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LETTERS

Heat activation of TRPM5 underlies thermal sensitivity of sweet taste

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TRPM5, a cation channel of the TRP superfamily, is highly expressed in taste buds of the tongue, where it has a key role in the perception of sweet, umami and bitter tastes[1,2]. Activation of TRPM5 occurs downstream of the activation of G-protein-coupled taste receptors and is proposed to generate a depolarizing potential in the taste receptor cells[2]. Factors that modulate TRPM5 activity are therefore expected to influence taste. Here we show that TRPM5 is a highly temperature-sensitive, heat-activated channel: inward TRPM5 currents increase steeply at temperatures between 15 and 35 °C. TRPM4, a close homologue of TRPM5, shows similar temperature sensitivity. Heat activation is due to a temperature-dependent shift of the activation curve, in analogy to other thermosensitive TRP channels[3]. Moreover, we show that increasing temperature between 15 and 35 °C markedly enhances the gustatory nerve response to sweet compounds in wild-type but not in *Trpm5* knockout mice. The strong temperature sensitivity of TRPM5 may underlie known effects of temperature on perceived taste in humans[4–6], including enhanced sweetness perception at high temperatures and 'thermal taste', the phenomenon whereby heating or cooling of the tongue evoke sensations of taste in the absence of tastants[7].

Temperature has a strong influence on how we taste. For example, the perceived sweetness of diluted sugar solutions increases strongly with temperature[4,5]. In addition, cooling or heating of the tongue by itself is sufficient to cause sensations of taste in ~50% of humans[7]. At present, the mechanisms underlying the thermal effects on taste are fully unknown. Given that several members of the TRP superfamily function as thermosensors[8–10], we tested whether TRPM5 is a temperature-sensitive component in the taste pathway.

TRPM5 was transiently expressed in HEK-293 cells and studied with the whole-cell patch-clamp technique. As previous studies have shown that TRPM5 functions as an intracellular Ca^{2+}-activated, voltage-dependent channel, we included 500 nM Ca^{2+} in the intracellular solution and measured currents during voltage steps to potentials ranging from −75 to +175 mV from a holding potential of +25 mV. Under these conditions, increasing the bath temperature from 14 to 38 °C led to a marked increase in current amplitude and the current relaxation rate (Fig. 1a), showing that TRPM5 is a heat-activated channel. The 10-degree temperature coefficient (Q_{10}) for current activation at −75 mV amounted to 10.3 ± 2.0 ($n = 6$) between 15 and 25 °C, which is in the same range as the Q_{10} values of other thermosensitive TRPs (thermoTRPs)[8,9]. The effect of temperature on current amplitude was strongly voltage dependent: inward current at −75 mV was undetectable at 14 °C, whereas at the same temperature robust outward currents were measured at +75 and +150 mV (Fig. 1b, c). Heat activation results from a strong shift of the voltage-dependent activation curve to negative potentials, with a change in the voltage for half-maximal activation (V_{act}) of −7.0 ± 0.6 mV per 1 °C increase in temperature (Fig. 1d, e). The maximal whole-cell conductance increased with temperature with an estimated Q_{10} of 1.17 ± 0.01, indicating that the temperature

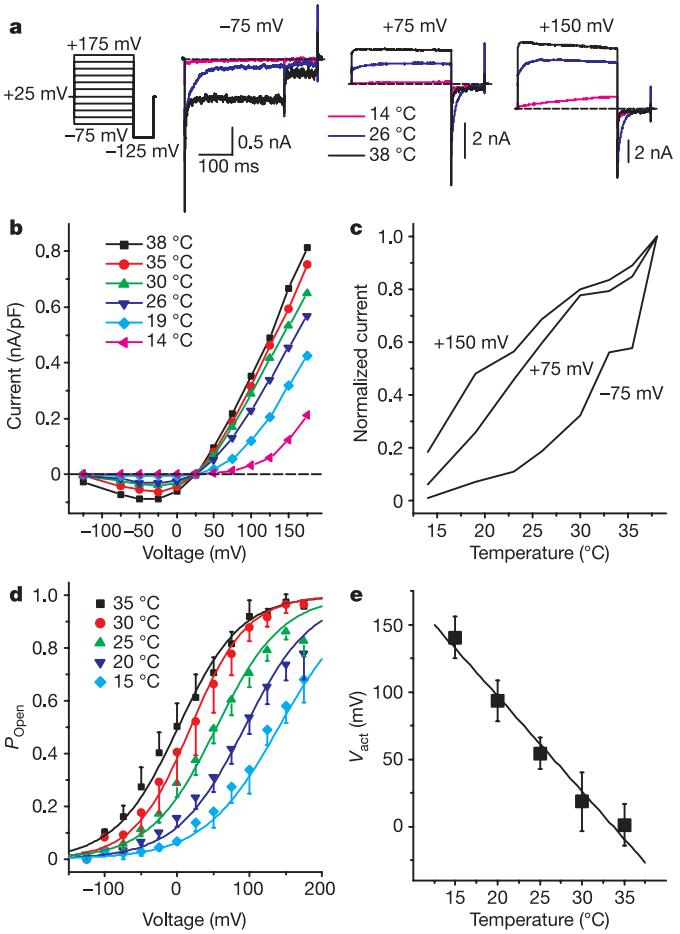

Figure 1 | Heat activation of TRPM5. **a**, Voltage protocol and current traces evoked at different voltages at 14, 26 and 38 °C. **b**, Current–voltage relationships at different temperatures. **c**, Temperature dependence of the current amplitudes at different potentials, normalized to the values at 38 °C. **d**, Average activation curves at different temperatures ($n = 4$–7). Lines are Boltzmann functions calculated with average values of the voltage for half-maximal activation (V_{act}) and slope factor (s_{act}) obtained for each temperature. **e**, Temperature dependence of V_{act}. Data are the mean ± s.e.m.

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dependence of the single-channel conductance resembles to that of aqueous diffusion[11].

Given that TRPM5 is modulated by intracellular Ca^{2+} (refs 12–14), we tested whether the effects of temperature on channel activation were Ca^{2+} dependent. Heat activation was abolished when 10 mM EGTA ($[Ca^{2+}]_i < 1$ nM) was included in the patch pipette (Fig. 2a), indicating that cytosolic Ca^{2+} is absolutely required for channel activation. In contrast, heat activation was fully preserved when intracellular Ca^{2+} was set at 100 μM, a concentration that is around 100-fold larger than needed for maximal Ca^{2+}-dependent activation of TRPM5 under our experimental conditions[14] (Fig. 2b). Heating still induced a shift of the activation curve to negative potentials (-7.9 ± 0.6 mV °C^{-1}; Fig. 2d, f) and an increase in the rate of current relaxation at every membrane potential (Fig. 2e). Together, these data indicate that heat activation of TRPM5 requires cytosolic Ca^{2+}, but is not due to temperature-dependent effects on the Ca^{2+}-dependent activation step. Heat activation of TRPM5 was also observed in cell-free inside-out patches, indicating that temperature modulation occurs in a membrane-delimited manner (see Supplementary Fig. S1).

TRPM4, which is the closest homologue of TRPM5, showing more

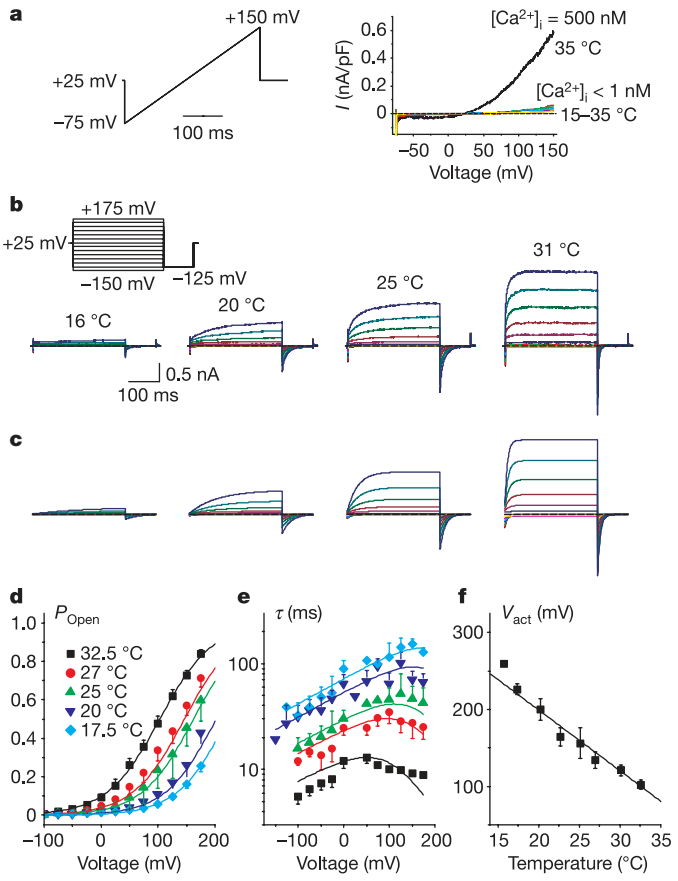

Figure 2 | Temperature dependence of TRPM5 in a high intracellular Ca^{2+} concentration. a, TRPM5 currents elicited by a voltage ramp (left) at an intracellular Ca^{2+} concentration buffered to 500 nM (35 °C, black) or below 1 nM (10 mM EGTA, colours) and at temperatures from 15 to 35 °C. **b**, Current traces elicited at different temperatures in response to the indicated voltage protocol at an intracellular Ca^{2+} concentration of 100 μM. **c**, Simulated current traces at different temperatures as predicted by the model for TRPM5 (see Supplementary Information). **d**, **e**, Average activation curves and voltage dependence of the time constant of current relaxation at different temperatures ($n = 3$–5). **f**, Temperature dependence of V_{act}. Continuous lines represent the fit of the two-state gating model in **e** and **f**, and the activation curves predicted by the model in **d**. Data are the mean ± s.e.m.

than 50% sequence similarity at the amino acid level and sharing the same functional hallmarks such as voltage dependence and activation by Ca^{2+}, may therefore show analogous temperature dependence. We measured TRPM4 currents in inside-out patches and at a saturating intracellular Ca^{2+} concentration of 1 mM to minimize current desensitization[15]. Under these conditions, heating strongly enhanced TRPM4 currents (see Supplementary Fig. S2a). Current amplitude at +25 mV showed a Q_{10} of 8.5 ± 0.6 between 15 and 25 °C. Heating shifted V_{act} towards physiological voltages (-5.6 ± 0.5 mV °C^{-1}; Supplementary Fig. S2c, e) and increased the rate of current relaxation at every membrane potential (Supplementary Fig. S2d). The maximal whole-cell conductance increased with temperature with a Q_{10} of 1.32 ± 0.08. Temperature had little effect on the Ca^{2+} dependence of channel activation (Supplementary Fig. S2f): the Ca^{2+} concentration for half-maximal activation decreased only slightly on heating from 15 °C to 37 °C, with an estimated Q_{10} of 1.36 ± 0.02 (Supplementary Fig. S2f, inset). Thus, as for TRPM5, the strong temperature dependence of TRPM4 is not due to a modulation of the Ca^{2+} dependency of the channel.

We have previously shown that thermal activation of the heat sensor TRPV1 and the cold sensor TRPM8 can be described by a simple two-state model for a voltage-gated channel[3]:

$$\text{Closed} \underset{\beta(V,T)}{\overset{\alpha(V,T)}{\rightleftharpoons}} \text{Open}$$

where α and β are the voltage- and temperature-dependent opening and closing rates, respectively. A fit of the experimental data (Fig. 2d–f and Supplementary Fig. S2c–e) allowed us to determine the enthalpies and entropies associated with the opening (ΔH_α and ΔS_α) and closing (ΔH_β and ΔS_β) of TRPM4 and TRPM5, along with the apparent gating valence (z) and the electrical coupling factor (δ; Fig. 3a–d). Simulations using these parameters yielded a close approximation of the experimental results for TRPM5 and TRPM4 (Fig. 2c, d, and Supplementary Fig. S2b, c). Opening of TRPM4 and TRPM5 is associated with a significant increase in both enthalpy and entropy (large positive values of $\Delta H^\ddagger$ and $\Delta S^\ddagger$; Fig. 3a, b), and thus obeys the same principle as the heat activation of TRPV1 (ref. 3). In contrast, opening of the cold-activated TRPM8 is associated with a large decrease in both enthalpy and entropy. The two-state kinetic model predicts that the midpoint of the activation curves is related to temperature as follows: $V_{act} = (\Delta H^\ddagger - T\Delta S^\ddagger)/zF$ (see Supplementary Information). TRPM4 and TRPM5, like TRPM8 and TRPV1 (ref. 3), combine a large absolute value of $\Delta S^\ddagger$ with a low gating valence z (Fig. 3b, c), which explains the large shifts in V_{act} on changing temperature (Fig. 3e). 'Classical' voltage-gated channels have a much larger z, and, accordingly, show a much less pronounced temperature sensitivity[16–18]. Notably, the structural determinants for the strong temperature dependence are conserved over TRPM and TRPV subfamilies, in contrast to those that determine whether these channels are heat or cold activated[19].

Measurable heat activation of inward TRPM5 currents at physiological potentials occurs above ~15 °C in a temperature range between that of significant activation of TRPM8 and that of TRPV1 (Fig. 3f). Notably, this range spans across room temperatures, in the apparent gap of temperature activation of thermoTRPs known so far[9]. Two other striking features set TRPM4 and TRPM5 apart from the other thermosensitive TRPs. First, TRPM4 and TRPM5 are the only Ca^{2+}-impermeable thermoTRPs described so far. As a consequence, heat activation of these channels leads to membrane depolarization but does not directly cause an increase in intracellular Ca^{2+}. Second, activation of TRPM4 and TRPM5 is abolished when intracellular Ca^{2+} is buffered to low levels. These channels thus have the potential to act as coincidence detectors of warm temperatures and stimuli that increase intracellular Ca^{2+}.

It is well known that temperature modulates human perception of

different taste modalities, including those involving TRPM5. The clearest example is the strong enhancement of perceived sweetness with increasing temperature[4,5]. To determine whether the temperature sensitivity of TRPM5 modulates taste perception *in vivo*, we carried out electrical recordings in chorda tympani nerves from wild-type and *Trpm5* knockout mice. These nerves innervate the anterior taste field of the tongue, which is preferentially responsive to sweet over bitter and umami tastes. Chorda tympani nerve stimulation in wild-type mice by two sugars (sucrose and glucose) and two non-caloric sweeteners (saccharin and SC45647) induced dose-dependent responses that increased ~3–10-fold on increasing the temperature from 15 to 35 °C (Fig. 4a–d). Similar temperature-dependent increments were observed with two other sugars (maltose, fructose) and the sweet amino acid glycine, but not with umami (monosodium glutamate), sour (HCl), salty (NaCl), or bitter (quinine hydrocholoride) stimuli (Fig. 4e).

In comparison to wild type, chorda tympani responses to sweet compounds were greatly diminished (all sugars, saccharin and glycine) or absent (SC45647) in *Trpm5* knockout mice (Fig. 4f). In marked contrast to wild type, the residual responses of the *Trpm5* knockout mice to all of the sweet compounds tested did not increase significantly on changing the temperature from 15 to 35 °C. Again, there were no temperature-dependent increments in the chorda tympani responses of the *Trpm5* knockout mice to monosodium glutamate, HCl, NaCl or quinine hydrochloride. Notably, integrated data from chorda tympani and glossopharyngeal nerves, together with results from behavioural assays, indicate that responses to sweet, bitter and umami tastes are severely diminished but not completely impaired in our *Trpm5* knockout model (S. Damak, M. Rong, K.Y., Z. Kokrashvili, C. Perez, N.S., R. Yoshida, B. Mosinger, J. Glendinning, Y.N. & R. Margolskee, unpublished data). Therefore, our results differ quantitatively from a previous study[2], which showed a complete loss of sweet, bitter and umami responses in a *Trpm5* knockout model. This discrepancy could be due to differences between the knockout targeting constructs (S. Damak, M. Rong, K.Y., Z. Kokrashvili, C. Perez, N.S., R. Yoshida, B. Mosinger, J. Glendinning, Y.N. & R. Margolskee, unpublished data).

In conclusion, we have identified TRPM4 and TRPM5 as two temperature-sensitive cation channels that are activated by heating in

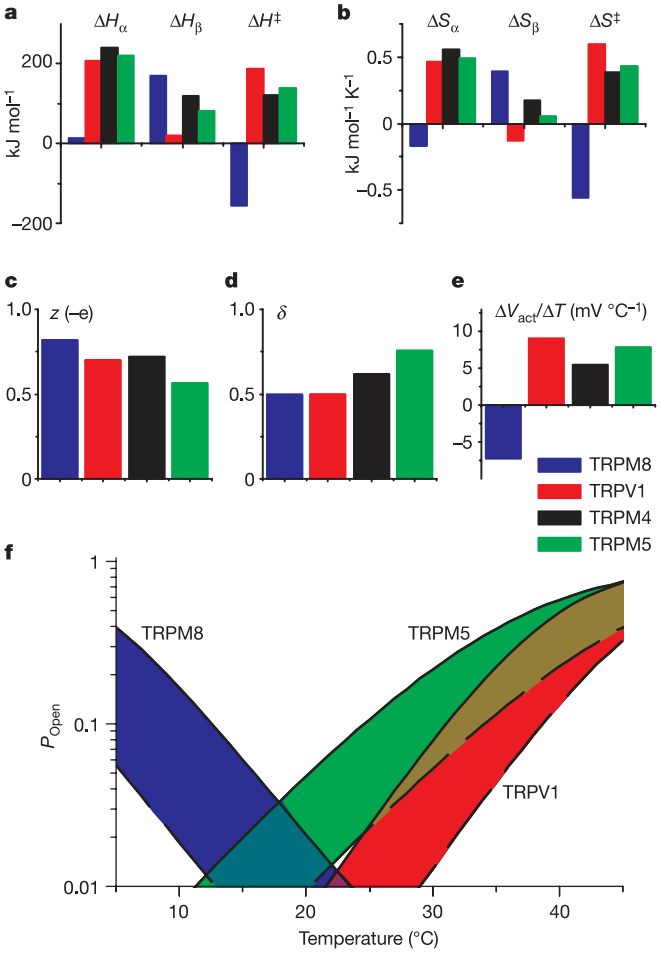

Figure 3 | Comparison of the properties of TRPM8, TRPV1, TRPM4 and TRPM5. **a**, **b**, Enthalpies and entropies associated with the opening (ΔH_α and ΔS_α) and closing (ΔH_β and ΔS_β) transitions and differences in enthalpy and entropy between the open and the closed states ($\Delta H^\ddagger$ and $\Delta S^\ddagger$). **c**, Effective valence of the gating charge (z). **d**, Coupling electric factor (δ). **e**, Shift of V_{act} per degree of temperature ($\Delta V_{act}/\Delta T$). **f**, Activity of TRPM8, TRPV1 and TRPM5 (Ca^{2+}500 nM) as a function of temperature. Coloured bands correspond to open probabilities in the range −100 mV (broken lines) to −30 mV (unbroken lines).

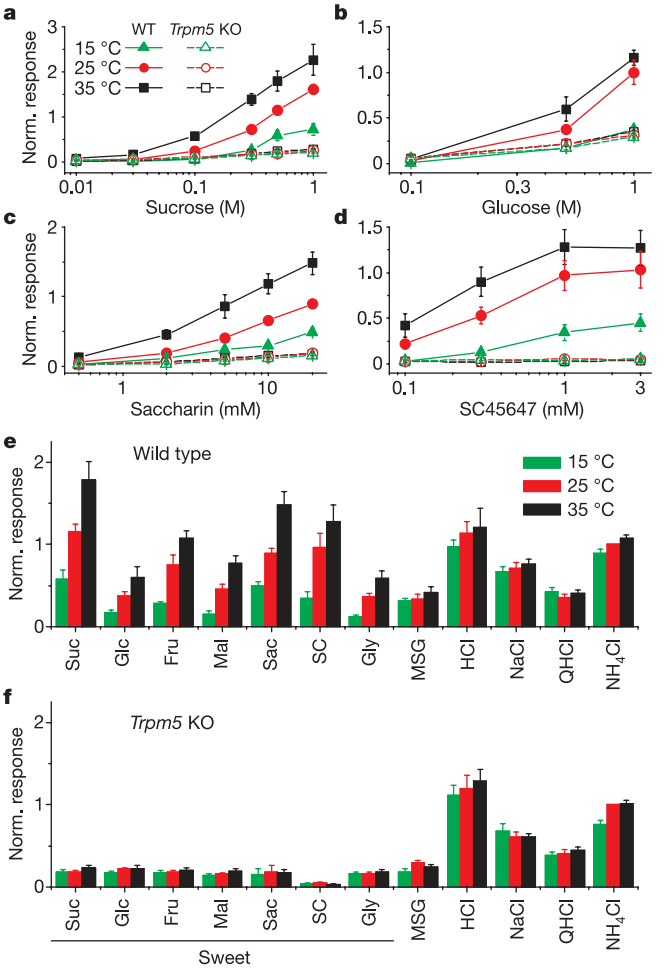

Figure 4 | Temperature dependence of chorda tympani nerve responses of wild-type and *Trpm5* knockout mice. **a**–**d**, Dose-dependent effects of the sweet compounds sucrose, glucose, saccharin and SC45647 on whole-nerve recordings from chorda tympani nerve of wild-type and *Trpm5* knockout mice at 15, 25 and 35 °C (n = 6–8). **e**, **f**, Whole-nerve responses from chorda tympani nerve of wild-type and *Trpm5* knockout mice stimulated by sweet, umami, sour, salty and bitter compounds at 15, 25 and 35 °C (n = 6–8). Sweet compounds: sucrose, 0.5 M (Suc); glucose, 0.5 M (Glc); fructose, 0.5 M (Fru); maltose, 0.5 M (Mal); saccharin, 20 mM (Sac); SC45647, 1 mM (SC); glycine, 0.3 M (Gly). Umami compound: monosodium glutamate, 0.1 M (MSG). Sour compound: HCl, 10 mM. Salty compound: NaCl, 0.1 M. Bitter compound: quinine hydrochloride, 20 mM (QHCl). Nerve responses were normalized to the response to 0.1 M NH_4Cl at 25 °C. Data are the mean ± s.e.m.

the temperature range between 15 and 35 °C. TRPM5 is involved in the transduction of taste, probably by providing a depolarizing current that is activated downstream of taste receptor activation[1,2]. Accordingly, we found that TRPM5-dependent taste responses in mouse gustatory nerves are strongly enhanced by heating. The thermal sensitivity of TRPM5 may also explain several known effects of temperature on taste perception in humans, such as the stronger perceived sweetness of sugar solutions at warmer temperatures[4–6]. Moreover, direct heat activation of TRPM5 could lead to activation of taste receptor cells in the absence of tastants, providing a straightforward explanation for the phenomenon of thermal taste[7].

METHODS

Patch-clamp experiments. *Trpm4b* and *Trpm5* were cloned in the pCAGGS–IRES–GFP vector and transiently transfected into HEK-293 cells by using Trans-IT-293 reagents (Mirus). Cell culture and patch-clamp recordings were done as described[20]. To minimize current rundown, TRPM4 and TRPM5 currents were recorded under inside-out and whole-cell conditions, respectively[14]. Holding potentials differed from zero and were such that no steady ionic flows occurred between test pulses (TRPM4, −50 mV, channels are closed; TRPM5, +25 mV, which equals the reversal potential). This allowed us to monitor leak currents and to avoid ionic depletion and accumulation in the intracellular milieu. The extracellular solution contained (in mM): 150 NaCl, 5 $CaCl_2$, 1 $MgCl_2$ and 10 HEPES (titrated to pH 7.4 with NaOH). For experiments with TRPM4, the intracellular solutions contained 150 mM NaCl and 10 mM HEPES (titrated to pH 7.2 with NaOH). For TRPM5, 50 mM NaCl was substituted with 100 mM $NMDG^+$. Intracellular Ca^{2+} was buffered by adding 2 mM EGTA (for 500 nM free Ca^{2+}) or 2 mM HEDTA (for 10–30 μM free Ca^{2+}) and total Ca^{2+} concentrations were calculated with the software Cabuf (G. Droogmans; ftp://ftp.cc.kuleuven.ac.be/pub/droogmans/Cabuf.zip). The temperature of the solutions perfusing the cells was controlled as described[3].

The patch-clamp data were analysed with WinASCD (G. Droogmans; ftp://ftp.cc.kuleuven.ac.be/pub/droogmans/winascd.zip) and Origin 7.0 (OriginLab Corporation). Whenever possible, linear background components of current traces were digitally subtracted before data analysis. Activation curves were determined from the amplitudes of tail currents elicited by sudden repolarization from different test potentials. These amplitudes were plotted as a function of the test potential and normalized to the maximal current value (I_{max}) obtained from the fit of the data with a Boltzmann function of the form:

$$I_{Tail}(V) = \frac{I_{max}}{1 + \exp(-(V - V_{act})/s_{act})}$$

where V_{act} is the potential of half-maximal activation and s_{act} is the slope factor. Time constants of current relaxation (τ) were obtained from the fit of current traces with a single exponential function.

***Trpm5* knockout mice.** Generation and characterization of *Trpm5* knockout mice have been described (ref. 21; and S. Damak, M. Rong, K.Y., Z. Kokrashvili, C. Perez, N.S., R. Yoshida, B. Mosinger, J. Glendinning, Y.N. & R. Margolskee, unpublished data). The *Trpm5* gene in the knockout mice has a deletion that removes 2.4-kb of the 5′-flanking region of the gene comprising the promoter and exons 1–4, including the translation start site in exon 2. By immunohistochemistry it has been shown that taste buds in these mice lack TRPM5 protein but retain the normal complement of taste receptor cells (S. Damak, M. Rong, K.Y., Z. Kokrashvili, C. Perez, N.S., R. Yoshida, B. Mosinger, J. Glendinning, Y.N. & R. Margolskee, unpublished data). The *Trpm5* knockout mice were maintained in the C57BL6/J background.

Nerve recordings. Whole-nerve responses to lingual application of tastants were recorded from the chorda tympani nerve as described[22]. Reservoirs of tastant solutions were adjusted to temperatures of 15, 25 or 35 °C. Before each stimulation, the tongue was pre-adapted for at least 1 min with distilled water at the temperature to be tested. The temperature of test solutions was monitored with a thermocouple placed on the tongue. Tastants were applied for 30 s. Integrated whole-nerve response magnitudes (time constant 1 s) were measured 5, 10, 15, 20 and 25 s after stimulus onset, averaged and normalized to responses to NH_4Cl at 25 °C. Significant effect of temperature on the responses to each compound was tested with analysis of variance (ANOVA). All data are presented as the mean ± s.e.m.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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