Carboxyl-terminal Domain of Transient Receptor Potential Vanilloid 1 Contains Distinct Segments Differentially Involved in Capsaicin- and Heat-induced Desensitization*

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John Joseph1, Sen Wang1, Jongseok Lee, Jin Y. Ro, and Man-Kyo Chung2

From the Department of Neural and Pain Sciences, School of Dentistry, Program in Neuroscience, University of Maryland, Baltimore, Maryland 21201

Background: Mechanisms of TRPV1 desensitization by heat are not fully understood.
Results: Distinct segments of the TRPV1 carboxyl-terminal domain are differentially involved in capsaicin- and heat-induced desensitization.
Conclusion: Capsaicin and heat desensitizes TRPV1 through a distinct structural basis.
Significances: Selective enhancement of desensitization could be a novel strategy for suppressing heat hyperalgesia.

Multiple Ca2+-dependent processes are involved in capsaicin-induced desensitization of transient receptor potential vanilloid 1 (TRPV1), but desensitization of TRPV1 by heat occurs even in the absence of extracellular Ca2+, although the mechanisms are unknown. In this study, we tested the hypothesis that capsaicin and heat desensitize TRPV1 through distinct mechanisms involving distinct structural segments of TRPV1. In HEK293 cells that heterologously express TRPV1, we found that heat-induced desensitization was not affected by the inclusion of intracellular ATP or alanine mutation of Lys155, both of which attenuate capsaicin-induced desensitization, suggesting that heat-induced desensitization occurs through mechanisms distinct from capsaicin-induced desensitization. To determine protein domains involved in heat-induced desensitization, we generated chimeric proteins between TRPV1 and TRPV3, a heat-gated channel lacking heat-induced desensitization. We found that TRPV1 with the carboxyl-terminal domain (CTD) of TRPV3 retained heat activation but was impaired in heat-induced desensitization. Further experiments using chimeric or deletion mutants within TRPV1 CTD indicated that the distal half of CTD regulates the activation and desensitization of TRPV1 in modality-specific manners. Within the distal CTD, we identified two segments that distinctly regulated capsaicin- and heat-induced desensitization. The results suggest that the activation and desensitization of TRPV1 by capsaicin and heat can be modulated differentially and disproportionally through different regions of TRPV1 CTD. Identifying the domains involved in thermal regulation of TRPV1 may facilitate the development of novel anti-hyperalgesic approaches aimed at attenuating activation and enhancing desensitization of TRPV1 by thermal stimuli.

Transient receptor potential vanilloid 1 (TRPV1)3 is a receptor for capsaicin and heat and is a reliable target for anti-hyperalgesic therapy (1). Noxious heat not only activates but also desensitizes TRPV1 (2). Considering the critical contribution of TRPV1 to thermal hyperalgesia, it is important to determine the contribution of specific domains or residues involved in heat-induced activity of TRPV1 for therapeutic targeting. Because the extent of channel activity, and thus the duration of nociceptor depolarization, is determined by the sum of activation and desensitization, understanding the structural basis of both is equally critical for understanding the overall heat responses of TRPV1 and its contribution to hyperalgesia. Activation of TRPV1 by heat is known to involve the functions of multiple regions of TRPV1(3–6). However, the mechanisms of heat-induced desensitization are poorly understood.

Upon activation by capsaicin, an influx of extracellular Ca2+ through TRPV1 triggers multiple Ca2+-dependent processes to desensitize TRPV1 (2, 7, 8). In contrast, heat-induced desensitization does not require Ca2+ influx, suggesting distinct underlying mechanisms (2, 9). Heat-induced desensitization also occurs in other heat-gated TRP channels such as TRPV4, although underlying mechanisms are not known (10, 11). Transient receptor potential melastatin 2 and 3 also undergo desensitization to repeated thermal stimuli (12, 13). A honeybee Hymenoptera-specific transient receptor potential A channel shows not only activation but also desensitization by heat (14). Among the heat-gated TRP channels, TRPV3 is a unique member in which repeated chemical and thermal stimuli evoke progressively increasing responses rather than desensitization (15). However, the sources of these differences between TRPV1 and TRPV3 are not known.

The TRPV1 carboxyl-terminal domain (CTD) is a cytosolic domain composed of ~150 amino acid residues and plays multiple roles in TRPV1 function such as channel gating, tetramerization, agonistic sensitivity, and interaction with phospholipids (16). The TRPV1 CTD is also implicated in cap-

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1 These authors contributed equally to this study.
2 To whom correspondence may be addressed: Dept. of Neural and Pain Sciences, School of Dentistry, University of Maryland, 650 W. Baltimore St., 8 South, Baltimore, MD 21201. Tel.: 410-706-4452; Fax: 410-706-0865; E-mail: mchung@umaryland.edu.
3 The abbreviations used are: TRPV, transient receptor potential vanilloid; CTD, carboxyl-terminal domain; CaM, calmodulin; ANOVA, analysis of variance.
saicin-induced desensitization through its ability to bind calmodulin (CaM) (17–19). The “TRP domain” within the TRPV1 CTD was also suggested to be involved in capsaicin-induced desensitization (20, 21). Given the fact that there are regions and residues of TRPV1 that are distinctly involved in activation by capsaicin and heat and that capsaicin and heat desensitize TRPV1 through apparently distinct mechanisms, we hypothesized that capsaicin and heat desensitize TRPV1 through distinct segments within the CTD. In this study, we tested this hypothesis by (i) further examination of distinct mechanisms of capsaicin and heat-induced desensitization of TRPV1 and (ii) delineation of segments of TRPV1 that are primarily involved in capsaicin- or heat-induced desensitization.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—HEK293 cells were cultured and transfected using Lipofectamine 2000 (Invitrogen) as previously described (9, 22). Plasmids containing cDNA encoding TRPV1 or TRPV1 mutants were co-transfected with cDNA encoding mCherry or GFP. Transiently transfected HEK293 cells were replated onto poly-L-ornithine-coated coverslips, encoding mCherry or GFP. Transiently transfected HEK293 cells were replated onto poly-L-ornithine-coated coverslips, kept at 32 °C, and used for electrophysiological experiments after 16–26 h.

Site-directed Mutagenesis—pcDNA3 vectors containing cDNA encoding TRPV1 (23) and TRPV3 (24) were used for generating chimeric or deletion mutants. Overlap extension PCR (25) was performed using a high fidelity polymerase (Takara Bio, Inc) and specific primers designed for generating chimeric or deletion mutants. The construct encoding TRPV1 CΔ5 in pcDNA3 was kindly provided by Dr. Viktorie Vlachova (Institute of Physiology, Academy of Sciences of the Czech Republic). TRPV1 K155A in pcDNA3 was generated by subcloning of SacII and BsmB1 fragments of TRPV1 K155A in pFB-cFLAG (a kind gift from Dr. Rachel Gaudet at Harvard University). Proper mutation of each construct and lack of unintended mutation was confirmed by sequencing. All electrophysiological experiments were performed using constructs in pcDNA3. For biochemical analysis, the amino terminus of TRPV1 was tagged with FLAG epitope. TRPV1 cDNA without a start codon was PCR-amplified and inserted into the HindIII and NotI sites of pFLAG-CMV2 (Sigma).FLAG-tagged chimeric or deletion mutants were generated by subcloning restriction-digested fragments into TRPV1 in pFLAG CMV2.

Whole Cell Patch Clamp—Whole cell voltage clamp techniques were performed as described previously (22). The recording pipettes (2–3 MΩ) were pulled from borosilicate glass using a P-97 (Sutter Instrument). The pipettes were filled with solution int A (Table 1). Unless otherwise indicated, the recording bath contained solution ext A. To examine the effects of intracellular ATP, solution int B contained ATP (int A plus 4 mM Na₂ATP). In this experiment, the intracellular solution of the control group contained additional Na⁺ (int C). To study the mechanisms of heat-induced desensitization that are not dependent on Ca²⁺, we evaluated heat-induced desensitization under several conditions minimizing the effects of Ca²⁺. In one condition, Ca²⁺ was replaced by an equimolar concentration of Ba²⁺ (ext B) based on a report that this would attenuate Ca²⁺-dependent desensitization (26). In another condition, Ca²⁺ was omitted, and EGTA was added (ext C). For testing capsaicin sensitivity in the absence of Ca²⁺, we used ext C containing only 5 mM EGTA. To estimate relative permeability of Ca²⁺ to Na⁺ (P_Ca/P_Na), we used an external solution containing 10 mM Ca²⁺ (ext D). To quantify the extent of desensitization in the presence of low concentration of extracellular Ca²⁺, we used an external solution containing 0.1 mM Ca²⁺ (ext E). The pH of solutions was adjusted with HCl or NaOH as necessary. Osmolarity of each solution was measured by a vapor pressure osmometer (Wescor Inc) and was adjusted with mannitol to 290–310 mOsm as necessary. Bath temperature was controlled using a combination of an in-line heater/cooler (Warner Instrument Inc) and in-line heater (Warner Instrument Inc). The actual temperature was recorded throughout the experiment with a thermocouple (Physitemp Instruments) that had been placed within 4 mm of the patch-clamped cell. When we evaluated the temperature-response relationship, the temperature of bath solution was increased from ~17 to 48 °C over ~45 s (τ = ~12 s). When we evaluated heat-induced desensitization, the bath temperature was increased from ~23 to 45 °C over ~10 s (τ = ~1.5 s).

Surface Biotinylation and Western Blot Analysis—HEK293 cells were plated into 6- or 12-well plates coated with poly-D-ornithine. The cells were transfected with TRPV1 or mutants in pFLAG CMV2. After 18–24 h, biotinylation of membrane protein followed by Western blot was performed as previously described (27). The cells were washed three times with PBS and incubated with 0.5 mg/ml EZ-Link Sulfo-NHS-LC-Biotin (Pierce) in PBS at 4 °C for 30 min. The reaction was quenched by washing the cells two times with cold PBS containing 100 mM glycine and incubated in PBS containing 100 mM glycine for 10 min at 4 °C. Next, the cells were lysed in lysis buffer containing protease inhibitor mixture and centrifuged at 1000 × g for 5 min. The 30–50 μg of collected lysate was incubated with streptavidin cross-linked to agarose beads (Pierce) for 90 min at 4 °C. The beads were then washed twice with lysis buffer and eluted with LDS loading buffer by heating at 100 °C for 5 min. The membranes were incubated with antibody against FLAG (1:500, monoclonal, anti-mouse; Sigma) overnight at 4 °C. To normalize the amount of protein loaded and to examine contamination of cytosolic components in the biotinylation assay, the stripped membranes were reprobed with GAPDH antibody (1:5000, monoclonal, anti-mouse; Calbiochem).

**TABLE 1**

The composition of recording solutions for electrophysiology

|        | Internal | External |
|--------|----------|----------|
| A      | B        | C        | D        | E        |
| NaCl   | 140      | 140      |          |          |
| NaOH   | 150      | 150      | 158      |          |
| KCl    | 5        | 5        |          |          |
| NMDG   |          |          |          |          |
| HCl    |          |          |          |          |
| MgCl₂  | 1        | 1        | 1        | 1        |
| CaCl₂  | 2        | 10       | 0.1      |          |
| BaCl₂  | 10       | 10       | 10       | 10       |
| HEPES  | 10       | 10       | 10       | 10       |
| EGTA   | 5        | 5        | 4        |          |
| Glucose| 10       | 10       |          |          |
| Na₂ATP |          |          |          |          |

* pH was adjusted to 7.4 by using the chemical indicated.
Data Analysis—To minimize variability caused by the differences in individual cell size, we determined current densities (current amplitude/membrane capacitance). Unless otherwise indicated, data are expressed as the means ± S.E. The mean values were compared by unpaired Student’s t test or ANOVA. The level of statistical significance was set at α = 0.05. Concentration-dependent relationships were fitted by a logistics function: Y = 1/(1 + 10^−(LogEC_{50} X) × H), where Y is the normalized current, EC_{50} is the half-maximal concentration, X is the log of concentration, and H is the Hill slope. P_{Ca}/P_{Na} was estimated as described previously (9) using the equation: P_{Ca}/P_{Na} = ([Na^{+}]_{o}/4[Ca^{2+}]_{o}) × [exp(ΔE_{rev}/F/RT) - 1] × [exp(E_{Ca}/F/RT) + 1], where [X]_{o} is the activity of ion X in the bath, F is the Faraday constant, R is the gas constant, E_{Ca} is the reversal potential (E_{rev}) in ext D containing 150 Na^{+} and 10 Ca^{2+}, and ΔE_{rev} is the shift in E_{rev} when ext D was replaced with ext C containing 150 Na^{+}. E_{rev} was corrected for junction potentials that were calculated using pClamp software. The temperature coefficient (Q_{10}) and temperature threshold of activation were determined following the methods previously described (28). An Arrhenius plot of logarithm of current amplitudes against the reciprocal of the absolute temperature (K) was generated. The data points were fitted by a straight line over the temperature range where the Arrhenius plot was linear. Q_{10} was calculated by a following function: Q_{10} = exp[10E_{r}/(RT_{1}T_{2})], where R is the gas constant, and E_{r} is the activation energy derived from the slope of the Arrhenius plot between absolute temperatures T_{1} and T_{2}. The thermal thresholds of activation were determined as temperatures in which the regression lines representing two different slopes intersect in Arrhenius plot. The enthalpy for channel opening (ΔH') was estimated by a following function: ΔH' = (RT^2lnQ_{10})/10 ≈ 20lnQ_{10} at physiological temperature (kcal/mol) (29). Statistical analysis, curve fitting, and plotting were performed using GraphPad Prism, Clampfit, or SigmaPlot. Protein sequences were analyzed aligned using a progressive alignment algorithm using CLC Sequence Viewer (CLC Bio).

RESULTS

Capsaicin and Heat Desensitize TRPV1 through Distinct Mechanisms—Interaction of CaM with TRPV1 plays important roles in capsaicin-induced desensitization (17, 18, 30, 31). Intracellular ATP has been demonstrated to compete with CaM for binding to the “multiligand binding sites” in the amino-terminal ankyrin repeat domain of TRPV1 and, hence, attenuate desensitization (30). To further investigate distinct mechanisms of heat- and capsaicin-induced desensitization of TRPV1, we tested whether inclusion of ATP in the intracellular side or mutation of the multiligand binding site differentially affected heat-induced desensitization. To assess the extent of desensitization, we applied two consecutive stimuli, capsaicin (1 μM) and heat (45 °C) (Fig. 1, A–D), and calculated the ratio of second/first responses (Fig. 1, E and F). In HEK293 cells transfected with TRPV1 WT, consecutive application of capsaicin showed a robust desensitization, and the ratio of second/first responses was less than 0.3 (Fig. 1, A and E). When ATP (4 mM)
was included in the recording pipette, desensitization was significantly reduced, and the ratio of second/first responses was ~0.7 (Fig. 1, A and E). These results confirmed the previous report that ATP attenuates the extent of capsaicin-induced desensitization (30). When TRPV1 was activated by two consecutive applications of heat, desensitization was also obvious; the second heat-evoked current was only 0.35 of the first heat-evoked current (Fig. 1, B and E). In heat-evoked responses, unlike capsaicin-evoked responses, the inclusion of ATP in the recording pipette resulted in only modest changes. The effects of inclusion of ATP on the ratio of second/first responses were significantly different in capsaicin and heat-evoked responses (Fig. 1E). These results suggest that heat-induced desensitization is distinctly unaffected by intracellular ATP.

Next we evaluated whether the alanine mutation of a multiligand binding site Lys\(^{155}\) affected heat-induced desensitization. We selected this residue because a single alanine mutation of Lys\(^{155}\), within one of the multiligand binding sites, was sufficient to prevent capsaicin-induced desensitization (30). Unlike WT, TRPV1 K155A showed little desensitization following repeated application of 1 \(\mu\)M capsaicin; the second capsaicin-evoked currents were slightly greater than the first capsaicin-evoked currents (Fig. 1, C and F). In contrast, the ratio of second/first heat responses in K155A was comparable to that of WT (Fig. 1, D and F). Thus, the effects of the K155A mutation on the ratio of second/first responses were significantly different between capsaicin- and heat-evoked responses. These results suggest that capsaicin and heat induced desensitization is differentially affected by K155A mutation and indicate that capsaicin and heat induce desensitization of TRPV1 through distinct mechanisms. In particular, the results suggest that heat-induced desensitization may not involve interaction of ATP or CaM at multiligand binding sites of TRPV1.

Chimeric Approach to Identify Heat-desensitizing Domains of TRPV1—To identify domains responsible for heat desensitization of TRPV1, we analyzed chimeric proteins composed of segments of TRPV1 and TRPV3 taking advantage of the fact that TRPV3 displays sensitization rather than desensitization in response to repeated heat stimulation (15) (Fig. 2A). We generated chimeric constructs by replacing the amino-terminal domain, pore loop, and CTD of TRPV1 with those of TRPV3, designated as TRPV1 ntV3, pV3, and cV3, respectively (Fig. 2B). These regions were selected because (i) these domains operate as functional modular units in TRP channels (32), (ii) the extent of homology between TRPV1 and TRPV3 over these regions is lower than in the transmembrane domains, and (iii) each region has been proposed to contain a segment playing a critical role in heat-induced activation (3–6) and is thus likely to contain heat-induced desensitization domains as well.

Initially, we examined whether the constructs were properly expressed and whether the proteins were localized to the plasma membrane using surface biotinylation followed by Western blot analysis. FLAG-tagged TRPV1 WT and chimeric mutants were detected in whole lysates as well as in biotinylated fractions. The extent of expression and surface localization of chimeric mutants was similar to that of TRPV1 WT (Fig. 2C). The functionality (current densities) of the chimeric proteins was tested following application of 45 °C heat followed by 10 \(\mu\)M capsaicin. TRPV1 WT was robustly activated by both heat (-173 ± 45 pA/pF at −60 mV, \(n = 16\)) and application of capsaicin (−898 ± 161 pA/pF). The heat-evoked currents were 20.7 ± 5.2% of capsaicin-evoked currents. In contrast, both TRPV1 ntV3 and TRPV1 pV3 showed significantly impaired heat- and capsaicin-evoked activation, such that the averaged current densities were less than 10% of WT (Fig. 2, D and E). Global functional impairment of TRPV1 ntV3 and pV3 was not surprising given the large extent of mutation in ntV3 and the common critical roles of the TRPV1 pore domain in TRPV1 activation by multiple agonists such as heat, a spider toxin, and capsaicin (4, 5, 33, 34). In the case of TRPV1 cV3, functional impairment was disproportional when heat and capsaicin were applied. TRPV1 cV3 showed profound impairment in capsaicin-evoked currents, whereas heat-induced activation of TRPV1 cV3 was less affected (Fig. 2, D and E).

We further characterized heat activation and desensitization properties of TRPV1 cV3. To evaluate the temperature-dependent activation profile, we applied a temperature ramp during which voltage ramps from −100 to +100 mV were applied every second. Current amplitudes were quantified at −60 mV. TRPV1 WT showed little activity at temperatures less than 40 °C. The current amplitude increased steeply at temperatures

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**FIGURE 2. Chimeric mutation of TRPV1 and TRPV3.** A, representative heat-evoked current traces from HEK293 cells expressing mouse TRPV3. B, schematics of chimeras of TRPV1 and TRPV3. In TRPV1 ntV3, TRPV1 Met1–Val430 were replaced by TRPV3 Met1–Ala337. In TRPV1 pV3, TRPV1 Asp601–Val658 were replaced by TRPV3 Lys622–Leu653. In TRPV1 cV3, TRPV1 Asp581–Lys599 were replaced by TRPV3 Glu682–Val697. C, Western blot analysis of biotinylated or soluble proteins in HEK293 cells expressing cDNAs as indicated. FLAG-tagged TRPV1 was detected by specific antibody against FLAG. GAPDH was used as a loading control. D and E, relative responses from TRPV1 and chimeras. The peak current amplitudes were normalized to the average of TRPV1 responses by heat (45 °C, 30 s; D) or capsaicin (10 \(\mu\)M; E). The recording solutions were int A/ext A. *, \(p < 0.05\); **, \(p < 0.005\); ***, \(p < 10^{-3}\) in Student’s t test. The numbers in parentheses represent the numbers of observations.
higher than 40 °C (Fig. 3, A, black, and B). In contrast, TRPV1 ctV3 showed substantial activity even at warm temperatures and showed an increase in activation as temperature increased (Fig. 3, A, gray, and C). To quantify temperature sensitivity, we calculated the enthalpy of heat activation (∆H°). Using this quantitative approach, TRPV1 ctV3 showed significantly smaller ∆H° than TRPV1 WT, suggesting reduced heat sensitivity of this mutant (Fig. 3D). These results suggest that TRPV1 CTD and TRPV3 CTD may contain a preserved segment required for heat activation.

To assess heat-induced desensitization properties, we applied two consecutive heat stimuli and evaluated the ratio of second/first heat responses. In these experiments, Ca2+ in the bath solution was replaced with Ba2+ to minimize the effects of Ca2+-dependent effects. The bath temperature was rapidly elevated from room temperature to ~45 °C and maintained for 30 s (Fig. 3, E and F). Using TRPV1 WT, this heat stimulus evoked a rapid increase of TRPV1 current, which was followed by a gradual decrease after peaking even when heat was maintained at the same level (Fig. 3E). A second application of an identical heat stimulus after 1 min evoked only modest activation such that the ratio of second/first heat response was ~0.5 (Fig. 3, E and G). TRPV1 ctV3 also showed robust activation by heat. In striking contrast to TRPV1 WT, however, the second heat stimulus evoked a current similar to the first stimulus (Fig. 3F); the ratio of second/first heat response of TRPV1 ctV3 was significantly different from TRPV1 WT (Fig. 3G). These results indicate that heat desensitization is impaired in TRPV1 ctV3, suggesting that the CTD plays an important role in heat desensitization. We focused our subsequent analysis on the roles of CTD in heat desensitization, although we do not exclude the possibility of a contribution of the amino-terminal and pore domains to heat-induced desensitization.

**Mutations of TRPV1 CTD Differentially Affect Capsaicin and Heat Activation Properties**—To further delineate segments of the CTD that contribute to heat-induced desensitization, we generated multiple TRPV1 mutants carrying mutations in the CTD. Because TRPV1 ctV3 exhibited impaired heat-induced desensitization, we generated two chimeric mutants replacing approximately half of the TRPV1 CTD with corresponding regions of the TRPV3 CTD (TRPV1 ctV3(A) and ctV3(B) in Fig. 4B). We also generated five mutants lacking portions of the CTD. We constructed serial CTD deletion mutants of ~30 amino acid residues (Fig. 4B). This size of mutation deletes one or two functional domains that have already been identified in the CTD (Fig. 4A), which may allow mechanistic interpretation of the data: The CA1 segment includes most of the TRP domain (35) and the PIP2 interaction domain 2 (20). The CA2 segment includes most of the implantable heat domain (36). The CA3 contains the tetrameric assembly domain (37). The CA4 includes most of the CaM-binding domain (18) and two-thirds...
of the PIP2 interaction domain 1 (38). The CΔ5 contains one-third of the PIP2 interaction domain 1 (Fig. 4, A and B).

The constructs encoding the TRPV1 mutants were well expressed in HEK293 cells, as verified by Western blot analysis. The TRPV1 mutants and the TRPV1 WT proteins were localized to the plasma membrane as demonstrated by surface biotinylation assay (Fig. 4C). In preliminary functional analysis, we found that TRPV1 ctV3(A), CΔ1, CΔ2, and CΔ3 exhibited no or extremely limited activation by heat and capsaicin, which precluded further analysis of these mutants. Because CΔ5 displayed a tendency toward attenuation of heat-induced desensitization, we generated two additional mutants in which only 20 or 10 amino acid residues are truncated from the 3’ end of CTD; designated CΔ6 and CΔ7, respectively (Fig. 4B). We examined the activation and desensitization properties of TRPV1 ctV3(B), CΔ4, CΔ5, CΔ6, and CΔ7 in further experiments.

Initially, we characterized activation properties of these CTD mutants. To test the effects of mutations on capsaicin sensitivity, a HEK293 cell expressing either TRPV1 WT or mutants was exposed to increasing concentrations of capsaicin (10 nM, 100 nM, 1 μM, and 10 μM; applied for 30 s with a 1-min interval prior to exposure to the next concentration). To prevent Ca2+–induced desensitization, we removed extracellular Ca2+ from the bath and added 5 mM EGTA. Under these conditions, TRPV1 WT exhibited increasing activation as the capsaicin concentration was increased and peaked at either 1 or 10 μM. All of the mutants also displayed current activation in a concentration-dependent manner (Fig. 5A). EC50 values of CTD mutants were not significantly

**FIGURE 4. Chimeric or deleting mutations of TRPV1 CTD.** A, aligned amino acid sequences of the CTD of TRPV1 and TRPV3. The numbers represent the amino acid numbers of TRPV1. Segments above the TRPV1 sequence represent approximate locations of previously identified functional domains: TRP domain (35), PIP2 interaction domain 2 (20), implantable heat domain (36), tetrameric assembly domain (37), CaM-binding domain (18), and PIP2 interaction domain 1 (38, 40). B, chimeric mutants, ctV3(A) and ctV3(B) were generated by replacing a portion of CTD of TRPV1 (gray) with the homologous portion of CTD of TRPV3 (black). In TRPV1 ctV3(A), TRPV1 Asn687–Ser776 were replaced with TRPV3 Glu682–Asn763. In TRPV1 ctV3(B), TRPV1 Leu777–Lys838 were replaced with TRPV3 Lys764–Val791. Deletion mutants, CΔ1 to CΔ5, were generated by deleting—30 amino acid residues serially as indicated by solid lines (CΔ1, Lys688–Arg717; CΔ2, Lys718–Val747; CΔ3, Asn748–Leu777; CΔ4, Ser779–Thr807; and CΔ5, Thr807–Lys838). In addition, CΔ6 and CΔ7 were generated by truncating 20 and 10 amino acid residues from the carboxyl terminus (CΔ6, Gly819–Lys838; and CΔ7, Phe829–Lys838). C, Western blot analysis of biotinylated or soluble proteins in HEK293 cells expressing cDNAs as indicated. FLAG-tagged TRPV1 was detected by specific antibody against FLAG. GAPDH was used as a loading control.

**FIGURE 5. Effects of mutations of CTD of TRPV1 on capsaicin-induced activation.** A and B, capsaicin concentration-response relationship of TRPV1 WT, CΔ4, and CΔ5 in A and TRPV1 WT, CΔ6, CΔ7, and ctV3(B) in B. Bath solution did not contain Ca2+ (recording solution int A/ext C with 5 mM EGTA). Progressively increasing concentrations of capsaicin (10 nM, 100 nM, 1 μM, and 10 μM) were applied in the same cells. Current amplitudes in each capsaicin concentration were measured at −60 mV and normalized to the maximal response in each cell. The numbers in parentheses represent the numbers of observations. The lines were derived from data fitting using sigmoidal logistics equation with fixed minimum and maximum value of 0 and 1, respectively. C and D, EC50 in C and Hill slope in D obtained from analysis in A and B. *, p < 0.05; **, p < 0.001; ***, p < 10−4 in Student’s t test.
different from that of TRPV1 WT (Fig. 5B). However, Hill slope values of CTD mutants were significantly smaller than WT except CΔ7 (Fig. 5C). These results suggest that TRPV1 CTD mutants only modestly alter responses to capsaicin.

To test the effects of CTD mutations on heat sensitivity, we recorded currents in response to the slow temperature ramp from ~17 to 47 °C over 45 s. TRPV1 WT and the CTD mutants were all activated by the heat ramp (Fig. 6, A and B). The peak current densities of all CTD mutants were comparable to that of WT except TRPV1 ctV3(B), which was significantly greater (by ~2-fold, 154 ± 28 pA/pF in WT and 317 ± 35 pA/pF in ctV3(B); p < 0.005; Fig. 6C). TRPV1 WT was activated by heat with a threshold temperature of ~39 °C. However, TRPV1 CTD mutants showed significantly lower threshold temperatures than WT (Fig. 6C; 39 ± 0.8 °C in WT, 33.8 ± 1.2 °C in ctV3(B), 32 ± 0.7 °C in CΔ4, 35.2 ± 1.4 °C in CΔ5, 34.8 ± 1.3 °C in CΔ6, and 35.8 ± 1.1 °C in CΔ7). Enthalpy of heat activation was reduced significantly in CΔ4, CΔ5, and CΔ6 but not in CΔ7 and ctV3(B) (Fig. 6D). These results indicate that regions in the distal half of the CTD modulate thermal sensitivity of TRPV1 and regulate the activation of TRPV1 in a modality-dependent manner.

Distinct Segments of CTD Contributes to Desensitization by Capsaicin and Heat—We examined the extent of capsaicin-induced desensitization of these CTD mutants by the consecutive application of capsaicin (1 μM) in TRPV1 WT and CTD mutants (Fig. 7A). We quantified the density of the current evoked by initial application of capsaicin (Fig. 7B) and the ratio of second/first capsaicin response (Fig. 7C). Among five mutants tested, the truncation mutants CΔ5, CΔ6, and CΔ7 showed no significant differences either in capsaicin-induced current density (Fig. 7B; ~704 ± 91 pA/pF in WT, ~806 ± 124 pA/pF in CΔ5, ~583 ± 135 pA/pF in CΔ6, and ~564 ± 116 pA/pF in CΔ7) or in the ratio of second/first capsaicin response (Fig. 7C; 0.17 ± 0.06 in WT, 0.22 ± 0.08 in CΔ5, 0.21 ± 0.04 in CΔ6, and 0.08 ± 0.02 in CΔ7). However, both TRPV1 ctV3(B) and CΔ4 exhibited significantly reduced capsaicin-induced current density (Fig. 7B; ~385 ± 46 pA/pF in CΔ4 and ~427 ± 69 pA/pF in ctV3(B)) and greater ratios of second/first capsaicin response (Fig. 7C; 0.53 ± 0.07 in CΔ4 and 0.42 ± 0.08 in ctV3(B)). To test whether the reduced current amplitude itself attenuated the extent of desensitization, we tested higher concentrations of capsaicin in CΔ4 and ctV3(B). In contrast to the sensitivity to capsaicin in the absence of external Ca2+ (Fig. 5), 1 μM capsaicin-induced currents were only approximately half of currents evoked by the following application of saturating concentration (30 μM) of capsaicin in the same cell in the presence of 2 mM extracellular Ca2+ (WT, 0.49 ± 0.09, n = 25; CΔ4, 0.50 ± 0.07, n = 23; and ctV3(B), 0.52 ± 0.10, n = 12). Consecutive application of capsaicin at 10 μM slightly increased current density, but the ratio of second/first responses remained significantly greater than that of WT (Fig. 7, B and C, gray).

Even when we compared the ratio of second/first responses of WT and CΔ4 only in the cells showing comparable capsaicin-evoked current density, the ratio of second/first responses of CΔ4 was significantly greater than that of WT (~553 ± 60 pA/pF in WT, ~545 ± 49 pA/pF in CΔ4, p > 0.8; and 0.15 ± 0.07 in WT, 0.40 ± 0.04 in CΔ4, p < 0.005). We also examined whether altered desensitization properties are due to the attenuated permeability of Ca2+ in mutants. We estimated Pca/Pna by measuring Erev of capsaicin-evoked currents in the presence of 10 mM external Ca2+ (ext D) followed by quenching the external Ca2+ (ext C). Pca/Pna of TRPV1 was 10.9 ± 0.5 (n = 7), which was not significantly different from those of CTD mutants (10.2 ± 0.2 in CΔ4, n = 7; 12.4 ± 1.1 in CΔ5, n = 7; 10.6 ± 0.6 in CΔ6, n = 6; 11.4 ± 0.6 in CΔ7, n = 7; and 12.3 ± 0.7 in ctV3(B), n = 5; p > 0.05 versus WT). These results indicate that the attenuated capsaicin-induced desensitization of TRPV1 ctV3(B) and CΔ4 cannot be fully explained by the altered extent of capsaicin-induced activation or Ca2+ permeability. To further support this, we tested the effects of reduced or enhanced influx of Ca2+ on desensitization by decreasing or increasing external Ca2+. In the presence of 0.1 or 10 mM extracellular Ca2+, we evaluated currents from TRPV1 WT, CΔ4, and ctV3(B) evoked by two consecutive application of 1 μM capsaicin following the same protocol as in Fig. 7A. In Fig. 7 (D and E), we plotted these results along with data obtained in the presence of 2 mM extracellular Ca2+ presented in Fig. 7 (B and C). Over the range of concentration of extracellular Ca2+ that we tested, the current density showed decreasing tendency as the concentration of extracellular Ca2+ increased (Fig. 7D). The ratio of second/first capsaicin response was also dependent upon the concentration of extracellular Ca2+ in TRPV1 WT. The higher extracellular Ca2+ resulted in a smaller ratio of second/first capsaicin response, indicating the Ca2+ dependent properties of capsaicin-induced desensitization. Capsaicin-evoked current density was significantly less in CΔ4 and ctV3(B) than WT (p < 0.001 in CΔ4 and p < 0.05 in ctV3(B) versus WT in two-way ANOVA). Capsaicin-induced desensitization of CΔ4 and ctV3(B) also occurred in a Ca2+ dependent manner (Fig. 7E). However, the second/first capsaicin response of CΔ4 and ctV3(B) was significantly greater than WT (p <
Effects of mutations of CTD of TRPV1 on capsaicin-induced desensitization. A, example traces of currents evoked by three consecutive applications of capsaicin (1 μM) (black bars above traces) in HEK293 cells transfected with TRPV1 WT and mutants as indicated. The recording solutions were int A/ext A. The scale bars represent 200 pA/pF in WT, ctV3(B), CΔ4 and CΔ7, 50 pA/pF in CΔ4, and 100 pA/pF in CΔ5. The time scale bars represent 1 min. B and C, box and whisker plots for current density measured at −60 mV (B) and second/first responses to capsaicin (C) of the TRPV1 WT and CTD mutants to capsaicin at 1 μM (white) and 10 μM (shaded). *, p < 0.05; **, p < 0.01; ***, p < 0.001 versus TRPV1 WT 1 μM (Student’s t test). The numbers of observations are indicated in parentheses. D and E, the relationship of extracellular Ca2⁺ concentration and capsaicin (1 μM)-evoked current density (D) or second/first capsaicin response (E) of TRPV1 WT, CΔ4, and ctV3(B). The recording solutions were int A (2 mM Ca2⁺) or ext D (10 mM Ca2⁺) or ext E (0.1 mM Ca2⁺); n = 12–19 in each point. **, p < 0.01; ***, p < 0.001 versus WT (Bonferroni post-test following two-way ANOVA). In E, the curves were fitted by a logistics function. WT, EC50 = 0.35 mM, H = −0.85; ctV3(B), EC50 = 1.7 mM, H = −0.79; CΔ4, EC50 = 2.4 mM, H = −1.2.

0.001 in CΔ4 and ctV3(B) versus WT in two-way ANOVA). When the sensitivity of desensitization to Ca2⁺ was evaluated by fitting the Ca2⁺ response relationships, EC50 of CΔ4 and ctV3(B) was 5–7-fold lower than that of WT (EC50 of WT, 0.35 mM; ctV3(B), 1.7 mM; and CΔ4, 2.4 mM). Because the current density of TRPV1 WT is greater than CΔ4 and ctV3(B) by no more than 3-fold (Fig. 7D), the extent of reduced Ca2⁺ sensitivity of capsaicin-induced desensitization in CΔ4 and ctV3(B) cannot be fully explained by the reduced current density.

Next, the extent of heat-induced desensitization of these CTD mutants was examined. To minimize Ca2⁺-dependent desensitization, the bath solution contained EGTA without the addition of Ca2⁺. Under this condition, two consecutive heat stimuli to −45°C were applied (Fig. 8A). TRPV1 WT and mutants were robustly activated by the initial application of heat (Fig. 8A). Among the CTD mutants, all except CΔ4 showed greater heat-evoked current density than WT (−133 ± 35 pA/pF in WT, −158 ± 78 pA/pF in ctV3(B), −182 ± 54 pA/pF in CΔ5, −560 ± 125 pA/pF in CΔ6, and −278 ± 47 pA/pF in CΔ7), but only CΔ6 and CΔ7 reached statistical significance (Fig. 8B). The heat-evoked current density of CΔ4 (−119 ± 30 pA/pF) was slightly smaller than WT but not significantly different. The second heat stimulus evoked smaller amplitudes of currents in TRPV1 WT (−69 ± 18 pA/pF) such that the second/first heat response ratio was 0.56 ± 0.06 (Fig. 8C). Likewise, the heat-evoked currents by the second heat stimulus were reduced in all CTD mutants (−50 ± 21 pA/pF in ctV3(B), −69 ± 21 pA/pF in CΔ4, −120 ± 40 pA/pF in CΔ5, −265 ± 62 pA/pF in CΔ6, and −218 ± 44 pA/pF in CΔ7), and the ratio of second/first heat responses was less than 1. The ratio of second/first heat responses of TRPV1 WT under this condition (0.56 ± 0.50) was significantly higher than were ratios in the presence of...
extracellular Ca\(^{2+}\) (Fig. 1A; 0.35 \(\pm\) 0.06, \(p < 0.05\)). TRPV1 ctV3(B) exhibited a smaller ratio of second/first heat response than WT, but it was not statistically significant (0.4 \(\pm\) 0.07, \(p = 0.09\)). All other CTD mutants exhibited greater ratios of second/first heat responses than WT (0.59 \(\pm\) 0.08 in C\(\Delta\)4, 0.74 \(\pm\) 0.08 in C\(\Delta\)5, and 0.61 \(\pm\) 0.12 in C\(\Delta\)6, 0.82 \(\pm\) 0.1 in C\(\Delta\)7), but only the C\(\Delta\)7 ratio of second/first heat response was significantly different from WT (Fig. 8B). These results suggest that two distinct segments of the CTD differentially modulate capsaicin- and heat-induced desensitization of TRPV1.

As demonstrated in Figs. 5–7, TRPV1 ctV3(B) and C\(\Delta\)4 showed similar activation and desensitization properties. To examine whether the common phenotypes of C\(\Delta\)4 and ctV3(B) are due to the common mutation of the C\(\Delta\)4 segment, we investigated the consequences of chimeric mutations over smaller extent. We generated two chimeric mutants: TRPV1 ctV3(B)-1 in which the C\(\Delta\)4 region is replaced with the equivalent region of TRPV3 and TRPV1 ctV3(B)-2 in which the C\(\Delta\)5 region is replaced with the equivalent region of TRPV3 (Fig. 9A). Both TRPV1 ctV3(B)-1 and ctV3(B)-2 were activated by capsaicin in a concentration-dependent manner. Interestingly, unlike TRPV1 ctV3(B) or C\(\Delta\)4 (Fig. 5C), the EC\(_{50}\) was significantly increased both in ctV3(B)-1 and ctV3(B)-2 (Fig. 9B). The Hill slope was also significantly reduced in both mutants (WT, 2.4 \(\pm\) 0.2, \(n = 10\); ctV3(B)-1, 1.4 \(\pm\) 0.2, \(n = 7\); p < 0.05 versus WT in Student’s t test; ctV3(B)-2, 1.7 \(\pm\) 0.2, \(n = 7\), p < 0.001). In contrast, heat activation properties were distinctly affected. The threshold of activation and \(\Delta H^o\) in TRPV1 ctV3(B)-1 was significantly reduced compared with WT (Fig. 9, D–F). However, TRPV1 ctV3(B)-2 displayed no significant changes in the threshold of activation and \(\Delta H^o\). The extent of capsaicin-induced desensitization was also distinctly affected. TRPV1 ctV3(B)-2 did not show significant differences in second/first responses (Fig. 9G) following consecutive application of capsaicin (1 \(\mu\)M) or current density (WT in 0.1 mM Ca\(^{2+}\), −1580 \(\pm\) 328 pA/pF, \(n = 9\); 2 mM Ca\(^{2+}\), −820 \(\pm\) 124 pA/pF, \(n = 9\); 10 mM Ca\(^{2+}\), −442 \(\pm\) 80 pA/pF, \(n = 8\); ctV3(B)-2 in 0.1 mM Ca\(^{2+}\), −1797 \(\pm\) 471 pA/pF, \(n = 12\); 2 mM Ca\(^{2+}\), −887 \(\pm\) 153 pA/pF, \(n = 10\); and 10 mM Ca\(^{2+}\), −323 \(\pm\) 37 pA/pF, \(n = 12\)). In contrast, TRPV1 ctV3(B)-1 showed significant differences in second/first responses compared with TRPV1 WT (p < 0.0001 in two-way ANOVA; Fig. 9G). The sensitivity of capsaicin-induced desensitization to Ca\(^{2+}\) was lower by approximately 100-fold (WT, 0.2 mM; ctV3(B)-1, 22 mM). Because the current density of TRPV1 WT was greater than ctV3(B)-1 by not more than 5-fold (ctV3(B)-1 in 0.1 mM Ca\(^{2+}\), −601 \(\pm\) 140 pA/pF, \(n = 9\); 2 mM Ca\(^{2+}\), −256 \(\pm\) 50 pA/pF, \(n = 9\); and 10 mM Ca\(^{2+}\), −89 \(\pm\) 17 pA/pF, \(n = 10\)), such reduction of Ca\(^{2+}\) dependence of desensitization cannot be solely attributed to the reduced current density. Furthermore, even when TRPV1 ctV3(B)-1 was activated by the consecutive application of 10 \(\mu\)M capsaicin in the presence of 10 mM external Ca\(^{2+}\) (green symbol in Fig. 9G), the extent of desensitization was significantly smaller than the conditions of WT in the presence of 2 or 10 mM following the application of 1 \(\mu\)M capsaicin.

There was no difference in \(P_{Ca}/P_{Na}\) (TRPV1 WT, 13.1 \(\pm\) 0.5, \(n = 13\); ctV3(B)-1, 12.1 \(\pm\) 3, \(n = 14\); p > 0.05). We also examined the extent of heat desensitization. The second/first responses following the consecutive application of heat stimuli were not significantly altered in TRPV1 ctV3(B)-1 (Fig. 9H). The extent of heat desensitization was even slightly enhanced in ctV3(B)-2. Compared with the results from Figs. 5–8, chime-
ric mutation or deletion of the CΔ4 region both produced qualitatively same changes in heat activation and capsaicin- and heat-induced desensitization. These results further confirmed the modality-specific regulation of activation and desensitization exerted by CΔ4 region.

**DISCUSSION**

In this study, we show that (i) heat-induced desensitization of TRPV1 is affected neither by the inclusion of ATP in the pipette solution nor by mutation of an ATP-binding site in TRPV1, (ii) replacement of the TRPV1 CTD with the TRPV3 CTD prevented heat-induced desensitization, and (iii) two segments of TRPV1 CTD are differentially involved in capsaicin- and heat-induced desensitization. These results support our hypothesis that desensitization of TRPV1 induced by capsaicin and heat occur through distinct segments of TRPV1.

TRPV1 CTD has been implicated in heat activation (6, 28, 39). The chimeric approach provided a clue as to the roles of TRPV1 CTD in heat-induced desensitization. However, our approach did not allow us to examine whether other regions of TRPV1 also contributed to heat-induced desensitization. Results from the chimeric mutants also suggested that different regions of CTD contributed to heat activation and desensitization. Both TRPV1 ctV3 and ctV3(B) showed reduced \( H^\circ \), suggesting that the altered heat sensitivity of ctV3 and ctV3(B) is commonly derived from the alteration of the distal half of CTD. Altered heat sensitivity conferred by truncation of the distal half of the CTD has been reported in rat TRPV1 and in tissue-specific splicing variants of TRPV1 in vampire bats (28, 39, 40). In contrast to activation, TRPV1 ctV3(B) displayed heat-induced desensitization similar to WT, whereas ctV3 lacked heat-induced desensitization. Therefore, attenuation of heat-induced desensitization in TRPV1 ctV3 is likely derived primarily from the mutation of the proximal half of the CTD. Results obtained with our chimeric and deletion mutations over the proximal half of TRPV1 CTD, however, did not permit further testing of this possibility. We plan to further...
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delineate smaller segments of TRPV1 involved in heat-induced desensitization within the proximal half of CTD. In this regard, regulatory effects of ctV3(B) were not uniform with respect to responses to capsaicin and heat: TRPV1 ctV3(B) exhibited significantly attenuated capsaicin-induced desensitization, whereas heat-induced desensitization was not affected. In contrast to the reduced heat threshold and heat sensitivity, capsaicin sensitivity was not altered. These results suggest that modality-specific regulatory effects on activation and desensitization of TRPV1 are mediated by the distal half of the CTD.

Among the deletion mutants, CΔ4 showed activation and desensitization properties similar to those of TRPV1 ctV3(B): reduced heat activation threshold, attenuated capsaicin-induced desensitization, and no changes in capsaicin sensitivity and heat-induced desensitization. Indeed, the CΔ4 segment of TRPV1 is poorly conserved in TRPV3 (Fig. 4). Because TRPV1 ctV3(B)-1, but not ctV3(B)-2, showed similar phenotypes to ctV3(B) and CΔ4, it is reasonable to conclude that CΔ4 region is critical for the common phenotypes of TRPV1 ctV3(B) and TRPV1 CΔ4. It is unclear how the CΔ4 segment induces these regulatory effects. Because the CΔ4 segment contains a domain suggested to be commonly regulated by CaM and PI(4,5)P2 (Fig. 4), we may speculate that both factors contribute. Because CaM negatively regulates TRPV1 (31), it is possible that the CΔ4 mutation enhances thermal activation of TRPV1. Likewise, loss of CaM interactions with the CΔ4 segment may attenuate capsaicin-induced desensitization. Our results are consistent with a previous report that deletion of the CaM-binding domain, a 35-amino acid segment that overlaps ~90% with CΔ4 (Fig. 4A), substantially attenuates capsaicin-induced desensitization (18, 41). Altered interactions with PI(4,5)P2 might contribute to the regulatory effects as well. Because the interaction of PI(4,5)P2 with the CΔ4 segment negatively regulates TRPV1 (38, 40), the loss of the interaction in the CΔ4 mutant may enhance thermal activation by decreasing threshold. Consistently, truncation of PI(4,5)P2-interacting domain 1, which includes the CΔ4 segment (Fig. 4A), shows markedly lowered heat threshold (38). Although depletion of PI(4,5)P2 following capsaicin-induced activation of TRPV1 is associated with acute desensitization and tachyphylaxis (20, 26, 30, 42), it is unlikely that altered PI(4,5)P2 interactions with distal CTD contribute to the attenuated capsaicin-induced desensitization in TRPV1 CΔ4 because the PI(4,5)P2 interaction with the CΔ4 segment is suppressive (38, 40). Any single unidirectional mechanism may not fully explain the differential regulation of capsaicin- and heat-induced activation and desensitization by the distal half of CTD. Elucidation of the mechanisms whereby the CTD contributes to modality-specific regulation of TRPV1 will require further study.

Supporting the differential mechanisms of heat-induced desensitization, the CΔ4 deletion did not affect heat-induced desensitization of TRPV1 in the absence of extracellular Ca2+. In contrast, we found that truncation of 10 amino acid residues from the 3’ terminus of CTD attenuated heat-induced desensitization without affecting capsaicin-induced desensitization. Again, these results suggest that distinct segments of the CTD of TRPV1 differentially contribute to capsaicin- and heat-induced desensitization. It is unclear how the distal end of the CTD affects heat-induced desensitization. A previous molecular modeling study based upon the structure of a homologous protein predicted that the distal end of the CTD contains an α-helical structure (28). We assume that the presumptive α-helix may be involved in heat-induced desensitization, which is affected by truncation of the CΔ7 segment. Because our chimeric approach suggested a role of the proximal half of the CTD in heat-induced desensitization, as discussed above, we speculate that the function of the distal end of the CTD may be associated with the functions of the proximal half of the CTD.

Although it is clear that heat-induced desensitization occurs without extracellular Ca2+, this does not preclude the possibility that Ca2+-dependent mechanisms contribute to heat-induced desensitization of TRPV1 under physiologically relevant conditions. Indeed, our data suggest that Ca2+ also contributes to heat-induced desensitization. The ratio of second/first heat response in the presence of Ca2+ (~35%; Fig. 1B) was significantly smaller than the ratio in the absence of Ca2+ (~56%; Fig. 8C). Therefore, both Ca2+-dependent and -independent mechanisms are likely to contribute to heat-induced desensitization. However, the relative contribution of Ca2+-dependent mechanisms appears to be smaller than the contribution of Ca2+-independent mechanisms. The minor contribution of Ca2+-dependent mechanisms could be due to quantitative and qualitative differences in Ca2+ permeation following the activation of TRPV1 by capsaicin and heat. The efficacy of thermal agonist was only ~20% that of 10 μM capsaicin. Moreover, PCa/PNa of TRPV1 in heat activation was found to be less than half that in capsaicin activation (2). Thus, Ca2+ influx following heat activation of TRPV1 could not be robust enough to induce the strong Ca2+-dependent processes as observed following capsaicin-induced activation.

An allosteric regulation model proposes that capsaicin and heat separately promote the TRPV1 gating process, which converges onto the TRPV1 pore (43). Likewise, despite the presence of distinct mechanisms, it is unlikely that capsaicin- and heat-induced desensitization are two completely segregated processes without any common mechanism. Desensitization of TRPV1 following the application of capsaicin also affects heat activation and vice versa (2, 26). Such cross-desensitization suggests common pathways or consequences of capsaicin- and heat-induced desensitization. Moreover, the rate of capsaicin-induced desensitization of TRPV1 is affected by temperature. The rate of capsaicin-induced desensitization increases as temperature increases (44). In a TRPV1 mutant that shows limited heat-evoked activation (TRPV1 N628K/N652T/Y653T), desensitization of capsaicin-induced current is enhanced by the superimposed application of heat, suggesting that heat accelerates the rate of capsaicin-induced desensitization even with little preceding heat activation (22). Heat can enhance agonist-induced desensitization not only in heat-gated channels but also in cold-gated channels. The activity of mammalian TRPA1 is enhanced by cold temperature (45). When mammalian TRPA1 is activated by chemical agonists, the rate and extent of desensitization is enhanced as the ambient temperature increases (46). Therefore, capsaicin- and heat-induced desensitization may occur through different modules but allosterically affects common steps, likely including the gating process.
Although it is unclear whether the TRPV1 CTD is involved in the common mechanisms of desensitization by heat and capsaicin, our results with truncation mutants indicate that the distal half of the CTD plays roles in distinct, rather than common, mechanisms of desensitization. It is possible that the proximal half of the CTD is involved in a common mechanism because our results from chimeric mutants suggest a role for the proximal half of the CTD in heat-induced desensitization (Figs. 2 and 8), and capsaicin-induced desensitization also involves the proximal half of CTD (20, 35). Further studies will be required to clarify whether heat and capsaicin-induced desensitization occurs through common or distinct segments or residues within the proximal half of the CTD.

In conclusion, we demonstrated that the TRPV1 CTD contains two distinct segments that differentially regulate capsaicin- and heat-induced activation and desensitization. From a therapeutic standpoint, investigating modality-specific modulation of TRPV1 is important. Although capsaicin is a prototypical agonist of TRPV1, capsaicin-related mechanisms do not represent every aspect of TRPV1 activity under pathophysiological conditions. Moreover, modality-specific targeting of TRPV1 induces differential expression of side effects, such as hyperthermia and distinct anti-hyperalgesic efficacy in different animal models (47, 48). Therefore, delineating the details of structural mechanisms mediating modality-specific regulation may allow rationalized design of compounds targeting modality-specific activity of TRPV1 to produce stronger anti-hyperalgesia with fewer adverse side effects.

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