TRPV2 has been proposed as a potential pain target, in part due to its relatedness to the nociceptor TRPV1 and to its reported activation by noxious high temperatures (>52 °C). However, TRPV2 responses to heat as well as to the nonselective agonist 2-aminoethoxydiphenyl borate (2-APB) have not been universally reproduced in other laboratories, leading to debate about the activation properties of this channel. Here, we report the expression of rat, mouse, and human TRPV2 in HEK293 cells and the differential properties of their responses to heat and 2-APB. Expression of mouse or rat TRPV2 in HEK293 cells resulted in robust channel activation when induced by either temperature (>53 °C) or 2-APB. By contrast, expression of human TRPV2 did not lead to detectable activation by either of these stimuli. Human TRPV2 protein was expressed at levels comparable with those of rat TRPV2, exhibited similar surface localization and responded to a novelly identified TRPV2 agonist, Δ9-tetrahydrocannabinol, indicating that human TRPV2 is functionally expressed on the cell surface. Studies using deletion mutants and chimeras between rat and human TRPV2 indicated that both amino- and carboxyl-cytoplasmic termini of rat TRPV2 are important for responses to heat and 2-APB but can be supplied in trans to form an active channel. The present study not only confirms and extends previous reports demonstrating that rat and mouse TRPV2 respond to 2-APB and noxious heat but also indicates that further investigation will be required to elucidate TRPV2 activation and regulatory mechanisms.

TRP (transient receptor potential) proteins comprise a large family of nonselective cation channels responsible for diverse cellular functions, including the transduction of thermal, chemical, and mechanical stimuli, as well as the regulation of cellular calcium levels. Named after the Drosophila phototransduction pathway mutant trp, with which they share common structural homology (1), these proteins are characterized by a six-transmembrane domain region, including a pore and cytosolically oriented NH2 and COOH termini, the former of which typically includes one or more ankyrin domain repeats (for a review, see Ref. 2). Recent studies have provided evidence that there are at least six distinct TRP channels that are temperature sensors (3). These thermo-TRPs are believed to be important transducers of temperature to the nervous system, including the sensation of pain due to noxious heat and cold. TRPV1, the most extensively characterized member of the TRP family, is activated by moderate heat (~43 °C), capsaicin, protons, and certain lipidic mediators, such as anandamide (4, 5). Moreover, numerous studies using a variety of experimental approaches have implicated TRPV1 in the transduction of pain signals in animal models of hyperalgesia (6, 7); hence, this TRP has been aggressively pursued as a target for the alleviation of certain types of pain.

The TRP vanilloid subfamily, type 2 channel (TRPV2; also known as vanilloid receptor-like 1, or VRL-1), is closely related to TRPV1 and was identified by homologous cloning from rats and humans (8) as well as mice (9). Unlike TRPV1, however, no selective activators of TRPV2 have been reported. Functional studies have revealed that rat TRPV2 responds to noxious heat, with an activation threshold above 52 °C (8) and that mouse TRPV2 responds to changes in osmolarity or to membrane stretch (10). The hypothesis that TRPV2 is an endogenous sensor of noxious heat and mechanical stretch is further supported by findings that it is expressed in medium to large diameter Aδ mechano- and thermosensitive neurons in the dorsal root ganglion (8, 11). Interestingly, translocation of mouse TRPV2 to the cell surface has been reported to be stimulated by insulin-like growth factor-I, suggesting an additional role in cell growth and promoting its alternate appellation as growth factor-regulated channel (9, 12). Recently, it was reported that TRPV2 protein levels were up-regulated in rat dorsal root ganglion after intra-plantar injection of complete Freund’s adjuvant to induce peripheral inflammation (13). In addition, TRPV2 protein expression was also up-regulated in rat sympathetic postganglionic neurons following nerve injury (14). Together, these studies suggest that TRPV2 may be involved in pain sensation.

However, the proposed role of TRPV2 as a nociceptor still remains a topic of some debate among researchers. Thus, although the mouse ortholog exhibited a mere 40% increase above basal current in response to a 54 °C stimulus in transfected CHO cells (9), TRPV2 activation by heat could not be reproduced at all in other laboratories (15). Furthermore, although TRPV2 is found in the sensory neurons of the dorsal root ganglion, it is also found in nonneuronal tissues, including the lung, spleen, and intestine (8), where it would seem unlikely...
to have a temperature-sensing role, particularly in the proposed range for TRPV2 (i.e. >52 °C). Little else is known about TRPV2 responses, in part because of the current lack of selective pharmacological tools and knock-out animals. Recently, it was reported that 2-aminoethoxydiphenyl borate (2-APB), a nonselective TRP modulator, was able to induce calcium influx (EC$_{50}$ ~ 130 μM) in HEK293 cells transfected with mouse TRPV2 (16). However, TRPV2 activation by 2-APB was not observed by other laboratories (17, 18). Here, we compare the functional expression of human, mouse, and rat TRPV2 in HEK293 cells and the properties of their responses to heat and 2-APB using calcium imaging and patch clamp electrophysiological assays. We demonstrate that there are species-specific differences in the activation properties of TRPV2 and show that both NH$_2$- and COOH-terminal domains of TRPV2 are involved in conferring responsivity to both 2-APB and noxious heat.

**EXPERIMENTAL PROCEDURES**

**Reagents**—2-APB was purchased from Sigma and stored at −20 °C as a 1 mM stock solution in 100% dimethyl sulfoxide. Δ$_9$-Tetrahydrocannabinol (Δ$_9$-THC) was from Sigma and was supplied as a 30 mg/ml stock in ethanol.

**Cloning and Expression**—Genes encoding human, mouse, and rat TRPV2 (accession numbers BC051305, BC005415, and BC089215, respectively) were obtained from Open Biosystems, Inc. (Huntsville, AL), and the open reading frames of each gene were subcloned into the expression vector, pCI-neo (Promega Corp., Madison, WI). For constructs containing a hemagglutinin (HA) tag, the DNA sequence tccaggttctatccatacgacgtacctg (encoding amino acids SRFYPYDVPDYA) was appended to the 3′ end by PCR. The generated fragment was digested with the appropriate restriction enzymes and then reinserted into the expression vector by ligation. Construction of the rat/human chimeras was performed by overlap PCR joining of DNA encoding portions of the TRPV2 open reading frame from each species to form full-length TRPV2 hybrids. For construct RRH, DNA encoding rat TRPV2 amino acids 1–646 was fused upstream of and in frame with DNAs encoding human TRPV2 amino acids 647–764. In a similar manner, construct RHR was created to express human TRPV2 amino acids 1–392 at the human amino terminus and rat TRPV2 amino acids 393–761. The open reading frames of all constructs were verified by DNA sequencing. A fragment HRR was created to express human TRPV2 amino acids 647–764. In a similar manner, constructs HRR and RHR were fused upstream of and in frame with DNAs encoding human TRPV2 amino acids 647–761 at the carboxyl terminus. The open reading frames of all constructs were verified by DNA sequencing. A HEK293 cell line that exhibited a low background response to 2-APB (a kind gift from Michael Zhu at Ohio State University) was used as the expression host. HEK293 cells were transiently transfected for adherent cell transfection using FuGene 6 reagent (Roche Applied Science), as recommended by the manufacturer. Stable TRPV2-expressing cell lines were prepared by transfecting HEK293 cells in the same manner and then selecting for G418 resistance (400 μg/ml) at 48 h post-transfection.

Clonal lines were then generated by limited dilution of surviving cells at ~5 days post-drug selection. Individual clones were expanded and evaluated by immunoblot and/or calcium mobilization assays to identify clones with the highest TRPV2 expression levels.

**Calcium Mobilization Assays**—Transfected cells were replated 24 h posttransfection into 96- or 384-well plates and analyzed ~24 h later using FLIPR or FLEX Station instruments with Calcium 3 Assay dye (Molecular Devices Corp., Sunnyvale, CA), as recommended by the manufacturer. FLIPR assays were conducted as previously described (19). Temperature activation studies were performed using a Flex Station in a 96-well plate format. After a 1-h incubation in 200 μl of 1× loading dye buffer at 37 °C, 160 μl of buffer was removed from each well prior to insertion of the cell plate into the Flex Station chamber. Temperature activation was triggered by the addition of 160 μl of heated (85 °C) isotonic HEPES buffer (same components as loading dye buffer minus the Calcium 3 dye) into the compound plate just prior to closing the chamber door and starting the run. All of the pipette tip rack and empty buffer plate were pre-equilibrated to 37 °C. Empirical testing showed that, after allowing for elapsed time before the buffer addition to the well (~30 s) and dilution with the sample buffer in each well, an initial peak temperature of ~53 °C was obtained.

**Electrophysiology**—Transfected cells were plated on glass coverslips, maintained in 5% CO$_2$ at 37 °C, and used 1–2 days after plating. Recordings from TRPV2-expressing HEK293 cells were performed using the whole-cell patch clamp technique. Control and test solutions were applied onto the cell at 0.5 ml/min via a gravity-fed perfusion system. Recordings were performed at 22 °C. Currents were sampled (Digidata 1322A), amplified, and filtered at 2 kHz (Axopatch 200B) and acquired and analyzed using pCLAMP 9.0 (all instruments/software from Molecular Devices). 2-APB-activated ramp currents were elicited with a 600-ms voltage ramp from −100 to +60 mV at a sampling rate of 10 kHz. The holding potential was −100 mV. The standard extracellular solution contained 132 mM NaCl, 1.8 mM CaCl$_2$, 5.4 mM KCl, 0.8 mM MgCl$_2$, 1 mM EGTA, 10 mM HEPES, and 10 mM glucose, pH 7.4.

**Cell Surface Expression**—Biotinylation and purification of cell surface proteins were performed using a biotinylation kit available from Pierce and used as reported previously (20). Briefly, 2.5 × 10$^6$ HEK293 cells were seeded onto 75-cm$^2$ poly-d-lysine-coated flasks (BD Biosciences) 24 h prior to transfection. At 48 h post-transfection, the cells were washed and biotinylated while they still adhered to the flask; the reaction was then quenched, the cells were lysed, and the surface labeled proteins were purified on avidin-immobilized resin. After extensive washing to remove unlabeled proteins, the biotinylated proteins were eluted, separated on 10–20% polyacrylamide gels (Invitrogen), and transferred to nitrocellulose as recommended by the manufacturer. Anti-HA staining utilized a hors eradish peroxidase-conjugated antibody (203819; Roche Applied Science) at a 1:500 dilution, which was detected after incubation and washing using the ECL Plus detection reagent (GE Healthcare Bio-Sciences Corp.), as recommended by the manufacturer.

Species-specific Differences in TRPV2 Activation

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2 The abbreviations used are: 2-APB, 2-aminoethoxydiphenyl borate; Δ$_9$-THC, Δ$_9$-tetrahydrocannabinol; HA, hemagglutinin; aa, amino acids.
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Antibody Staining—An antibody directed to the carboxyl terminus of native rat TRPV2 (AB5398P) was purchased from Chemicon International Inc. (Temacula, CA). Anti-human TRPV2 antibody was developed in rabbits using an immunogen of a synthetic peptide (EDGASEENYVPQLLQSN), which corresponds to amino acids 747–764 of the carboxyl terminus of human TRPV2. The peptide was synthesized and conjugated to keyhole limpet hemocyanin, and the antibody was raised and affinity-purified by Quality Controlled Biochemicals, Inc. (Hopkinton, MA). The resulting affinity-purified antibody was used for Western blotting analysis. Immunofluorescent staining of HA-labeled proteins was performed using fluorescein-conjugated anti-HA antibody (1988506; Roche Applied Science) at a concentration of 1 μg/ml. Cells for immunofluorescence studies were plated on poly-D-lysine-coated slides (BD Biosciences) 24 h prior to staining and were fixed and permeabilized by a 10-min treatment with 10% formalin fixative solution (Sigma) containing 0.1% Triton X-100.

Microscopy and Imaging—Fluorescent Mounting Medium (Dako Corp., Carpinteria, CA) was added to the fixed cell-coated slides to minimize bleaching of the fluorescent signal. Cells were viewed on an Eclipse E600 fluorescence microscope equipped with a Photometrics CoolSnap cf camera (Nikon Corp., Melville, NY), and the images were captured using Image Pro Plus software (MediaCybernetics, Inc., Silver Spring, MD). Black and white images were then pseudocolored green to better reflect the staining by the fluorescein dye-conjugated anti-HA antibody.

RESULTS

Rodent but Not Human TRPV2 Responds to 2-APB—Responses of HEK293 cells transiently transfected with TRPV2-expressing or vector control plasmids to 2-APB were evaluated using FLIPR calcium mobilization assays. As shown in Fig. 1, A and B, 2-APB elicited a robust, concentration-dependent response in HEK293 cells expressing rat or mouse TRPV2, with EC50 values of 18 and 22 μM, respectively. At higher concentrations (i.e. >100 μM), 2-APB also elicited an endogenous response in vector-transfected HEK293 cells, with an EC50 value of 239 μM. The endogenous response was transient (lasting less than 3 min) compared with the response in rodent TRPV2-transfected cells, which was sustained throughout the assay. This background response is probably the result of 2-APB-mediated mobilization of internal calcium stores, which would also be detected by this assay, since 2-APB is a nonselective TRP channel modulator. When human TRPV2-transfected HEK293 cells were evaluated, there was no response to 2-APB above that observed for vector-transfected cells over a >105-fold range in 2-APB concentration.

A similar response to 2-APB was observed in patch clamp studies of TRPV2-transfected cells. The responses of HEK293 cells transiently transfected with rat or human TRPV2 to the application of 2-APB (300 μM) were also compared using a voltage ramp protocol from −100 mV to +60 mV. In cells expressing rat TRPV2 (Fig. 2A), 2-APB elicited a large current response with a reversal potential near 0 mV. The current-voltage relationship of 2-APB-activated rat TRPV2 current showed dual rectification, characteristic of this channel, as reported by others (8, 16). By contrast, in human TRPV2-expressing HEK293s (Fig. 2B), the same 300 μM 2-APB concentration did not activate any measurable current. There also was no measurable background response to 2-APB in HEK293 cells alone, which is consistent with the premise that 2-APB may be causing low level internal calcium mobilization that would be detected fluorescently using FLIPR but not electrophysiologically.

Rodent but Not Human TRPV2 Responds to Heat—Rat TRPV2 was previously shown to be activated by temperatures of >52 °C (8). This was confirmed in cells transiently transfected with mouse or rat TRPV2 (Fig. 3A). In this experiment, the assay temperature was quickly raised to ~53 °C by the direct addition of heated assay buffer to each well. The increases in Ca2+-activated fluorescent signal observed in

![Figure 1. Activation of TRPV2 by 2-APB.](image)

**Figure 1. Activation of TRPV2 by 2-APB.** HEK293 cells transiently transfected with pcDNA3 plasmid DNA alone (gray dashed line) or TRPV2 of rat (solid black line), mouse (gray solid line), or human origin (black dashed line) were evaluated for responses to 2-APB using FLIPR. Data points are presented as the average of four wells. A, mean response at 100 μM 2-APB. B, concentration-response relationships; data are mean ± S.E.

![Figure 2. Current-voltage (I-V) relationships of TRPV2 to 2-APB.](image)

**Figure 2. Current-voltage (I-V) relationships of TRPV2 to 2-APB.** Shown is differential activation of rat (A) and human (B) TRPV2 expressed in HEK293 cells by 2-APB (300 μM). The currents were elicited with a continuous voltage ramp from −100 to +60 mV. Gray dashed line, control current (no 2-APB); black solid line, current in the presence of 2-APB.
either rat or mouse TRPV2 transfected cells upon exposure to elevated temperature was not due to changes in cell viability, since the cells were still able to respond to a second addition of 2-APB after heat treatment (Fig. 3A). Furthermore, the response to elevated temperature in a rat TRPV2-expressing cell line could be completely inhibited by 300 μM ruthenium red (Fig. 3B), a nonselective TRP channel blocker. However, under the same conditions in HEK293 cells transiently transfected with human TRPV2, no significant response above vector control levels was observed when the temperature was elevated to 53 °C (Fig. 3A). This lack of response to elevated temperature by human TRPV2 was further confirmed using cells that stably expressed human TRPV2, which were evaluated in an attempt to maximize any potential responses, since presumably, the number of expressing cells/expression levels would be higher in these cells than in transiently transfected cells. As shown in Fig. 3C, the human TRPV2 cell line did not respond to elevated temperature above controls despite detectable levels of TRPV2 product present in cell lysates (inset).

TRPV2 Is on the Cell Surface and Is Functionally Expressed—One possible explanation for the lack of activity by human TRPV2 could be that human TRPV2 is not expressed as well as
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To evaluate TRPV2 cell surface expression levels, equal numbers of cells transiently transfected with either rat or human TRPV2-HA were surface-biotinylated, and the biotin-labeled proteins were purified, immunoblotted, and stained with an anti-HA antibody. As shown in Fig. 3D, human TRPV2-HA was well expressed on the cell surface relative to rat TRPV2-HA. Interestingly, one of the doublet bands present in the Western blot of total cell lysate (Fig. 4C) was absent in lanes containing avidin-purified protein for both human and rat TRPV2-HA (Fig. 4D), suggesting that this form is not localized on the cell surface, a finding that was previously observed for rat TRPV2 by others (21). Further confirmation of human TRPV2 cell surface expression was demonstrated by immunofluorescence microscopy. HEK293 cells transiently transfected with rat or human TRPV2-HA exhibited comparable levels of HA-specific staining on the cell surface (Fig. 4, E and F, respectively). Thus, human TRPV2-HA appears to be abundantly expressed on the cell surface.

To determine whether human TRPV2 is fully functional, a compound screen was performed to identify additional activators of TRPV2. One such compound, Δ9-THC, was identified that activates both rat and human TRPV2, causing robust responses above that observed for the parental HEK293 rodent TRPV2 in HEK293 cells. To facilitate the direct evaluation of the relative expression levels of both rat and human TRPV2, an HA tag was appended to the COOH terminus of rat and human TRPV2. The addition of this tag did not appear to affect the response of rat TRPV2 to 2-APB or noxious heat (Fig. 4, A and B). When immunoblots of cell lysates from human and rat TRPV2-HA were stained with an anti-HA antibody, human TRPV2 was shown to be expressed at higher levels relative to rat TRPV2 (Fig. 4, D). Hence, human and rat TRPV2-HA were stained with an anti-HA antibody. As shown in Fig. 3C, human TRPV2-HA was well expressed on the cell surface relative to rat TRPV2-HA. Interestingly, one of the doublet bands present in the Western blot of total cell lysate (Fig. 4C) was absent in lanes containing avidin-purified protein for both human and rat TRPV2-HA (Fig. 4D), suggesting that this form is not localized on the cell surface, a finding that was previously observed for rat TRPV2 by others (21). Further confirmation of human TRPV2 cell surface expression was demonstrated by immunofluorescence microscopy. HEK293 cells transiently transfected with rat or human TRPV2-HA exhibited comparable levels of HA-specific staining on the cell surface (Fig. 4, E and F, respectively). Thus, human TRPV2-HA appears to be abundantly expressed on the cell surface.

TRPV2 Chimera Responses—In an attempt to understand which regions of rat TRPV2 are responsible for 2-APB and heat activation, a series of chimeras were prepared between rat and human TRPV2. The proteins were divided into three domains: 1) the NH2-terminal intracellular domain; 2) the six-transmembrane domain, including the pore; and 3) the COOH-terminal intracellular domain (Fig. 6A). These domains were then exchanged to create full-length TRPV2 of mixed species origin. As shown in Fig. 6C, when the human transmembrane domain was used to replace the comparable region of rat TRPV2 (designated chimera RHR), the resulting protein was found to be highly responsive to 2-APB. To further assess the impact of intracellular regions on 2-APB responses, two additional chimeras were constructed: chimera RRH, which is composed of rat NH2-terminal and transmembrane domains and...
a human COOH terminus, and chimera HRR, which is composed of a human NH2-terminal domain and rat transmembrane and COOH-terminal domains. Neither of these chimeras responded to 2-APB (Fig. 6C), although their expression levels were comparable with that of rat and human TRPV2 (Fig. 6B). However, when both of these inactive chimeras, RRH and HRR, were co-expressed in HEK293 cells, a full response to 2-APB was restored, albeit with a more rapid drop in fluorescent signal after activation. Furthermore, coexpression of equal levels of full-length, nonchimeric human and rat TRPV2 led to 2-APB responses that were comparable with those of rat TRPV2 alone (data not shown). By contrast, each of the chimeras appeared to be largely unresponsive to activation by heat (Fig. 6D). In particular, neither chimera RHR nor the combination of chimera RRH and chimera HRR had significant responses to temperature elevation in this assay.

**TRPV2 Deletion Mutant Activities**—Since studies using the above chimeras implicated both the NH2- and COOH termini as being responsible for the activity differences observed between rat and human TRPV2, a series of deletion mutants of each intracellular domain of rat TRPV2 was prepared (Figs. 7A and 8A) and evaluated to more precisely define the regions conferring responsivity to 2-APB and heat. NH2-terminal mutants lacking as much as the first 65 aa (mutant ΔN65) were still responsive to 100 μM 2-APB (Fig. 7C) and 53 °C (Fig. 7D). However, when NH2-terminal deletions were 83 or 120 aa (mutants ΔN83 and ΔN120, respectively), the mutants were inactive (Fig. 7A), although they were well expressed (Fig. 7B). Immunofluorescence microscopy revealed that both of these mutants appeared not to be well surface-localized (data not shown). Mutants of rat TRPV2 lacking the COOH-terminal 11, 23, or 32 aa (Fig. 8A) were also responsive to 100 μM 2-APB (Fig. 8C) and 53 °C (Fig. 8D), despite the removal of a potential protein kinase C phosphorylation site at Ser240 in the −23 and −32 mutants. The ΔC55 mutant displayed only minimal activation by 2-APB and heat, but the decreased responses may be due to protein instability, since considerably less protein was detected on Western immunoblots (Fig. 8B).

**DISCUSSION**

We confirm here previous findings (16) that 2-APB activates murine TRPV2 and extend that observation to the rat ortholog. By contrast, we find that human TRPV2 does not respond to 2-APB...
Species-specific Differences in TRPV2 Activation

activated by 2-APB when expressed in COS-7 or CHO cells, neither of which is of human origin (data not shown). Post-translational modification of human TRPV2, such as phosphorylation, which could regulate channel activity, has been reported previously for TRPV2 in resting mast cells and was shown to be increased upon treatment with forskolin (23). Other modifications, such as changes in glycosylation, could alter activation properties, as was shown for TRPV1 (24). Thus, such modifications may block a binding domain or prevent the association of monomers to form a functional human TRPV2 channel. In addition, it is conceivable that human TRPV2 simply does not respond to 2-APB. Such species differences have been noted previously for other TRP channels. For instance, resiniferatoxin and capsaicin, two potent activators of rodent and human TRPV1, were shown to have minimal effect on TRPV2 of rabbits (25) and chickens (26), respectively, with residues critical for these activity differences being localized to the transmembrane domain. TRPV2 response to extreme heat, on the other hand, might be expected in all mammals if TRPV2 is indeed a nociceptive transducer. However, this apparent lack of response by human TRPV2 to noxious heat could mean that the human form has a higher temperature threshold than that of rodents. It is technically difficult to generate temperatures much above 53 °C with our plate-reading equipment, so it is possible that human TRPV2 can only be activated by temperatures higher than what we were able to achieve in our assays.

Finally, the possibility cannot be excluded that an alternate, as yet unidentified variant of TRPV2 exists that is the high temperature-sensing channel in humans. Variants of other TRP channels having different activation properties have been reported (27, 28), including variants of TRPV1 that do not respond to known stimuli of TRPV1 (29) and others that appear to act as dominant negative channels (30).

To better define what regions of rat TRPV2 are responsible for activation by 2-APB and heat, our studies with rat TRPV2 chimeras and mutants demonstrate that both the NH2- and COOH-terminal regions appear to be important for responsiveness to 2-APB. This is consistent with the findings of others, who demonstrated that individual amino acids present in both cytosolic tails of TRPV1 were key to full activation by capsaicin (31). Interestingly, although both termini are necessary for TRPV2 activation, we show that they may not have to be provided on the same molecule (i.e. in cis). This novel, chimeric trans complementation of an active channel by two inactive chimeras shown here suggests that monomeric forms of TRPV2, which by themselves are inactive, are required to oligomerize in order to form a functional channel complex. Furthermore, this intermolecular association between chimeras provides further evidence that native TRPV2 interacts in a multimeric way to form a functional channel, of which a tetramer is the most likely form (23). It is thus tempting to speculate that the promiscuity in subunit associations, which was demonstrated by the chimeric complementation experiments, presages the potential for the formation of fully functional heteromeric channels, including other TRP subtypes, perhaps each with unique biophysical and pharmacological properties.

Although both termini of rat TRPV2 appear important for channel activation, the transmembrane domain region can be substituted with the comparable region of human TRPV2 without losing 2-APB responses (see chimera RHR in Fig. 6 C). This indicates that the sequences responsible for activation by 2-APB are either not found in this region or are present in human TRPV2 as well but are blocked/made inaccessible by its intracellular regions. This is in agreement with the results of other investigators (32), who showed that chimeras composed of TRPV1 and TRPM8 channels were functional and that the temperature-sensing properties of the chimeras were determined predominantly by the source of the COOH-terminal sequence. Thus, these findings would suggest that the transmembrane domain might play a more general gating role in the
activation of at least some TRP channels. However, since we observed different responses to 2-APB and heat with chimeric RHR (Fig. 6, and C and D), this indicates that thermo-TRP channel gating by chemical and thermal stimuli may involve distinct, separable mechanisms for chemical and temperature activation, as was previously observed for TRPM8 mutants (22).

Finally, we demonstrated that at least the NH2-terminal 65 amino acids were not required for activation of rat TRPV2 by 2-APB or high temperature. NH2-terminal deletion of 83 or 120 aa of rat TRPV2 resulted in proteins that were well expressed in HEK293 cells, but each failed to respond to either activator, probably due, at least in part, to poor cell surface localization. Deletion of the COOH-terminal 32 aa of rat TRPV2 appeared not to affect 2-APB or high temperature responses, despite the removal of a potential protein kinase C phosphorylation site. Interestingly, this potential site is also present in mouse but absent in human TRPV2, initially piquing our interest as a possible explanation for the lack of response by human TRPV2. However, since removal of the protein kinase C site had no effect on rat TRPV2 activation, its absence in human TRPV2 is unlikely to be the reason for the lack of activity.

Thus, although these studies eliminated many of the sequence differences between rat and human TRPV2 as being responsible for 2-APB and heat activation, the challenge still remains to identify which residues in rat TRPV2 enable these responses. For instance, rat TRPV2 residues 65–83, a region with sequence differences compared with human TRPV2, was shown to be necessary for responses to 2-APB and heat, but whether substitution of these sequences into human TRPV2 would modulate activity is unclear. Complicating conclusions of these studies is our finding that both NH2- and COOH-terminal intracellular domains of rat TRPV2 are required for activity, suggesting that the sites of 2-APB interaction are discontinuous and come together in the context of either a fully folded TRPV2 monomer or multimer. One approach that may provide some further insight in this regard would be to profile the responsivity and amino acid sequence of TRPV2 from a number of different species in an attempt to identify a consensus sequence for activation, as successfully employed for TRPV1 previously (26).

In conclusion, we demonstrate that rodent but not human TRPV2 is a noxious heat- and 2-APB-activated channel and that its amino- and carboxyl-terminal regions are both important in conferring responsiveness to these stimuli. The explanation(s) for this lack of response by human TRPV2 will require further investigation, which would greatly benefit from the future development of specific pharmacological tools and transgenic models. In the meantime, caution is warranted in reaching any conclusions about the endogenous role(s) of TRPV2 in humans. Finally, the novel trans complementation of interspecies TRPV2 chimeras demonstrated here not only provides a useful experimental approach toward better understanding the properties of this channel but also suggests the potential for even greater heterogeneity of TRP channel structure and function than previously imagined.

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