Lack of potentiating effect of increasing temperature on responses to chemical activators in vagal sensory neurons isolated from TRPV1-null mice

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Submitted 17 July 2008; accepted in final form 29 August 2008

Ni D, Lee L-Y. Lack of potentiating effect of increasing temperature on responses to chemical activators in vagal sensory neurons isolated from TRPV1-null mice. Am J Physiol Lung Cell Mol Physiol 295: L897–L904, 2008. First published August 29, 2008; doi:10.1152/ajplung.90385.2008—Our recent study (Ni D, Lee LY. Am J Physiol Lung Cell Mol Physiol 294: L563–L571, 2008) demonstrated that the responses of rat pulmonary sensory neurons to transient receptor potential vanilloid (TRPV1) activators were enhanced by increasing temperature, but the role of the TRPV1 channel in this potentiating effect could not be definitively evaluated. In the present study, we used whole cell perforated patch-clamp technique to compare the responses of isolated nodose/jugular sensory neurons to chemical activators and increasing temperature between wild-type (WT) and TRPV1-null (TRPV1–/–) mice. Our results showed that, in voltage-clamp mode, the peak inward current evoked by hyperthermia was not different between WT and TRPV1–/– neurons; however, the inward current evoked by 2-aminoethoxydiphenyl borate (2-APB), a common activator of TRPV1–3 channels, was greatly potentiated by increasing temperature from 36 to 40.5°C in WT neurons (n = 9; P < 0.01) but was not affected by the same change in temperature in TRPV1–/– neurons (n = 9; P = 0.54). Similarly, the inward current evoked by acid (pH 5.5), an activator of both TRPV1 channel and the acid-sensing ion channel, was enhanced by increasing temperature (n = 7; P < 0.05) in WT neurons, and this potentiating effect was absent in TRPV1–/– neurons (n = 13; P = 0.11). These results demonstrated that deletion of the TRPV1 channel does not significantly alter the stimulatory effect of hyperthermia on nodose/jugular neurons but eliminates the potentiating effect of increasing temperature on the responses of these neurons to nonselective TRPV1 channel activators. This study further suggests that a positive interaction between these chemical activators and increasing temperature at the TRPV1 channel is primarily responsible for the hyperthermia-induced sensitization of these neurons.

C fibers; exercise; inflammation; airways; transient receptor potential vanilloid channel

HYPERTERMIA CAN OCCUR in the body during strenuous exercise, severe fever, or inflammation. It has been reported that body core temperature can increase to near 42°C in humans (20) and −43°C in rats (3) during heavy exercise. A recent study reported a significant increase in the exhaled air temperature (ΔT = 2.7°C) in asthmatic patients (30), indicating an increase in airway tissue temperature during inflammatory reaction.

Transient receptor potential vanilloid (TRPV) channels are known to be involved in polymodal sensory transduction, including thermal sensitivity. Thermal-sensitive TRPV channels, namely, TRPV subtypes 1–4 (TRPV1–4), are activated in different temperature ranges and have been shown to act as temperature sensors in various sensory systems (4, 29). In addition to their role in thermal sensation, these TRPV receptors can be also activated and/or sensitized by nonthermal stimuli, such as endogenous chemical mediators (7, 14), which in turn may lead to augmented reflex responses.

It has been demonstrated that TRPV1-4 channels are expressed on the cell bodies of vagal sensory neurons both in rats (25) and in mice (40). Our previous study (25) showed that hyperthermia exerts a direct stimulatory effect on rat pulmonary sensory neurons, and the thermal-sensitive TRPV channels, especially the TRPV1 channel, are involved in this action. A more recent study further demonstrated a potentiating effect of hyperthermia on the responses of pulmonary sensory neurons to chemical activators and a potential role of the TRPV1 channel in mediating this effect (26). All these previous studies seem to suggest that TRPV1 plays an important role in the temperature-sensitive properties of pulmonary sensory neurons. However, these findings were based on results obtained from experiments using the TRPV1 antagonists, of which the pharmacological specificities have been repeatedly challenged and definitive evidence is still lacking. Furthermore, because of the polymodal sensitivities of the TRPV1 channel, the relative potency and efficacy of a given antagonist are known to vary substantially between different types of TRPV1 activators (e.g., capsaicin vs. temperature), probably related to different activation sites of the receptor (6, 23). In view of the background information and unanswered questions, we used TRPV1-null (TRPV1–/–) mice as the animal model to determine the role of TRPV1 in mediating both the stimulatory and sensitizing effects of hyperthermia on vagal sensory neurons.

MATERIALS AND METHODS

This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and was approved by the University of Kentucky Institutional Animal Care and Use Committee.

Labeling vagal pulmonary sensory neurons with Dil. In the preliminary experiments, pulmonary sensory neurons were identified by retrograde labeling from the lungs by using the fluorescent tracer 3,3′-dioctadecylindocarbocyanine (DiI). Young adult C57BL/6J mice (~20 g, Jackson Laboratory) were anesthetized with continuous inhalation of isoflurane administered by a nose cone connected to a vaporizing machine (AM Bickford). A small (~0.5 cm) midline incision was made on the neck skin to expose the trachea with the mouse lying in the supine position and the head tilted upward at ~60°. A needle (30 gauge) was inserted into the lumen of the trachea to instill DiI (0.2 mg/ml; 0.025 ml) into the lungs, and the incision was then closed. DiI was initially sonicated, dissolved in ethanol, and diluted in saline [1% ethanol (vol/vol)]. Animals were used for the study after 5–7 days to allow time for the dye to reach the cell body...
located in the nodose and jugular ganglia. Results obtained from these preliminary experiments showed no detectable difference in the responses between Dil-labeled neurons and those isolated from unlabelled animals; the data collected from both groups were therefore pooled for analysis.

Isolation of vagal sensory neurons. Mice were decapitated after being anesthetized by halothane inhalation. The head was immediately immersed in ice-cold Dulbecco’s modified Eagle’s medium (DMEM)-F-12 solution, followed by quick extraction of nodose and jugular ganglia under a dissecting microscope. Each ganglion was then desheathed, cut into about three pieces, placed in a 0.04% type IV collagenase digestion, and incubated for 1 h in 5% CO2 in air at 37°C. After collagenase digestion, the ganglion suspension was centrifuged at 150 g for 5 min, followed by resuspension in a modified DMEM-F-12 solution (DMEM-F-12 supplemented with 10% [vol/vol] heat-inactivated fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 100 µM MEM nonessential amino acids). Cells were then dissociated by gentle trituration with a small-bore fire-polished Pasteur pipette. Myelin debris was separated and discarded after centrifugation of the dispersed cell suspension (500 g at 8 min) through a layer of 15% bovine serum albumin. The pellets were resuspended in the modified DMEM-F-12 solution, plated onto poly-L-lysine-coated glass coverslips, and then incubated overnight (5% CO2 in air at 37°C).

Electrophysiology. Vaginal sensory neurons with spherical shape and smooth membrane and identified by the fluorescent intensity of Dil (in Dil-labeled mice only) were chosen for the study. Patch-clamp recordings were performed in a small-volume (0.2 ml) perfusion chamber that was continuously perfused by gravity feed (VC-6, Warner Instruments, Hamden, CT) with extracellular solution (ECS) at 1 ml/min. Recordings were made in the whole cell perforated patch (50–80 mV) clamp mode, the resting membrane potential was held at −70 mV. Borosilicate glass electrodes had tip resistance of 2–4 MΩ. The series resistance was usually in the range of 6–10 MΩ and was not compensated. The intracellular solution contained (in mM) 92 potassium gluconate, 40 KCl, 8 NaCl, 1 CaCl2, 0.5 MgCl2, 10 EGTA, and 10 HEPES, pH at 7.2. ECS contained (in mM) 136 NaCl, 5.4 KCl, 1.8 CaCl2, 1 MgCl2, 0.33 Na2HPO4, 10 glucose, and 10 HEPES, pH at 7.4. For solutions with pH ≤ 6, MES was used instead of HEPES for pH buffering.

PCR. Four TRPV1+/− breeding pairs (purchased from Jackson Laboratory, Bar Harbor, ME) and two pairs (female and male) of the offspring (1 pair each from the 2 generations) were selected for genotyping. A small piece of tissue punched from the ear was collected from each mouse selected. Tissues were treated with 100 µl of 50 mM NaOH solution at 100°C for 1 h until homogenized. After brief spinning, 50 µl of the supernatant was taken and 5 µl of 1 M Tris (pH 8.0) was added, followed by centrifugation at 10,000 rpm. PCR was performed with the AccuPrime Tag DNA Polymerase System (Invitrogen) according to the manufacturer’s protocol. The amplification program consisted of an initial denaturation at 94°C for 3 min; 50 cycles of denaturation at 94°C for 30 s, annealing at 64°C for 1 min, and elongation at 72°C for 1 min; and a final 2-min extension at 72°C. The amplification products were analyzed by electrophoresis in 1.5% agarose gels and were detected by ethidium bromide staining.

Chemical applications. Chemical solutions were applied by using a three-channel fast-stepping perfusion system (SF-77B, Warner), with its tip positioned within 500 µm from the cell recorded to ensure that the cell was fully within the stream of the perfusate (width of stream: ~700 µm). In the series of experiments studying the stimulatory effect of hyperthermia on these neurons, the temperature of the ECS was increased in a ramp pattern from room temperature (RT; ~22°C) to hyperthermic temperature (HT; ~40.6°C) in ~20 s; in those experiments studying the sensitizing effect of hyperthermia on these neurons, the temperature of the ECS solution perfusing the neurons was maintained (TC-344B and SHM-6, Warner) at a constant level of either resting body temperature (BT; ~36°C) or HT for >60 s before application of the chemical solution at the same temperature. The actual temperature was measured by a microtemperature probe (time constant = 5 ms) (Harvard Apparatus, Holliston, MA) positioned within 100 µm downstream from the cell being perfused and recorded on computer.

Q10 calculations. The temperature coefficient Q10 was derived from an Arrhenius plot in which the common logarithm of current (I) was plotted against the reciprocal of the absolute temperature (T). In the temperature range above 30°C and where the plot was linear (correlation coefficient r > 0.99), Q10 was determined by the formula Q10 = exp[10Ea/(RT1T2)], where Ea = −2.303R[ln(I/T1)/ln(I/T2) − (1/T1)]; Ea, activation energy; R, gas constant (36).

Chemicals. Capsaicin, 2-aminoethoxydiphenyl borate (2-APB), acid (pH 5.5, 6.0, and 6.5), acetaldehyde (ACh), and adenosine 5′-triphosphate (ATP) are known activators of pulmonary sensory neurons, and they were chosen in this study for the following reasons: capsaicin is a selective TRPV1 agonist (6); 2-APB is a nonselective activator of TRPV1, V2, and V3 (12); acid activates both TRPV1 and acid-sensing ion channels (ASICs) (6, 37); in contrast, ACh and ATP are not known to directly activate the TRPV1 channel.

All chemicals were obtained from Sigma Chemical (St. Louis, MO), except for 2-APB (Tocris, Ellisville, MO) and dispase II (Roche, Indianapolis, IN). A stock solution of capsaicin (1 mM) was prepared in 1% Tween 80, 1% ethanol, and 98% saline. A stock solution of 2-APB was prepared in dimethyl sulfoxide (DMSO) at a concentration of 0.1 M. Solutions of these chemical agents at desired concentrations were then prepared daily by dilution with ECS.

Data analysis. Data were analyzed with a one-way ANOVA analysis, followed by a post hoc Newman-Keuls test. A P value <0.05 was considered significant. Data are presented as means ± SE.

RESULTS

Experimental protocols were completed in a total of 155 and 98 nodose/jugular ganglion neurons of wild-type (WT) and TRPV1−/− mice, respectively. The whole cell capacitances of these neurons were in the range of 7.5–35.5 pF (19.2 ± 0.5 pF; n = 155) for WT neurons and 10.2–31.8 pF (18.7 ± 0.6 pF; n = 98) for TRPV1−/− neurons.

Genotyping of TRPV1−/− mouse colony. To confirm that TRPV1 gene knockout was maintained through generations of offspring in our breeding colony, a PCR experiment was performed. Our results confirmed the absence of TRPV1 gene expression in the PCR reaction product obtained from the tissue of both breeding pairs and offspring of TRPV1−/− mice (Fig. 1). In contrast, the PCR reaction product from WT mouse tissue contained the 984-bp fragment corresponding to the TRPV1 allele (Fig. 1).

Responses to increase in temperature of vagal sensory neuron isolated from WT and TRPV1−/− mice. When the temperature of the ECS surrounding the neuron was elevated from RT (~23°C) to HT (~40.6°C) in ~20 s in a ramp pattern, whole cell inward current was evoked in both WT and TRPV1−/− neurons (Fig. 2A). The current densities of all vagal neurons tested were 4.26 ± 1.08 and 2.94 ± 0.5 pA/pF for WT (n = 66) and TRPV1−/− (n = 41) neurons, respectively, and no significant difference was found between these two groups of neurons (P = 0.37) (Fig. 2B).

In our preliminary experiments, only nodose and jugular neurons labeled with Dil (pulmonary sensory neurons) were studied in both WT and TRPV1−/− groups. There was no
significant difference in the responses of current density to the same increase in temperature between pulmonary (DiI labeled) neurons isolated from WT (4.35 ± 0.96 pA/pF) and TRPV1−/− (3.03 ± 0.44 pA/pF) mice (P = 0.15) (Fig. 2B).

Within the WT group, hyperthermia-induced inward current density in capsaicin-sensitive (Cap+) neurons (5.75 ± 1.78; n = 33) was not significantly different from the capsaicin-insensitive (Cap−) group (2.87 ± 0.48; n = 32) (P = 0.13, Fig. 2B). Q10 was also measured in order to quantitatively determine the neuron sensitivity to temperature increase; a Q10 value >3 is considered to be temperature sensitive (11). There was no significant difference in Q10 in the temperature range of 30–40.5°C between the WT (n = 45) and TRPV1−/− (n = 29) neurons (Q10 = 4.52 ± 1.34 and 2.43 ± 0.35, respectively; P = 0.22; Fig. 2B). The lack of difference in peak current response and Q10 between WT and TRPV1−/− mice was probably due to the large variability within the WT group, because the Q10 value of the hyperthermia-evoked inward current in WT Cap+ neurons (n = 22) was significantly higher than that in WT Cap− neurons (Q10 = 7.16 ± 2.59 and 1.87 ± 0.23, respectively; P < 0.05; Fig. 2B).

Responses to TRPV1 activators of isolated vagal sensory neurons. Inward current was evoked by capsaicin (0.3 or 1.0 µM, 1–6 s) application in nodose/jugular neurons isolated from WT (52.2 ± 9.7 pA/pF; n = 29) mice, but not in those from TRPV1−/− mice (n = 45) (Fig. 3, A and B). Furthermore, 2-APB (0.3 mM, 6 s)-evoked inward current in WT neurons (20.14 ± 4.43 pA/pF; n = 49) was significantly larger than that in TRPV1−/− neurons (3.98 ± 0.45 pA/pF; n = 45) (P < 0.01; Fig. 3, C and D). Very similar responses to both capsaicin and 2-APB were recorded from DiI-labeled neurons in both WT and TRPV1−/− mice (Fig. 3, B and D).

Vagal sensory neurons from WT mice were concentration-dependently activated by low pH at different acidity; acid (6 s)-evoked response was significantly augmented when the pH of the ECS was lowered: I = 1.74 ± 0.79, 4.16 ± 1.08, and 13.45 ± 5.40 pA/pF at pH = 6.0, 5.5 (n = 13, P < 0.01 vs. response at pH 6.0), and 5.0 (n = 13, P < 0.01 vs. response at pH 6.0), respectively (Fig. 4). However, acid (6 s)-evoked response in TRPV1−/− neurons was not significantly changed with decrease in ECS pH: I = 0.71 ± 0.18, 1.47 ± 0.30, and 2.61 ± 0.51 pA/pF at pH = 6.0 (n = 24), 5.5 (n = 24; P = 0.28 vs. response at pH 6.0) and 5.0 (n = 24, P = 0.16 vs. response at pH 6.0), respectively (Fig. 4). In addition, acid (6 s)-evoked response at pH 5.0 in WT neurons (13.45 ± 5.40 pA/pF; n = 13) was significantly larger than that in TRPV1−/− neurons (2.61 ± 0.51 pA/pF; n = 24) (P < 0.05).

Responses to non-TRPV1 activators of isolated vagal sensory neurons. To determine whether TRPV1 gene knockout affects cell response to non-TRPV1 activators, we investigated the inward current evoked by ATP and ACh, activators of the P2x/P2y and nicotinic/muscarinic ACh receptors, respectively.
ATP (1 μM, 6 s)-evoked current density was 8.24 ± 2.8 (n = 25) and 8.24 ± 2.1 (n = 34) pA/pF in WT and TRPV1−/− mice, respectively, and no significant difference was found between them (P = 0.72) (Fig. 5, A and B). Similarly, there was no significant difference between the responses to ACh (100 μM, 6 s) in WT (13.6 ± 4.5 pA/pF; n = 17) and TRPV1−/− (10.3 ± 5.4 pA/pF; n = 20) neurons (P = 0.69) (Fig. 5, C and D).

**Effect of increasing temperature on responses of vagal sensory neurons to 2-APB.** Our previous study (26) showed that 2-APB-evoked response was potentiated by increasing temperature in rat vagal pulmonary sensory neurons. In the present study, we compared the responses of vagal sensory neurons to 2-APB between WT and TRPV1−/− mice at three different temperatures in order to determine 1) whether hyperthermia has a similar potentiating effect on 2-APB-evoked response in mouse vagal sensory neurons and 2) if so, whether the TRPV1 channel plays a dominant role in mediating such an effect. Our results showed that in WT neurons 2-APB (0.3 mM, 6 s)-evoked inward current was clearly augmented when temperature of the ECS surrounding the cells was elevated from RT (22.3 ± 0.11°C) to BT (35.9 ± 0.07°C) and then to HT (40.4 ± 0.05°C); 2-APB-evoked current density was 9.0 ± 4.2 pA/pF at RT, 16.4 ± 6.1 pA/pF at BT, and 50.6 ± 17.7 pA/pF at HT (P < 0.01 and 0.05, respectively, comparing the responses at BT and HT with that at RT), and it recovered when the temperature was returned to RT (Fig. 6, A and B). However, in TRPV1−/− mice, neuron response to 2-APB (0.3 mM, 6 s) was not changed when the temperature of the ECS was elevated: 2.7 ± 0.6 pA/pF at RT, 4.0 ± 1.3 pA/pF at BT, and 5.0 ± 2.6 pA/pF at HT (P = 0.18 and 0.24, respectively, comparing the responses at BT and HT with that at RT) (Fig. 6, C and D).

**Effect of increasing temperature on responses of vagal sensory neurons to acid.** In rat pulmonary sensory neurons, acid-evoked response has been shown to be mediated through the activation of both ASICs and the TRPV1 channel (25), and our previous study (26) showed that hyperthermia exerts a potentiating effect on TRPV1-mediated acid-evoked currents in those neurons. This study series was carried out to determine 1) whether the TRPV1 channel plays a role in mediating the acid-evoked response in mouse vagal sensory neurons and 2) whether a similar potentiating effect of increasing temperature on this TRPV1-mediated acid-evoked response also exists in mice. When the temperature of the ECS was increased, the acid (pH 5.5, 6 s)-evoked current was significantly aug-
FIG. 5. Responses to non-TRPV1 activators in vagal sensory neurons isolated from WT and TRPV1−/− mice. A: experimental record illustrating inward currents evoked by adenosine 5′-triphosphate (ATP; 1 μM, 6 s) in a WT (21.4 pF) and a TRPV1−/− (22.8 pF) neuron. B: group data for the ATP (1 μM, 6 s)-evoked current density in WT and TRPV1−/− neurons. No significant difference ($P = 0.72$) in the current response was found between WT and TRPV1−/− neurons. C: experimental record illustrating inward currents evoked by acetylcholine (ACh; 100 μM, 6 s) in a WT (33.3 pF) and a TRPV1−/− (31.8 pF) neuron. D: group data for the ACh (100 μM, 6 s)-evoked current density in WT and TRPV1−/− neurons. No significant difference ($P = 0.69$) in the current response was found between WT and TRPV1−/− neurons.


discussed, 6.2 ± 2.9 pA/pF at RT, 24.0 ± 10.6 pA/pF at BT, and 39.5 ± 17.3 pA/pF at HT (both $P < 0.05$ comparing the responses at BT and HT with that at RT) in WT neurons (Fig. 7, A and B), but was not significantly changed in TRPV1−/− neurons, 2.4 ± 0.6 pA/pF at RT, 2.5 ± 0.5 pA/pF at BT, and 2.3 ± 0.5 pA/pF at HT ($P = 0.45$ and $P = 0.37$, respectively, comparing the responses at BT and HT with that at RT) (Fig. 7, C and D).

DISCUSSION

Results of this study demonstrated that increasing temperature to 40.5°C evoked inward currents in isolated mouse vagal sensory neurons. The whole cell current densities evoked by the temperature increase were not significantly different between neurons isolated from WT and TRPV1−/− mice, suggesting that the TRPV1 channel does not play an essential role in the response of vagal sensory neurons to hyperthermia. However, the cell response to capsaicin was absent in both WT and TRPV1−/− neurons, suggesting that the TRPV1 channel does play a role in the response of vagal sensory neurons to heat. In contrast, cell responses to non-TRPV1 activators, ATP and ACh, were not different between WT and TRPV1−/− mice. Furthermore, increasing temperature generated a pronounced potentiating effect on the responses of vagal sensory neuron to 2-APB and ACh in WT mice, similar to that previously observed in neurons isolated from rats (26); in sharp contrast, the same increase in temperature had no effect on the responses of these neurons to the same stimuli in TRPV1−/− mice, indicating that the sensitizing effect of hyperthermia on these sensory neurons is almost exclusively mediated through the TRPV1 channel.

Hyperthermia can occur in the lungs and airways under either physiological (e.g., exercise) or pathophysiological (e.g., fever or inflammation) conditions. It has been reported previously that intrathoracic hyperthermia directly stimulates bronchopulmonary C-fiber sensory terminals (34). A follow-up study on isolated sensory neurons innervating the airways and lungs reveals that the stimulatory effect of hyperthermia on these sensory afferents is mainly mediated through direct activation and not due to a secondary effect caused by hyperthermia-evoked release of inflammatory mediators or local changes of tissue mechanics (25). Application of a specific TRPV1 antagonist, either capsazepine (CPZ) or (E)-3-(4-t-butylphenyl)-N-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acrylamide (AMG9810), attenuated the hyperthermia-evoked response by 52–77%, indicating that such response is primarily mediated by TRPV1 (25). A more definitive conclusion could not be reached in that study because of the uncertainty about the selectivity of these TRPV1 antagonists on other TRPV channels and/or other receptors (9, 22, 32).

In the present study, we found no significant decrease in hyperthermia-evoked inward current density in vagal sensory neurons isolated from TRPV1−/− mice compared with that of the WT mice (Fig. 2). The lack of difference was also seen in Dil-labeled (pulmonary) sensory neurons from WT and TRPV1−/− mice (Fig. 2B). This finding was somewhat surprising, and it suggested that TRPV1 may not play as a dominant role in mediating the direct stimulatory effect of hyperthermia on these sensory neurons as we previously suggested (25). Interestingly, recent studies have also reported that TRPV1 is not essential for the thermal sensitivity of dorsal root ganglion (DRG) sensory neurons; it has been shown that TRPV1 and TRPV2 are not required for nociception of heat (38). The lack of difference may be due to an extensive overlap of the temperature range among these different TRPVs, since the temperature activation thresholds of heterologously expressed TRPV3 and TRPV4 are 34–39°C and 25–34°C, respectively (4), whereas the threshold temperature of the hyperthermia-evoked inward current in rat pulmonary sensory neurons is ~34.4°C (25). It also seems reasonable to speculate that changes in expression and/or sensitivity of other thermosensitive TRPV channels and other unidentified receptors in TRPV1−/− mice may have contributed to the lack of significant difference, although to our knowledge there is no evidence in support of this possibility in the literature. Whether an alteration (either downregulation or upregulation) in gene expression of other TRPV channels exists in vagal sensory neurons isolated from TRPV1−/− mice remains to be determined. Nevertheless, results obtained in the present study clearly show that TRPV1 is not essential for triggering the direct stimulatory effect of hyperthermia on vagal sensory neurons. This finding seems to be in general agreement with a recent report that, although the TRPV1 antagonist induces hyperthermia, the signal essential for thermoregulation is not mediated by the TRPV1 channel (35).

In isolated rat pulmonary sensory neurons, 2-APB-evoked response was attenuated by ~60% after application of CPZ (10). In comparison, the response to 2-APB in vagal sensory neurons to the same stimuli in TRPV1−/− mice, indicating the sensitizing effect of hyperthermia on these sensory neurons is almost exclusively mediated through the TRPV1 channel.
neurons isolated from TRPV1−/− mice was only 20% of that in WT mice (Fig. 3, C and D). This discrepancy in the relative contribution of TRPV1 to the response to 2-APB could be related partially to the fact that the previous study was carried out only in pulmonary sensory neurons, whereas all vagal sensory neurons were tested in this study. However, this possibility seems less likely because the 2-APB-evoked response in TRPV1−/− mice was not significantly different between Dil-labeled (pulmonary) neurons (17.0%) and all vagal neurons tested (19.8%) (Fig. 3 D). It is also conceivable that CPZ may not be completely effective in blocking the TRPV1-mediated cell response to 2-APB, despite the fact that it completely blocked the stimulatory effect of capsaicin on these neurons. Alternatively, the difference may be related to the species difference (mice vs. rats).

In a recent study (26), we reported that the responses of pulmonary sensory neurons to chemical activators of the TRPV1 channel were potentiated by hyperthermia. Furthermore, treatment with CPZ or AMG9810 significantly attenuated but did not abolish this potentiating effect, suggesting an important role of TRPV1 in mediating the sensitizing effect of hyperthermia on pulmonary sensory neurons (26). In the present study, the potentiating effect of hyperthermia on the responses of these neurons to 2-APB (Fig. 6, C and D) and to acid (Fig. 7, C and D) was completely abolished in the absence of the TRPV1 channel, clearly indicating an essential role of TRPV1.

![Figure 6](http://example.com/fig6.png)  
**Fig. 6.** Effect of increasing temperature on response to 2-APB in vagal sensory neurons isolated from WT and TRPV1−/− mice. A: experimental record illustrating that the 2-APB (0.3 mM, 2 s)-evoked current was augmented in a WT neuron (16.8 pF) when the temperature was increased from 22.8°C to 36.0°C and then to 40.3°C; the responses recovered when the temperature was returned to 36.1°C. B: group data for the 2-APB (0.3 mM, 2–6 s)-evoked current density tested at the temperatures in the order shown in A: RT, room temperature (23.6 ± 0.07°C); BT, body temperature (35.8 ± 0.07°C); HT, hyperthermic temperature (40.4 ± 0.09°C). *P < 0.05 compared with the response at RT.  

![Figure 7](http://example.com/fig7.png)  
**Fig. 7.** Effect of increasing temperature on response to acid in vagal sensory neurons isolated from WT and TRPV1−/− mice. A: experimental record illustrating that the acid (pH 5.5, 2 s)-evoked current was augmented in a WT neuron (16.8 pF) when the temperature was increased from 22.9°C to 35.9°C and then to 40.5°C; the responses recovered when the temperature was returned. B: group data for the acid (pH 5.5, 2–6 s)-evoked current density tested at the temperatures in the order shown in A: RT, room temperature (22.2 ± 0.07°C); BT, body temperature (36.0 ± 0.05°C); HT, hyperthermic temperature (40.3 ± 0.08°C). *P < 0.05 compared with the current response at RT. †P < 0.05 compared with the current response at BT. C: experimental record illustrating that the acid (pH 5.5, 6 s)-evoked current was not changed in a TRPV1−/− neuron (23.6 pF) when the temperature was increased. D: group data for the acid (pH 5.5, 6 s)-evoked current density tested at the temperatures in the order shown in C: RT (21.7 ± 0.08°C); BT (35.9 ± 0.06°C); HT (40.4 ± 0.05°C). No significant difference of the current response was found either between RT and BT or between BT and HT.
It has been suggested that TRPV1 is required for sensitization of nociceptive afferents, especially when an inflammatory reaction is involved. For example, thermal hyperalgesia was impaired in TRPV1−/− mice (5, 24), and TRPV1 expression is critical for maintenance of inflammatory hyperalgesia (1, 13). The possible mechanisms underlying the tissue hyperthermia-induced potentiation of the TRPV1-mediated response are still not clear. However, several possible explanations have been proposed. First, it has been demonstrated that hyperthermia activates protein kinase (PKC) and PKA (16): phosphorylation of the TRPV1 channel by intracellular signaling pathways involving PKC and PKA may lead to the sensitization of the TRPV1 channel (18, 31). Furthermore, TRPV channels are known to mediate the transmembrane influx of calcium (8), which is a well-known intracellular second messenger that is involved in many signaling pathways. Thus it is reasonable to expect that influx of Ca²⁺ may trigger the activation of other receptors and/or pathways, which in turn may result in augmented cell response to activators during hyperthermia. In addition, increasing evidence has demonstrated a shift in voltage-dependent gating of TRPV1 channel with increasing temperature (21, 27), and such shift may directly contribute to the hyperthermia-induced hypersensitivity of the pulmonary sensory neurons. Furthermore, tissue hyperthermia is known to induce the release of inflammatory mediators, such as tumor necrosis factor-α, prostaglandin E₂, etc. (2, 15, 33). The inflammatory mediator-mediated potentiating effect of hyperthermia could be due to an interaction between these mediators and the TRPV1 channel, which in turn sensitizes the TRPV1 channel and ultimately elicits an augmented response of sensory neurons to TRPV1 activators. Finally, the augmented response under hyperthermic condition may be due to an increased functional expression of the TRPV1 protein on cell surface. Previous studies have reported an increase in TRPV1 expression on sensory neuron somata in rat DRG resulting from either nerve injuries or inflammation, which led to the development of hyperalgesia in those animals (17, 19, 39).

In conclusion, results of this study indicate that the TRPV1 channel plays an essential role in the sensitizing effect of hyperthermia on isolated mouse vagal sensory neurons, which lends further support to the suggestion of the unique “integrator” function of the TRPV1 for various physiological stimuli (28). This sensitizing effect may lead to augmented reflex responses to TRPV1 activation when hyperthermia develops under either normal physiological or pathophysiological conditions. However, deletion of TRPV1 does not seem to significantly alter the normal physiological or pathophysiological conditions. How-to TRPV1 activation when hyperthermia develops under either normal physiological or pathophysiological conditions. How-to TRPV1 activation when hyperthermia develops under either

ACKNOWLEDGMENTS

The authors thank Michelle E. Wiggers for technical assistance.

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grants HL-58686 and HL-67379.

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