Molecular Cloning of an N-terminal Splice Variant of the Capsaicin Receptor

LOSS OF N-TERMINAL DOMAIN SUGGESTS FUNCTIONAL DIVERGENCE AMONG CAPSAIN Receptor Subtypes

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Recently a cDNA clone, vanilloid receptor subtype-1 (VR1), was isolated and found to encode an ion channel that is activated by both capsaicin, the pain producing compound in chili peppers, and by noxious thermal stimuli. Subsequently, two related cDNAs have been isolated, a stretch inactivating channel with mechanosensitive properties and a vanilloid receptor-like protein that is responsive to high temperatures (52–53 °C). Here, we report the isolation of a vanilloid receptor 5’-splice variant (VR.5’sv) which differs from VR1 by elimination of the majority of the intracellular N-terminal domain and ankyrin repeat elements. Both VR.5’sv and VR1 mRNA were shown to be expressed in tissues reportedly responsive to capsaicin including dorsal root ganglion, brain, and peripheral blood mononuclear cells. Functional expression of VR.5’sv in Xenopus oocytes and mammalian cells showed no sensitivity to capsaicin, the potent vanilloid resiniferatoxin, hydrogen ions (pH 6.2), or noxious thermal stimuli (50 °C). Since VR.5’sv is otherwise identical to VR1 throughout its transmembrane spanning domains and C-terminal region, these results support the hypothesis that the N-terminal intracellular domain is essential for the formation of functional receptors activated by vanilloid compounds and noxious thermal stimuli.

Capsaicin, the pungent main ingredient of hot chili peppers, selectively activates the peripheral termini of small diameter sensory neurons (nociceptors) and evokes the sensation of burning pain (1). The activation of nociceptors by capsaicin and other vanilloids is thought to be mediated by their binding to and activation of a ligand-gated ion channel (2, 3). Recently a functional capsaicin receptor termed vanilloid receptor subtype-1 (VR1),1 was cloned (4). The inferred amino acid sequence of VR1 predicts an ion channel subunit with six transmembrane-spanning segments flanked by large intracellular N-terminal and C-terminal domains (4). Thus far, VR1 has been reported to have a pattern of mRNA expression by in situ hybridization and immunohistochemical studies that is restricted to small diameter dorsal root and trigeminal and vagal sensory neurons (4–6). In addition to its response to vanilloids, at least three additional functions for VR1 have been demonstrated as follows: the transduction of noxious thermal stimuli, its potentiation or activation by hydrogen ions (low pH) (4, 6), and most recently its activation by anandamide, an endogenous ligand of the cannabinoid receptor (7). The response properties of individual nociceptive neurons to these stimuli are, however, more complex than those responses characterized from the expression of VR1 in either Xenopus oocytes or transfected HEK293 cells (8–14). One explanation for this heterogeneity is that individual nociceptors express multiple vanilloid receptor subtypes (10, 11, 14). Thus far, evidence for molecular heterogeneity of vanilloid receptors includes the isolation of cDNAs encoding a stretch-inactivating channel (15) and a vanilloid receptor-like protein, which is insensitive to capsaicin but responds to high temperatures (52–53 °C) (16). To test this hypothesis, we used a segment of the VR1 cDNA to screen a sensory ganglion cDNA library for other subtypes of the capsaicin receptor. A cDNA was isolated that diverges with VR1 at its 5’ end and is termed VR.5’sv. It is predicted to encode a capsaicin receptor subunit with a truncated intracellular N-terminal domain. Comparison of VR.5’sv and VR1 mRNA expression by semi-quantitative RT-PCR revealed that they are differentially expressed in dorsal root ganglion (DRG) and brain and also present in peripheral blood mononuclear cells. Furthermore, functional expression experiments demonstrate that VR.5’sv does not direct inward currents in response to vanilloid compounds or noxious thermal stimuli. These experiments support the hypothesis that the N-terminal intracellular domain encoded by VR1 is essential for the formation of receptors activated by capsaicin and noxious heat.

EXPERIMENTAL PROCEDURES

Southern Blot and Filter Hybridization—A cDNA library was constructed from adult rat dorsal root (DRG) and trigeminal ganglion RNA by Stratagene® with cDNA packaged into λ ZAP Express® as 10 independent pools (50,000 independent recombinants/pool). We then converted each pool into a plasmid (phagemid) pBk-CMV form by “mass excision” according to the manufacturer’s protocol (Stratagene). In or-
order to screen the cDNA library, each pool was digested with BamHI and NotI and electrophoresed on a 1% agarose gel with resultant fragments transferred to a Hybond N®, nylon membrane (Amersham Pharmacia Biotech). The library was initially screened using a cDNA fragment generated from a BglII/SacII restriction digest of VR1 (nucleotides 405–1430) which was gel-purified with Qiagen Ionex containing final concentration in (in max) NaCl (90), KCl (1), NaHCO3 (2.4), HEPES (10), MgCl2 (1), CaCl2 (2) at 94 °C × 4 min, 94 °C × 30 s; 58 °C × 1 min; 72 °C × 1 min at 35 cycles/22 and 390–410) were then used to screen each cDNA pool for reactivity with the resultant isolation of a cDNA, VR.5 sv terminal VR1 PCR product. Pool 3 was subsequently screened by filter hybridization with the resultant isolation of a cDNA, VR.5 sv, containing a 2.7-kb insert which hybridized to the radiolabeled VR1 N-terminal fragment (not shown).

sequenced directly or subcloned into pGEM T Easy® (Invitrogen) and then sequenced using either SP6- or TT-based sequencing primers.

**RT-PCR—**Oligodeoxynucleotide primers for reverse transcription-polymerase chain reaction (RT-PCR) experiments were designed based on cDNA sequences using GeneWorks® and MacVector® primer selection software (Oxford Molecular Inc.) and were custom-synthesized and cartridge-purified (Life Technologies, Inc.). RT-PCR conditions are as follows: first strand synthesis from total neonatal or adult rat DRG RNA 0.8 µg/15 µl was primed with random hexamers and reverse-transcribed with SuperScript II® (Life Technologies, Inc.) using the manufacturer’s protocol. Adult rat brain RNA was derived from the following four subregions: 1) cerebral cortex; 2) hypothalamus, midbrain, and hippocampus; 3) cerebellum; 4) medulla oblangata and pons as described (17). Rat peripheral blood mononuclear cells were prepared by centrifugation through a Percoll gradient as described previously (18). All RNA samples used for RT-PCR or 5’-RACE were treated with DNase (Promega) to remove trace contamination of genomic DNA.

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**OCYTES**—**Electrophysiology—**Xenopus oocytes were injected with cRNA (10–20 ng/50 nl of diethyl pyrocarbonate-treated water) using a Nanocjetor® (Drummond Scientific) as described previously (4). Two electrode voltage clamp analysis (E_mem = –80 mV) was performed using a Geneclamp® 500 (Axon Instruments) 2 days following the cRNA injection. Recording solutions were prepared with a mix containing NaCl (90), KCl (1), NaHCO3 (2.4), HEPES (10), MgCl2 (1), CaCl2 (2) at pH 7.5 and superfused the oocytes at a rate of 3–5 ml/min. Experiments were performed at room temperature (23 °C) unless otherwise indicated. Thermal stimuli were applied by application of heated recording solution controlled by an adjacent water bath held at constant temperature. Recording chamber temperature was determined by use of a Sensors® model BAT-12 thermocouple (Physitemp Instruments Inc) placed within 2 mm of the oocyte. Statistical analysis of oocyte currents in response to capsaicin, RTX, and noxious heat (50 °C) was performed with StatView® (SAS Institute Inc.) using analysis of variance with Fisher’s post hoc test.

RESULTS

**Southern Blot Analysis**—**Identifies a Variant Form of the Capsaicin Receptor cDNA**—By using a cDNA fragment derived from the N-terminal region of VR1 (nucleotide 405–1330), the plasmid sub- pools of the rat sensory ganglion cDNA library were screened by Southern blot hybridization (see under “Experimental Procedures”). This resulted in the identification of a 1.4–1.6-kb band in sub-pool 3 (Fig. 1) that was not present in other pools. cDNA library pools 4–8 showed a strongly hybridizing band of approximately 2.5 kb in size, and pool 10 showed both a 2.0- and 2.5-kb band. Primer pairs derived from the N-terminal region of VR1 (VR1 nucleotide positions 200–220 and 390–410) were then used to screen each cDNA pool for
A predicted VR1, N-terminal PCR product. Pools 4–8 and 10 showing the large, intensely hybridizing bands were found to direct the amplification of the N-terminal VR1 PCR product. However, pool 3 did not generate such a PCR product (not shown). We focused subsequent efforts on the isolation and characterization of the weakly hybridizing clone in pool 3. Based on the restriction map of VR1, the uniquely hybridizing BamHI/NotI fragment in pool 3 suggested that it diverged at the 5′ end of the cDNA. Following three rounds of filter hybridization and colony purification, a 2.7-kb cDNA was isolated that continued to hybridize to the N-terminal VR1 probe.

Sequence Characterization of a 5′-Splice Variant (VR.5sv) of the Capsaicin Receptor—As shown in Fig. 2A, the cDNA sequence referred to as VR.5sv for vanilloid receptor-5′-splice variant, GenBank(TM) accession number AF158248, was determined and aligned to the VR1 cDNA sequence (GenBank(TM) accession number AF029310) using the program ALIGN® (Geneworks, Oxford Molecular). VR.5sv was found to be 2768 bp in length and to share regions of DNA sequence identity beginning at VR1 nucleotide sequence 588. VR.5sv is identical to VR1 from nucleotides 106 to 246. Following this is a divergent region completely unique to VR.5sv (nucleotides 247–347). This is followed by another region of sequence identity (nucleotides 348–647) followed by an “in-frame” deletion (nucleotides 648–921) that lies within the open reading frame. Beyond this point, both VR.5sv and VR1 cDNAs continue to have identical DNA sequence (except for a single base substitution at VR.5sv nucleotide 746, T versus C at VR1 nucleotide position 1396 which encodes the same amino acid, valine) until the termination of the VR1 sequence within its 3′-untranslated region, nucleotide 2847. VR.5sv then continues an additional 575 bp to include a polyadenylation signal sequence AATAAA (see Fig. 2A, bold) that precedes the terminal poly(A)+ tail.

Analysis of the nucleotide sequence of VR.5sv identified an open reading frame beginning at the codon ATG (nucleotide 528) encoding an inferred 471 amino acid polypeptide with an estimated molecular mass of 54,416 which continues in-frame to nucleotide 1941 where the stop codon TAA is found for both VR.5sv and VR1 cDNA sequences. As illustrated in Fig. 2B, the open reading frame of VR.5sv represents a nearly complete deletion of the intracellular N-terminal domain of the VR1 receptor subunit. Furthermore, the open reading frame also includes an additional 60 amino acid deletion (see Fig. 2B, carets) which partially truncates an ankyrin repeat domain identified in VR1. However, the sequence continues in-frame and is identical to the predicted transmembrane spanning domains and the intracellular C-terminal domain of VR1 (4). Therefore, VR.5sv should maintain the same secondary structure as was predicted for VR1 by hydrophilicity analysis (4) with the exception of its truncated and modified N-terminal intracellular domain. The possibility that VR.5sv represents the incomplete splicing of introns was considered; however, no consensus sequences were found at points of cDNA divergence between the subtypes that would fulfill the requirements of a splice donor/acceptor site (19). Interestingly, comparison of VR.5sv to the National Center for Biotechnology Information data base (8) revealed DNA and amino acid sequence identity with a “stretch-inhibitable nonelective channel” derived from mouse kidney (GenBank(TM) accession number AB015231) (15), which has an identical translational start site and initial N-terminal protein sequence as VR.5sv (see Fig. 2B). Other sequences known to be related to VR1 and VR.5sv include a “growth factor-regulated calcium channel” (GenBank(TM) accession number AB021665), a “vanilloid receptor like protein” (GenBank(TM) accession number AF129911) for rat and
AF129112 for human) (16) and an “epithelial calcium channel” (GenBank™ accession number AJ133128) (20). In addition, several sequences contain similarity to ankyrin repeats (GenBank™ accession number AF045639) (21) and an olfactory channel in *Caenorhabditis elegans* (GenBank™ accession number AF031408) (22).

**RT-PCR and 5' RACE Experiments Confirm 5' Divergence of Capsaicin Receptor mRNA in Dorsal Root Ganglion**—To confirm that VR.5'sv is not an artifact of cDNA library construction, we performed both RT-PCR and 5'-RACE experiments using RNA derived from rat DRG. First, using total DRG RNA and VR.5'sv-specific primers (P1 and P2, Fig. 2A) PCR amplified an approximately 300-bp fragment of the expected size (see Fig. 3A, lane 1), whereas no such band was seen when an identical reaction was performed in the absence of reverse transcriptase (Fig. 3A, lane 2). DNA sequence of this RT-PCR fragment was identical to that derived from the cDNA clone VR.5'sv (not shown). In addition, we performed 5’-RACE experiments (see “Experimental Procedures”) to generate additional 5’ nucleotide sequence encoding capsaicin receptors beginning from a point common to both VR1 and VR.5'sv (see Fig. 2A, reverse primer P3). DNA sequence analysis of subcloned 5’-RACE fragments demonstrated two independent sequences, one identical to VR.5'sv (nucleotides 66–320 Fig. 2A) and the other identical to VR1. No additional sequence variants were identified. 5’-RACE reactions based on more upstream sequence of VR.5'sv failed to provide additional cDNA sequence (not shown). It is therefore likely that VR.5'sv represents most if not all of the 5’ sequence of this transcript.

**Semi-quantitative RT-PCR Demonstrates Differential Expression of VR.5'sv and VR1 in Neuronal and Non-neuronal Cells**—To begin to investigate the tissue distribution and relative abundance of VR.5'sv mRNA expression as compared with VR1 and VR.5'sv (see Fig. 2A, reverse primer P3). DNA sequence analysis of subcloned 5’-RACE fragments demonstrated two independent sequences, one identical to VR.5'sv (nucleotides 66–320 Fig. 2A) and the other identical to VR1. No additional sequence variants were identified. 5’-RACE reactions based on more upstream sequence of VR.5'sv failed to provide additional cDNA sequence (not shown). It is therefore likely that VR.5'sv represents most if not all of the 5’ sequence of this transcript.

**Fig. 2—continued**
capsaicin-induced currents (hypothalamus) (23), or in the case of peripheral blood mononuclear cells, capsaicin was reported to enhance the release of the neuropeptide substance P (24, 25). Therefore, we constructed additional primers (see "Experimental Procedures") that amplified fragments unique to VR.5\textsuperscript{sv} (primer pair P5 and P6) and VR1 (primer pair P5 and P7 (see Fig. 2A)). By using these primers in parallel reactions with ones directed to amplify the housekeeping gene GAPDH, RT-PCR reactions were performed at 25, 30, and 35 cycles of amplification for each sample. Subsequently, 30 cycles of PCR amplification was selected for semi-quantitative comparison as this represented linear amplification of VR.5\textsuperscript{sv}, VR1, and GAPDH in DRG and brain. As shown in Fig. 3, B and C, primers directed to amplify both VR.5\textsuperscript{sv} and VR1 were found to be expressed in the RNA derived from DRG, brain, and peripheral blood mononuclear cells and were of the predicted size. In addition, VR.5\textsuperscript{sv} fragments were subcloned, and their DNA sequence identity was confirmed (not shown). Parallel RT-PCRs run in the absence of reverse transcriptase or DNA template showed no ethidium bromide-stained products after 35 cycles of amplification (not shown). When the ethidium bromide-stained VR.5\textsuperscript{sv} and VR1-directed products were compared by densitometry (see "Experimental Procedures"), approximately a 2-fold difference in expression of VR1 over VR.5\textsuperscript{sv} was observed within the DRG RNA. However, VR1 and VR.5\textsuperscript{sv} expression was essentially equal when expression was compared in the brain subregion containing hypothalamus, midbrain, and hippocampus. Similar results were found when the remaining brain subregions (cerebral cortex, cerebellum, medulla oblongata, andpons) were compared (not shown). Finally, RNA derived from peripheral blood mononuclear cells expressed both capsaicin receptor subtype mRNAs but at substantially lower levels as their predicted products were not observed until 35 cycles of PCR amplification.

Expression of VR.5\textsuperscript{sv} in Xenopus Oocytes and Mammalian Cells—Since VR1 has been shown to direct the functional expression of a nonselective cation channel activated by capsaicin, resiniferatoxin (RTX), noxious thermal stimuli (45–50 °C), (4) and more recently by protons (low pH 6.3, at 37 °C) (6), we sought to express functionally VR.5\textsuperscript{sv} to determine whether it could direct similar pharmacologic and physiologic properties in oocytes and mammalian cells. cRNA transcribed \textit{in vitro}, encoding VR.5\textsuperscript{sv}, was microinjected into Xenopus oocytes, cultured for 48 h, and then studied using two electrode voltage clamp technique for electrophysiological study at a holding potential of −80 mV. Initially, the responses to maximal concentrations of capsaicin (10 μM) and RTX (100 nM) were tested (4). No inward current responses were observed when compared with oocytes injected with VR1 cRNA or water-injected controls (see Fig. 4, A and B). Similar results were found following longer periods of culture (5 days) or performing electrophysiologic measurements at more positive holding potentials (not shown).

When VR.5\textsuperscript{sv} cRNA-injected oocytes were stimulated with heat in the noxious range (50 °C), only small inward currents were observed, of equal magnitude to the water-injected controls (see Fig. 4B). Given the recent description of a "vanilloid receptor-like protein" (VR1L-1) which is reported to have a higher threshold for thermal activation (approximately 52–53 °C) (16), we also compared the response of VR.5\textsuperscript{sv}-injected oocytes compared with water-injected controls to a thermal stimulus of 53 °C (n = 6). Although this higher temperature produced currents of 1–2 μA in the VR.5\textsuperscript{sv}-injected oocytes, they also produced currents of similar magnitude in the water-injected controls (not shown). Although we were able to observe proton-induced (pH 6.2 at 37 °C) inward currents in oocytes injected with VR1 cRNA, oocytes injected with VR.5\textsuperscript{sv} cRNA failed to respond to low pH (6.2) at either room temperature (23 °C) or at 37 °C (not shown). We also transiently transfected mammalian cells (HEK293) using the calcium-phosphate method with the plasmids pBK-CMV/VR.5\textsuperscript{sv}, pBK-CMV vector, and pcDNA3/VR1. Following transfection and 48 h of culture on coverslips, cells were loaded with the calcium dye, Fura II® (Molecular Probes), and were imaged for changes in intracellular calcium in response to capsaicin, thermal stimuli (48 °C), and protons (pH 6.2 at 37 °C). VR.5\textsuperscript{sv} and the vector control failed to respond to any of these stimuli, whereas VR1-transfected cells showed responses to capsaicin, heat, and low pH (n = 4, not shown).

DISCUSSION

We have used a portion of the VR1 cDNA to isolate a novel N-terminal splice variant from a rat sensory ganglion cDNA library. This divergent subtype, termed VR.5\textsuperscript{sv}, contains unique BamHI restriction sites in the 5′ region distinguishing it from VR1. When VR.5\textsuperscript{sv} and VR1 cDNA sequences were compared (Fig. 2A), VR.5\textsuperscript{sv} was found to share alternating regions of DNA sequence identity beginning at nucleotide position 588 of VR1. A search for an open reading frame for VR.5\textsuperscript{sv} identified an ATG, which was preceded by several in-frame stop codons. Comparison of the sequences upstream of the ATG showed partial agreement (positions −1 (C), −3 (A), and −5 (C) from the ATG) with the consensus sequence described by Kozak (26) that correlates with \textit{in vitro} initiation of translation for eukaryotic organisms (27). The designated open reading frame of VR.5\textsuperscript{sv} encodes a truncated intracellular N-terminal coding region homologous to VR1. VR.5\textsuperscript{sv} also remains in-frame following a 60 amino acid deletion (see Fig. 2B) to encode amino acid sequence identical with VR1 for the remaining transmembrane spanning domains and intracellular C-terminal coding region (4). Since the VR.5\textsuperscript{sv} cDNA is identical in the region encoding the transmembrane spanning domains and inferred C-terminal region, it is likely that VR1 and VR5\textsuperscript{sv} are both variants of a common gene. Variants splicing of genes encoding the N-terminal domain of proteins is used to generate structural and functional diversity within ion channel families. For example, TRPC1A, to which VR1 and VR.5\textsuperscript{sv} are structurally related (28), is an N-terminal splice variant of the human homologue Htrp-1, a cation channel activated by depletion of intracellular calcium stores (29, 30). Furthermore, the expression of different N-terminal splice variants of potas-
sium channels has been shown to determine such diverse functions as arachidonic acid-mediated inhibition in the renal K⁺ channel, ROMK1 (31), or control the rate of inactivation in voltage-gated K⁺ channels (32).

The identification of ankyrin repeat domains, present in the intracellular N-terminal region of VR1 (4) and VR.5'sv, are contained in a diverse range of proteins, receptors, and ion channels including trp and trpl, which are also structurally related to VR1 (33). The function of the ankyrin repeats are believed to be for interconnection of integral membrane proteins with spectrin-based cytoskeletal elements. Neural-specific forms of ankyrin have been demonstrated to participate in maintaining and localizing specific ion channels to subcellular regions such as Nodes of Ranvier (34). VR.5'sv diverges from VR1 by containing deletions that virtually eliminate the ankyrin domains except for a portion of the third ankyrin repeat element of VR1 (see Fig. 2B). If the presence of ankyrin domains serves to affect distribution through cytoskeletal associations, modulation of capsaicin receptor channel location in nociceptor terminals could have a profound effect on how pungent compounds such as capsaicin and noxious thermal stimuli are detected. Alternatively, the reduction or elimination of ankyrin repeat domains as occurs in VR.5'sv could represent the loss of specific subunit protein-protein interactions.

Several lines of evidence support the presence of VR.5'sv mRNA expression in the dorsal root ganglion. When primer pairs unique to VR.5'sv sequences were used to amplify RT-PCR products, the resultant fragments were of the predicted size and, when sequenced, showed identity to VR.5'sv cDNA. In addition, when 5'-RACE experiments were performed using a primer that was common to both subtypes (Fig. 2A, primer P3), the resultant subcloned products were found to have DNA sequences identical to VR.5'sv and VR1. The use of total versus poly(A)⁺ RNA did not change this result (not shown). In addition, when RT-PCR (Fig. 3A) and 5'-RACE experiments were performed in the absence of reverse transcriptase, no products were observed. These findings are consistent with previous results employing Northern blot analysis indicating the presence of VR1 in DRG and trigeminal ganglion RNA (4).

We also investigated, using semi-quantitative RT-PCR, the relative level of VR.5'sv expression compared with VR1, in tissues in which vanilloids produce physiologic effects. As shown in Fig. 3B, VR1 mRNA was found to be expressed at approximately a 2-fold greater level than VR.5'sv in adult rat DRG. Since both subtypes have essentially identical mRNA sequence following the N-terminal divergence, previous experiments that utilized these common sequences for study of receptor regulation or distribution would have been unable to distinguish between VR1 and VR.5'sv mRNA. If VR.5'sv can be shown to modify capsaicin receptor activity, the differential expression of each subtype could have significant implications for the physiologic responses of nociceptive neurons. As shown in this study, VR1 expression was essentially equal to VR.5'sv in brain (Fig. 3B). Although expression of the capsaicin receptor mRNA has been reported to be restricted to neurons in peripheral sensory ganglia (4, 6), we found both VR.5'sv and VR1 mRNA expression throughout the rat brain. Potential sources of mRNA encoding these subtypes in brain include the hypothalamus, a region reported to express capsaicin-sensitive neurons involved with thermoregulation (25). However, expression of VR.5'sv and VR1 was not restricted to this brain subregion. Peripheral blood mononuclear cells and mast cells, pervasive throughout the central nervous system, should also be considered as a source of the RT-PCR products, as they have been recently reported to express functional capsaicin receptors based on substance P release, calcium influx experiments, and binding studies (24, 25, 35). We have found that peripheral blood mononuclear cells contain mRNA encoding both VR1 and VR.5'sv but at substantially lower levels than those found in DRG or brain (Fig. 3B). Nevertheless, the presence of mRNA encoding VR1 or VR.5'sv is in contrast to the previous report that showed no evidence for mRNA expression of VR1 in brain by Northern blot or the hypothalamic region as determined by in situ hybridization methods (4). This discrepancy may very well be due to the greater sensitivity of RT-PCR-based detection versus hybridization methods, even under the limited cycles of PCR amplification used in our study.

Another splice variant of the capsaicin receptor, termed “stretch-inactivated channel” (SIC), has been isolated from kidney and found to have mechanosensitive properties (15). Although VR1, VR.5'sv, and SIC all share identical transmembrane domains, each subtype diverges in either its N-terminal
or C-terminal intracellular region. For example, VR.5’sv and the SIC channel share an identical translation start site, ATG (not shown), and have similar truncated N-terminal domains. However, SIC contains a divergent C-terminal domain. In contrast, VR1 and VR.5’sv share identical C-terminal domains but diverge in their N-terminal region. Functionally, only VR1 has been shown to be activated by vanilloids and noxious heat. Recently it has been shown that capsaicin and its ultra-potent analog, resiniferatoxin, activates the capsaicin receptor at an intracellular site (36). Since the predicted polypeptide of VR1 encodes both a large N- and C-terminal intracellular domain, it is plausible that either region could serve as an agonist-binding site for vanilloid compounds such as capsaicin or participate in the activation of the channel by noxious heat. VR.5’sv lacks the majority of the large N-terminal intracellular domain but encodes a C-terminal intracellular domain identical to VR1. Interestingly, when VR.5’sv was expressed in oocytes or mammalian cells, it was unresponsive to maximal concentrations of capsaicin, resiniferatoxin, or to noxious heat (50 °C). These observations support the hypothesis that the N-terminal intracellular domain as found in VR1 is essential for the noxious chemical (capsaicin and resiniferatoxin) and noxious thermal (50 °C) activation of the vanilloid receptor. Future studies examining the distribution and possible interaction of these subtypes may reveal novel physiologic interactions. Differential expression of VR.5’sv, a subtype insensitive to vanilloids and heat, may represent a novel mechanism whereby nociceptive transduction could be modulated. The fact that both VR1 and VR.5’sv are not only expressed in sensory ganglia but also in other neuronal and non-neuronal tissues suggests they may serve functions in addition to nociceptive transduction.

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