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Plenary Article

Temperature dependence of rapidly adapting mechanically activated currents in rat dorsal root ganglion neurons

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HIGHLIGHTS

- Thermal sensitivity of rapidly adapting mechanically activated channels was studied.
- The temperature coefficient (Q10) value was >5 and activation energy >30 kcal/mol.
- Thus, the activation of these channels is highly temperature-dependent.

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ABSTRACT

Rapidly adapting mechanically activated channels (RA) are expressed on somatosensory neurons and thought to play a role in mechanical transduction. Because mechanical sensations can be significantly affected by temperatures, we examined thermal sensitivity of RA currents in cultured dorsal root ganglion (DRG) neurons to see if RA channel activity is highly temperature-dependent. RA currents were evoked from DRG neurons by membrane displacements and recorded by the whole-cell patch-clamp recording technique. We found that RA currents were significantly enhanced by warming temperatures from 22 to 32 °C and reduced by cooling temperatures from 24 to 14 °C. RA channel activation exhibited steep temperature-dependence with a large temperature coefficient (Q10 > 5) and a high activation energy (Ea > 30 kcal/mol). We further showed that RA channel activation by mechanical stimulation led to membrane depolarization, which could result in action potential firing at 22 °C or 32 °C but not at 14 °C. Taken together, our results provide the measurements of thermal dynamics and activation energy of RA channels, and suggest that a high energy barrier is present for RA channels to open. These findings are in agreement with temperature sensitivity of mechanical sensations in mammals.

1. Introduction

The sense of touch can be significantly affected by skin temperatures in mammals. For example, in humans the sensitivity to punctuate pressure [16], vibration [6], tactile spatial acuity [15], and roughness [7] is decreased at cooling temperatures. In fact, tactile function can be completely impaired to result in numbness when skin temperature falls well below normal [11]. On the other hand, raising skin temperature above normal levels can increase the sensitivity to high-frequency vibration [6] and enhance the apparent roughness [7]. Mechanisms by which temperature affects mechanical sensations remain unclear.

The transduction of mechanical stimulation is believed to be mainly mediated by mechanically activated ion channels (MA) that are expressed on sensory organs including primary afferent fibers derived from dorsal root ganglions (DRG) and trigeminal ganglions. McCarter et al. [13] first recorded MA currents from rat DRG neurons in culture by using the whole-cell patch-clamp recording technique. At least two types of whole-cell MA currents have been observed in DRG neurons, the rapidly adapting (RA) and the slowly adapting currents (SA) [4]. The RA currents are found to be mediated by large-pore mechanically activated channels nonselective for cations but impermeable to anions [12]. Single channel recordings from DRG neuron membrane patches also revealed two types of MA channels with either low- or high-threshold to pressure stimulation [1]. These two types of MA channels detected by single channel recordings may give rise to the observed MA whole-cell currents in sensory neurons. Molecular identities of MA channels in sensory neurons have recently been explored; RA currents in DRG neurons appear to be mediated by Piezo2, one of the two

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mammalian MA channels recently cloned from a mouse neuroblastoma cell line [3]. Piezo2 as a molecular substrate of RA currents is supported by the findings that Piezo2 is expressed on some DRG neurons and that knockdown of Piezo2 in DRG neurons specifically reduced RA currents without affecting SA currents [3].

One mechanism by which temperature affects mechanical sensations may be through thermal dynamic influence on activation energy of MA channels. This is because MA channels would expose to different temperatures if they are located at nerve terminals under the skin. Most ion channels have low to moderate thermal dynamics with Q10 values of 2–4 [8] and thereby are not highly temperature dependent for their activation. In contrast, thermal receptors such as TRPV1 and TRPM8 channels have Q10 > 9 [18]. For MA channels in mammalian sensory neurons, previous studies were usually performed at a single temperature and temperature dependence of their MA channel activation was not determined. In the present study, we set out to determine RA channel activation on DRG neurons at warming and cooling temperatures, and to measure thermal dynamics (Q10) and activation energy (Ea) of RA channels on DRG neurons. Our study provides evidence indicating that mechanical transduction through RA channels is highly temperature-dependent.

2. Materials and methods

Adult Sprague Dawley rats (100–250 g, both genders) were used. Animal care and use conformed to National Institutes of Health guidelines for care and use of experimental animals. Experimental protocols were approved by the University of Cincinnati Institutional Animal Care and Use Committee. DRG neuron cultures were prepared as described previously and maintained in MEM culture medium that also contained nerve growth factor (10 ng/ml) [17]. Cells were used at the days in culture between 5 and 12 days.

Cultured neurons were continuously perfused with a normal bath solution at 2 ml/min. The normal bath contained (in mM) 145 NaCl, 5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 glucose, 10 HEPES, pH 7.3 and 330 mOsm. Unless otherwise indicated, bath solution was maintained at a room temperature of 22 °C. Electrode internal solution contained (in mM) 135 K-Gluconate, 5 KCl, 2.4 $MgCl_2$, 0.5 $CaCl_2$, 5 EGTA, 10.0 Hepes, 5.0 Na2ATP, 0.33 GTP-Tris salt, pH was adjusted to 7.35 with NaOH and osmolarity was adjusted with sucrose to 320 mOsm. Electrode resistance was 3–6 MΩ, and membrane access resistance in the whole-cell configuration was about 10 MΩ. Junction potential was calculated to be 16 mV and was corrected for in the data analysis. Current and voltage signals were recorded with an Axopatch 200B amplifier, and signals were filtered at 2 kHz and sampled at 5 kHz using pCLAMP 9.0 (Axon Instruments).

Cells were either under voltage-clamp or current-clamp configuration. Unless otherwise indicated, voltage-clamp experiments were performed with cells held at −76 mV. Mechanical stimulation was applied to DRG cell bodies using a heat-polished glass pipette as a probe (tip diameter approximately 4 μm); the probe was controlled by a piezo-electric device (Physik Instruments, Auburn, MA). The probe was positioned at an angle of 45° to the surface of the dish. The tip of the probe and the recorded cell were visualized as live images on a monitor. The images were captured continuously through a camera that was connected to the microscope with 40× objective. The tip was positioned so that a 2 μm movement did not contact the cell, 3 μm had a visible contact but little membrane movement, and a 4 μm stimulus produced an observable membrane deflection. Therefore, tip forward steps of 2 μm and 3 μm were assigned as position −1 and 0 μm, respectively; a 4 μm forward step was recorded as a 1 μm membrane displacement. The probe was moved at a speed of 0.5 μm ms^{-1}. Membrane displacement steps were applied at 1 μm increments each with 500 ms duration. Membrane input resistance was continuously monitored during each recording by applying testing pulses at a voltage step of 5 mV.

The temperatures of the bath solution were controlled by a peltier cooling device (Warner Instrument, CT). The bath solution was delivered to recorded cells from a short tube (0.2 cm L, 500 μm ID) with the outlet 500 μm from the recorded cells. The temperatures at the recording sites were continuously recorded with a thermal probe. Temperature ramp was applied either from 22 °C to 32 °C (6 min) or from 24 °C to 14 °C (10 min). During the temperature ramps, MA currents were evoked at every 2 °C temperature interval.

Recordings from voltage- and current-clamp experiments were analyzed using Clampfit 9 software. Q10 was calculated using the following equation: $Q10 = (X_{T2}/X_{T1})^{(T2-T1)/10}$; where X_{T1} and X_{T2} are the current amplitudes (scaled with input resistance) at the temperatures of T2 and T1, respectively [9]. To calculate activation energy, the scaled current amplitudes were plotted as Arrhenius plots with the equation of $X = Ae^{-Ea/RT}$ [9] where X is the scaled currents, A is a constant, Ea is the activation energy, R is the gas constant, and T is the temperature in the Kelvin scale. Plots of $\ln X$ versus $1000/T$ were fitted by linear regression to give a slope of $-Ea/R$, from which Ea was obtained. Data are reported as mean ± SEM. Statistical significance ($p < 0.05$) was assessed by Student's t test or Analysis of Variance (ANOVA, one way) followed by Student–Newman–Keuls post hoc.

3. Results

We first characterized mechanically activated currents at room temperature of 22 °C. Under voltage-clamp with cells held at −76 mV, displacements of DRG neuron membranes by using a fire-polished glass probe (Fig. 1A) could elicit inward currents in many cells. Rapidly adapting (RA) currents were usually encountered (Fig. 1B). Slowly adapting currents were sometimes observed but were not further characterized in this study because we intended to focus on MA currents that were likely to be mediated by Piezo2 channels [3].

RA currents after peaking decayed rapidly to baseline level although stimulation was maintained at the same degree (Fig. 1B). In a sample of 14 cells tested with a membrane displacement of 6 μm, we fitted the falling phase of RA currents with a single exponential equation (Fig. 1B), which yielded a decay time constant of $3.9 \pm 0.1\ ms^{-1}$ ($n = 14$). The RA currents were enhanced with increases of membrane displacement distances up to 8 μm (Fig. 1C, $n = 12$). Membrane displacements of >8 μm could further increase RA currents in some cells but in other cells could result in the loss of patch (not shown). Therefore, we only reported MA currents with membrane displacement distances of ≤8 μm. We tested RA currents elicited by 6 μm membrane displacement at several different holding potentials (Fig. 1D), which revealed a reversal potential near 0 mV (−6.7 ± 3.6 mV, $n = 12$) based on RA current–voltage relationship (Fig. 1D).

We tested effects of warming temperatures on RA currents (Fig. 2A and B). In this set of experiments, a membrane displacement of 6 μm was used to elicit RA at warming temperatures from 22 to 32 °C. Membrane input resistance was continuously recorded in each cell during an experiment (Fig. 2A). This served as a quality control on one hand and on the other hand it was for scaling RA currents to equivalent input resistance because temperature changes affected membrane input resistance (see below). As an example shown in Fig. 2A, a 6-μm membrane displacement elicited a small RA current. The RA current amplitude was increased by several folds at 32 °C and brought back to the original level upon returning to 22 °C (Fig. 2A). Warming temperatures from 22 to

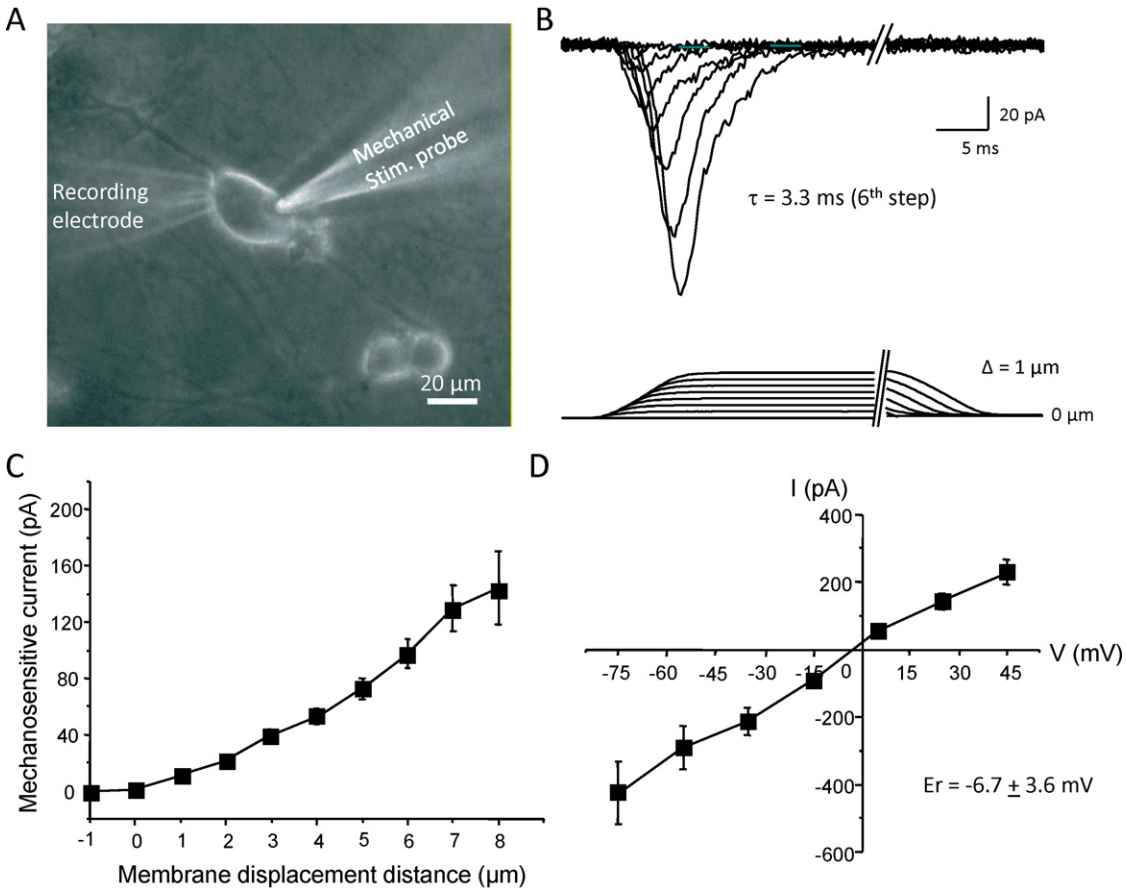

Fig. 1. Characterization of rapidly adapting mechanically activated currents on DRG neurons at 22 °C. (A) Image shows a DRG neuron with a patch-clamp recording electrode and a mechanical stimulation probe and illustrates the relative positions of recording electrode tip and stimulation probe tip for each cell tested in the present study. The recording electrode tip and stimulation probe tip were positioned at ~1/8 of the cell from left side and ~1/4 of cell from right side, respectively. (B) Sample traces show the rapidly adapting mechanically activated currents (RA) evoked by membrane displacements at different distances. The τ value is decay time constant obtained by fitting with a single exponential equation the current evoked by 6 μm membrane displacement. Membrane displacement distances with increments of 1 μm are indicated below the current traces. (C) Summary of RA currents evoked by membrane displacement at different distances. (D) I–V curve of RA currents evoked by membrane displacement at the distance of 6 μm. All experiments were performed at a room temperature of 22 °C.

32 °C increased RA current amplitude in a temperature-dependent manner (Fig. 2B). At 32 °C, the highest temperature tested in this study, RA current amplitude was ~4-fold (374 ± 47%, n = 20) of that at 22 °C (Fig. 2B). Warming temperatures from 22 °C to 32 °C also resulted in a temperature-dependent reduction of cell membrane input resistance (Fig. 2C). We scaled RA currents by input resistance values so that Q10 would not be underestimated due to the change of input resistance (Fig. 2D). In the warming temperature range, Q10 was calculated to be about 5 (5.2 ± 0.6, n = 20) and activation energy (Ea) was 32.7 ± 2.0 kcal/mol (n = 20, Fig. 3A).

In contrast to warming temperatures, cooling temperatures from 24 to 14 °C resulted in a progressive decease of RA current amplitude (Fig. 2E). For example, RA current amplitude at 14 °C was only ~17% (16.8 ± 6%, n = 9) of that at 24 °C. Cooling temperatures increased membrane input resistance significantly (Fig. 2F). After scaling by input resistance, cooling temperature-dependent reduction of RA became steeper (Fig. 2G). At the cooling temperatures ranging from 24 to 14 °C, Q10 was calculated to be about 7 (6.7 ± 1.5, n = 9) and Ea was 47.1 ± 5.8 kcal/mol (n = 9, Fig. 3B).

We examined membrane depolarization in response to mechanical stimulation at different temperatures (Fig. 4). Fig. 4A and B is an example showing RA currents (Fig. 4A) and membrane depolarization (Fig. 4B) examined under voltage-clamp and current-clamp, respectively. Responses to membrane displacements were tested at 22 °C (left panels of Fig. 4A and B), cooling temperature of 15 °C (middle panels of Fig. 4A and B), and warming temperature of 32 °C (right panels of Fig. 4A and B). As shown in this example, the degree of membrane depolarization was lower at 15 °C in comparison with the responses at 22 °C or 32 °C (Fig. 4B), corresponding to RA currents elicited by the same membrane displacements (Fig. 4A). In this cell, membrane displacement at 6 μm led to action potential firing at 32 °C but not at other two temperatures; increasing membrane displacement to 7 μm resulted in action potential firing at both 22 and 32 °C but not at 15 °C. Fig. 4C summarizes the degree of membrane depolarization and numbers of cells that fire action potentials at the three temperatures. Membrane depolarization was significantly higher at 22 and 32 °C than at 15 °C. Due to action potential firing in some cells it is not applicable to make a statistical comparison between 22 and 32 °C for the mount of membrane depolarization. At the membrane displacement of 6 μm, action potentials were elicited in 1 of 5 cells at 22 °C, 2 of 5 cells at 32 °C, but no cell (n = 0/5) at 15 °C. With a further increase of membrane displacement to 7 μm, action potentials were elicited in 2 of 5 cells at 22 °C, 3 of 5 cells at 32 °C, but still no cell at 15 °C (n = 0/5).

4. Discussion

RA currents recorded at room temperatures in the present study have kinetics and reversal potentials similar to those reported in previous studies [3,4] and are likely to be mediated by

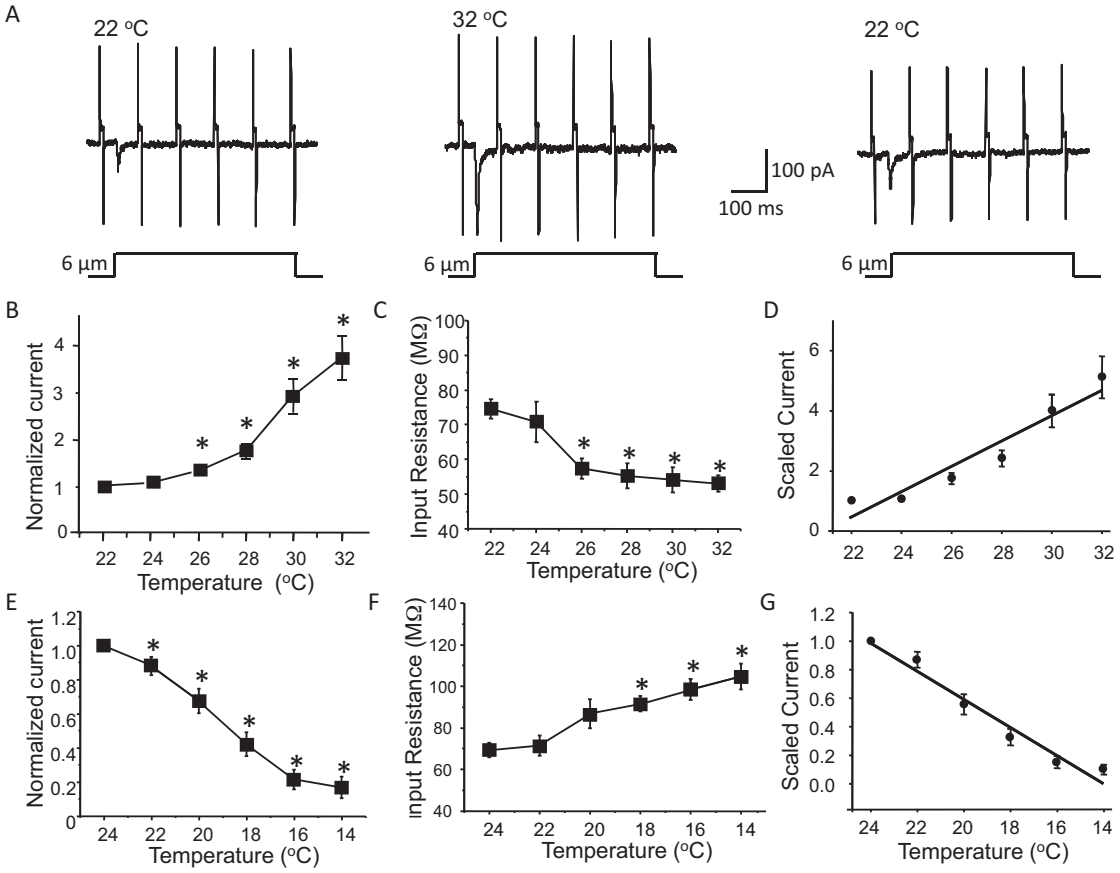

Fig. 2. Effects of warming and cooling temperatures on RA currents in DRG neurons. (A) Sample traces show RA currents elicited by 6 μm membrane displacement at 22 °C (left panel and right panel) and 32 °C (middle panel). Membrane displacement step is shown below each sample current trace. The sample traces also include membrane input resistant tests, which were achieved by applying multiple testing pulses each at a voltage step of 5 mV. (B) Summary of RA currents evoked by 6 μm membrane displacement at different warming temperatures. RA Currents were normalized to the values at 22 °C. (C) Summary of membrane input resistance at different warming temperatures. (D) Normalized currents in (C) are further scaled by input resistance and fitted by linear regression. (E) Summary of RA currents evoked by 6 μm membrane displacement at different cooling temperatures. Currents were normalized to the values at 24 °C. (F) Summary of membrane input resistance at different cooling temperatures. (G) Normalized currents in (E) are further scaled by input resistance and fitted by linear regression.

Piezo2 channels identified recently [3]. The present study further characterized temperature dependence of RA currents by performing experiments in both warming and cooling temperatures. This allowed us to reveal the thermal dynamic properties of RA channel activation. RA channel activation in the present study shows highly temperature-dependent, as is evidenced by high values of Q10. The Q10 values are ~5 in warming temperatures of 22–32 °C, and ~7 in cooling temperatures of 24–14 °C. These Q10 values are very high for an ion channel, suggesting that a very high free energy may be required in order to change RA channel

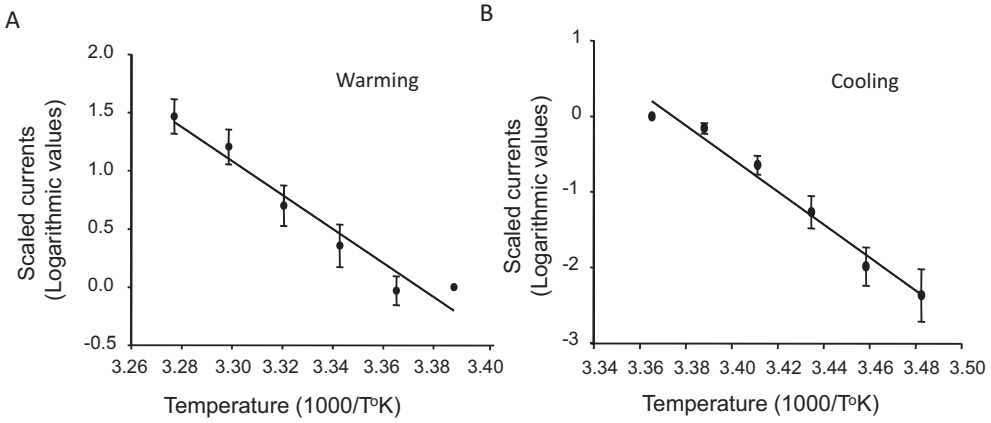

Fig. 3. Arrhenius plot of RA currents of DRG neurons at warming and cooling temperatures. (A) and (B) Arrhenius plot of scaled RA currents evoked by 6 μm membrane displacement at warming (A, 22 °C to 32 °C) and cooling temperatures (B, 24 °C to 14 °C). Solid lines are the best linear fits to the data points. The slopes of the fitted lines correspond to activation energies of 33 kcal/mol in the warming temperatures and 47 kcal/mol in the cooling temperatures. Logarithmic values with base number e are used for scaled currents. Temperature is at the Kelvin scale (°K).

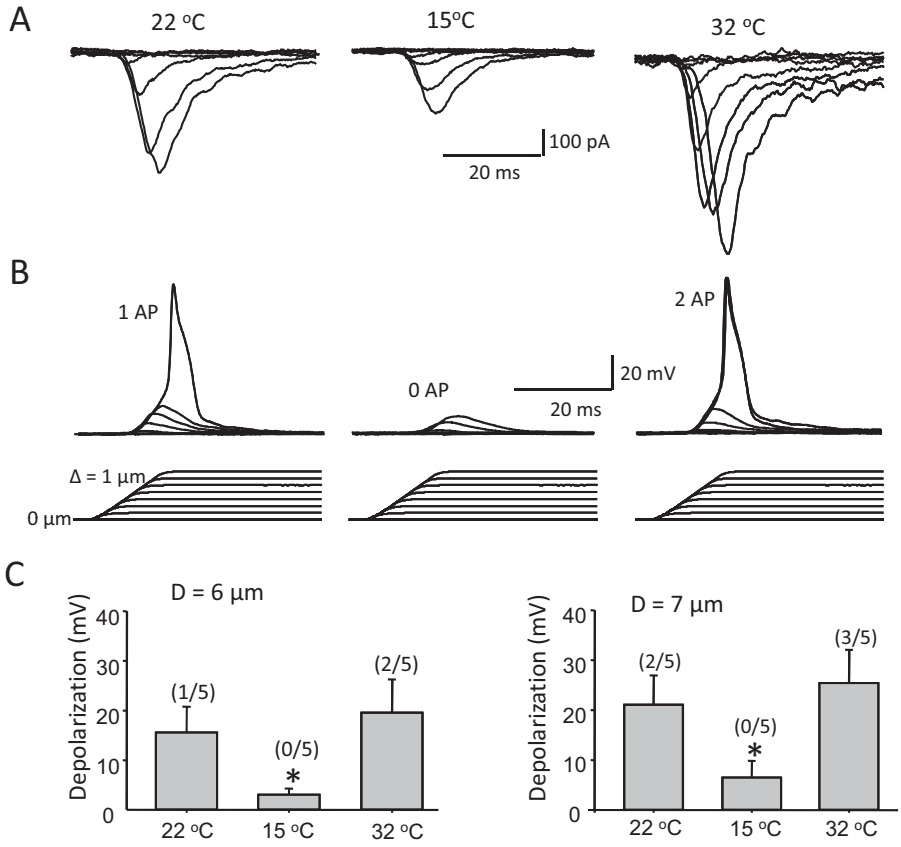

Fig. 4. Membrane depolarization and action potential firing of DRG neurons in response to mechanical stimulation at different temperatures. (A) and (B) Sample traces show RA currents (A) and membrane depolarization (B) elicited by membrane displacements in a same DRG neuron. The tests were performed at 22 °C (left panels), 15 °C (middle panels), and 32 °C (right panels). Membrane displacement steps are indicated in (B) below traces of membrane depolarization. (C) Summary of membrane depolarization evoked by membrane displacement of 6 μm (left) and 7 μm (right) at different temperatures. Numbers above each bar indicate numbers of cells that fired action potentials in total numbers of cells tested. For cells that fired action potentials, the differences between action potential threshold and resting membrane potentials were used as estimated values of membrane depolarization in response to mechanical stimulation at 22 °C and 32 °C.

conformation to open RA channels in response to mechanical stimulation.

While temperature affects channel gating of almost all types of ion channels, most of ion channels including voltage-gated Na^+, Ca^{2+} and K^+ channels are known to have low to moderate temperature dependence with Q10 values in a range of 2–4 [8]. However, thermo-TRP channels have exceptionally high temperature sensitivity with Q10 between 6 and 30 [19]. RA channels shown in the present study have significantly higher Q10 value than most of other ion channels. With Q10 near the lower end of thermal-TRP channels, it is conceivable that mechanical transduction mediated by RA channels should be highly susceptible to temperature changes.

We have shown in this study that RA channels in DRG neurons have activation energy between 33 and 47 kcal/mol in a temperature range between 14 and 32 °C. To the best of our knowledge, this is the highest temperature sensitivity yet observed for mechanotransduction in animal kingdoms. Temperature sensitivity of mechanotranduction has been studied previously in cockroach tactile spine [5], bullfrog saccular hair cells [2], VS-3 neurons of a spider mechanoreceptor [9], and cat Pacinian corpuscles [10]. Activation energy in these sensory organs has been shown to be between 12 and 23 kcal/mol, much smaller than that of RA channels in rat DRG neurons. The relatively high activation energy of RA channels in comparison with other mechanical sensory organs suggests that the RA channels of DRG neurons have a relatively high-energy barrier for activation and thereby may have a high mechanical threshold in comparison with the previously characterized mechanoreceptors.

We have shown that stimulation of RA channels can result in membrane depolarization and action potential firing in a temperature-dependent manner. For example, at cooling temperature of 15 °C, the degree of membrane depolarization was much smaller than that at 22 °C and 32 °C. At 15 °C, action potentials were not elicited by mechanical stimulation although the same stimulation could elicit action potentials at 22 °C and 32 °C. The failure of eliciting action potentials 15 °C appears to be because of too small depolarization to reach action potential threshold at the cooling temperature. This result may explain why mechanical sensations become blunted or even numbed by cooling temperatures [11]. Conduction block due to the inhibition of voltage-gated Na^+ channels has been suggested to contribute to the cooling effects on mechanical sensitivity [20]. However, the Q10 value of voltage-gated Na^+ channels were less than 2 at cooling temperatures [14], much lower than that of RA channels. Therefore, effects of cooling temperatures on RA-mediated mechanical sensation would be more at a mechanical transduction level and less at a signal conduction level.

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References

[1] H. Cho, J. Shin, C.Y. Shin, S.Y. Lee, U. Oh, Mechanosensitive ion channels in cultured sensory neurons of neonatal rats, Journal of Neuroscience 22 (2002) 1238–1247.

[2] D.P. Corey, A.J. Hudspeth, Kinetics of the receptor current in bullfrog saccular hair cells, Journal of Neuroscience 3 (1983) 962–976.

[3] B. Coste, J. Mathur, M. Schmidt, T.J. Earley, S. Ranade, M.J. Petrus, A.E. Dubin, A. Patapoutian, Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels, Science 330 (2010) 55–60.

[4] L.J. Drew, J.N. Wood, P. Cesare, Distinct mechanosensitive properties of capsaicin-sensitive and -insensitive sensory neurons, Journal of Neuroscience 22 (2002) RC228.

[5] A. French, Transduction mechanisms of mechanosensilla, Annual Review of Entomology 33 (1988) 39–58.

[6] B.G. Green, The effect of skin temperature on vibrotactile sensitivity, Perception & Psychophysics 21 (1977) 243–248.

[7] B.G. Green, S.J. Lederman, J.C. Stevens, The effect of skin temperature on the perception of roughness, Sensory Processes 3 (1979) 327–333.

[8] B. Hille, Ion Channels of Excitable Membranes, Sinauer Associates, Inc., Sunderland, MA., 2001, 814 pp.

[9] U. Hoger, A.S. French, Temperature sensitivity of transduction and action potential conduction in a spider mechanoreceptor, Pflugers Archiv 438 (1999) 837–842.

[10] N. Ishiko, W.R. Loewenstein, Effects of temperature on the generator and action potentials of a sense organ, Journal of General Physiology 45 (1961) 105–124.

[11] N.H. Mackworth, Finger numbness in very cold winds, Journal of Applied Physiology 5 (1953) 533–543.

[12] G.C. McCarter, J.D. Levine, Ionic basis of a mechanotransduction current in adult rat dorsal root ganglion neurons, Molecular Pain 2 (2006) 28.

[13] G.C. McCarter, D.B. Reichling, J.D. Levine, Mechanical transduction by rat dorsal root ganglion neurons in vitro, Neuroscience Letters 273 (1999) 179–182.

[14] J.R. Schwarz, The effect of temperature on Na currents in rat myelinated nerve fibres, Pflugers Arch 406 (1986) 397–404.

[15] J.C. Stevens, Temperature and the two-point threshold, Somatosensory and Motor Research 6 (1989) 275–284.

[16] J.C. Stevens, B.G. Green, A.S. Krimsley, Punctate pressure sensitivity: effects of skin temperature, Sensory Processes 1 (1977) 238–243.

[17] K. Tsuzuki, H. Xing, J. Ling, J.G. Gu, Menthol-induced Ca^{2+} release from presynaptic Ca^{2+} stores potentiates sensory synaptic transmission, Journal of Neuroscience 24 (2004) 762–771.

[18] T. Voets, G. Droogmans, U. Wissenbach, A. Janssens, V. Flockerzi, B. Nilius, The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels, Nature 430 (2004) 748–754.

[19] T. Voets, K. Talavera, G. Owsianik, B. Nilius, Sensing with TRP channels, Nature Chemical Biology 1 (2005) 85–92.

[20] K. Zimmermann, A. Leffler, A. Babes, C.M. Cendan, R.W. Carr, J. Kobayashi, C. Nau, J.N. Wood, P.W. Reeh, Sensory neuron sodium channel Nav1.8 is essential for pain at low temperatures, Nature 447 (2007) 855–858.