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Diverse contributions of Tas1r2/Tas2rs within the rat and mouse soft palate to sweet and bitter neural responses

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HIGHLIGHTS

- The GSP responds robustly to sweet stimuli in rat and bitter stimuli in mouse.
- Threshold of the sweet response in rat and bitter response in mouse is lower than in other tested species.
- The number of Tas2rs cells is twice larger than that of Tas1r2 cells in rat SP.
- The number of Tas1r2 cells is similar to that of Tas2rs cells in mouse SP.
- Sweet/bitter GSP responses correlate with the respective receptor density in taste cells.

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ABSTRACT

Neural responses to sweet and bitter stimuli in the rat and mouse are compared to the expression of the molecular taste receptors, Tas1r2/Tas2rs. Integrated taste responses from the greater superficial petrosal nerve (GSP) innervating the soft palate (SP) and the chorda tympani (CT) nerve innervating the fungiform papillae (FF) were recorded in C57BL mice and SD rats. The sum of the phasic and tonic response magnitudes (SRM) was calculated by summating all relative mean responses to a concentration series of QHCl (10^{-6}–10^{-2} M) or Suc (10^{-4}–1.0 M). Molecular expression was analyzed by double-colored *in situ* hybridization for Gα-gustducin with Tas1r2 or Tas2rs in the SP and FF. The vast majority of cells expressing Tas1r2 or Tas2rs were included in Gα-gustducin-expressing cells in the SP of both species. Unexpectedly, a comparison between species revealed that the SRM from the GSP is not positively correlated with receptor expression in the SP. In the rat SP, the percentage of Tas2rs with Gα-gustducin (Tas2rs/gust, 65%) was twice larger than that for Tas1r2/gust (33%), while the SRM to Suc in the rat GSP was 1.5 times (tonic and phasic) larger than that to QHCl. In the mouse SP, the percentage of Tas2rs/gust (46%) was less than that in the rat and similar to that of Tas1r2/gust (40%). However, the SRM to QHCl in the mouse GSP was 2.4 (phasic) and 4.7 (tonic) times larger than to Suc. On the other hand, threshold to Suc in the rat GSP was 10^{-3} M, one log unit lower than in mouse, and the threshold to QHCl in the mouse GSP was 10^{-6} M, one log unit lower than in rat. These results suggest that the robust GSP response to Suc in rat and to QHCl in mouse likely do not depend upon a large number of taste cells expressing the taste receptors Tas1r2 for Suc or Tas2rs for QHCl, but upon a higher density of Tas1r2/Tas2rs within the respective taste cells of the two species.

1. Introduction

In rat and hamster, sweet compounds produced more robust taste activity from the greater superficial petrosal nerve (GSP) that innervates the soft palate (SP) taste buds [5,6,11] than from the chorda tympani nerve (CT) that innervates taste buds within the fungiform papillae (FF). Also, behavioral experiments revealed a contribution of the GSP to the taste of sucrose (Suc) [4,9]. Although robust taste responses to sweet stimuli were obtained from the GSP in both rat and hamster, the GSP of the mouse produced huge neural responses to quinine-HCl (QHCl) [14].

Gα-gustducin, an alpha subunit of the G-protein expressed in type II taste cells, is involved in both sweet and bitter signal transduction in C57BL mouse [3,7,14,15]. Due to the remarkable sweet sensitivity of the GSP in the rat and hamster, Stone et al. [13] examined the expression of Tas1rs and Gα-gustducin in palatal taste

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buds of the mouse and found that Gα-gustducin was coexpressed more with Tas1r2 than with either Tas1r1 or Tas1r3. In keeping with this expression pattern, GSP responses to bitter and sweet compounds were markedly reduced in Gα-gustducin knockout mice [14].

A regional-dependent selective expression of sweet and bitter taste receptor cells on the tongue was suggested [8,10]. The Tas2r family serves as bitter taste receptors [1,2]. In the circumvallate papillae (CV) of mouse, Gα-gustducin is coexpressed predominantly with bitter receptors and rarely with sweet receptors [1,8]. In contrast, Gα-gustducin is co-expressed with bitter receptors (SP 87%, FF 88%) or sweet receptors (SP 89–91%, FF 85–90%) in mouse [14]. However, the proportional differences of sweet receptor cells *versus* bitter receptor cells in these regions between rat and mouse were not examined.

The mechanisms accounting for these highly divergent response properties in the GSP between the two rodents are unknown. The present report compares the rat and mouse GSP responses to Suc and QHCl with respect to regional differences of expression in the SP of the taste receptors, Tas2rs and Tas1r2.

2. Materials and methods

2.1. Animals

Adult male mice (C57BL) and male rats (Sprague Dawley) were studied. Each animal was anesthetized deeply with sodium pentobarbital (50 mg/kg) for neurophysiology and with an overdose (250 mg/kg) for the histological experiments. All animal experimentation was conducted at Kagoshima University, and all experimental procedures were approved by the institutional animal care and use committee.

2.2. Electrophysiological recordings

The GSP and CT were dissected as previously described [9,10]. Responses from the whole nerve were amplified, integrated and displayed on a thermal array recorder at a speed of 1 mm/s as described [6].

Taste stimuli were applied to a deionized water (DW) rinse (1 ml/s) through polyethylene tubing (2 mm id. for the rat, 1 mm id. for mice). The constantly flowing rinse water was switched to the stimulus solution for 10 s. The concentration of the stimulus solution reached its maximum within one second. Four basic taste stimuli tested were 0.1 M NaCl, 0.01 M quinine hydrochloride (QHCl), 0.5 M sucrose (Suc), 0.01 M HCl, and sequential 1/2 log step increases in concentration of sucrose (10^{-4}–1.0 M), and QHCl (10^{-6}–10^{-2} M). Stimuli and rinse solutions were presented at 20 ± 1 °C.

The height of the peak of the initial phasic response and the height of the tonic portion at 10 s after stimulus onset were measured. The phasic response to the standard stimuli (0.1 M NaCl) was used as the standard response magnitude. Response magnitudes for each stimulation were calculated relative to the magnitude of the standard phasic response. Although the response magnitude at a particular concentration provided a simple response index, the responses of each nerve are represented by the slope and curvature of the concentration-response-curve rather than by the peak response magnitude or some other parameter at a particular concentration for each taste. Therefore, to obtain a better measure for comparison of the response ratio, the sum of the phasic and tonic response magnitudes (SRM) to QHCl and Suc were calculated individually by summating all relative response magnitude to each stimulus at each concentration between 10^{-6}–10^{-2} M for QHCl and 10^{-4}–1.0 M for Suc.

2.3. In situ hybridization

The SP and FF of adult male SD rats were excised and frozen in embedding compound. Sagittal sections of whole SP and coronal section of anterior one-fourth of tongue (FF) were made at 5 μm. Antisense complementary RNA (cRNA) probes were generated with digoxigenin- or fluorescent-UTP from the following rat cDNA: Tas1r2 (1123-1623: Genbank X65747), Tas2rs [T2R1 (1-1008: AF227140), T2R3 (1-900: AF227141), T2R7 (1-918: AF227144), T2R9 (1-930: AF227146), gift from Dr. Mark Hoon], and Gα-gustducin (1123-1623: X65747, gift from Dr. Keiko Abe). A mixture of 4 Tas2r cRNA probes was used to detect Tas2rs.

Double-colored *in situ* hybridization was performed as described previously [14]. The sections were hybridized with the combination of two cRNA probes of Tas1r2, Tas2rs and Gα-gustducin differently labeled with digoxigenin- or fluorescent-UTP. Fluorescent images were captured with a Retiga EX camera (Rooper, Retiga-LM) and a DM-IRB microscope (Leica Microsystems). Each signal with an unstained nuclear profile in every fourth section was analyzed in order to avoid any double counting of the gene-expressing cells. Double-color images were merged using Photoshop (Adobe Systems). No co-localization of Tas1r2 and Tas2rs were observed, and no specific signals were found with any of the sense probes.

3. Results

3.1. Responses from the GSP and CT of rat and mouse to the four basic taste stimuli

All four basic taste stimuli produced in the GSP and CT of the rat and mouse robust phasic and tonic responses (Fig. 1). The GSP evoked robust responses to Suc in rat and QHCl in mouse. The largest response ratio of both phasic and tonic responses to Suc occurred in the rat GSP and to QHCl in the mouse GSP. The response ratio for the phasic GSP response to 0.3 M Suc in the rat was 29.9%, 2.3 times larger than that in the mouse; that for the tonic GSP response was 27.2%, 2.6 times larger than that in the mouse. In contrast, the ratio for 0.01 M QHCl for the phasic GSP response in mouse was 33.3%, 1.4 times larger than that in the rat; the tonic response ratio for the response of the mouse GSP to QHCl was 44.8%, 1.9 times larger than that in the rat (Fig. 2). On the other hand, the huge phasic and tonic CT responses both in rat and mouse (37.5–41.6%) were exclusively produced by 0.1 M NaCl (Fig. 2).

Concentration-response curves (standard: 0.1 M NaCl) to QHCl and Suc were plotted in Fig. 3. The threshold in the mouse GSP to QHCl was 10^{-6} M, one log unit lower than that in rats. In contrast in rats, the threshold in the GSP to Suc was 10^{-3} M, one log unit lower than that in mice. In the CT, the thresholds for Suc and QHCl were not different between the two species (Fig. 3). The SRM to Suc in the rat GSP was 1.5 (tonic and phasic) times larger than to QHCl (Table 1). Conversely, the SRM to QHCl in the mouse GSP was 2.4 (phasic) – 4.7 (tonic) times larger than to Suc. The SRM to QHCl in the rat CT was 2.3 (phasic) and 0.7 (tonic) times larger than to Suc. The SRM to Suc in the mouse CT was 0.7 (phasic) and 1.2 (tonic) times larger than to QHCl.

In summary, the largest proportion of taste responses among the four classical taste stimuli tested occurred in the GSP to Suc in the rat and to QHCl in the mouse (Fig. 2). Species differences in neurophysiological thresholds to QHCl and Suc occurred only in the GSP. The larger proportion of SRM in the GSP between QHCl and Suc occurred to the particular stimulus in the species that possessed the lower neural threshold.