) cells (Boughter et al. 1997) and is mainly localized in apical microvilli (Yang et al. 2000). The regional difference of α-gustducin expression in the same subpopulation (such as the FF papillae region) are not well illuminated, although there has been some attentions directed toward the expression difference of α-gustducin in taste buds among the subpopulations in the oral cavity in postnatal rat and mouse (El-Sharaby, Ueda, Wakisaka 2001; El-Sharaby, Ueda, Kurisu, Wakisaka 2001; Kim et al. 2003).

However, our previous work indicated a pronounced anterior to posterior gradient difference in FF taste bud density, volume, and α-gustducin expression (Zhang et al. 2006). Here, we reevaluate the related previous works and regional difference of the taste buds distribution pattern and α-gustducin expression pattern in mouse FF taste buds. The current study provides a background well elucidate the regional functional difference of the FF taste buds.

Materials and methods

All experiments were reviewed and approved by Changshu Institute of Technology of Life Sciences Intramural Use and Care Committee before the study.

Animals

Adult Institute of Cancer Research male mice (8 weeks old) were purchased from Zhejiang Academy of Medical Science (China) and sacrificed at the postnatal age of 9 weeks. Eleven mice tongues were used in this test, among which 5 tongues were used for statistical analysis by ordinary hematoxylin-eosin (HE) staining, 3 for the immunohistochemical investigation of α-gustducin using the immunofluorescence-staining procedure, and the remaining 3 mice tongue's data were discarded for the disconnected sections or unclear staining.

Histological procedures for quantification of taste buds

The procedure of making tissue blocks embedded in paraffin by Harada et al. (2000) was followed with slight modification. Mice were anesthetized with CO2, and then the heads were removed after dislocation of cervical vertebrae. Each head was placed directly in 4% paraformaldehyde in 0.1 M phosphate buffer to minimize shrinkage by fixation. The tissue blocks (tongues) containing FF papillae were embedded. Complete serial coronary sections were cut at 10-μm thicknesses, mounted on glass slides, and stained with routine HE staining for the statistical analysis. Each section was examined carefully by a light microscope (40–200X, BH2, Olympus, Shanghai, China), and the existence of a taste pore representing structural maturation were recorded for each taste bud (survey by Scopephoto soft, Zhejiang Technology Inc., China). To distinguish individual taste bud and to avoid counting the same taste bud twice, the image was digitized by a high-resolution digital camera (A550, Canon, Beijing, China) and stored on line as a picture file (1280 × 1000 pixels, 32 bits/pixel color) on a microcomputer (NVIDIA GeForce 7300 LE, Lenovo, Beijing, China). The digitized image was processed by Photoshop software (6.0J, Adobe Systems Inc., San Jose, CA) and printed out at 600 dpi resolution (Deskjet 900, Hewlett Packard, Palo Alto, CA). By observing the sequence of photographs of each section, we can check and identify each taste bud. The total counts of taste buds and pored taste buds of anterior and intermediate region were counted; we can clearly distinguish between papillae and buds; and also the taste pore can be discerned in serial sections and this can avoid the overcounting error.

To calculate taste bud volumes, the perimeters of the taste bud in serial sections were outlined and the corresponding area was computed by Scopephoto image analysis system. The areas were multiplied by section thickness and summed to derive an estimate of the total taste bud volume (Krimm and Hill 1999). At the same time, 20 taste buds were randomly selected at different regions of the tongue per animal to count cells in a single taste bud, and 1 nucleus stands for 1 cell on the section, so the number of nucleus on the serial sections of a single taste bud stands for the cell counts in a single taste bud.

Immunofluorescence histochemistry procedures for quantification of α-gustducin expression

Paraffin serial sections were cut at a thickness of 7 μm and mounted on poly-L-lysine–subbed glass slide. For the immunohistochemical experiments, these paraffin sections were deparaffinized in xylene and rehydrated in a graded series of ethanol. Sections were incubated for 10 min with hydrogen peroxide in methanol to inhibit endogenous peroxidases, followed by 3% normal goat serum diluted in phosphate buffer saline (PBS)/bovine albumin serum 1.5% (pH 7.4) for 15–20 min. Afterward, the sections were incubated overnight at 4 °C with the primary antibody anti–α-gustducin (Santa Cruz Biotechnology Inc., CA, dilution 1:200), washed in PBS, and incubated with a secondary antibody (biotinylated goat anti-rabbit, Wuhan Boster Biological Technology Ltd., Wuhan, China, dilution 1:100). A Strept–Avidin–Biotin complex (SABC) technique was used to reveal sites of antigen–antibody reaction. For the SABC method, a commercial kit (SABC-Cy3, Wuhan Boster Biological Technology Ltd, dilution 1:100) was used. Kit instructions were followed with regard to dilution and incubation times.

Controls consisted of 1) omission of the primary antibody and 2) omission of the secondary antibody in the immunolabeling steps. No controls exhibited immunolabeling.

For numerical analysis, all taste buds containing α-gustducin–immunoreactive cells were thoroughly traced in all examined sections, and in each region (anterior or intermediate region), 20 α-gustducin–immunoreactive taste buds were randomly selected per animal to count α-gustducin–immunoreactive cells in single taste bud (i.e., every immunoreactive cell profile containing a nucleus was counted once). The total counts of immunoreactive cells/taste buds were recorded in 2 regions examined. The positive reaction was detected by the laser confocal scanning microscope (Leica-SP2, Leica Ltd., Germany).

Data were analyzed quantitatively by cell counts. Using single-labeled protocols, positive cells were typically observed in all taste buds. Individual buds were selected for analysis, and labeled cells were counted. Taste buds of large cross-sectional area were usually chosen because these buds contain more cells with both obvious apical processes and perinuclear areas, both of which are required to discern positive labeling. It is assumed that the possibility of encountering single-labeled cells is unrelated to the cross-sectional diameter of the sectioned taste bud because taste buds could be cross-sectioned at any angle and cells within the center of the bud or at the edges of the bud could be present equally. Hence, choosing taste buds of large cross-sectional area should not impose any systematic bias on the quantitative results. As adult mice taste buds are roughly 40–50 μm in

diameter, to ensure that a taste bud was not chosen twice, adjacent sections were never chosen for analysis and sections were spaced by greater than 50 μm.

Definition of anterior and intermediate region on the tongue

The FF taste buds are located on the anterior two-thirds of the tongue. The first millimeter and the posterior 4 mm of the adult mice tongue were defined to be anterior region and intermediate region, respectively (Figure 1).

Statistical analysis and image process

Results are presented as mean ± standard error of the mean. Paired *t*-test was carried out to compare regional difference by Origin 7.0 software (OriginLab, Northampton, MA). Significance level was taken as $P < 0.05$.

Results

The regional differences in the number and maturation of FF taste buds

In adult mice, taste buds had a characteristic onion-like appearance (Figure 2 A,B) with an average number of 127.80 ± 1.07 FF taste buds across the anterior 5 mm of tongue, and there was a noticeable anterior to posterior gradient in taste bud densities. The very anterior tongue had a much greater density of taste buds; the first millimeter of tongue (anterior region) had a mean of 72.00 ± 0.84 taste buds, whereas the last 4 mm only had a mean of 55.80 ± 0.86 taste buds, there was a significant regional difference on the counts of taste buds between the anterior and intermediate region on the tongue ($t = 12.28$, $P = 0.00025 < 0.001$; Figure 3A).

The number of the pored taste buds arrived at a mean of 117.60 ± 2.52, accounting for about 92% of the total FF taste buds (117.60 ± 2.52 of 127.8 ± 1.07). A significant difference in the number of the pored taste buds was discovered between the 2 regions ($t = 6.29$, $P = 0.00326 < 0.01$; Figure 3B) with the anterior region containing significantly more pored taste buds (66.40 ± 1.89) than the intermediate region (51.20 ± 1.59).

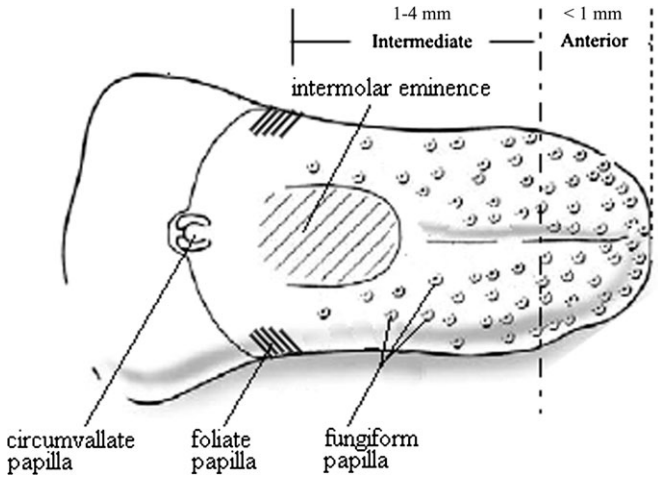

Figure 1 Partition of anterior and intermediate regions on mouse tongue. The first millimeter and the posterior 4 mm of the adult mouse tongue were defined to be anterior region and intermediate region, respectively.

The regional differences in the volume and cell counts of a single FF taste bud

The mean taste bud volume across the anterior 5 mm of tongue of adult mice was 28416.80 ± 1453.90 μm^3. To compare the regional differences in taste bud size along the anterior–posterior axis, a paired *t*-test was performed on the mean volume of taste buds on the most anterior tip region of the tongue (the first millimeter) and the mid-region of the tongue (Figure 1). The first millimeter of the tongue, which will be referred to as the anterior tongue, contained significantly smaller taste buds (24635.68 ± 1049.88 μm^3, $t = 6.88$, $P = 0.00234 < 0.01$; Figure 3C) than the more posterior region, which will be referred to as the intermediate tongue (32197.92 ± 1123.18 μm^3).

Moreover, there was a significant regional difference in the cell counts of the single FF taste bud with the anterior region of the tongue containing less cells (31.37 ± 1.62,

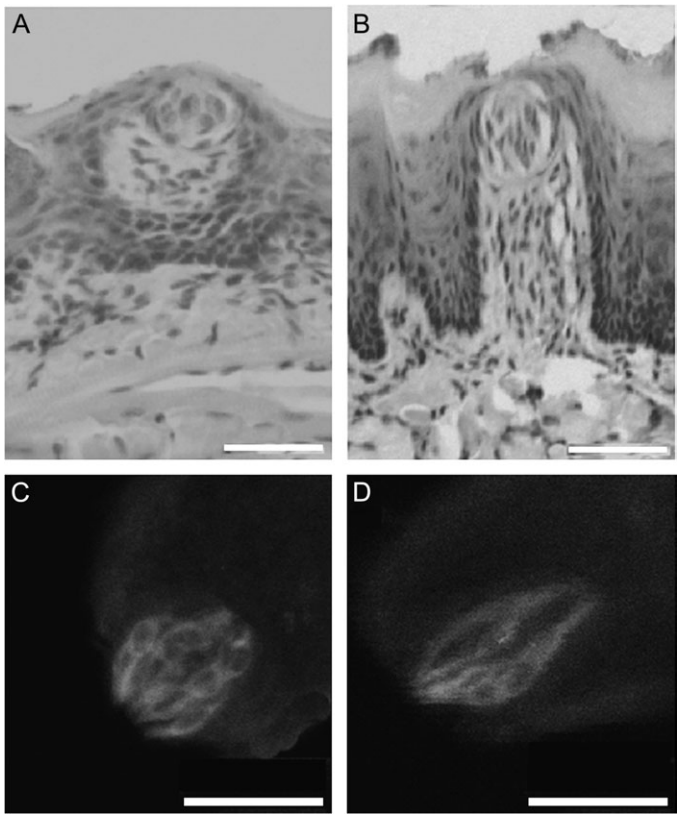

Figure 2 Coronary section (10-μm thickness) of the mouse tongue at different regions (Anterior region: **A** and **C**; Intermediate region: **B** and **D**) with HE staining indicating the taste buds (A and B) and fluorescence immunohistochemistry staining indicating α-gustducin expression (C and D) in the mouse tongue. Scale bars = 40 μm.

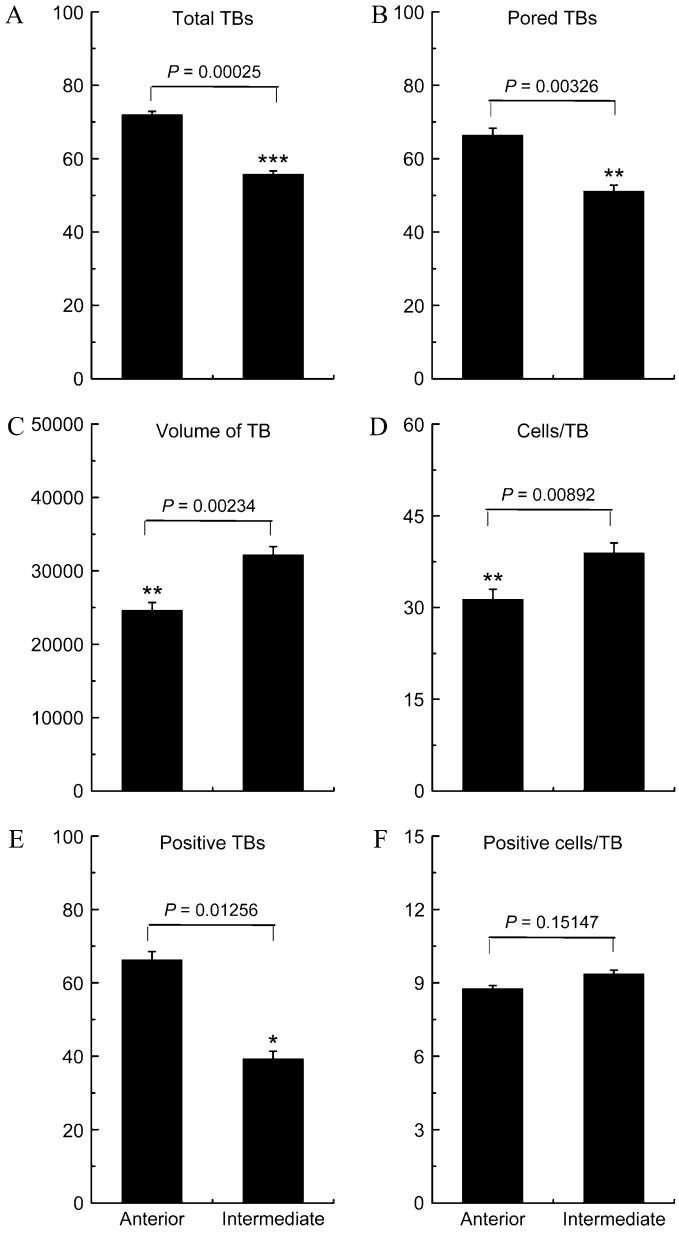

Figure 3 Regional differences of different indexes between the anterior and intermediate regions of the adult mouse tongue. **(A)** Comparison of total taste buds number; **(B)** Comparison of pored taste buds number; **(C)** Comparison of the mean volumes of taste buds; **(D)** Comparison of cell counts of a single taste bud; **(E)** Comparison of the number of taste buds containing α-gustducin–immunoreactive cells; **(F)** Comparison of the number of α-gustducin–immunoreactive cell counts in a single taste bud. Paired *t*-test was performed between the anterior and intermediate region, and significance level was taken as $P < 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

$t = 4.76$, $P = 0.00892 < 0.01$; Figure 3D) than the intermediate region did (38.95 ± 1.63).

The regional differences of α-gustducin immunoreactivity of FF taste buds

To investigate whether regional difference of the distribution pattern in mouse taste buds occurred concomitantly with regional difference in other properties of taste cells, such as the expression of specific proteins involved in the sensory transduction, we used immunohistochemistry to quantify the distribution of α-gustducin immunoreactivity. The α-gustducin is the α-subunit of a G-protein considered to be a potent marker of chemosensitive cells (Boughter et al. 1997; Sbarbati et al. 1999). We evaluated the number of taste buds which contained α-gustducin–positive cells at the anterior and intermediate region of adult mice tongue. As shown in Figure 2 (C,D), the immunoreactive cells shared a similar morphology in all examined taste buds at different regions which were spindle shaped with elongated processes process extending from the base to the pore of taste buds. Intense immunoreaction was found throughout the cytoplasm while the nuclei were negatively stained. Quantitative analysis of α-gustducin expression revealed a significant difference in the number of immunoreactive taste buds in different regions, with the anterior region containing significantly more immunoreactive taste buds (66.33 ± 2.19, $t = 8.84$, $P = 0.01256 < 0.05$; Figure 3E) than the intermediate region (39.33 ± 2.03). However, there was no significant regional difference in the number of α-gustducin–positive cells in single taste bud between the anterior and intermediate region of tongue ($t = 2.27$, $P = 0.15147 > 0.05$; Figure 3F), reaching 8.77 ± 0.12 and 9.37 ± 0.15, respectively.

Discussion

These experiments provided a description of mouse FF taste bud shape, density, size, α-gustducin expression pattern, and the regional difference that is essential for future research using the mouse model.

In the present study, we found that the first millimeter of mouse tongue (the anterior region) had an average number of 72.00 ± 0.84 taste buds, whereas the last 4 mm only had a mean of 55.80 ± 0.86 taste buds. Statistical analysis indicated a significant regional difference in the counts of taste buds between the anterior and intermediate region on the mice tongue. These results were in agreement with Miller (1986) who showed that the average taste bud densities on the tip region and the mid-region of the mouse tongue were 116/cm^2 and 25.2/cm^2, respectively, and that the taste bud density was 4.6 times higher on the tip than on the mid-region. Similar result was also reported by Cheng and Robinson (1991) that a mean of 193 taste buds were on FF papillae of the human tongue and that 87% of them are located in the anterior 2 cm.

In addition, the size of taste buds and the count of cells within a single taste bud on the mid-region portion of the tongue were bigger than those on the tip region portion of tongue in our test. These data were similar to those found in the rat (Krimm and Hill 1998) and hamster (Whitehead et al. 1999). Krimm and Hill (1998) found that tip region taste buds were smaller than mid-region taste buds. In the rat and hamster, these differences were related with differences in innervation patterns, with larger taste buds innervated by more

ganglion cells than smaller ones (Krimm and Hill 1998; Whitehead et al. 1999). Also, these regional differences in density and size of taste bud could be ascribed to regional differences in cellular/molecular factors related to taste bud cell death. Sun and Oakley (2002) found in the developing mouse tongue that the tongue on the anterior-most millimeter was dependent on epidermal growth factor (EGF) for normal development, whereas the mid-region of the tongue developed normally in the absence of EGF receptors. Conversely, brain-derived neurotrophic factor (BDNF) null mutant mice had more taste buds remaining on the tip of the tongue than on the more posterior regions (Mistretta et al. 1999).

Noteworthy to say, the diverse distribution pattern of FF on the tongue of mice and human indicates the functional differences, these are in agreement with the taste sensitivity diversity of the tongue. Physiological studies have shown that there was regional taste sensitivity to NaCl at different tongue zone of young people (Matsuda and Doty 1995). By using a signal detection procedure and a microprocessor-controlled gustometer to measure some subjects' sensitivity to 3 concentrations of NaCl on the tongue tip and on a region 3.0 cm posterior to the tongue tip, they found that these young subjects were more sensitive to NaCl on the tongue tip than on the more posterior stimulation site and no sex differences.

Taste-specific G-protein was first demonstrated in rats (McLaughlin et al. 1992) and then confirmed in man (Takami et al. 1994). The α-gustducin is believed to be a reliable marker of chemosensitive cells, and the observation of the α-gustducin immunoreactivity in the anterior and intermediate region of the tongue could provide information about the functional differences between the 2 regions. By tracing such immunoreactivity for α-gustducin we found that immunoreactive taste bud count was about 2 times higher on the anterior region than on the intermediate region, although there was no significant regional difference in the number of α-gustducin–positive cells in a single taste bud between the 2 regions of tongue. Gustducin is selectively expressed in 20–30% of taste receptor cells (TRCs) throughout the oral cavity (McLaughlin et al. 1992; Boughter et al. 1997; Kim et al. 2003; Shen et al. 2005). Because gustducin is itself a subset of TRCs that also express essential taste signal transduction molecules, such as phospholipase C-beta2, inositol 1,4,5-trisphosphate receptor type 3, and transient receptor potential cation channel, subfamily M, member 5 (Clapp et al. 2001; Pérez et al. 2003), the gustducin-positive cells in FF is essential for transducing the gustation signal, such as sweet or bitter. This regional α-gustducin expression pattern is consistent with the taste buds distribution pattern mentioned above.

Our experiments suggest that the taste buds on the anterior two-thirds of the mouse tongue demonstrate some anatomical and functional regional differences. These differences may be brought by the following potential causes, such as the different innervated scale of geniculate ganglion cells and regional differences in cellular/molecular factors (EGF or BDNF) related to taste bud cell death.

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