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Biophysical properties of menthol-activated cold receptor TRPM8 channels

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Abstract

The temperature-sensitive transient receptor potential channel, TRPM8, was recently cloned and found to be activated by cold and menthol. Whole-cell recordings show that TRPM8 is permeable to multiple cations and exhibits a strong outward rectification. Here, we examine the mechanism underlying menthol-evoked current rectification of TRPM8 transiently expressed in tsA-201 cells at room temperature (~25 °C). Whole-cell currents (ruptured, bath: Na^+, K^+, Ca^{2+}, or Ba^{2+}; pipette: KCl) exhibited a strong outward rectification in the presence of menthol, consistent with previous studies. The outward K^+ current was reduced in the presence of external Ca^{2+} or Ba^{2+}. Single-channel recordings (cell-attached) showed that menthol induced brief channel openings with two conducting states in the voltage range between −80 and +60 mV. The small current (i_S) conducted both monovalent and divalent ions, and the large one (i_L) predominantly monovalent ions. The i–V plot for Ca^{2+} was weakly outward rectifying, whereas those for monovalent ions were linear. The i_S may result in the divalent ion-induced reduction of the whole-cell outward current. The open probability (P_o) in all ion conditions tested was low at negative voltages and increased with depolarization, accounting for the small inward currents observed at the whole-cell level. In conclusion, our results indicate that menthol induced steep outward rectification of TRPM8 results from the voltage-dependent open channel probability and the permeating ion-dependent modulation of the unitary channel conductance.

Keywords: Recombinant DNA; Patch clamp recording; Macroscopic and unitary channel activity; Outward rectification; Non-selective cation channel; Ion permeability; Channel open probability; Fura-2 calcium imaging; Cold sensation

Thermoception is the physiological process of sensing heat or cold. Recent studies reveal at least six members of the transient receptor potential (TRP) ion channel family involved in the mediation of thermosensation [1]. Similar to other members of the TRP family, the temperature-activated channels belong to the class of non-selective cationic channels [2]. They are composed of six putative transmembrane segments with intracellular amino and carboxyl termini. Analogous to the voltage-dependent potassium channels, the predicted pore region is located between transmembrane helices five and six [3–6].

TRPM8 (also known as the cold and menthol receptor: CMR1) is a member of the TRPM subfamily, which has the highest homology with the melastatin receptor (TRPM1). It was cloned independently by its responsiveness to cooling or menthol [7] or by screening of a human genomic DNA database [2]. The channel is expressed in neurons located at the dorsal root ganglion [2,8] and trigeminal ganglion [2], as well as prostate cells [9]. Whole-cell voltage-clamp recordings show that temperatures below 25–28 °C or menthol activates the channel and results in a non-selective cationic current with outward rectification [1,7,10,11]. The channel is permeable to sodium (Na^+), potassium (K^+), cesium (Cs^+), and calcium (Ca^{2+}), leading to a rise in cytoplasmic Ca^{2+} levels [2,7]. In addition, activation of TRPM8 channels localized on intracellular stores may also be involved in elevating the calcium signal [8]. Recent evidence suggests that the rectification property from cold-elicited TRPM8

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channels arises from a voltage-dependent gating process [11,12]. However, whether the gating and unitary properties of the menthol-induced current are also altered by voltage has not been tested in detail. In addition, it is unclear whether the gating mechanism is dependent on the permeable ion. Furthermore, all the single-channel studies were previously carried out at strong depolarization voltages (>+60 mV) [11,12].

Here, we take advantage of the transient expression system of recombinant TRPM8 to examine the menthol-induced channel activities at both the macroscopic and unitary levels under different ionic conditions at room temperature (~25 °C). We found that current activities of single-TRPM8 channels elicited by menthol at a voltage range between −80 and +60 mV exhibited a small (i_S) and a large (i_L) conductance to monovalent ions K^+ or Na^+, and only a small conductance to divalent ions, Ca^{2+} or Ba^{2+}. The open probability of the channel appeared low in all conditions, and increased with depolarization. Our findings provide fundamental insight into the steep outward rectification properties of the macroscopic current of the channel.

Materials and methods

Tissue culture and transient transfection. Human embryonic kidney tsa-201 cells were maintained in a 37 °C CO_2 incubator in standard DMEM (Gibco) supplemented with 10% fetal bovine serum, 200 U/ml penicillin, and 0.2 mg/ml streptomycin [13]. At 85% confluency, the cells were split with trypsin EDTA and plated on glass coverslips at 20% confluency. The cells were allowed to recover for 12 h at 37 °C. The medium was then replaced, and the cells were transiently transfected with cDNAs encoding for TRPM8 in pcDNA3 (a kind gift from Dr. David Julius of the University of California, San Francisco, California, USA) and green fluorescent protein (gWIZ-GFP) at a 4:1 molar ratio, using a standard calcium phosphate protocol. After 12 h, the medium was replaced with fresh DMEM. The cells were allowed to recover for an additional 1–2 days prior to recording.

Chemicals and solutions. All chemicals used in the cell culture were purchased from Gibco (Invitrogen). Chemicals used for physiological recordings were purchased from Sigma.

Electrophysiology and data analysis. Single-channel (cell-attached patch) and whole-cell (ruptured) recordings were performed using an Axopatch 700A amplifier (Axon Instruments, Foster City, CA) linked to a personal computer equipped with pClamp9. Patch pipettes (Sutter borosilicate glass, BF 150-86-15) were pulled using a Sutter P-87 microelectrode puller and subsequently fire polished using a Narashige microforge, as described previously [13].

For whole-cell current recordings, pipettes (2–4 MΩ) were filled with internal solution containing (mM) 140 cesium methanesulfonate or KCl, 4 $MgCl_2$, 9 EGTA, and 9 Hepes (pH 7.2, adjusted with CsOH or KOH). The cells were transferred to a 3 cm culture dish containing recording solution composed of (mM) 140 NaCl or KCl, 1 $MgCl_2$, 10 Hepes, and 10 glucose (pH 7.2 adjusted with NaOH or KOH). Perfusions consisted of the above recording solution with 0.01–1 mM (±) menthol or a solution composed of (mM) 20 $BaCl_2$ (or $CaCl_2$), 1 $MgCl_2$, 10 Hepes, 10 glucose, and 100 NaCl. Currents were elicited by stepping from a holding potential of −100 mV to various test potentials using Clampex (Axon Instruments). The external recording solution was perfused onto the cells using a gravity-driven perfusion system. Data were filtered at 1 kHz (−3 dB) using a 4-pole Bessel filter and digitized at a sampling frequency of 2 kHz. Data were analyzed using Clampfit (Axon Instruments). All curve fittings were carried out using Origin 7 (Microcal).

For single-channel recordings, pipettes (10–20 MΩ) were coated with sylgard and filled with solution containing (mM) 100 KCl (or NaCl, $BaCl_2$, $CaCl_2$), 10 Hepes, and 0.01–1 (±) menthol (pH 7.2). Cells were transferred to a 3 cm culture dish containing solution composed of (mM) 140 NaCl or KCl, 1 $MgCl_2$, 10 Hepes, 10 EGTA, and 10 glucose (pH 7.2). Currents were elicited by holding at the test voltage. The data were sampled at 25 kHz and filtered at 10 kHz (−3 dB, 4-pole Bessel filter), using an analog-to-digital interface Digidata 1322 (Axon Instruments, Foster City, CA). All experiments were performed at room temperature (22–26 °C).

The single-channel data were filtered at 6 kHz and a segmental k-means (SkM) approach was used for event detection and idealization [14] using QuB software [15]. The algorithm is based on hidden Markov Modelling and takes into account a priori information on both channel current and background noise. It assumes a Markovian signal superimposed with Gaussian noise, and thus is capable of distinguishing events even at low signal-to-noise ratios. A fixed dead-time of 80 μs was imposed and corrected for in the idealized data. The time constants were estimated with the QuB software MIL (Maximum Interval Likelihood) as previously described [16]. The current amplitude was determined by direct measurement from the recording traces. The open probability of the channel (P_o) was determined by normalizing the net current to the product of the maximal current amplitude and length of recording time.

Ratiometric Fura-2 calcium imaging. Intracellular calcium ($[Ca]_i$) was measured using a Fura-2 ratiometric Ca^{2+} imaging system, as described previously [17]. The cells were incubated with 4 μM Fura-2 AM (Molecular Probes Inc.) for 30 min and washed with recording solution three times prior to imaging. The experiments were carried out in dark to prevent photobleaching of the dye. Fura-2 fluorescence was excited at 340 and 380 nm generated by UV light from a 100 W Hg/Xe-arc lamp, alternately passed through 340 and 380 nm excitation filters, which were controlled by Image Pro 5 (PTI). The fluorescence was sampled at a frequency of 5 Hz. The fluorescence signal, generated after the excitation light, was reflected via a 430 nm dichroic mirror, passed through a 510 nm emission filter, and detected and digitized by an intensified charged-coupled device (ICCD) camera (PTI) in Image Pro 5. The fluorescence intensity ratio of images acquired at 340 and 380 nm was calculated on a pixel-by-pixel basis using Image Pro 5. Mean values for the fluorescence intensity ratio of any given region of interest were measured after a correction for a background signal recorded in the absence of the fluorescent dye.

Statistics. The data were presented as means ± SEM. Error propagation was determined as described by Abramowitz and Stegun [18]. Statistical analysis was carried out using SigmaStat 2.0 (Jandel Scientific). Differences between mean values from each experimental group were tested using a Student's t test for two groups and one-way analysis of variance (ANOVA) for multiple comparisons. Differences were considered significant if $p < 0.05$.

Results

Divalent ions reduce the whole-cell current activity of recombinant TRPM8 channels

Whole-cell current recordings were carried out in TRPM8-expressing tsA201 cells in the presence of 10–100 μM (±) menthol. The current–voltage (I–V) relation