Acta histochemica **109** (2007) 486–490

Available online at www.sciencedirect.com
ScienceDirect

acta histochemica
www.elsevier.de/acthis

Differentiation of α-gustducin in taste buds of the mouse soft palate and fungiform papillae

Gen-Hua Zhang[a,*], Hai-Yun Zhang[a], Shao-Ping Deng[b], Yu-Mei Qin[b]

[a]*Laboratory of Sensory Science, Department of Biological Science, Changshu Institute of Technology, Nansanhuan Road, Changshu City, Jiangsu Province 215500, China*
[b]*Zhejiang Gongshang University, Hangzhou 310035, China*

Received 19 March 2007; received in revised form 9 May 2007; accepted 16 May 2007

KEYWORDS
α-gustducin;
Immuno-histochemistry;
Taste buds;
Soft palate;
Fungiform papillae;
Mice

Summary

We used α-gustducin, a type II taste-cell-specific G protein, to investigate the onset of taste transduction and its relation to the development of the soft palate (SP) and fungiform (FF) papillae taste buds in the mouse. Paraffin wax embedded sections were prepared from the SP and anterior region of the tongue of the mouse from birth until postnatal day (PD) 63. No α-gustducin-immunoreactive cells were observed on the day of birth. One day later, α-gustducin was immunolocalised in taste buds with pores with a relatively higher frequency recorded in the SP as compared with the FF papillae. The immunoreactive cells were spindle shaped with elongated processes extending from the base to the pore of the taste buds. On PD 7, the number of taste buds containing α-gustducin-immunoreactive cells in the SP was three times greater than that of FF papillae. Our results indicate that taste transduction is essentially acquired from the time of birth. Moreover, the onset of taste transduction by the SP taste buds developed earlier than that achieved by taste buds in the FF papillae.

Introduction

Taste perception is a critical sense for basic food appraisal and confers the organism with valuable discriminatory power. In mammals, taste perception is an oral chemical sense actuated by the receptor cells congregated within different subpopulations of taste buds, which become functionally mature a few days after birth (Hosley and Oakley, 1987; Miller and Smith, 1988; Farbman and Mbiene, 1991; Witt and Reutter, 1996; Harada et al., 2000). However, age-dependent changes in the structure and function of taste buds may influence taste perception. In fact, a histological studies in newborn rats showed that taste pores (the entrance of taste substances to taste bud cells (TBCs)) existed only in 14% of fungiform (FF) taste

*Corresponding author. Tel.: +86 512 52767806.
E-mail address: zgh@cslg.edu.cn (G.-H. Zhang).

0065-1281/$ - see front matter © 2007 Elsevier GmbH. All rights reserved.
doi:10.1016/j.acthis.2007.05.003

buds (taste buds on the anterior two-thirds of the tongue), but they were open in 53% or more in taste buds of the soft palate (SP), suggesting the importance of SP taste buds in neonates (Harada et al., 2000). A single taste bud consists of approximately 50 TBCs that are classified into four cell types (types I–IV) based on their ultrastructural features. Type II cells possess taste receptor molecules for sweet, bitter and umami and taste transduction-related proteins, e.g. α-gustducin, the α subunit of trimeric G-protein complex, which was first demonstrated in rats (McLaughin et al., 1992) and later confirmed in humans. Therefore, gustducin is considered to be a potent marker of mature chemosensitive cells (Boughter et al., 1997; Yang et al., 2000). However, more investigations are required to clarify the ontogeny of the gustatory epithelium. The present study aimed to trace the functional maturation time course of taste buds from birth to adults by monitoring α-gustducin immunolocalisation of type II cells in the taste buds of the SP and FF epithelium of the mouse.

Material and methods

Pregnant ICR mice were purchased from the Zhejiang Academy of Medical Science, and the day of birth was designated as postnatal day 0 (PD 0). The procedure employed to prepare tissue blocks embedded in paraffin wax was routine and as described previously (Harada et al., 2000). About 7 μm-thick serial sections were cut by microtome and mounted on poly-L-lysine-coated glass slides. Sections were deparaffinized in xylene and dehydrated in a graded series of ethanol. Sections were incubated for 10 min with 0.3% hydrogen peroxide in methanol to inhibit endogenous peroxidases. They were then incubated for 15–20 min with 3% normal goat serum (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), diluted in phosphate buffered saline (pH 7.4) with 1.5% bovine serum albumin (PBS/BSA). Afterwards, the sections were incubated overnight at 4 °C with the primary antibody, anti-α-gustducin (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), diluted 1:200. An avidin–biotin complex (ABC) technique was used to reveal sites of antigen–antibody reaction. For this, a commercial kit was used (Biotin SP-HRP, Dingguo Biotechnology Inc., Beijing, China). Kit instructions were followed with regard to dilution and incubation times. Peroxidase activity was revealed by incubating the slides with phosphate buffered saline (pH 7.4) containing 0.04% diaminobenzidine (DAB, Sigma; St. Louis, MO, USA) and 0.003% H_2O_2. Controls consisted of (1) omission of the primary antibody, (2) alteration of the sequence of primary antibody application or (3) omission of the second antibody in the immunolabeling steps. No controls exhibited immunolabeling. The immunolabeled sections were dehydrated through a graded series of ethanol, cleared in xylene, coverslipped with Permount (Fisher Scientific, New Jersey, NJ), and examined using a light microscope.

Mice at different postnatal ages were studied (day 0, day 1, or weeks 1, 2, 3, 7 and 9; $n = 3$ for each age). For numerical analysis, all profiles containing immunoreactive cells for α-gustducin were thoroughly traced in all examined sections, serially reconstructed on schematic drawings of the SP and the FF regions, and their numbers graphically represented (El-Sharaby et al., 2001a). Furthermore, 20 immunopositive taste buds were randomly selected on the SP and the most anterior tip-region of the tongue (FF papillae of the first millimeter) of each animal to count α-gustducin-immunoreactive cells. Every cell profile containing a nucleus was counted once. The total counts of α-gustducin-immunoreactive cells per taste bud in both regions were recorded at different ages.

To analyze differences between the two developmental curves (SP and FF), the two-sample Kolmogorov–Smirnov test was used, and results presented as means ± SEM.

Results

Using an immunohistochemical method, we investigated the number of taste buds containing α-gustducin-immunoreactive cells and the number of α-gustducin-immunoreactive cells in taste buds in the SP and FF of mice at different postnatal developmental stages.

At PD 0, no α-gustducin-immunoreactive cells were observed in the SP and FF. However, solitary ovoid or bipolar α-gustducin-immunoreactive cells were found among FF and SP at PD1 (Fig. 1a, d), and the SP contained a substantially higher number of taste buds containing immunoreactive cells (42.0 ± 1.7) compared with the FF (12.0 ± 1.2). At the same time, the immunoreactive cells within the taste buds in the SP (2.2 ± 0.1) were higher compared to the FF (1.5 ± 0.2). The immunoreactive cells were only demonstrated in the pored taste buds, while the taste buds without pores completely lacked evidence of immunoreactivity. The immunoreactive cells had a similar morphology in all taste bud populations examined. They were spindle shaped with elongated processes extending from the base to the pore of the taste buds. Intense