Multiple sweet receptors and transduction pathways revealed in knockout mice by temperature dependence and gurmarin sensitivity

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Ohkuri T, Yasumatsu K, Horio N, Jyotaki M, Margolskee RF, Ninomiya Y. Multiple sweet receptors and transduction pathways revealed in knockout mice by temperature dependence and gurmarin sensitivity. Am J Physiol Regul Integr Comp Physiol 296: R960–R971, 2009. First published February 11, 2009; doi:10.1152/ajpregu.91018.2008.—Sweet taste transduction involves taste receptor type 1, member 2 (T1R2), taste receptor type 1, member 3 (T1R3), gustducin, and TRPM5. Because knockout (KO) mice lacking T1R3, gustducin’s Gα subunit (Ggust), or TRPM5 exhibited greatly reduced, but not abolished responses of the chorda tympani (CT) nerve to sweet compounds, it is likely that multiple sweet transduction pathways exist. That gurmarin (Gur), a sweet taste inhibitor, inhibits some but not all mouse CT responses to sweet compounds supports the existence of multiple sweet pathways. Here, we investigated Gur inhibition of CT responses to sweet compounds as a function of temperature in KO mice lacking T1R3, Ggust, or TRPM5. In T1R3-KO mice, responses to sucrose and glucose were Gur sensitive (GS) and displayed a temperature-dependent increase (TDI). In Ggust-KO mice, responses to sucrose and glucose were Gur-insensitive (GI) and showed a TDI. In TRPM5-KO mice, responses to glucose were GS and showed a TDI. All three KO mice exhibited no detectable responses to SC45647, and their responses to saccharin displayed neither GS nor a TDI. For all three KO mice, the lingual application of pronase, another sweet response inhibitor, almost fully abolished responses to sucrose and glucose but did not affect responses to saccharin. These results provide evidence for 1) the existence of multiple transduction pathways underlying responses to sugars; a T1R3-independent GS pathway for sucrose and glucose, and a TRPM5-independent temperature sensitive GS pathway for glucose; 2) the requirement for Ggust in GS sweet taste responses; and 3) the existence of a sweet independent pathway for saccharin, in mouse taste cells on the anterior tongue.

sweet taste transduction; gurmarin sensitivity; temperature-dependent increase; knockout mice; chorda tympani nerve

A MAJOR ADVANCE IN OUR UNDERSTANDING of sweet taste signaling is the discovery of two G protein-coupled receptors, taste receptor type 1, member 2 (T1R2) and taste receptor type 1, member 3 (T1R3), which dimerize to form a broadly tuned sweet taste receptor (T1R2+T1R3). Binding of sweet compounds to T1R2+T1R3 leads to activation and dissociation of the subunits of the coupled heterotrimeric G protein, probably gustducin (Ggust) but possibly other G proteins too. The dissociated βγ subunits of the Ggust activate phospholipase C-β2 (PLCβ2), which hydrolyzes phosphatidylinositol bisphosphate into diacylglycerol and inositol trisphosphate (IP3). Subsequently, IP3 activates the type 3 IP3 receptor (IP3R3), leading to the release of Ca2+ from intracellular stores. Rapid increases in [Ca2+]i open basolaterally located transient receptor potential melanin 5 (TRPM5) channels, leading to the Na+ influx, membrane depolarization, and generation of action potentials, which might be required for the neurotransmitter release (31, 38). Importantly, mice lacking T1R2, T1R3, T1R2+T1R3, Ggust, PLCβ2, IP3R3, or TRPM5 display markedly diminished or even absent behavioral and nerve responses to sweet compounds, suggesting that these molecules are principal mediators of sweet signal transduction (6, 7, 8, 13, 39, 46, 48). However, multiple studies using knockout (KO) mice for each of these key molecules for sweet have raised the possibility that there exist additional receptor and transduction pathways for sweet taste responses, especially to the compounds (e.g., sucrose, glucose, saccharin) to which chorda tympani (CT) nerve responses do not fully abolish (30–80% of control) in the T1R3-, Ggust-, or TRPM5-KO mice (6, 7, 8, 39), and substantial residual taste-guided behavior was observed in the T1R3 or Ggust KO mice (9, 10). Yet, it remains unclear whether the residual responses to sweet compounds occur through activation of sweet taste receptors [e.g., T1R2 dimers (48)] and their downstream signaling molecules or via other nonsweet receptor systems [e.g., bitter taste receptors (16)].

In rodents, it has been shown that the peptide gurmarin (Gur) inhibits CT nerve responses by 30–80% to multiple sweet compounds (e.g., sucrose, glucose, saccharin, SC45647) but does not affect CT responses to salty, sour, or bitter compounds (15, 21, 26, 27, 28, 29, 33, 44). Gur inhibition was observed in the CT nerve but not in the glossopharyngeal nerve innervating the posterior tongue (27). Responses of both taste nerves to sucrose were fully abolished by the lingual application of the proteolytic enzyme pronase (27). In C57BL mice, sweet-responsive CT fibers could be classified into two distinct groups, Gur-sensitive (GS) and Gur-insensitive (GI). These data suggest that there may be corresponding GS and GI receptor subtypes for mouse sweet responses (29, 44). Moreover, a recent study demonstrated that heat activation of TRPM5 explains the enhancement of sweet taste perception by warm temperature (39). The CT nerve responses to sweet compounds sucrose, glucose, saccharin, and SC45647 showed a temperature-dependent increase (TDI) between 15 and 35°C in wild-type (WT) mice but not in TRPM5-KO mice. This suggests that the TDI may be indicative of sweet responses dependent on TRPM5.
To gain further insights into the number and nature of transduction pathways underlying sweet taste, we examined Gur inhibition, pro- nase inhibition, and temperature-dependent enhancement of CT responses to sugars (sucrose and glucose) and artificial sweeteners (saccharin and SC45647) in WT and KO mice lacking T1R3, Gogust, or TRPM5. Our results indicate that sweet taste responses to sugars but not to artificial sweeteners use additional transduction pathways independent of T1R3, Gogust, and TRPM5.

MATERIALS AND METHODS

Experimental manipulation. All experimental procedures were approved by the committee for Laboratory Animal Care and Use at Kyushu University (Fukuoka, Japan) and were in accordance with the Animal Care Guidelines of Kyushu University. T1R3-, Gogust-, and TRPM5-KO mice used were developed in the C57BL/6 background at the Mount Sinai School of Medicine (6, 7, 8). Both T1R3- and TRPM5-KO mice were produced by homologous recombination in C57BL/6 embryonic stem cells and maintained in this background (6, 7). Gogust-KO mice, homozygous null for the Gnt3 allele, were created by homologous recombination in 129/Sv background embryonic stem cells and genotyped as previously described (43). These mice were backcrossed eight or more times to WT C57BL/6 mice,
then heterozygous mice from the last backcross generation were used to establish the colony. The estimated C57BL/6 contribution to the background of this C57BL/6–129/Sv mixed strain is >99.6%. C57BL/6 WT mice were obtained from the Charles River Japan (Tokyo, Japan). Both male and female mice of each genotype were studied; they were 8 to 12 wk old at testing. Animals were housed in plastic cages under a 12:12-h light-dark cycle at 20–22°C and had free access to tap water and food pellets (MF; Oriental Yeast, Tokyo, Japan).

Recordings of responses from the CT nerve. The procedures of dissection and recordings have been described previously (30). Briefly, under pentobarbital sodium anesthesia (50–60 mg/kg ip; Somnopentyl; Schering-Plough, Kenilworth, NJ), the trachea of each animal was cannulated, and the mouse was then fixed in the supine position with a head holder to allow dissection of the CT nerve. The right CT nerve was sutured, and the mouse was then fixed in the supine position with a head holder to allow dissection of the CT nerve. The right CT nerve was exposed at its exit from the lingual nerve and cut near its entrance to the bulla. For whole-nerve recording, the entire nerve was placed on a silver holder to allow dissection of the CT nerve. The right CT nerve was stimulated with a time constant of 1.0 s and recorded on a computer (Osaka, Japan). These chemicals were dissolved in distilled water. During stimulation of the tongue with test solutions, the solutions were flowed for ~30 s at the same flow rate and temperature as the distilled water used for rinsing the tongue (~0.1 ml/s). The tongue was rinsed with distilled water during an interval of ~1 min between successive stimulations. To examine Gur inhibition of the CT nerve responses, we treated the tongue with 30 μg/ml (~7.1 μM) Gur dissolved in 5 mM phosphate buffer (pH 6.8; made with Na2HPO4 12 H2O and NaH2PO4 2 H2O) for 10 min in the same manner as that described by Ninomiya and Imoto (26). This concentration of Gur had been shown to have a near maximal effect in C57BL/6 mice to suppress CT nerve responses to sucrose (26, 27, 29). Application of the phosphate buffer alone without Gur has no inhibitory effect on CT responses to all taste stimuli tested. In addition, to examine specific inhibition of the CT nerve responses to sweet compounds, the tongue was treated for 15 min with another sweet inhibitor: Pronase E (2%), a proteolytic enzyme that has been shown to act as a specific inhibitor for responses to sweet compounds (12, 25, 27, 29, 32). Pronase E was dissolved in 50 mM phosphate buffer (pH 7.0, 37°C) immediately before application to the tongue [as described by Ninomiya et al. (27)]. Adaptation of the tongue to phosphate buffer without pronase had no effect on the responses to taste stimuli. To examine the temperature dependency of the CT nerve responses, the test solutions were applied to the tongue at three different temperatures, 35 ± 2°C.

Table 1. ANOVA results for the CT nerve responses to sweet compounds in WT and KO mice lacking T1R3, Gagust, or TRPM5

<table>
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<th>Effect</th>
<th>WT</th>
<th>T1R3-KO</th>
<th>Gagust-KO</th>
<th>TRPM5-KO</th>
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<tr>
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<tr>
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<td>1,61 47.9 ‡</td>
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<tr>
<td>Concentration 5,335 820.4 ‡ 3,78 128.2 ‡ 3,69 107.1 ‡ 3,183 229.9 ‡</td>
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<td>6,183 2.02</td>
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<tr>
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<td>Concentration 2,124 883.0 ‡ 2,26 233.8 ‡</td>
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<td>Sac, response magnitude</td>
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<tr>
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<tr>
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<tr>
<td>Gurmarin 1,52 96.2 ‡</td>
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<tr>
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<tr>
<td>Temperature × Concentration 2,52 11.6 ‡</td>
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<td>ND</td>
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Response magnitudes were analyzed using 3-way ANOVA assessing effects of temperature (a between-group factor), gurmarin, and concentration (within-group factors) and their 2- and 3-way interactions. Table based on data shown in Figs. 2–5. DF, degree of freedom; F, F values; ND, not done; *P < 0.05, ‡P < 0.01, †P < 0.001, ANOVA.
25 ± 2°C, and 15 ± 2°C in the same manner as that described by Talavera et al. (39). In control experiments, we obtained temperature response curves for five taste stimuli (see Fig. 1). The curve for 0.5 M Suc shows a minimal response at 15°C and near maximal response at 35°C. During temperature dependency experiments, solutions were maintained at room temperature (25°C), in a cooling box at 15°C or in a water bath at 35°C. Before each stimulation, the tongue was preadapted for 2 min with distilled water of the appropriate temperature.

Data analysis. For the analysis of whole nerve response to each stimulus, the magnitudes of the integrated response from 5 to 25 s (every 5 s) after stimulus onset were measured and averaged. Relative response magnitude (averaged) for each test stimulus was calculated, with the response magnitude to 0.1 M NH₄Cl at 25°C defined as unity (1.0), and used for statistical analysis. Repeated-measures ANOVA and post hoc Tukey-Kramer test or Student’s t-test were performed to statistically evaluate the effects of temperature and Gur on responses. Calculations were performed using the statistical software packages Statcel (OMS, Tokyo, Japan) or Statistica (StatSoft Japan, Tokyo, Japan).

RESULTS

CT nerve responses of WT mice. Figure 2A shows sample recordings of the integrated responses of the CT nerve of a WT mouse to 0.5 M Suc before and after the treatment with Gur, and at temperatures of 15, 25, and 35°C. Before or after treatment with Gur, responses to Suc increased with increasing temperature from 15 to 35°C. Treatment with Gur suppressed responses to Suc at all three temperatures. Figure 2B shows concentration-response relationships for sweet compounds before and after treatment with Gur at 15, 25, and 35°C. Responses to all of the sweet compounds were significantly increased with increasing temperature from 15 to 35°C and suppressed by Gur (Table 1). Gur inhibition of CT nerve responses varied according to the sweet stimulus: SC (inhibited by ~70% after treatment with Gur) > Sac (~60%) > Suc (~50%) > Glc (~30%) (Fig. 2B). As shown in Fig. 2C, responses of WT mice to 0.5 M Suc, 0.5 M Glc, 20 mM Sac, and 3 mM SC exhibited significant TDI (shown by an asterisk in Fig. 2C) before and after treatment with Gur, and Gur inhibition (shown by a plus sign in Fig. 2C) at all three temperatures.

CT nerve responses of T1R3-KO mice. The CT nerve responses of T1R3-KO mice to all four tested sweet compounds (Fig. 3, A–C) were greatly diminished compared with those of
WT mice (Fig. 2, A–C). It should be noted that the vertical axis scale is much lower in the figures for the KO mice (Fig. 3–5B) relative to Fig. 2B for WT mice. At 25°C, responses of T1R3-KO mice were reduced compared with those of WT by ~80% (Suc or Sac), ~40% (Glc), or even ~95% (SC). Similar effects were observed at each temperature, in particular, responses to SC were almost fully abolished at each temperature tested. Figure 3A shows sample recordings of the integrated responses of the CT nerve of a T1R3-KO mouse to 1 M Suc before and after the treatment with Gur at 15, 25, and 35°C. The responses to Suc increased with increasing temperature from 15 to 35°C and were suppressed by Gur at two temperatures (15 and 25°C). Figure 3B shows concentration-response relationships of T1R3-KO mice for sweet compounds before and after the treatment with Gur at 15, 25, and 35°C. Responses of T1R3-KO mice to Suc and Glc, but not Sac, increased significantly with increasing temperature and were suppressed by Gur (Tables 1 and 2). T1R3-KO mice exhibited significant TDI before and after treatment with Gur in their responses to 1 M Suc and 1 M Glc (shown by an asterisk in Fig. 3C). Gur inhibited responses of T1R3-KO mice to 1 M Suc (at 15 and 25°C) and 1 M Glc (at 25°C) (shown by a plus sign in Fig. 3C). No such effects of temperature or Gur (TDI or GS) were evident in the responses of T1R3-KO mice to 20 mM Sac (Tables 1 and 2).

CT nerve responses of Gagust-KO mice. The CT nerve responses of Gagust-KO mice to all four tested sweet compounds (Fig. 4, A–C) were greatly diminished compared with those of WT mice (Fig. 2, A–C). At 25°C, responses of Gagust-KO mice were reduced compared with those of WT by ~80% (Suc and Sac), by 40% (Glc), and by ~95% (SC). Similar effects were observed at each temperature, as with the T1R3-KO mice, responses to SC were almost fully abolished at each temperature tested. Figure 4A shows sample recordings of the integrated responses of the CT nerve of a Gagust-KO mouse to 1 M Suc before and after the treatment with Gur at 15, 25, and 35°C. In contrast to the results with WT and T1R3-KO mice, Gur did not suppress the responses of Gagust-KO mice to Suc at any temperature tested (15, 25, and 35°C). Figure 4B shows concentration-response relationships of Gagust-KO mice for sweet compounds before and after the treatment with Gur (30 μg/ml) at three different temperatures (15, 25, and 35°C). Values indicated are expressed as means ± SE. Numbers of subjects are 7–13 Gagust-KO mice. C: CT nerve responses of Gagust-KO mice to sweet compounds (1 M Suc, 1 M Glc, 20 mM Sac, 3 mM SC) before (top) and after (bottom) Gur treatment at three different temperatures (15°C, open bars; 25°C, gray bars; 35°C, solid bars). Tukey-Kramer test for differences in responses to sweet compounds among three temperatures: *P < 0.05, t-test for differences in responses to sweet compounds before and after treatment with Gur: +P < 0.05.
treatment with Gur at 15, 25, and 35°C. Responses of Ggust-KO mice to Suc and Glc, but not Sac, increased significantly with increasing temperature from 15 to 35°C (Table 1). In contrast to the results with WT and T1R3-KO mice, Gur did not suppress CT responses of Ggust-KO mice to Suc, Glc, or Sac (Tables 1 and 2). Ggust-KO mice also showed TDI before and after treatment with Gur (shown by an asterisk in Fig. 4C), but no significant suppression by Gur of responses to 1 M Suc or 1 M Glc at any temperature tested. No such effects of temperature or Gur (TDI or GS) were evident in the responses of Ggust-KO mice to 20 mM Sac (Tables 1 and 2).

CT nerve responses of TRPM5-KO mice. Using a TRPM5-KO mouse model different from our own, Zhang et al. (46) showed that taste responses to sweet, bitter, and umami stimuli are completely impaired. However, it is important to note that our TRPM5-KO mouse model is quantitatively different, as it shows decreased, but not totally abolished, behavioral and gustatory nerve responses to these stimuli (7, 39). The CT nerve responses of TRPM5-KO mice to all four tested sweet compounds (Fig. 5, A–C) were greatly diminished compared with those of WT mice (Fig. 2, A–C). At 25°C, responses of TRPM5-KO mice were reduced compared with those of WT by ~80% (Suc and Sac), by 40% (Glc), and by ~95% (SC). Similar effects were observed at each temperature, as with the T1R3-, and Ggust-KO mice, responses to SC were almost fully abolished at each temperature tested. Figure 5A shows sample recordings of the integrated responses of the CT nerve of a TRPM5-KO mouse to 1 M Suc before and after the treatment with Gur at 15, 25, and 35°C. The responses of TRPM5-KO mice to Suc were unaltered by temperature from 15 to 35°C. The responses of TRPM5-KO mice to Suc were not suppressed by Gur at 15 and 35°C but were suppressed at 25°C. As shown in Fig. 5C, only the responses to Glc increased significantly with increasing temperature from 15 to 35°C (also see Table 1). Gur suppressed responses of TRPM5-KO mice to Suc and Glc but did not suppress responses to Sac (Tables 1 and 2). TRPM5-KO mice showed TDI before Gur only in the responses to 1 M Glc (shown by an asterisk in Fig. 5C), and Gur inhibition in the responses to 1 M Suc (at 25°C) and 1 M Glc (at 35°C) (shown by a plus sign in Fig. 5C). No such
effects of temperature or Gur (TDI or GS) were evident in the responses of TRPM5-KO mice to 20 mM Sac (Tables 1 and 2).

Inhibition by pronase of CT nerve responses to sweet compounds in KO mice. CT nerve responses to sweet compounds in WT mice were greatly suppressed by Pronase E (Pro E) (data not shown), as was shown in previous studies (25, 27, 32). Figures 6A, 7A, and 8A shows sample recordings of the integrated CT nerve responses of the three types of KO mice to 1 M Suc, 1 M Glc, 20 mM Sac, and 3 mM SC before and after the treatment with 2% Pro E on the tongue for 15 min at room temperature (25°C). With all three KO mice, the treatment with Pro E greatly suppressed the responses to 1 M Suc and 1 M Glc but did not affect the responses to 20 mM Sac. Figures 6B, 7B, and 8B show concentration-response relationships in the three KO mice for sweet compounds before and after treatment with Pro E. With all three KO mice, suppression by Pro E was observed for the responses to Suc (to <20% of control) and Glc (<30%) [ANOVA, effect of Pro E: F(1,10–12) = 15.7, P < 0.01 in all three KO mice] but was not found with the CT responses to Sac (~100%) [ANOVA, effect of Pro E: F(1,10–12) ≤ 1.08, P > 0.05 in all three KO mice]. The suppressive effects of Pro E in all three KO mice were observed in responses to Suc and Glc at 0.3 M or more of concentrations (t-test, P < 0.05) (Table 2). In marked contrast, the responses to Sac at any concentration in all three KO mice were not suppressed by treatment with Pro E (t-test, P > 0.05) (Table 2).

DISCUSSION

In the present study, we examined GS and TDI of the CT nerve responses to four sweet compounds at three temperatures (15, 25, and 35°C) in WT and KO mice lacking T1R3, Gogust, or TRPM5. The results with WT mice indicated that Gur maintained its suppressing effects on CT responses to sweet compounds at the three different temperatures tested. Gur suppressed responses to the four compounds by 30–70% (Fig. 2). The present results and previous studies (26, 29, 44) indicate that the GS and GI taste pathways vary in their responsiveness to sweet compounds. For example, the GS system appears to respond more broadly to nonsugar sweet

Fig. 6. A: sample recordings of the integrated responses of the CT nerve of T1R3-KO mice to 1 M Suc, 1 M Glc, 20 mM Sac, and 3 mM SC before and after treatment with 2% Pronase E (ProE). B: concentration-response relationships of the CT nerve responses of T1R3-KO mice for Suc, Glc,Sac, and SC before (open) and after (solid) treatment with ProE. The CT responses were normalized to the response to 0.1 M NH4Cl. Values indicated are expressed as means ± SE. Numbers of subjects are 6 T1R3-KO mice. Significant difference between responses before and after treatment with ProE: t-test, *P < 0.05; ***P < 0.001.
compounds than does the GI system: the CT response to D-phenylalanine, and its enhancement by Sac were totally abolished by Gur (26). Moreover, Yasumatsu et al. (44) found that the GS proportion of the sweet response (the GS component) varied among the compounds tested, in the following order: SC (70%) > Sac, Suc (60%) > Glc and sorbitol (30%) in C57BL/6 mice. The present study found that the GS and GI components of CT responses of WT (C57BL/6) mice to Suc, Glc, Sac, and SC were similar to that observed in a previous study (44) [GS component: SC (70%) > Sac, Suc (60%) > Glc (30%)]. In addition, CT nerve responses to the four sweet compounds before and after treatment with Gur significantly increased with increasing temperature from 15 to 35°C (Fig. 2, A–C and Table 1). These findings indicate that both GS and GI components exhibit the TDI.

In comparison to CT nerve responses of WT mice, the responses of the three different KO mice to the sweet compounds were all diminished, and each in the same order: SC > Sac ≥ Suc > Glc, as had been shown in previous studies (6, 7, 8). SC is a very specific sweet compound, whose responses were almost fully abolished at any temperature in all three KOs (Figs. 3–5). This indicates that responses to SC may occur solely through the main pathway involving T1R3, G_gust, and TRPM5. This is supported by a recent study (13) showing that mice lacking IP3R3, which is likely to be involved in the main sweet pathway, exhibited sweet taste responses similar to those of the three KOs that we examined here, with only responses to SC among various sweet compounds being abolished. In addition, the relative order in the magnitude of decrease of sweet taste responses in IP3R3 KO mice was comparable to what we observed here with the three KOs examined (i.e., SC > Sac ≥ Suc > Glc). It is interesting to note that this relative order of decreased response from genetic knockout was similar to that found pharmacologically in WT mice by the addition of Gur [SC (inhibited by ~70% after treatment with Gur) > Sac (~60%) ≥ Suc (50%) > Glc (30%)]. This may indicate that the main pathway, including T1R3-containing receptor(s), is more important for the GS than for GI response component. Full abolition of responses to SC in these KO mice, however,
indicates that both GS and GI components of responses to SC are mediated by this pathway, although the GI component of SC responses is much smaller than the GS component. On the basis of current knowledge, one possible explanation for segregation of the two SC response components may be difference in GS of T1R2/H11001 T1R3 heteromers vs. other hypothetical receptors (e.g., T1R3 homomers). Additional studies are needed to clarify this possibility.

We found that the CT nerve responses to Suc and Glc remaining in T1R3-KO mice exhibited TDI and were weakly but significantly suppressed by Gur at different temperatures (Fig. 3, Tables 1 and 2). This indicates the potential existence of T1R3-independent but TRPM5-involving pathway for GS and/or GI components of Suc and Glc responses in addition to the main pathway. Zhao et al. (48) demonstrated that high concentrations of sugars (Suc and Glc) elicited modest but detectable attractive responses in both T1R2- and T1R3-KO mice, although behavioral and neural sweet taste responses of T1R2/T1R3 double KO mice were fully abolished. If receptors for sweet taste are exclusively composed of T1R2 and T1R3 molecules, then differentially sensitive receptors may only be produced by monomer and dimer combinations of these subunits. Thus, T1R3-independent (TRPM5-involving) GS and/or GI sweet response pathways for Suc and Glc may occur through the homodimers of T1R2 (T1R2/T1R2).

In G9ust-KO mice, the remaining CT responses to all sweet compounds were not suppressed by Gur at any temperature (Fig. 4, Tables 1 and 2). This indicates that G9ust-KO mice possess only the GI sweet response component and lack the GS component, suggesting that G9ust is only involved in the GS pathway. Shigemura et al. (35) showed that coexpression of T1R2, T1R3, and G9ust in the fungiform papillae was significantly lower in Gur weakly sensitive BALB mice than in GS C57BL/6 mice. In addition, the lowest levels of coexpression of these taste-signaling proteins in both of these mouse strains was found in circumvallate papillae (innervated by the
glossopharyngeal nerve, whose sweet taste responses were not suppressed by Gur). These findings suggest that Gustog may play an indispensable role in the GS transduction pathway for sweet taste responses in mice. Recent molecular research revealed that most taste cells expressing T1R2 and T1R3 on the GI posterior tongue coexpress Ga14 instead of Gustog (36, 40). It also has been shown that in brief access behavioral experiments that Gustog-KO mice exhibited only a slight decrease in appetitive licking to various sweet taste stimuli, including sugars and artificial sweeteners (10). The substantial sweet-guided behavior remaining in Gustog-KO mice may occur through the residual GI sweet response component of the CT nerve and the glossopharyngeal nerve. Suc and Glc responses of Gustog-KO mice exhibited TDI (Fig. 4, Tables 1 and 2). Presumably another G protein may function in the TRPM5-dependent sweet pathway for sugars. Go12 and PLCβ2 are coexpressed in the same subset of taste cells, and a subset of taste cells expressing PLCβ2 also expresses T1R2 and IP3R3 (1, 2). This indicates that IP3R3, PLCβ2, and TRPM5 are part of a Ca2+ signaling pathway downstream of T1Rs, and Go12 may also be part of this pathway. It seems possible that GI responses to Suc and Glc may occur through a pathway comprising T1R2+T1R3, Go12, PLCβ2, IP3R3, and TRPM5.

In TRPM5-KO mice, the residual responses to Suc and Glc were weakly but significantly suppressed by Gur at each temperature (Fig. 5, Tables 1 and 2). This suggests the existence of TRPM5-independent but Gustog-dependent GS pathway (because Gustog must be involved in the GS pathway). Damak et al. (7) suggested three possibilities of the TRPM5-independent pathways: 1) Ca2+ release or Cr2+ entry acts on another Ca2+-activated Trp channel expressed in taste receptor cells, leading to cation influx, depolarization, and neurotransmitter release; 2) the TRPM5-independent pathways may not utilize Trp channels at all: if sufficient Ca2+ enters the taste receptor cell or is released from stores, then neurotransmitter may be released independently of Trp channels, and finally; 3) some taste receptor cell signaling pathways may not rely on mobilization of calcium. Several lines of evidence suggest that the signal transduction pathways for sweet stimuli involve cAMP and IP3 (3, 4, 17, 18, 22, 37, 41, 42). An increase in cAMP levels has been proposed to activate taste receptor cells either by opening a cyclic nucleotide-gated channel (20) or by activating protein kinase A to phosphorylate K+ channels (5, 19). It is probable that cAMP and IP3 pathways for sweet stimuli might underlie TRPM5-independent and TRPM5-dependent pathways, respectively.

With regard to Glc, the CT responses in TRPM5-KO mice exhibited TDI, which disappeared after treatment with Gur, that is, Glc responses in TRPM5-KO mice are both temperature-sensitive and gustmarin-sensitive. This argues for a TRPM5-independent, temperature-sensitive, GS pathway for responses to Glc. A previous electrophysiological study reported that TRPM5-KO mice showed no significant TDI of the CT nerve responses to Glc (39). However, in that report, a nonsignificant tendency (P = 0.09) for TDI was seen, but only six TRPM5-KO mice had been tested for responses to Glc. Thus, this apparent discrepancy between our past electrophysiological results and those in the current paper may be due to the greater power in this study: here, we tested 30 mice (data for 24 mice were added to 6 from the previous study) vs. six in the previous study. Note that TDI was similar in the previous and the present study. On the other hand, Talavera et al. (39) demonstrated that inward TRPM5 currents increased steeply at temperatures between 15 and 35°C in the HEK-293 cell heterologous expression system, and TRPM4, a close homologue of TRPM5, showed similar temperature sensitivity. Moreover, it has been shown that taste cells of TRPM5 KO mice express a channel with characteristics similar to those of TRPM4 (47). One potential explanation may be that Glc responses to GS component in TRPM5-independent temperature-sensitive pathway may involve TRPM4.

With regard to Sac, residual responses of all three KOs exhibited neither TDI nor GS (Figs. 3–5, Tables 1 and 2). Moreover, in all three KO mice, lingual application of Pro E, a specific inhibitor of responses to sweet compounds, almost fully abolished responses to Suc and Glc but did not affect the responses to Sac (Figs. 6–8, Table 2). These results suggest that the residual responses to Sac in the KO mice may occur through a pathway that does not involve sweet reception, e.g., bitter. Previous studies in humans have demonstrated that as Sac concentration increases, the taste perception shifts from pleasant (sweet) toward unpleasant (bitter/metallic) (11, 14, 34). In addition in heterologous assays, Sac activates two members of the human TAS2R family (hTAS2R43 and hTAS2R44) (16). In mice, salty-, bitter-, or other electrolyte-responsive CT fibers and taste cells may respond to Sac (24, 45). Thus, for responses to Sac there may exist not only sweet-dependent reception, but also sweet-independent reception. In summary, the present study examined Gur-inhibition and TDI of the CT nerve responses to sweet compounds at different temperatures in WT and KO mice lacking T1R3, Gustog, or TRPM5. Our results in these KO mice indicate that remaining responses to sugars (Suc and Glc) but not artificial sweeteners (Sac and SC) exhibited GS and/or TDI (Table 2). These findings suggest the existence of multiple receptors and pathways for GS and GI sweet taste reception of sugars in the
mouse taste cells on the anterior tongue innervated by the CT nerve.

**Perspectives and Significance**

There is growing evidence that there may be multiple receptors and transduction pathways underlying sweet taste. However, little is known about the specific properties or differential responses of these inferred pathways. The present study provides new findings suggesting that sugars such as sucrose and glucose have a transduction pathway that does not require T1R3, gustducin’s G protein, and TRPM5. We also found that the CT response to the artificial sweetener, SC45647, was dependent on all three proteins because the CTS from all genotypes of KO mice were unresponsive to this compound. Although all of these KO mice displayed a degree of responsiveness to the artificial sweetener saccharin, this was likely due to a specific AVT receptor component.

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**REFERENCES**


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**MUTLIPLE SWEET RECEPTORS AND TRANSDUCTION PATHWAYS**

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**Abstract:**

Sweet taste transduction in vertebrate taste bud cells.

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**References:**


