Intracellular Ca\textsuperscript{2+} and the phospholipid PIP\textsubscript{2} regulate the taste transduction ion channel TRPM5

Dan Liu and Emily R. Liman*

Department of Biological Sciences and Program in Neuroscience, University of Southern California, Los Angeles, CA 90089

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The transduction of taste is a fundamental process that allows animals to discriminate nutritious from noxious substances. Three taste modalities, bitter, sweet, and amino acid, are mediated by G protein-coupled receptors that signal through a common transduction cascade: activation of phospholipase C β2, leading to a breakdown of phosphatidylinositol-4,5-bisphosphate (PIP\textsubscript{2}) into diacylglycerol and inositol-1,4,5-trisphosphate, which causes release of Ca\textsuperscript{2+} from intracellular stores. The ion channel, TRPM5, is an essential component of this cascade; however, the mechanism by which it is activated is not known. Here we show that heterologously expressed TRPM5 forms a cation channel that is directly activated by micromolar concentrations of intracellular Ca\textsuperscript{2+} (K\textsubscript{1/2} = 21 μM). Sustained exposure to Ca\textsuperscript{2+} desensitizes TRPM5 channels, but PIP\textsubscript{2} reverses desensitization, partially restoring channel activity. Whole-cell TRPM5 currents can be activated by intracellular Ca\textsuperscript{2+} and show strong outward rectification because of voltage-sensitive gating of the channels. TRPM5 channels are nonselective among monovalent cations and not detectably permeable to divalent cations. We propose that the regulation of TRPM5 by Ca\textsuperscript{2+} mediates sensory activation in the taste system.

ion channels in the transient receptor potential (TRP) family conduct second messenger-gated currents that play diverse roles in cellular physiology. Many are known to mediate vertebrate and invertebrate sensory transduction, including visual transduction (1), pheromone detection (2–4), thermal reception (5–8), and mechanoreception (9, 10). TRP channels fall into the TRPM class on the basis of structural similarity to the founding member (melastatin) (12). Some of these channels are activated by varied stimuli, including ADP-ribose (TRPM2) (13, 14), Ca\textsuperscript{2+} (TRPM4) (15), and cold or menthol (TRPM8) (6, 7), whereas others are nonselective among monovalent cations and not detectably permeable to divalent cations. We propose that the regulation of TRPM5 by Ca\textsuperscript{2+} mediates sensory activation in the taste system.

TRPM5 is coexpressed in taste receptor neurons with G protein-coupled taste receptors (19, 20) and PLCβ2, and targeted deletion of TRPM5 in mice leads to the specific loss of taste sensitivity to bitter, sweet, and amino acids (20). Identification of the second messenger that activates TRPM5 will, thus, provide a framework with which to understand taste transduction. A recent study showed that depletion of Ca\textsuperscript{2+} stores by thapsigargin induced a current in TRPM5-expressing cells (19). However, a separate group reported instead that activation of TRPM5 is independent of release of Ca\textsuperscript{2+} from intracellular stores (20).

Here we show that TRPM5 forms a cation channel activated by micromolar concentrations of Ca\textsuperscript{2+}. After activation, TRPM5 channels undergo rapid Ca\textsuperscript{2+}-dependent desensitization, which is partially reversed by PIP\textsubscript{2}. These data allow us to propose a model for taste transduction that links known receptor signaling events to membrane depolarization.

**Methods**

**Molecular Cloning and Heterologous Expression.** TRPM5 was amplified from mouse vomeronasal organ cDNA with plaque-forming unit DNA polymerase (Stratagene) as described (27) and was cloned into pBlueScript KS+ (Stratagene). The coding sequence was identical to that previously reported (AAF98120) (28) except for a deletion of three nucleotides, resulting in the deletion of a threonine at position 130. The coding sequence was subcloned into pEGFP-C2 (Clontech) to generate an N-terminal fusion of enhanced GFP (EGFP) and into an EGFP dual promoter vector (pHGCX). Plasmid encoding TRPM5 was transiently transfected into cells [COS-7, Chinese hamster ovary (CHO)-K1, or HEK-293 M1, a cell line stably expressing the muscarinic M1 receptor (29)] by using Effectene (Qiagen, Valencia, CA) or FuGene (Roche Molecular Biochemicals) as suggested by the manufacturer. Transfected cells were identified by green fluorescence. Recordings were performed 24–72 h after transfection at room temperature.

**Electrophysiology.** All recording were made with an Axopatch 200B amplifier, digitized with a Digidata 1322A, acquired with PCLAMP 8.2 and analyzed with CLAMPFIT 8.2 (Axon Instruments, Union City, CA). Records were sampled at 5 kHz and filtered at 1 kHz. For measurements of the voltage dependence of activation, series resistance compensation (85–95%) was used. Patch pipettes (1.5–3 Mohm) were fabricated from borosilicate glass. For excised patch recordings, pipettes were coated with Sylgard. Solution exchange was achieved by placing the patch or cell in front of a linear array of micropipetion pipettes under computer control (Warner Instruments, Hamden, CT).

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Abbreviations: TRP, transient receptor potential; PIP\textsubscript{2}, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; IP\textsubscript{3}, inositol 1,4,5-trisphosphate; CHO, Chinese hamster ovary; OAAG, oleoyl-2-acyl-sn-glycerol; ACh, acetylcholine; PLC\textsubscript{β2}, phospholipase C β2; EGFP, enhanced GFP.

*To whom correspondence should be addressed at: Department of Biological Sciences, 3641 Watt Way, Los Angeles, CA 90089-2520. E-mail: liman@usc.edu.

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Results

TRPM5 Forms a Ca2+-Activated Channel. To determine possible mechanisms by which taste transduction leads to activation of TRPM5 channels, we performed patch clamp recording from CHO-K1 cells transfected with TRPM5. An N-terminal GFP-tagged TRPM5 construct (referred to as TRPM5) was generated to allow identification of cells expressing the TRPM5 protein. To test for second messenger activation of TRPM5 channels, we added calcium to excised cell patches during cell perfusion. In whole-cell recording of Ca2+-activated currents, the intracellular solution was 140 mM CsCl/2 mM MgCl2/10 mM Hepes/160 mM NMDGCl. For all other whole-cell experiments, the external solution contained 10 mM NaCl, 145 mM CsCl, or 145 mM KCl. For determination of Ca2+ permeability, the external solution contained 10 mM Hepes and 50 mM CaCl2, 90 mM N-methyl-D-glucamine (NMDG), or 2 mM CaCl2 and 160 mM NMDGCl. For all other whole-cell experiments, the external solution contained 145 mM NaCl, 10 mM Hepes, and 2 mM CaCl2. For excited patch recording the pipette contained 150 mM NaCl, 10 mM Hepes, 2 mM EGTA, and 1.8 mM CaCl2 (±500 μM free Ca2+); the low Ca2+ solution was not important for these recordings). Immediately after seal formation and before excision, the external solution was replaced with a 0 Ca2+ solution: 150 mM NaCl/10 mM Hepes/2 mM HEDTA. Solutions with micromolar concentrations of free Ca2+ were obtained by adding Ca2+ to this HEDTA-based solution as follows: 0.5 mM CaCl2 for 1 μM free Ca2+, 1.2 mM CaCl2 for 5 μM free Ca2+, 1.6 mM CaCl2 for 12 μM free Ca2+, and 1.9 mM CaCl2 for 40 μM free Ca2+. Solutions containing 100 and 500 μM Ca2+ were made by adding 0.1 and 0.5 mM CaCl2 to 150 mM NaCl/10 mM Hepes. All Ca2+ concentrations are reported as calculated with MAXCHELATOR (www.stanford.edu/bioscience and Assay Designs, Ann Arbor, MI), 100 μM diC8-PIP2 (Echelon Bioscience, Salt Lake City)/1:200 anti-PIP2 antibody (Echelon Bioscience and Assay Designs, Ann Arbor, MI), 100 μM 1 -oleoyl-2-acetetyl-sn-glycerol (OAG) (Avanti Polar Lipids, Alabaster, AL), 10 μM IP3 (Research Biochemicals International, Natick, MA), 50 μM calmidazolium (Calbiochem), 5 μM thapsigargin (Calbiochem), and 20 μM U73122 (Calbiochem).

Data Analysis. All data were analyzed by CLAMPFIT (Axon Instruments) and curve fitting and statistical analyses were done in Origin (Microcal, Northampton, MA). For excised patches, we classified recordings according to whether the patch was desensitized and grouped data before analysis. This was necessary because occasionally a patch was inadvertently exposed to Ca2+ before running the stimulus protocol. For each experiment, we determined the ratio of current evoked by 12 and 40 μM Ca2+; this ratio was 0.22 ± 0.02 (n = 21) for the first recording and 0.05 ± 0.01 (n = 9) in patches that were desensitized intentionally. If the ratio was <0.10, the patch was considered desensitized. Leak current in excised patches was determined from the magnitude of the current in 0 Ca2+ before and after each stimulus application. Capacitive current was subtracted from whole-cell I–V curves. Permeability ratios of monovalent cations with respect to the permeability of Cs+ were determined from the equation P/P0 = exp(F/RTΔψ), where Δψ is the change in membrane potential in ND96 containing external solution to an external solution with an equal concentration of cation ρ. Measured liquid junction potentials for all solutions were <3 mV and were not corrected. Dose–response curves were fit with a Hill equation of the form y = Vmax (x^n/(k^n + x^n)). All data are presented as mean ± SEM.

We quantified the sensitivity of TRPM5 channels in response to Ca2+ concentrations ranging from 5 to 500 μM (Fig. 1c). In patches that had not been previously exposed to Ca2+, dose–response relations could fit with a K1/2 of 21.5 ± 1.8 μM and a Hill coefficient of 2.4 ± 0.2 (n = 3). In performing these experiments, we noted that the sensitivity of the channel to Ca2+ varied according to whether the patch had previously been exposed to Ca2+. Indeed, as shown in Fig. 1c and d, repeated application of a dose–response protocol led to a reduction in the peak amplitude of the current as well as a change in the sensitivity of the current to Ca2+. The shift in the dose–response relation stabilized after repeated exposure to Ca2+. After desensitization, the current in response to 40 μM Ca2+ was reduced to 8 ± 2% of its initial magnitude (n = 7). Assuming no change in the shape of the dose–response relation, our data are consistent with an ~4-fold reduction in sensitivity to Ca2+ and an ~4-fold reduction in the peak amplitude (Fig. 1d).

Desensitization of TRPM5 Is Ca2+-Dependent. Desensitization of TRPM5 channels could be caused by a steady loss of a signaling molecule after patch excision or by a process that is specifically stimulated by Ca2+. To distinguish between these possibilities, we repeatedly elicited TRPM5 currents by application of 40 μM intracellular Ca2+ and intermittently exposed patches to 500 μM Ca2+. A gradual decline in the current was observed in response to application of 40 μM Ca2+, whereas activation of the current by 500 μM Ca2+ led to a dramatic reduction in the current (Fig. 2a), indicating that the decline in the current is caused by a Ca2+-dependent process. To confirm this result, we measured the response of TRPM5 currents to prolonged Ca2+ exposure (Fig. 2b). TRPM5 currents decayed with a time course that could...
be fit with a single exponential and the rate of decay was linearly related to the concentration of Ca\(^{2+}\), indicating that Ca\(^{2+}\) mediates desensitization ([Fig. 2c]). A number of TRP channels show inactivation or desensitization in response to entry of extracellular Ca\(^{2+}\) (e.g., refs. 6 and 31). For the Drosophila light-activated channel TRP, Ca\(^{2+}\)-dependent inactivation appears to be mediated by calmodulin, which directly binds to the channel (32). We were unable to block desensitization or activation of TRPM5 currents by the calmodulin inhibitor calmidazolium (50 \(\mu\)M; \(n = 4\)), although this does not rule out a role for calmodulin in either of these processes.

Desensitization of TRPM5 is Partially Reversed by PIP_2. These data suggest that, in response to a prolonged increase in intracellular Ca\(^{2+}\), TRPM5 channels will desensitize. What is the mechanism by which the sensitivity of the channels is then restored? We reasoned that because levels of PIP_2 are depleted during PLC signaling and restored after termination of PLC signaling (18, 33–35), PIP_2 might act to restore the sensitivity of TRPM5 channels. To test this hypothesis, we used a short chain synthetic PIP_2, DiC_5Pl(4,5)P_2, hereafter referred to as PIP_2. Application of PIP_2 alone did not elicit any current in patches excised from TRPM5-expressing cells (\(n = 3\)), and, before desensitization of the channels, PIP_2 had no effect on the magnitude of the current elicited by a range of Ca\(^{2+}\) concentrations ([Fig. 3a and b; for 40 \(\mu\)M Ca\(^{2+}\), \(P > 0.05\), paired \(t\) test]). In contrast, after desensitization, application of 10 \(\mu\)M PIP_2 led to a dramatic enhancement of the current in response to Ca\(^{2+}\) ([Fig. 3a]). In these experiments, the response to a 2-s application of 40 \(\mu\)M Ca\(^{2+}\) was enhanced ~4-fold when PIP_2 and Ca\(^{2+}\) were applied together ([Fig. 3b; \(P < 0.01\), paired \(t\) test]). Longer application of PIP_2 led to slightly larger enhancement of the current, with a maximal effect reached after ~6 s. Dose–response relations, measured from desensitized channels in the absence or presence of PIP_2, showed that PIP_2 partially restored the sensitivity of the channel to Ca\(^{2+}\) ([Fig. 3c]). Neither application of IP_3 (\(n = 4\)) nor application of OAG (\(n = 4\)) led to any change in the magnitude of the current after desensitization.

The observation that PIP_2 enhances TRPM5 currents only after they have desensitized suggests that desensitization may involve the loss of bound PIP_2 through a Ca\(^{2+}\)-dependent mechanism. One possibility is that Ca\(^{2+}\) activates native phospholipases (36), and that this leads to a depletion of PIP_2 in the patch. However, application of neither the PLC inhibitor U73122 (20 \(\mu\)M; \(n = 8\)) nor IP_3 (10 \(\mu\)M; \(n = 6\)) (which can displace PIP_2 from the binding site of some PLCs) changed the rate or extent of Ca\(^{2+}\)-dependent desensitization. We were also unable to promote desensitization through application of two different anti-PIP_2 antibodies, suggesting that if PIP_2 is bound to the channel, it is not readily accessible.

Whole-Cell TRPM5 Currents in Response to Elevation of Intracellular Ca\(^{2+}\) by Ionophore and G Protein-Coupled Receptor Signaling. To confirm results obtained from excised patches and to study the
properties of TRPM5 currents in more detail, we measured electrical responses in whole-cell recording mode from HEK-293 M1 cells transfected with TRPM5. Perforated-patch recording was used in initial experiments to maintain intracellular signaling components intact. Upon attaining electrical access, little current was present in transfected cells, indicating that TRPM5 is not constitutively active (Fig. 4a). Introduction of the membrane-permeant Ca\(^{2+}\) ionophore A23187 in the presence of 2 mM Ca\(^{2+}\) induced a large outwardly rectifying current in TRPM5-expressing cells (Fig. 4a). In total, 12 of 14 TRPM5-transfected cells responded to the Ca\(^{2+}\) ionophore (peak induced \(I = 547 \pm 140\) pA at +80 mV and \(-182 \pm 62\) pA at −80 mV, \(n = 14\)), whereas none of the untransfected cells responded to the Ca\(^{2+}\) ionophore (peak induced \(I = 7.5 \pm 4\) pA at +80 mV and \(0 \pm 1\) pA at −80, \(n = 7, P < 0.01\)). Rundown of the TRPM5 current was consistently observed in these recordings and is reminiscent of the Ca\(^{2+}\)-induced desensitization of channels observed in excised patches. To determine whether TRPM5 currents can be activated downstream of a G protein-coupled receptor, we measured electrical responses in HEK-293 M1 cells transfected with TRPM5 to stimulation with acetylcholine (ACh). The M1 muscarinic ACh receptor expressed by these cells couples to PLC–Ca\(^{2+}\)–CAG protein, G16z44 (37). In whole-cell recording, with no Ca\(^{2+}\) buffer in the pipette, we observed a large outwardly rectifying current in response to application of 100 μM ACh (two of three cells responded, Fig. 4b). In contrast, we did not observe activation of a current in response to ACh when the patch pipette contained a Ca\(^{2+}\) buffer, EGTA (10 mM; \(n = 3\)). These data suggest that physiological concentrations of Ca\(^{2+}\) elicited by stimulation of a G protein-coupled receptor that couples to PLC can activate TRPM5.

![Figure 3](image_url) **Fig. 3.** PIP\(_2\) partially restores TRPM5 channel activity after desensitization. (a) Responses to 12 and 40 μM Ca\(^{2+}\) in the presence and absence of 20 μM PIP\(_2\) before and after desensitization. Desensitization was induced by a 30-s exposure to 40 μM Ca\(^{2+}\) (V\(_{m} = −80\) mV). PIP\(_2\) enhances the current in response to Ca\(^{2+}\) after, but not before, desensitization. (b) Enhancement of the current in response to 40 μM Ca\(^{2+}\) by PIP\(_2\) before (\(n = 6\)) and after desensitization (\(n = 8\)). Current amplitudes are the averaged peak magnitude recorded within 2 s from the start of Ca\(^{2+}\) exposure. The asterisk indicates that the difference between the enhancement of control and desensitized currents was significant at \(P < 0.05\). (c) Dose-response relations before desensitization (open triangles), after desensitization (filled circles), and after desensitization in the presence of 10 μM PIP\(_2\) (open circles) (mean ± SEM, \(n = 3\) patches). In these experiments, PIP\(_2\) was present before and during a 6-s application of Ca\(^{2+}\). Currents are normalized to the maximum current obtained in each patch. Solid lines are the same fits as in Fig. 1d. The dashed line shows the expected relationship if 25% of the current recovered full sensitivity. The data could not be well fitted by assuming that either only the maximum current or the sensitivity was restored by PIP\(_2\).

![Figure 4](image_url) **Fig. 4.** Electrophysiological properties of TRPM5 expressed in HEK-293 M1 cells. (a) Application of the Ca\(^{2+}\) ionophore A23187 (20 μM) in the presence of 2 mM extracellular Ca\(^{2+}\) in perforated-patch recording mode elicits a large outwardly rectifying current. (Inset) The current in response to a ramp depolarization (1 V/s) at the times indicated. (b) Application of ACh (100 μM) induced a transient current in HEK-293 M1 cells cotransfected with TRPM5 and G16z44. (Inset) The current in response to a ramp depolarization (0.44 V/s) at the times indicated. The pipette solution contained no Ca\(^{2+}\) buffer. (c) In whole-cell recording mode, 40 μM Ca\(^{2+}\) in the pipette elicited a large rectifying current. Recording began shortly after break into the whole-cell mode. (Inset) The current in response to a ramp depolarization (1 V/s). (d) Currents in response to a family of step depolarizations and the resulting I–V relationship for the peak current at each voltage. Steps are to 0–100 mV from a holding potential of −80 mV with repolarization to −50 mV. Note the prominent relaxation of the current upon depolarization, consistent with voltage-dependent gating of the channels. A small fraction of the current showed a fast increase, indicating that there is low, but nonzero, probability of opening at the resting potential (−80 mV).
patch recording, the TRPM5 current inactivated over the time course of tens of seconds (Fig. 4c). Ion substitution experiments revealed that TRPM5 is equally permeable to K⁺, Na⁺, and Cs⁺ (Pₐₕ/Pₖₐ = 1.1, Pₐₕ/Pₖₛ = 1.2, n = 5; Fig. 6, which is published as supporting information on the PNAS web site). We did not detect any permeability to Ca²⁺ (Eᵢₑᵢ = −43.6 ± 3.4 mV in 50 mM Ca²⁺, 90 mM NMDG⁺ as compared with Eᵢₑᵢ = −44.2 ± 3.9 mV in 2 mM Ca²⁺, 160 NMDG⁺; n = 5). Removal of external Ca²⁺ (with EGTA), external Mg²⁺ (with EDTA), or both (with EDTA) did not lead to a significant increase in the magnitude of the inward current or to a change in the rectification of the current (n = 4), indicating that TRPM5 channels are not blocked by divalent cations. We also did not observe any block of TRPM5 currents by La³⁺ (200 μM, n = 4).

The pronounced rectification of TRPM5 currents could be due to an inherent voltage sensitivity of the channels. Indeed, whole-cell currents in HEK-293 M1 cells expressing TRPM5 evoked in response to intracellular dialysis of 40 μM Ca²⁺ showed a clear relaxation to a higher current level with depolarizing voltage steps (Fig. 4d; similar results were obtained in 5 cells). Voltage-sensitive gating has also been observed for TRPM8 and TRPM4 currents (6, 38). A comparison of the fourth transmembrane domain (S4) of TRPM5, TRPM4, and TRPM8 with that of voltage-sensitive K⁺ channels shows a striking similarity, suggesting that similar structural elements underlie voltage-dependent gating in these disparate channels (39). For any of the TRP channels, the physiological significance of this additional level of regulation is not known.

Discussion

Our data show that TRPM5, which is essential for taste transduction (20), forms a nonsensitive cation channel that is directly gated by micromolar concentrations of Ca²⁺. In addition, we show that TRPM5 is regulated by PIP₂, which enhances sensitivity of the channels to Ca²⁺. These data support a model for sensory transduction of bitter, sweet, and amino acid stimuli in taste cells, shown in Fig. 5. In this model, taste receptors signal through PLCβ2 to release Ca²⁺ from intracellular stores, which rapidly activates TRPM5 channels. This is consistent with physiological data and with results from targeted deletion of taste transduction molecules. (20, 23–26, 40–42)

Mechanism of TRPM5 Activation. The mechanism of activation of TRPM5 has been controversial, with conflicting data published in two previous reports (19, 20). Although there is general agreement that TRPM5 is activated downstream of PLC, the nature of the second messenger that activates TRPM5 has eluded previous investigations. One report showed that prolonged treatment with thapsigargin, which inhibits uptake of Ca²⁺ into intracellular stores, leads to the development of a linear conductance in TRPM5-expressing cells (19). These data were used to argue that TRPM5 is activated by depletion of Ca²⁺ stores. The conductance described in this report does not resemble the TRPM5 currents that we recorded, either in rectification properties, Ca²⁺ permeability, or sensitivity to La³⁺ block. Furthermore, we were unable to observe significant activation of TRPM5 by short-term thapsigargin treatment, presumably because of the relatively slow elevation of intracellular Ca²⁺ (Fig. 7, which is published as supporting information on the PNAS web site). A second study showed that G protein-coupled receptors could activate TRPM5 currents, but that the response was not occluded when intracellular Ca²⁺ was heavily buffered (19). These results were used to argue that TRPM5 activation is independent of release of Ca²⁺ from intracellular stores. In similar experiments, we found that TRPM5 currents could be activated downstream of a G protein-coupled receptor; however, we failed to observe activation of this current if the intracellular pipette solution contained a Ca²⁺ buffer. Importantly, neither previous study directly tested whether Ca²⁺ activates TRPM5 channels. By using excised patch and whole-cell recording, we directly demonstrate that TRPM5 is Ca²⁺-activated. Similar results were recently obtained by Hofmann et al. (43).

Ca²⁺ levels in taste cells in response to taste stimuli reach only high nanomolar levels (24–26); however, we report that TRPM5 has micromolar sensitivity to Ca²⁺, a result that might appear incompatible with an essential role of TRPM5 in taste transduction (20). However, as noted by the authors of one of these studies, these measurements underestimate true Ca²⁺ levels because of spatial and temporal averaging (25). In restricted microdomains of cells, Ca²⁺ levels can reach low micromolar concentrations (44), which would be sufficient to activate a TRPM5 current. It will be interesting to determine whether TRPM5 channels are localized in taste cells in proximity to sites of Ca²⁺ release or entry, as might be expected if they are to be efficiently gated by Ca²⁺. An alternative explanation for this apparent discrepancy is that TRPM5 forms a heteromultimer with other channel subunits in taste cells, leading to an alteration in Ca²⁺ sensitivity. Study of native Ca²⁺-activated nonselective channels from taste cells will be needed to address this possibility.

Our study raises a number of questions concerning the mechanisms of activation and inactivation of TRPM5. The Ca²⁺ dependence of activation may be conferred on the channel by auxiliary subunits, such as calmodulin, as is the case for small conductance Ca²⁺-activated K⁺ channels (45) or it may be an inherent property of the channel, as is the case for large conductance Ca²⁺- and voltage-activated K⁺ channels (46). Ca²⁺-dependent desensitization may similarly involve direct binding of Ca²⁺ to the channel or may be mediated by associated or nonassociated proteins. One attractive model is that Ca²⁺ induces an allosteric change in the TRPM5 protein that lowers its affinity for PIP₂. We cannot rule out the possibility that desensitization of the channel is independent of PIP₂ binding, and that the desensitized channel is more sensitive to PIP₂ modulation.

A Molecular Basis for Ca²⁺-Activated Nonsensitive Cation Channels. Ca²⁺-activated nonsensitive cation channels have been described in a large number of cell types (47); however, their physiological functions are not well understood and their molecular identity remains obscure. A previous report demonstrated that a related channel, TRPM4, encodes a Ca²⁺-activated cation channel with sensitivity to Ca²⁺ in the high nanomolar range (ref. 15, but see
also ref. 38). Our data show that TRPM5 also encodes a Ca\(^{2+}\)-activated nonselective ion channel. In addition to lingual epithelium, TRPM5 is detected in stomach, intestine, uterus, and tests (19), and it is likely that on closer examination it will be found in other tissues and cell types. We cloned TRPM5 from the vomeronasal sensory epithelium, where it shows low levels of expression (D.L. and E.R.L., unpublished data). TRPM5 does not match properties of Ca\(^{2+}\)-activated nonselective channels that have been described in vomeronasal neurons (48), suggesting that it might form a heteromultimer with additional subunits.

**TRPMs and PIPs.** PIP\(_2\), once regarded simply as the substrate from which second messengers are generated, has recently been shown to be an important regulator of transporters and ion channels, including several TRP channels (18, 49, 50). PIP\(_2\) inhibits gating of TRPV1, and receptor-stimulated hydrolysis of PIP\(_2\) relieves the channel from inhibition leading to a lower threshold for activation by protons, capsaicin, and heat (49, 50).

For TRPM5, PIP\(_2\) appears to play an opposite role in modulating channel activity: PIP\(_2\) promotes gating of TRPM5 channels, which, after desensitization, are activated by lower concentrations of Ca\(^{2+}\) in the presence of PIP\(_2\). Similarly, PIP\(_2\) promotes gating of TRPM7, which is constitutively active and inactivated by PIP\(_2\) hydrolysis (18). Our *in vitro* data suggest that PIP\(_2\) might play a significant role in modulation of taste transduction. Further defining that role should be a major goal of future studies.

**Note Added in Proof.** After acceptance of our manuscript, we learned about a complementary paper (51) published in this issue. The main results and conclusions of that study also support the notion that TRPM5 indeed represents an intracellular Ca\(^{2+}\)-activated cation channel.

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