Cellular/Molecular

Extracellular Cations Sensitize and Gate Capsaicin Receptor TRPV1 Modulating Pain Signaling

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Transient receptor potential (TRP) channels detect diverse sensory stimuli, including alterations in osmolarity. However, a molecular detector of noxious hypertonic stimuli has not yet been identified. We show here that acute pain-related behavior evoked by elevated ionic strength is abolished in TRP vanilloid subtype 1 (TRPV1)-null mice and inhibited by iodoresiniferatoxin, a potent TRPV1 antagonist. Electrophysiological recordings demonstrate a novel form of ion channel modulation by which extracellular Na\(^{+}\), Mg\(^{2+}\), and Ca\(^{2+}\) ions sensitize and activate the capsaicin receptor, TRPV1. At room temperature, increasing extracellular Mg\(^{2+}\) (from 1 to 5 ms) or Na\(^{+}\) (+50 ms) increased ligand-activated currents up to fourfold, and 10 mM Mg\(^{2+}\) reduced the EC\(_{50}\) for activation by capsaicin from 890 to 450 ms. Moreover, concentrations of divalent cations >10 ms directly gate the receptor. These effects occur via electrostatic interactions with two glutamates (E600 and E648) formerly identified as proton-binding residues. Furthermore, phospholipase C-mediated signaling enhances the effects of cations, and physiological concentrations of cations contribute to the bradykinin-evoked activation of TRPV1 and the sensitization of the receptor to heat. Thus, the modulation of TRPV1 by cationic strength may contribute to inflammatory pain signaling.

Key words: TRPV1; VR1; nociception; cations; ionic strength; pain

Introduction

Mammals vigorously defend ionic homeostasis, and alterations in ionic strength and osmolarity are often perceived as painful (Steinbrocker et al., 1953; Alessandri-Haber et al., 2003). Furthermore, elevated ionic strength (for example, MgSO\(_4\) or NaCl) is used in models of muscle and visceral “pain” (Fukawa et al., 1980; Gyires and Torma, 1984; Graven-Nielsen and Mense, 2001). Indeed, injections of MgSO\(_4\) in the clinic are reported as being intensely painful (Agarwal et al., 2004), and NaCl produces a very familiar “burning” pain when applied to injured tissue, hence the phrase “rubbing salt into wounds.” These disturbances in extracellular ion concentrations are perceived as painful because they activate nociceptive sensory neurons. However, little is known about the molecular mechanisms or identity of receptors that detect these stimuli. Understanding this process is significant not only in the context of hyperosmotic stimuli, but also because it may reveal fundamental information about general nociceptive signaling.

Elevations in extracellular ions such as NaCl may stimulate sensory neurons either directly by regulating membrane ion channels or indirectly by osmotic deformation of the cell membrane. The burning sensation evoked by salt is similar to that evoked by capsaicin, heat, extracellular protons, and ethanol, all of which are known to act through the capsaicin receptor [transient receptor potential vanilloid subtype 1 (TRPV1)] (Caterina et al., 1997; Tominaga et al., 1998; Trevisani et al., 2002). We therefore hypothesized that ionic strength may regulate the function of TRPV1. TRPV1 is a nonselective “cation” channel expressed in a subset of sensory C-fibers. Targeted deletion of the TRPV1 gene in mice has demonstrated an essential role for this ion channel in inflammatory thermal hyperalgesia (Caterina et al., 2000; Davis et al., 2000). We demonstrate here that cations gate TRPV1 directly and that this mechanism may contribute to the nociceptive response to elevated ionic strength. Moreover, we show that under inflammatory conditions, physiological levels of cations contribute to the activation of TRPV1.

Materials and Methods

Behavioral analysis. All experimental procedures were approved by the Georgetown University Animal Care and Use Committee and conformed to National Institutes of Health guidelines. Animals from the following strains were obtained from The Jackson Laboratory (Bar Harbor, ME): CBA/J, C57BL/6, and mice lacking the TRPV1 receptor (B6.129S4-TRpv1tm1Jul/J). Mice were either bred in house or allowed at least 10 d to acclimate before behavioral analysis. Male mice 4–6 weeks of age were allowed to habituate to the testing room for 1 h and to the observation chamber for 10 min before testing. Wild-type and TRPV1 null mice received an intraperitoneal injection (10 ml/kg) of MgSO\(_4\) heptahydrate (120 mg/kg) (Mogil et al., 1999), MgCl\(_2\), CaCl\(_2\), or SrCl\(_2\) (50 ms) in distilled water. Writhing (lengthwise stretch of the torso with a concomitant concave arching of the back) was monitored over a 40 min period. To test pharmacological inhibition of TRPV1 in the MgSO\(_4\) writhing test, we used CBA mice, because this strain performs better in this assay (Mogil et al., 1999). CBA mice writhed for only 10–15 min and did not demonstrate a secondary phase, unlike C57BL/6 mice (see Fig 1A, B). CBA/J mice received a subcutaneous injection (10 ml/kg) of either 1 μmol/kg iodoresiniferatoxin (I-RTX) or vehicle (1% ethanol in 0.9% saline) 1 h before an intraperitoneal injection (10 ml/kg) of anhydrous MgSO\(_4\). Writhing was monitored over 10 min.

Oocyte electrophysiology. Defolliculated Xenopus laevis oocytes (harvested from adult females anesthetized with 0.5 g/L tricaine methanesul-
were clamped at −20 mV (the chloride reversal potential) and injected with 100 mM BAPTA (Sigma, St. Louis, MO) prepared in 10 mM HEPES (brought to pH 7.3 with KOH). BAPTA was pressure injected (20 psi) 15–60 min before the experiment with 10 pulses of 5 s duration.

For heat activation, bath temperature was raised from ~22 to 50°C over ~100 s using an in-line solution heater (Warner Instruments, Hamden, CT). The temperature was continuously monitored with a probe placed within 2 mm of the oocyte. The temperature-activation threshold was defined as a 10% deviation from baseline.

Human embryonic kidney cell and sensory neuron electrophysiology. Human embryonic kidney 293F (HEK293F) cells (Invitrogen, Carlsbad, CA) were cultured in DMEM supplemented with 1% nonessential amino acids and 10% fetal calf serum. Cell cultures were maintained at 37°C with 5% CO2. Cells were transfected with rat TRPV1 (a gift from David Julius) and green fluorescent protein (GFP) cDNA using Lipofectamine (Invitrogen) according to the instructions of the manufacturer and used 24–48 h after transfection.

Nodose ganglia were obtained from adult mice (C57BL/6 and TRPV1-null), cut, digested with collagenase, and cultured in Neurobasal plus 2% B-27 medium (Invitrogen), 0.1% l-glutamine, and 1% penicillin/streptomycin on poly-D-lysine-coated glass coverslips at 37°C in 5% CO2. Neurons were used within 24–36 h of culture.

Whole-cell and single-channel patch-clamp recordings were performed using an EPC 8 amplifier (HEKA Elektronik, Lambrecht/Pfalz, Germany). The current signal was low-pass filtered at 1–3 kHz and sampled at 4 kHz. Currents were further filtered for display purposes. For whole-cell and excised patch recordings, the bath solution contained the following (in mM): 10–100 MgCl2/CaCl2, 0–140 NaCl, 140 NaCl, 5 EGTA, 10 HEPES, and 10 glucose, pH 7.3 (290 mOsm). Divalent solutions (295 ± 5 mOsm) contained the following (in mM): 10–100 MgCl2/CaCl2/SrCl2, 0–140 NaCl, and 10 HEPES, pH 7.3. The pipette solution contained the following (in mM): 140 CsCl, 10 NaCl, 10 HEPES, 5 EGTA, 2 MgATP, and 0.03 GTP, pH 7.3. For measurements of divalent cation permeability, the bath contained the following (in mM): 110 MgCl2/CaCl2, 5 EGTA, and 10 HEPES, pH 7.3, buffered with 2 Mg(OH)2 or Ca(OH)2; the pipette solution contained the following (in mM): 150 NaCl, 5 EGTA, and 10 HEPES, pH 7.3, buffered with NaOH. Measured liquid junction potentials were <1 mV. Permeability ratios were calculated as follows: 

\[
P_{\text{I}^+/\text{Na}^+} = \frac{[\text{Na}^+]}{[\text{I}^+]} \cdot \exp(\Delta V_{\text{f}/RT})/(1 + \exp(\Delta V_{\text{f}/RT})/4)[Y^{2+}]_0,
\]

where \(Y\) represents a divalent cation. Ion activity coefficients were 0.75 for monovalents and 0.25 for divalents.

Chemicals. Capsazepine, phorbol-12,13-dibutyrate (PDBu), and pephylarsine oxide (PAO) were obtained from Sigma. Anandamide (AEA), N-arachidonoyl dopamine (NADA) was obtained from Cayman Chemicals (Ann Arbor, MI), and 1-oleoyl-2-acetyl-sn-glycerol was from Calbiochem (La Jolla, CA). Drugs were prepared as stock solutions in DMSO or ethanol and diluted into a physiological solution before experiments.

Statistical analysis. Data are given as mean ± SEM, and statistical significance was evaluated using unpaired Student’s t test.

Results

TRPV1 is essential for acute Mg2+-induced pain

Intraperitoneal infusion of MgSO4 elicits writhing in mice (Gyires and Torma, 1984), and we investigated whether TRPV1 is necessary for this response. As reported previously (Mogil et al., 1999), intraperitoneal injection of MgSO4 (120 mg/kg) evoked writhing responses in wild-type mice (C57BL/6) that occurred shortly after injection with a duration of ~5–10 min (Fig. 1A–D). This effect was independent of osmolarity, because we observed similar effects with the heptahydrate and anhydrous solutions of MgSO4 in water (~75–110 mOsm) or 0.9% saline (345 mOsm). In addition to this acute writhing response, we identified a slower secondary phase of writhing with an onset ~20 min after injection that may represent a non-neurogenic response. Strikingly, we found that the acute phase of writhing was completely absent in TRPV1−/− mice, whereas the latter phase was unaffected (Fig. 1A–C). Moreover, this acute writhing was inhibited by the specific TRPV1 antagonist iodoresiniferatoxin (Wahl et al., 2001) (Fig. 1D). Thus, these data show that TRPV1 is essential for the acute noxious effects of Mg2+ and are consistent with a non-inflammatory mechanism; writhing after MgSO4 injection is not inhibited by nonsteroidal anti-inflammatory drugs and is not accompanied by an increase in prostaglandins in the peritoneum (Gyires and Torma, 1984).
Elevated extracellular Mg\(^{2+}\) or Ca\(^{2+}\) could directly gate TRPV1. LiCl (100 mM) activated outwardly rectifying currents (1 \pm 0.7\% of 10 \mu M capsaicin; \(n = 5\)) that were enhanced after PKC stimulation (11 \pm 5\% of 10 \mu M capsaicin; \(n = 5\)), but NaCl at concentrations up to 400 mM was without effect in both oocytes and HEK293 cells (data not shown). This lack of effect with hypertonic NaCl is consistent with a previous report (Caterina et al., 1997). Submillimolar concentrations of trivalent cations Gd\(^{3+}\) and La\(^{3+}\) completely blocked capsaicin-evoked currents (\(n = 3–4\)), suggesting that these cations produce an open-channel block. Thus, cation activation of TRPV1 requires cation permeation and appears to correlate with charge and charge density (Ca\(^{2+}\) = Mg\(^{2+}\) \gg Li\(^+\) \gg Na\(^+\)).
Mg$^{2+}$ and Ca$^{2+}$ ions directly gate TRPV1 in sensory neurons
To test whether cations regulated native TRPV1 channels, we turned to voltage-clamped sensory neurons. We found that 10–100 mM Mg$^{2+}$ and Ca$^{2+}$ evoked large inward currents in capsaicin-sensitive neurons (Fig. 3B–D) (n = 23) but not in capsaicin-insensitive neurons (Fig. 3A, top) (<0.1 nA; n = 13). Similarly, no cation-evoked currents were seen in neurons cultured from TRPV1-null mice (Fig. 3A, bottom) (n = 6). All of these neurons exhibited ATP-evoked currents reflecting expression of P2X channels and confirming their status as nociceptors. Figure 3D shows that Mg$^{2+}$ (10–100 mM) acted in a dose-dependent manner, with 100 mM concentrations eliciting a slightly greater response than 100 nm capsaicin. Capsazepine (5 mM) inhibited these Mg$^{2+}$-evoked currents by ~90% (Fig. 3D). These responses are in agreement with those observed in HEK293 cells and oocytes. This indicates that, in sensory neurons, Mg$^{2+}$ and Ca$^{2+}$ primarily act at TRPV1 with little contribution from other cation-sensing receptors.

Direct cation activation of TRPV1 was observed at the single-channel level in cell-free patches (n = 6). Figure 3E shows that 10 mM Mg$^{2+}$ activated single channels at +50 mV that were identified as TRPV1 by subsequent sensitivity to capsaicin (data not shown). High [Mg$^{2+}$] also caused a small reduction in single-channel conductance at positive potentials (from ~90 to 80 pS). Thus, cations can activate TRPV1 in a membrane-delimited manner, and this is consistent with cations interacting directly with extracellular glutamate residues (see Fig. 7).

Cation-induced writhing correlates with activation of TRPV1
Our data show that divalent cations (>10 mM) directly activate TRPV1 and suggest a mechanism for Mg$^{2+}$-induced writhing. However, it remains possible that other cation-sensitive pathways contribute to writhing. For example, millimolar concentrations of divalent cations are known to activate the Ca$^{2+}$-sensing receptor (CaR), a G-protein-coupled receptor that regulates Ca$^{2+}$ homeostasis (Hofer and Brown, 2003). In turn, activation

Figure 3. Mg$^{2+}$ and Ca$^{2+}$ directly activate TRPV1 in sensory neurons. Neurons were voltage clamped at ~60 mV in a nominally Ca$^{2+}$-free medium with a CsCl pipette solution. A, Response of a capsaicin (Cap)-insensitive neuron (top) to 100 mM Mg$^{2+}$, 100 mM Ca$^{2+}$, and 100 mM capsaicin and response of a neuron cultured from a TRPV1-null mouse (bottom) to 100 mM Mg$^{2+}$ and 100 mM Ca$^{2+}$, 100 mM capsaicin, and response of a neuron cultured from TRPV1-null mice (Fig. 3). B, Mg$^{2+}$ (30 and 100 mM)-evoked inward currents in a neuron that responded to capsaicin (100 nM). Ca$^{2+}$ (100 mM)-evoked currents that exhibited desensitization. C, Dose-dependent activation of currents by 30–100 mM Mg$^{2+}$ (n = 4–9 for each point) and comparison with 100 mM capsaicin (n = 14; left). Block of 30 mM Mg$^{2+}$ with 5 μM capsazepine (Czp; n = 3; right); **p < 0.01. E, Mg$^{2+}$ (10 mM)-activated TRPV1 channels in an outside-out patch (holding potential, +50 mV). Error bars represent SEM.
of CaR or another cation-sensitive pathway could indirectly lead to activation of TRPV1. Therefore, to examine whether cation writhing is directly related to the activation of TRPV1, we tested whether the magnitude of acute writhing correlated with the activation of TRPV1 currents. Figure 4A shows representative responses of voltage-clamped sensory neurons to 100 mM concentrations of MgCl₂, CaCl₂, or SrCl₂. As described above, Mg²⁺ produced a sustained inward current, whereas Ca²⁺ evoked a rapidly desensitizing response. In addition, Sr²⁺ evoked somewhat smaller desensitizing currents (similar responses were seen in TRPV1-expressing HEK293 cells). Thus, high concentrations of Sr²⁺ can activate TRPV1, but, like Ca²⁺, also promote TRPV1 desensitization. Next, we compared the acute writhing responses induced by intraperitoneal injections of these cations. Figure 4B shows that Ca²⁺ and Sr²⁺ elicited significantly less writhing than Mg²⁺, consistent with the reduced ability of these cations to activate sustained TRPV1 current. In contrast, all of these cations activated the second phase of writhing in a statistically indistinguishable manner (data not shown). These concentrations of Ca²⁺ and Sr²⁺ are sufficient to robustly activate CaR (Hofer and Brown, 2003; Coulombe et al., 2004). Thus, the acute phase of writhing evoked by cations corresponds with the direct activation of TRPV1 and not via another cation sensing pathway.

Cations sensitize TRPV1 currents in oocytes and neurons

We next asked whether monovalent or divalent cations at concentrations below the threshold for direct activation (<10 mM) could modulate agonist-evoked TRPV1 responses. Elevated extracellular MgCl₂ (1–10 mM) enhanced the capsaicin-activated currents in a whole-cell recording (~60 mV) and in an outside-out patch (~100 mV). Mg²⁺ lowers the TRPV1 temperature threshold in oocytes. Current versus temperature relationships are plotted for TRPV1-expressing oocytes in solutions containing 1–10 mM MgCl₂ before (A) and after PDBu treatment (B). The mean temperature thresholds of three to seven oocytes are shown under stated conditions (***p < 0.001 compared with 1 mM Mg control).

Figure 6. Physiological cation concentrations enhance TRPV1 activity during inflammatory conditions. A, B, MgCl₂ (3 mM) enhances BK (100 mM)-evoked activation of TRPV1 in a whole-cell recording (~60 mV) and in an outside-out patch (~100 mV). C–E, Mg²⁺ lowers the TRPV1 temperature threshold in oocytes. Current versus temperature relationships are plotted for TRPV1-expressing oocytes in solutions containing 1–10 mM MgCl₂ before (G) and after PDBu treatment (H). The mean temperature thresholds of three to seven oocytes are shown under stated conditions (***p < 0.001 compared with 1 mM Mg control).

Physiological concentrations of cations contribute to bradykinin and heat-evoked TRPV1 activation

Our data show that a modest increase in [Mg²⁺] (4 mM) is capable of sensitizing TRPV1 to a variety of agonists. In addition, cation regulation of TRPV1 is enhanced by PLC signaling (Fig. 2D,E). We therefore asked whether normal concentrations of cations can contribute to the activation of TRPV1 and, in particular, under conditions that simulate inflammatory pain signaling. The inflammatory mediator bradykinin (BK) plays a key role in signaling during tissue injury. BK sensitizes and activates TRPV1 currents via PLC, PKC, and lipoxygenase-derived products (Premkumar and Ahern, 2000; Huang et al., 2002; Shin et al., 2002). We tested the response of sensory neurons to brief applications of BK (100 nM) in either 1 or 3 mM MgCl₂ solution, which approximates the total physiological concentration of divalent cations (i.e., ~2 mM Ca²⁺ and 1 mM Mg²⁺). Figure 6, A and B, shows that BK evoked substantially greater responses in 3 mM compared with 1 mM Mg²⁺ solutions, and this was apparent in both whole-cell (Fig. 6A) and single-channel (Fig. 6B) recordings from three separate neurons.

TRPV1 is a heat-gated channel (Cesare and McNaughton, 1996; Caterina et al., 1997), and a reduction in the temperature threshold for activation by PLC signaling is considered a hallmark of inflammatory hyperalgesia. We therefore explored the
effects of cations on heat activation of TRPV1 expressed in oocytes (Fig. 6C–E). Under control conditions, 10 mM Mg²⁺ did not significantly alter the temperature threshold of ~47°C (Fig. 6C,E). However, after the stimulation of PKC, the threshold decreased in an Mg²⁺-dependent manner (Fig. 6D,E). Significantly, a marked sensitization occurred with 3 mM Mg²⁺ (~40°C). Importantly, these experiments with bradykinin and heat suggest that physiological concentrations of divalent cations can contribute to the sensitization and activation of TRPV1 during inflammatory pain signaling.

Cations act at TRPV1 proton-binding residues E600 and E648

We next sought to determine the molecular mechanism(s) underlying cation modulation of TRPV1. Elevations in cation concentrations (in particular divalents) will change the membrane surface potential, and it is possible that this might activate/sensitize TRPV1 via its voltage-sensitive properties (Ahern and Premkumar, 2002; Voets et al., 2004). However, the reduction in membrane surface potential produced by cations would oppose TRPV1 via its voltage-sensitive properties (Ahern and Premkumar, 2002; Voets et al., 2004). Thus, these receptors still exhibited Mg²⁺ blockade of proton-evoked currents (Fig. 7A,B), whereas capsaicin-evoked currents were enhanced over the same range of [Mg²⁺] (Fig. 5). To confirm an action at proton-binding sites, we used mutant receptors lacking acidic extracellular amino acids identified as a key for proton activation and modulation. E600 is believed to be important for proton sensitization of agonist-evoked responses, whereas E648 is important for direct proton activation (Jordt et al., 2000).

First, we tested the responses of these mutants to sensitization by cations. Mutation of E600 (E600Q) did not significantly affect the Mg²⁺ enhancement of capsaicin-evoked currents (Fig. 7C). Furthermore, these receptors still exhibited Mg²⁺ blockade of proton currents (Fig. 7B), although the effect was smaller compared with wild-type receptors. In contrast, potentiation by Mg²⁺ was completely inhibited by mutation of E648 (E648A) (Fig. 7C). (Note that these receptors are not activated by protons, and we therefore could not test Mg²⁺ blockade of proton currents.) Second, we tested whether mutants retained the ability to be directly activated by Ca²⁺ and Mg²⁺. Mutation of E600 inhibited Ca²⁺- and Mg²⁺-evoked currents (Fig. 7D,E) without affecting responses to 10 μM capsaicin (applied with 70 mM MgCl₂ or CaCl₂). Mutation of E648 substantially inhibited Ca²⁺-evoked currents. This mutation also reduced the current activated by Mg²⁺, this being significant after PKC stimulation (note that PDBu treatment was used here to reduce variability in the control data). Thus, these data support a model (Fig. 7F) whereby E600 and, to a lesser degree, E648 mediate cation activation of TRPV1, whereas E648 selectively supports cation sensitization of agonist-evoked responses. Interestingly, this is the converse of the response seen with protons, in which E648 mediates proton sensitization of agonist-mediated currents (Jordt et al., 2000). Like protons, activation by cations may occur via charge neutralization of these residues. Divalent cations are presumably more potent agonists, because they impart a greater net positive charge at these sites. This is consistent with observations that substitution of acidic residues with basic residues can produce constitutively open channels (Jordt et al., 2000).

Discussion

Cations directly gate TRPV1

Our results demonstrate that extracellular cations can directly gate and sensitize the TRPV1 channel. This represents a new mechanism for modulating TRPV1 function and extends the list of TRPV1 activators that includes heat, protons, capsaicin (Cate-
Cations interact with extracellular glutamate residues

We showed that cations modulate TRPV1 by interacting with two glutamates (E600 and E648) located between transmembrane domains 5 and 6 near the channel pore. Sensitization with Mg\(^{2+}\) was occluded in the E648A mutant, whereas activation by Mg\(^{2+}\) and Ca\(^{2+}\) was inhibited in both E600Q and E648A mutants. However, the responses to Ca\(^{2+}\) were more markedly inhibited compared with Mg\(^{2+}\), suggesting that additional acidic residues may also contribute to Mg\(^{2+}\) activation. E600 and E648 have been implicated previously in the activation and sensitization of TRPV1 by protons (Jordt et al., 2000). Acidosis to pH 6 sensitizes TRPV1 to agonists including heat and capsaicin, whereas acidosis <pH 6 directly gates the receptor. Similarly, extracellular cations sensitize and directly gate TRPV1 in a concentration- and charge-dependent manner. At room temperature, 1–10 mM divalent cations sensitize TRPV1, whereas divalent cations >10 mM directly gate the receptor. Monovalent cations require concentration increases of ~50 mM to exert significant effects on activity. Nevertheless, all of these processes are likely to be enhanced at 37°C, given the temperature dependence of TRPV1 gating. Although proton regulation of TRPV1 is likely to be manifest only during tissue acidosis, sensitization of TRPV1 by cations can occur under normal physiological conditions. Thus, extracellular glutamates might serve interchangeably as both proton- and cation-binding sites. Indeed, we found that cations could inhibit proton-gated responses presumably because of competitive binding at these sites. However, a significant block of proton responses only occurred at 5–10 mM Mg\(^{2+}\), suggesting that this effect will not play a general role under normal physiological conditions. Interestingly, competitive cation–proton interactions also occur in acid-sensitive ion channels (Immmke and McCleskey, 2003) and may subserve gating of these channels.

TRPV1 may transduce nociceptive responses to elevated ionic strength

Elevations in ionic strength are often perceived as painful and are used in models of visceral and muscle pain (Fukawa et al., 1980; Gyires and Torma, 1984; Graven-Nielsen and Mense, 2001). Cation regulation of TRPV1 may explain why high concentrations of exogenous Mg\(^{2+}\), salt, and seawater (~11 mM Ca\(^{2+}\), 55 mM Mg\(^{2+}\), and 460 mM NaCl) cause pain and why this response is more marked in the setting of tissue injury. Moreover, concentrations of cations sufficient to directly gate TRPV1 are found in various tissues and extracellular microenvironments. For example, sensory neurons innervate the epithelial tissues of skin and airways that commonly encounter elevated ionic strength (Anderson, 1984), as well as bone, in which the [Ca\(^{2+}\)] surrounding resorbing osteoclasts approaches 20 mM (Silver et al., 1988). TRPV1 activation in these settings may contribute to the pain of exercise-induced asthma (Anderson, 1984; Wiens et al., 1992) and bone cancer (Walls et al., 1995). In addition, cation regulation of TRPV1 may participate in non-nociceptive functions. In the urinary bladder, TRPV1 channels are present in both sensory nerves and the bladder epithelium, in which they contribute to voiding behavior (Birder et al., 2001, 2002). Interestingly, in the tongue, TRPV1-positive fibers innervate the taste papillae (Ishida et al., 2002; Kido et al., 2003), and amiloride-insensitive salt responses in the chorda tympani are abolished in TRPV1-null animals (Lyall et al., 2004). Thus, TRPV1 or a splice variant may transduce some forms of salt taste perception.

Cation sensitization of TRPV1 may contribute to pain signaling

Although direct activation of TRPV1 may occur under extreme or pathophysiological cation concentrations, a more significant effect of cations may occur under normal physiological conditions. Our data show that millimolar increases in cation concentrations (1–5 mM) are sufficient to sensitize TRPV1 to various ligands including capsaicin, AEA, and NADA. This sensitization is quite marked; in sensory neurons, Mg\(^{2+}\) produced an approximately fourfold increase in capsaicin-evoked current, whereas in oocytes, increasing Mg\(^{2+}\) from 1 to 10 mM halved the capsaicin EC\(_{50}\) equivalent to the shift produced by acidosis (pH 6.4) (Tomina et al., 1998). In addition, we demonstrate that physiological concentrations of cations are capable of regulating TRPV1 in the setting of tissue injury and inflammation. Tissue damage leads to the generation of chemical mediators, such as bradykinin, which activate and sensitize TRPV1 (Premkumar and Ahern, 2000; Chuang et al., 2001). In turn, this manifests as a marked reduction in the threshold for heat activation (Cesare and McNaughton, 1996; Tomina et al., 2001; Vellani et al., 2001) and accounts for inflammatory thermal hyperalgesia in animals (Caterina et al., 2000; Davis et al., 2000). Importantly, our data show that normal levels of divalent cations contribute to both of these processes. Thus, cations may act in a constitutive manner to regulate TRPV1 sensitization. Even small changes in cation concentrations may therefore influence nociception by modulating the magnitude of TRPV1 responses.

References


