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Analysis of aging-dependent changes in taste sensitivities of the senescence-accelerated mouse SAMP1

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ABSTRACT

To investigate aging-dependent changes in taste sensitivities, we performed behavioral tests regarding taste sensitivity among young and old SAMP1 mice. In this senescence-accelerated mice model, dramatic changes in taste sensitivities were observed at least 70 weeks old. As for in a brief access test, old mice showed significantly increased taste sensitivity to bitter, salty, sweet, and umami tastes. On the other hand, in a two-bottle test, avoidance of bitter and salty tastes increased, while preference for umami decreased with aging. To investigate the participation of peripheral taste detection systems in the observed changes, we analyzed both the expression of representative taste-related molecules and also turnover rates of taste bud cells. The mRNA expressions of the bitter taste receptor *Tas2r105* and its coupled G protein *gustducin* were significantly decreased with aging. However, the majority of molecules tested did not show significant expression changes. In addition, no significant differences in the turnover rates of taste bud cells were observed between the two age groups. These results suggest that the changes in taste sensitivity of SAMP1 mice due to aging are caused by factors other than the deterioration of taste detection systems in the oral cavity.

1. Introduction

Taste is a vital sense for nutritional intake and consequently for maintenance of health and longevity. In general, the pleasure derived from eating food diminishes with age; this is partly due to deterioration of smell and taste sensations. Elucidating the aging-dependent changes in taste cognition is important for better understanding the factors that govern changes in taste perception during an individual's lifespan.

The taste of food is classified into five basic categories: salty, sweet, bitter, sour, and umami. Taste is detected by taste cells; these cells transmit information to the gustatory nerves, which connect to the central nervous system (Yarmolinsky et al., 2009). Recent studies have identified taste receptors and taste-related molecules in taste bud cells. Tas1r2/Tas1r3, Tas1r1/Tas1r3, and Tas2rs act as the receptors for sweet, umami, and bitter tastes, respectively. Transient receptor potential channel type M5 and phospholipase Cβ2 (Plcβ2) are known to be the downstream signaling effectors of these taste receptors. Polycystic kidney disease (Pkd) 2 l1/Pkd1l3 and epithelial sodium channel (ENaC) are candidates for the sour and salty taste receptors, respectively (Yarmolinsky et al., 2009). Mammals show attractive behavior towards low concentrations of salty taste, but exhibit aversive behavior to its high concentration (Chandrashekar et al., 2010; Oka et al., 2013). Although the mechanism for perception of salty taste remains a matter of debate, it is generally accepted that ENaC-expressing taste cells detect low concentrations of salt, whereas cells that express the bitter taste receptor Tas2rs and sour taste receptor Pkd2l1 detect high salt concentrations (Chandrashekar et al., 2010; Oka et al., 2013).

Several studies have investigated the correlation between aging and taste sensation in rodents. Thaw reported increases in taste threshold for sucrose and NaCl in old rats (Thaw 1996). Tordoff reported an increase in palatability for NaCl in 125-week-old mice (Tordoff 2007). Conversely, Shin et al. reported that although sweet sensitivity decreases in 18-month-old mice, there are no other age-related changes in taste perception (Shin et al., 2012). Inui-Yamamoto et al. reported that in rats, the preference for sucrose and MSG decreases with age, whereas the preference for quinine-HCl increases (Inui-Yamamoto et al., 2017). Although these reports produced inconsistent results, they all suggest that aging modifies taste sensitivity. As one of the reasons for the aging dependent changes in taste sensitivities, it has been suggested that the number of taste papillae and/or taste buds decreases with aging (Arey et al., 1935; Shimizu 1997). However, several studies have reported that the number of taste papillae and/or taste buds do not drastically

Abbreviations: BrdU, 5-bromo-2′-deoxyuridine; CvP, circumvallate papillae; ENaC, epithelium sodium channel; FuP, fungiform papillae; IMP, inosine 5′-monophosphate; MSG, monosodium glutamate; Pkd, polycystic kidney disease; Plcβ2, phospholipase Cβ2; SAM, senescence-accelerated mouse

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change with age (Arvidson 1979; Bradley et al., 1985; Mistretta and Baum 1984). As such, the factors affecting changes in taste sensitivity remain poorly understood. We have previously compared the peripheral taste detection systems of young and old C57BL/6 (B6) mice, in order to investigate the causes of aging-dependent changes in taste sensitivity (Narukawa et al., 2017). Although an aging-associated change was observed, we concluded that this was not caused by aging-related degradation of peripheral taste organs.

Rodents, such as rats and mice, are frequently used in aging studies. They have approximately a two and a half year lifespan, which although shorter than that of a human being, still requires a long wait to reach an aged state. Therefore, in aging studies, shortening the aging period is advantageous. The senescence-accelerated mouse (SAM) is an inbred strain that shows hastened aging, compared to a general mouse strain; SAM median survival time is 9.7 months (Takeda 1999). It is known that senile amyloidosis, hyperinflation of the lungs, hearing impairment, and hypertensive vascular disease are characteristic pathobiological phenotypes in SAMP1 mice (Takeda 1999). It has been reported that these phenotypes appear at different stages of breeding. For example, the incidence of amyloidosis is recognized after 5 months of age (Takeshita et al., 1982), while morphometric changes in the lungs begin starting at 2 months of age (Kurozumi et al., 1994). Thus, by using the SAM strain, we can expect any effect of aging on taste sensitivity to be observed within a shorter experimental period. Furthermore, by comparing results obtained from B6 and SAMP1 mice, we aim to pinpoint the factors that elicit aging-associated changes in taste sensitivity.

In this study with SAMP1, we first used behavioral tests to investigate whether taste sensitivities were changed due to aging or not. We then analyzed the expression of taste-associated molecules and turnover rates of taste bud cells to identify the cause of taste sensitivity changes. Finally, to investigate other aging-related factors affecting taste sensitivity, we measured the concentrations of several serum components that modify taste responses.

2. Materials and methods

2.1. Materials

Citric acid, NaCl, and sucrose were purchased from Kanto Chemical (Tokyo, Japan). Denatonium benzoate (denatonium), monosodium glutamate (MSG), and inosine 5′-monophosphate (IMP) were purchased from Sigma. All other reagents were of analytical grade and obtained from standard suppliers.

2.2. Animals

The SAM is consisting of 9 senescence-prone inbred strains (SAMP). Among the commercially available SAMP1, P6, P8, and P10 strains, SAMP1 strain was used for this study, because it had the highest grading score of senescence (8.72) at 8 months of age (Takeda et al., 1994). Seven-weeks male mice were purchased from Japan SLC (Hamamatsu, Japan), and were housed at The University of Tokyo Animal Care Facility until the beginning behavioral experiments. The mice had *ad libitum* access to a standard laboratory chow 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 0800 h). We divided the mice into two age groups: young group aged 8–20 weeks and old group aged 69–83 weeks. In our preliminary study using SAMP1 mice, because apparent change to taste sensitivities was observed using 70 weeks of aged mice, the behavioral experiments were initiated using about 70 weeks of aged mice. SAMP1 mice with normal shapes and feeding behaviors were used. All experiments were performed in accordance with protocols approved by The University of Tokyo Animal Care Committee (Approval Number: P10–457).

2.3. Grip strength test

Before the beginning at the behavioral tests, the grip strength for forelimb was measured by Grip Strength Meter (GPM-101B; Melquest, Toyama, Japan). As a mouse grasped the bar, the peak pull force in grams was recorded on a digital force transducer. Measurements were repeated three times, and the maximum tension from the three measurements was used in the analyses.

2.4. Behavioral experiments

Each age group was divided into three subgroups. One performed a brief access test, followed by a 48-h two-bottle preference test. The other performed the 48-h two-bottle preference test, followed by the brief access test. Subsequently, taste bud and blood samplings were performed. For immunohistochemistry, we prepared another group.

2.4.1. Brief access test

Young mice (n = 10–11) and old mice (n = 5–6) were used for the brief access test, which was performed for 2 weeks. The numbers of licks to aversive and attractive taste substances were measured in the first and second weeks, respectively. The mice were applied both training procedures to assess both aversive and attractive components of salty taste.

Evaluation of aversive taste substances in the first week: Each animal with 23-h water deprivation was placed in a test cage on day 1 of training and given free access to distilled water during a 1-h session. The number of licks per 5 s was measured by a lickometer (Med associates, Fairfax, VT). Days 2–3 were the training session. During this period, the animal was trained to drink distilled water on an interval schedule, consisting of 5-s periods of presentation of distilled water with 30-s intervals. Days 4–6 were the test session. The numbers of licks for denatonium, citric acid, NaCl (aversive component), and distilled water by each animal were counted during the first 5 s after the animal's first lick. After the test session, the mice were rested.

Evaluation of attractive taste substances in the second week: Each animal was limited in its water intake to the average intake of water during the normal breeding period. Days 8–10 were the training session. On days 11–13, the numbers of licks for sucrose, MSG + IMP, and NaCl (attractive component) by each animal were counted during the first 5 s after the animal's first lick.

The taste sensitivities to tastants were expressed as lick ratios. The lick ratios of the tastants were calculated as follows: number of licks of tastant/number of licks of water. When the lick ratios to attractive taste substances were calculated, the average lick numbers for water during aversive taste test sessions were utilized. To avoid restriction effects, data for mice whose body weight fell below 80% of the *ad libitum* normal free-feeding value were excluded from the analyses. Tastant solutions for the brief access test were (in mM): 0.1–100 citric acid, 0.03–10 denatonium, 10–1000 NaCl, 0.3–300 sucrose, and 1–300 MSG + 0.5 IMP.

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

Young mice (n = 10–11) and old mice (n = 4–9) 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 each other for positional effects. The preference ratios of the tastants were calculated as follows: tastant intake/total fluid intake (tastant intake + water intake). The tastant solutions for the two-bottle preference test were (in mM): 1–10 citric acid, 0.3–3 denatonium, 10–500 NaCl, 10 and 30 sucrose, and 0.1–100 MSG + 0.5 IMP.

2.5. Real-time RT-PCR

The liver and tongue of the mice were removed after the behavioral