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Research Paper

Aging does not affect the proportion of taste cell types in mice

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ABSTRACT

Generally, taste sensitivity is known to change with age. However, the molecular mechanisms underlying this phenomenon remain unclear. Mammalian taste buds are classified into type I, II, III, and IV cells; among them, type II and III cells have an important role in the taste detection process. We hypothesized that age-related changes in the proportion of taste cell types would be a factor in changes in taste sensitivity. To test this hypothesis, we compared the expression patterns of type II and III cell markers in taste buds obtained from the circumvallate papillae of young and old mice. Gustducin, SEMA3A, PLCβ2, and CAR4 were used as type II and III cell markers, respectively. When we performed double-fluorescence staining using antibodies for these molecules, Gustducin and SEMA3A immune-positive cells were 22.7 ± 1.2% and 27.6 ± 0.9% in young mice and 22.0 ± 0.7% and 25.9 ± 1.1% in old mice, respectively. PLCβ2 and CAR4 immune-positive cells were 30.3 ± 1.5% and 20.7 ± 1.3% in young mice and 29.1 ± 0.8% and 21.1 ± 1.2% in old mice, respectively. There were no significant differences in the percentage of immunopositive cells for all antibodies tested between young and old mice. These results suggest that the proportion of type II and III cells does not change with aging.

1. Introduction

In countries where the aging population has progressed, maintaining the health of the elderly is an important social issue. A well-balanced diet is essential for maintaining good health. Taste is a chemical sense that not only detects nutrients but also potentially harmful non-nutritive substances. Therefore, preserving taste function is significant for maintaining a balanced diet habit.

Several reports have indicated that taste sensitivity gradually changes in an age-dependent manner (Bartoshuk et al., 1986; Narukawa et al., 2020; Narukawa et al., 2017; Thaw, 1996); e.g., we have reported that old B57BL/6 J (B6) mice (over 100 weeks old) exhibited significant changes in their taste sensitivities for bitter and salty tastes (Narukawa et al., 2020; Narukawa et al., 2017). However, the molecular mechanisms underlying this phenomenon remain unclear.

Taste is classified into five categories: sweet, sour, salty, bitter, and umami. Taste processing begins with molecular events that occur at the surface membranes of taste cells, which are organized in taste buds within the fungiform, foliate, and circumvallate papillae (CvP) on the tongue. Mammalian taste buds are classified into four cell types (Type I - IV cells) with distinct morphological and functional characteristics (Roper, 2013); Glial-like type I cells are involved in maintaining overall taste bud structure. Type II cells play a role in transducing sweet, umami, and bitter tastes. G protein-coupled receptors, sweet receptor Tas1r2 +Tas1r3, umami receptor Tas1r1 +Tas1r3, and bitter receptor Tas2rs, are involved in detecting these tastes. Each of these receptors is exclusively expressed. Sweet, umami, and bitter taste signal via a G protein-coupled transduction cascade. When taste stimuli activate their receptors in type II cells, phospholipase Cβ2 (PLCβ2) is activated, leading to increased cytosolic inositol trisphosphate (IP_3) levels. IP_3 binds to the IP_3 receptor isoform 3 (IP_3R3) expressed on the endoplasmic reticulum, which elicits Ca^{2+} release from intracellular stores. Type II cells typically do not form conventional synapses with nerve terminals in taste buds, but they are thought to use adenosine triphosphate (ATP) as a transmission signal to primary afferents. Type III cells are also called presynaptic cells and respond to sour taste. Recently, otopetrin 1 has been identified as a sour taste receptor (Teng et al., 2019). Type III cells typically synapse directly with afferent nerve fibers from cranial nerves and can release serotonin upon depolarization. Type III cells express proteins associated with synaptic transmission, including SNAP 25, voltage-gated Ca^{2+} channels, and biosynthetic enzymes for serotonin. Type IV cells are progenitors that can differentiate into the other three cell types. Taste cells turn over throughout life. The average turnover rate for taste cells has been reported to be approximately 8–12 days (Beidler and Smallman, 1965; Farbman, 1980). Even if taste cells are replaced continuously, we can still taste food properly, which means

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that the differentiation mechanism is definitely controlled.

Here, we hypothesized that age-related changes in the proportion of taste cell types would be a factor in changes in taste sensitivity. To test this hypothesis, we compared the expression patterns of type II and III cell markers in taste buds obtained from the circumvallate papillae of young and old mice. The G protein which is coupled with the bitter taste receptor, Gustducin (Clapp et al., 2001), the guidance molecule produced by bitter taste-responsive cells, Semaphorin 3 A (SEMA3A) (Lee et al., 2017), and the signaling enzyme in bitter, umami, and sweet signaling, PLCβ2 (Zhang et al., 2003), were used as type II cell markers. the enzyme associated with the taste of carbonation, carbonic anhydrase IV (CAR4), was used as a type III cell marker (Chandrashekar et al., 2009). First, we confirmed the decrease in bitter sensitivity, which is a typical taste phenotype of aging (Narukawa et al., 2020; 2017). Next, we performed double fluorescent immunostaining using combinations of anti-Gustducin and SEMA3A antibodies and/or anti-PLCβ2 and CAR4 antibodies.

2. Materials and methods

2.1. Animals

The study population comprised male B6 mice (CLEA Japan, Tokyo, Japan). The animals were housed at the Kyoto Women's University Animal Care Facility and had ad libitum access to standard laboratory chow (CE-2, CLEA Japan) and distilled water. The surrounding temperature and humidity were maintained at 23 °C and 55%, respectively, with a 12-h/12-h light/dark cycle (lights switched on at 0700 h). We divided the mice into young (aged 11 to 24 weeks, n = 4) and old groups (aged 108 to 121 weeks, n = 5). Mice of normal sizes and with typical feeding behaviors were used.

2.2. Forty-eight-hour two-bottle preference test

Mice were caged individually and given 48 h of access to two bottles, one containing deionized water and the other containing a tastant solution. After 24 h, the bottle positions were switched to avoid positional effects. The ratio of tastant volume to total liquid consumed was recorded. The preference ratios of the tastants were calculated as follows: tastant intake/total fluid intake (tastant intake + water intake). Denatonium benzoate (0.3 mM) was used as a tastant solution. Mice were habituated by presenting two bottles that contained water for only one week before starting the preference test, and it was confirmed that the mice drank water evenly from both bottles containing water.

2.3. Double fluorescent immunostaining

For the CvP preparations, mice were sacrificed by an overdose of intraperitoneal sodium pentobarbital and transcardially perfused with ice-cold phosphate-buffered saline (PBS), followed by treatment with 4% paraformaldehyde (PFA) in PBS. The CvPs were dissected, postfixed in 4% PFA/PBS at 4 °C overnight, cryoprotected in 20% sucrose/PBS at 4 °C at least until they sink, and frozen in an O.C.T. compound (Sakura Finetek, Tokyo, Japan). The frozen blocks containing CvP were stored at –80 °C until use. The CvPs were sectioned at 10 μm with a cryostat (Cryostar NX70; Thermo Scientific, Waltham, MA). The sections were mounted onto MAS-coated glass slides (Matsunami Glass, Osaka, Japan) and stored at –80 °C until further use.

The CvP sections were washed with PBS and incubated in antigen retrieval solution for 20 min at 80 °C (Dako Target Retrieval Solution, pH 9, Agilent Technologies, Santa Clara, CA). For double-staining using anti-Gustducin and SEMA3A antibodies, the sections were incubated in MOM Mouse Ig Blocking Reagent (Vector MOM Immunodetection Kit; Vector Laboratories, Burlingame, CA) for 1 h at room temperature. The sections were washed twice with PBS, blocked in MOM diluent for 5 min at room temperature, and incubated overnight at 4 °C with rabbit anti-Gustducin (1:500; sc-395, Santa Cruz Biotechnology) and mouse anti-SEMA3A antibodies (1:300; sc-74554, Santa Cruz Biotechnology, Santa Cruz, CA). After washing with PBS, the sections were incubated with the secondary antibodies Alexa Fluor 488 donkey anti-rabbit IgG (1:500; A21206, Thermo Fisher Scientific, Waltham, MA) and Alexa Fluor 555 donkey anti-mouse IgG (1:500; A31570, Thermo Fisher Scientific) for 1 h at room temperature. For double-staining using anti-PLCβ2 and CAR4 antibodies, the slides were then blocked with PBS containing Blocking One Histo (Nacalai Tesque, Kyoto, Japan) and incubated overnight at 4 °C with rabbit anti-PLCβ2 (1:500; sc-206, Santa Cruz Biotechnology) and goat anti-CAR4 antibodies (1:500; AF2414, R&D Systems, Minneapolis, MN). The sections were then washed with PBS and incubated with the secondary antibodies Alexa Fluor 488 donkey anti-rabbit IgG and Alexa Fluor 555 donkey anti-goat IgG (1:500; A21432, Thermo Fisher Scientific) for 1 h at room temperature. After the secondary antibody step, the sections were washed with PBS and incubated with 4′,6-diamidino-2-phenylindole (DAPI) (1 μg/ml) for 5 min. The sections were mounted with Fluoromount G (SouthernBiotech, Birmingham, AL). The fluorescent and differential interference contrast (DIC) images were captured using a BX60 microscope (Olympus, Tokyo, Japan) equipped with an AdvanCam-E3R digital camera (Advan Vision, Tokyo, Japan). The brightness and contrast of the images were adjusted, and/or each image was merged using Adobe Photoshop Elements 14 (Adobe Systems, San Jose, CA).

The cells with immunoreactive signals were counted in three or four sections (every 50 μm) per the CvP sample. Taste bud cells were identified from DIC and DAPI images. The number of immunoreactive cells was determined by comparing DIC, DAPI, and fluorescence images. The percentage of immunoreactive cells was expressed as the number of immunopositive cells per taste bud cell in a taste bud. The total number of taste cells counted is as follows: for young mice, 3405 cells for anti-Gustducin and SEMA3A antibodies, and 3387 cells for anti-PLCβ2 and CAR4 antibodies; for old mice, 5052 cells for anti-Gustducin and SEMA3A antibodies, and 4750 cells for anti-PLCβ2 and CAR4 antibodies.

2.4. Statistical analysis

The statistical significance of differences between the groups was analyzed by Welch's *t* test using Prism 6 software (Graph Pad Software, San Diego, CA, USA). The data are expressed as the mean ± standard error of the mean (SEM). For all analyses, differences were considered to be significant at $p < 0.05$.

3. Results

3.1. Change in bitter preference due to aging

First, we assessed the decrease in taste sensitivity to bitter taste. The preference ratio for 0.3 mM denatonium benzoate was significantly increased in old mice compared to that in young mice ($p < 0.01$, Fig. 1).

3.2. Comparison with Gustducin and SEMA3A expression in taste bud cells of the CvPs

Fig. 2A shows the staining images for anti-Gustducin and SEMA3A antibodies. The percentages of Gustducin-positive cells per taste bud in young and old mice were 22.7 ± 1.2% and 22.0 ± 0.7%, respectively ($p = 0.82$, Fig. 2B). The percentages of SEMA3A-positive cells per taste bud in young and old mice were 27.6 ± 0.9% and 25.9 ± 1.1%, respectively ($p = 0.09$, Fig. 2B). The percentages of cells coexpressing Gustducin and SEMA3A were 15.1 ± 0.9% and 14.3 ± 0.8%, respectively ($p = 0.63$, Fig. 2B). There were no significant differences in either percentage between young and old mice.

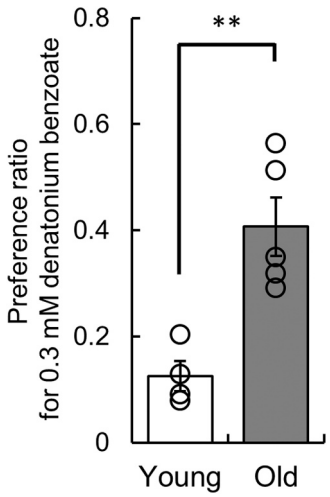

Fig. 1. Comparisons of the preference ratio for bitter taste between young and old mice. ** indicates $p < 0.01$ (n = 5, Welch's *t* test).

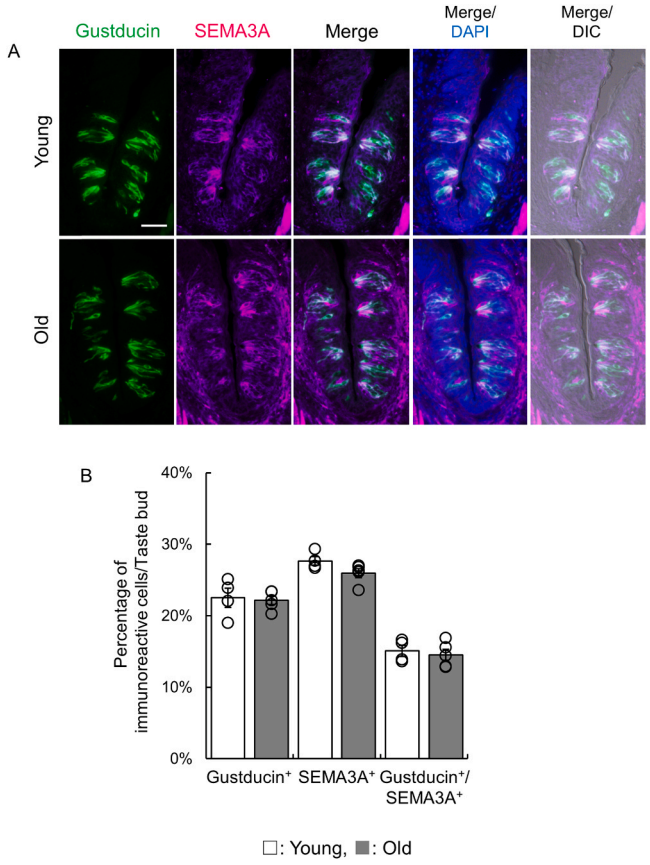

Fig. 2. Expression of Gustducin and SEMA3A in the circumvallate papillae of young and old mice. A) Double-fluorescent immunostaining was examined using antibodies against Gustducin (green) and SEMA3A (magenta). Scale bar is 50 μm. B) Percentage of Gustducin- and/or SEMA3A-immunoreactive cells per taste bud.

3.3. Comparison with the expression of PLCβ2, a type II cell marker, and CAR4, a type III cell marker, in taste bud cells of the CvPs

Fig. 3A shows the staining images for anti-PLCβ2 and CAR4 antibodies. The percentages of PLCβ2-positive cells per taste bud in young and old mice were 30.3 ± 1.5% and 29.1 ± 0.8%, respectively

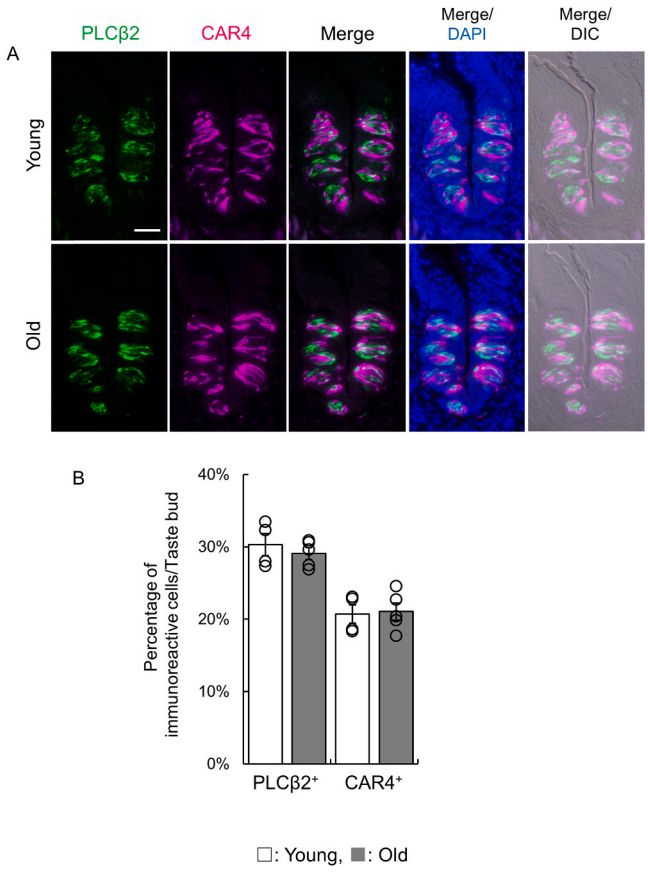

Fig. 3. Expression of PLCβ2 and CAR4 in the circumvallate papillae of young and old mice. A) Double-fluorescent immunostaining was examined using antibodies against PLCβ2 (green) and CAR4 (magenta). Scale bar is 50 μm. B) Percentage of PLCβ2- or CAR4-immunoreactive cells per taste bud.

($p = 0.52$, Fig. 3B). The percentages of CAR4-positive cells per taste bud in young and old mice were 20.7 ± 1.3% and 21.1 ± 1.2%, respectively ($p = 0.85$, Fig. 3B). There were no significant differences in the percentage of PLCβ2- and CAR4-positive cells between young and old mice.

4. Discussion

Generally, taste sensitivity is known to change with age. A decrease in appetite due to decreased taste sensitivity leads to malnutrition, which in turn causes a decrease in muscle mass and physical activity level. Decreased taste sensitivity affects not only appetite but also health problems, and it is important to maintain taste function throughout life. In this study, to investigate the cause of the change in taste sensitivity due to aging, we observed whether the proportion of taste cell types changes with age.

Taste cells are classified into types I to IV. Type II cells are involved in sweet, umami, and bitter tastes, whereas type III cells are involved in sour taste (Roper, 2013). The detection of salty taste continues to be a matter of debate. Salty taste is divided into two components: amiloride-sensitive (AS) and amiloride-insensitive (AI) components (Kasahara et al., 2022). The epithelial sodium channel and transmembrane-like channel 4 are thought to be involved in the detection of AS and AI components, respectively (Kasahara et al., 2022). It has been suggested that type II cells are involved in the detection of AS component (Ohmoto et al., 2020), while bitter-sensing and sour-sensing taste cells are involved in the detection of AI component (Oka et al., 2013); this means that type II and III cells play an important role in taste detection. Therefore, type II and III cell markers were used in this study.

The three papillae are innervated by the chorda tympani (CT) or

glossopharyngeal (GL) nerve. The fungiform papillae are innervated by the CT, the CvP is innervated by the GL nerve, and the foliate papillae are innervated by both (Yamamoto and Kawamura, 1975). Since it is known that the bitter response in the GL nerve was observed to be stronger than that in the CT nerve (Danilova and Hellekant, 2003), we analyzed taste bud cells of the CvP in this study.

Semaphorins play a role in neuronal wiring (Tran et al., 2007). It has been reported that SEMA3A is highly enriched in bitter-sensing cells and acts positively to promote the formation of connections with bitter neurons (Lee et al., 2017). The decrease in bitter sensitivity is a typical taste phenotype of aging in B6 mice (Narukawa et al., 2020; Narukawa et al., 2017). Considering the possibility that the decrease in bitter sensitivity is caused by a mismatch in the expression pattern of SEMA3A, we investigated the expression pattern between SEMA3A and Gustducin, which is a G protein that couples with bitter taste receptors (Clapp et al., 2001). A decrease in bitter sensitivity was observed in the old mice used (Fig. 1). However, no significant differences were observed in the percentage of cells that expressed SEMA3A and/or Gustducin or the percentage of cells that expressed both SEMA3A and Gustducin (Fig. 2). Therefore, it was considered that the decrease in bitter sensitivity was not caused by a mismatch in SEMA3A expression.

Simultaneously, using representative type II and III cell markers, PLCβ2 and CAR4, we examined whether the proportion of taste cell types was changed by aging (Fig. 3). Fig. 4 shows the composition of taste cells in young and old mice. In Fig. 4, the cells categorized as others correspond to type I cells. There was no significant difference in the proportion of any cell type between the two groups. It is known that type I, II, and III cells make up approximately 50%, 30%, and 20% of the taste buds, respectively (Kinnamon and Margolskee, 2008). This result indicates that the proportion of taste cell types does not change with aging.

In our previous study using B6 mice, aging-induced changes in taste sensitivity did not affect all taste qualities, indicating the existence of certain taste quality-specific changes, such as bitter and salty taste. In that study, the mRNA expression of taste-related genes in taste buds was investigated, but there were no significant differences in the expression levels of taste receptors. Although the mRNA expression of signaling effectors was significantly decreased in the old mice, the degrees of the decrease were slight (Narukawa et al., 2017). PLCβ2 is a common signaling enzyme in bitter, umami, and sweet taste signaling (Zhang et al., 2003). Thus, although we expected that the sensitivities to umami and sweet tastes may be affected by aging as well as the sensitivity to bitter taste, the behavioral responses to umami and sweet tastes were free of the influence of aging. On the other hand, in other research using SAMP1 mice, although taste sensitivity to bitter and salty tastes increased with aging, the mRNA expression of the bitter taste receptors Tas2r105 and PLCβ2 decreased with aging in SAMP1 mice (Narukawa et al., 2018). Thus, the expression levels of peripheral taste-related molecules and changes in taste behavior do not necessarily correspond. This suggests that the aging-related changes in taste sensitivity do not involve the tested main taste-related molecules.

What is the cause of age-dependent changes in taste sensitivity? We are focusing on brain function and saliva components. It has been reported that age-related changes in taste function are associated with changes in neuronal circuits (Iannilli et al., 2017). On the other hand, it is thought that salivary secretion decreases with age (Vissink et al., 1996). In fact, we observed that the protein concentration in saliva and taste behaviors in response to basic taste were significantly changed by salivary gland removal (Narukawa et al., 2024). We are investigating whether these factors contribute to age-related changes in taste sensitivity.

5. Conclusions

In this study, we observed whether the proportion of taste cell types changes with age. There was no significant difference in the proportion of taste cell types between young and old mice, which suggests that aging did not affect the proportion of taste cell types. Our results provide useful information for understanding the mechanisms of age-related changes in taste sensitivity.

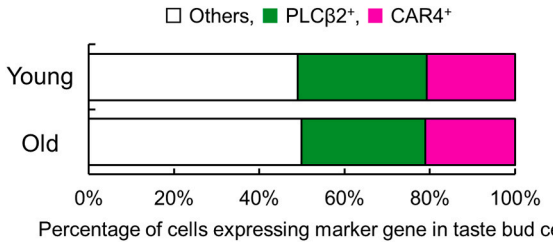

Fig. 4. Populations of PLCβ2 (type II cells) and CAR4 (type III cells) immunoreactive cells. The remaining PLCβ2- and CAR4-immunoreactive cells were categorized as others.

Ethical statement

Animal experiments were conducted in accordance with protocols approved by The Kyoto Women's University Animal Care Committee (Approval Number: 2022-7). Every effort was made to minimize animal suffering, discomfort, and the total number of animals needed to obtain reliable results.

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CRediT authorship contribution statement

M. Narukawa designed the research; H. Oka and M. Narukawa performed research and analyzed data; M. Narukawa wrote the paper.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data Availability

Data will be made available on request.

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