Regulation of TRPV1 by a novel renally expressed rat TRPV1 splice variant

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Regulation of TRPV by a novel renally expressed rat TRPV1 splice variant. Am J Physiol Renal Physiol 290: F117–F126, 2006. First published August 9, 2005; doi:10.1152/ajprenal.00143.2005.—The capsaicin receptor and transient receptor potential channel TRPV1 senses heat, protons, and vanilloid agonists in peripheral sensory ganglia. Abundant data have suggested the presence of potentially novel splice variants in the kidney. We report a novel rat TRPV1 splice variant, TRPV1VAR, cloned from kidney papilla. TRPV1VAR cDNA was identified in multiple kidney tissues. Its sequence was fully compatible with potential splice donor and acceptor sites in the TRPV1 gene. TRPV1VAR is predicted to encode a truncated form of TRPV1 consisting of the NH2-terminal 248 residues of TRPV1 (all within the NH2-terminal intracellular domain) followed by five non-consensus amino acids (Arg-Glu-Ala-Met-Trp) and a stop codon. The variant utilizes the same consensus Kozak sequence as canonical TRPV1. A band of the appropriate molecular mass was identified in the NH2-terminal intracellular domain of TRPV1. Interestingly, when expressed in the COS-7 epithelial cell line, TRPV1VAR functioned in a dominant-negative acting capacity, partially blocking TRPV1-dependent resiniferatoxin responsiveness. We conclude that TRPV1VAR is one of perhaps several TRPV1 splice variants expressed in rat kidney and that it may serve to modulate TRPV1 responsiveness in some tissues.

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ATCTGGTTGGGCTGCA) between nucleotides 821 and 822 of NM_039182; 2) the novel variant was absent 11 bp (GTCAAGGC-GAG) spanning nucleotides 2414 through 2424 of NM_039182 (at position 2456/2457 of TRPV1VAR); 3) T-to-A substitution at nucleotide 145 of NM_039182 (nucleotide 87 of TRPV1VAR); 4) A-to-C substitution at nucleotide 1348 of NM_039182 (nucleotide 1391 of TRPV1VAR); and 5) A-to-G substitution at nucleotide 1791 of NM_039182 (nucleotide 1834 of TRPV1VAR). The locations of these single-nucleotide mismatches are shown in Fig. 1B. The 101-bp cassette in TRPV1VAR but absent from canonical TRPV1 was shared with a single cDNA in GenBank [AF158248, a previously reported rat TRPV1 splice variant (23)]. Fidelity of the clone to predicted intron/exon boundaries of the rat TRPV1 genomic sequence (rat chromosome 10 genomic contig NW_042663) was tested with Spidey (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov/spidey). Of note, at the time of this writing, NW_042663 is no longer accessible electronically although the same information is contained in NW_047336 (TRPV1 gene ~ nucleotides 1108661 to 113444) and in rat chromosome 10 sequence NC_00105 (TRPV1 gene ~ nucleotides 60122797–60148562). For RT-PCR-based generation of additional clones only in the region of the 3′ 11 bp deletion (see Fig. 1B), the following primer pair was used: rVR1–2374–5′ (5′-actgtgaggctgtcag-3′); matching nucleotides 2374–2391 of NM_031982 and nucleotides 2417–2434 of TRPV1VAR) and rVR1–2568–3′ (5′-tgaaactcagctctctg-3′); matching nucleotides 2568–2548 of NM_031982 and nucleotides 2600–2580 of TRPV1VAR). Clones harboring this deletion were identified in kidney cortex, medulla, and papilla. Full-length TRPV1VAR was initially subcloned into pcDNA3.1/V5-His-TOPO with its native stop intact such that no epitope tag was expressed. For other studies, only the open-reading frame (ORF) encoding the conceptual translation of TRPV1VAR was PCR-subcloned into this vector, absent the native stop such that a carboxy-terminal V5 epitope tag was expressed. RNA was treated with the TURBO DNA-free system (Ambion) in accordance with the manufacturer’s directions, under the most stringent conditions possible (“rigorous DNase treatment,” in the manufacturer’s parlance).

**Confirmation of the novel splice variant using “mismatched” primer pairs.** Further RT-PCR-based confirmation of TRPV1VAR was accomplished by amplification of additional clones from RT product of total RNA using upstream primers designed to hybridize with a non-coding portion of TRPV1 VAR common to only AF158248, and “downstream” primers common to only NM_031982, such that only TRPV1VAR cDNA could generate product. Primer pairs consisted of P3–812–5′ (5′-ccggagccctgctagg-3′) and P3–1346–3′ (5′-tgactgctgcttattgct-3′), and P3–744–5′ (5′-gggctgctgcttattgct-3′) and P3–1234–3′ (5′-cgccgagccctgctagg-3′); template consisted of PCR product from reverse-transcribed RNA harvested from dorsal root ganglion and from kidney cortex, medulla, and papilla (see RESULTS).

**Transient transfection.** Transient transfection of HEK cells was performed using Lipofectamine PLUS (Life Technologies) according to the manufacturer’s directions. ΔTRPV1, consisting of only the amino-terminal intracellular portion of rat TRPV1 (absent the membrane-spanning domains), in conjunction with a V5 epitope tag, was used in some experiments where indicated. ΔTRPV1 was amplified from rat TRPV1 using “top” and “bottom” primers 5′-aggagggaa-ccgctgctgtatgcag-3′ and 5′-gggctgctgcttattgctgct-3′, respectively, and subcloned into pcDNA3.1/V5-His-TOPO. For rat TRPV1 devoid of the V5 epitope tag, TRPV1-V5 in pcDNA3.1/V5-His-TOPO was subjected to site-directed mutagenesis (QuikChange; Stratagene) with the following primer pairs: 5′-gggctgctgcttattgctgct-3′ and 5′-ctgggagccctgctgtatgcag-3′ and 5′-gctgctgcttattgctgct-3′ and 5′-gggctgctgcttattgctgct-3′, which underpase represents the label make-up, but the resultant construct expressed only the full-length native rat TRPV1 protein. TRPV1VAR, containing a carboxy-terminal FLAG epitope tag, was generated by annealing oligonucleotides encoding restriction site-tailed FLAG epitope followed by a stop codon (5′-ctgggagccctgctgtatgcag-3′ and 5′-gggctgctgcttattgctgct-3′)
samples using raw data, or for independent samples using normalized data (VassarStats; http://faculty.vassar.edu/lowry/VassarStats.html).

RESULTS

Identification of TRPV1VAR. Using primers designed to amplify full-length rat TRPV1, we identified a novel splice variant of rat TRPV1 that we initially called TRPV1-P3 and, later, TRPV1VAR. This variant was detected repeatedly from multiple preparations and sources of rat kidney RNA using several different primer pairs (see EXPERIMENTAL PROCEDURES).

After fully sequencing the variant clone, we compared it with the rat genomic sequence to determine whether it represented a rational splice product of the rat TRPV1 gene. Using the public-domain mRNA analysis tool Spidey (www.ncbi.nlm.nih.gov/spidey), we aligned TRPV1VAR with rat chromosome 10 genomic contig (NW_042663) and found it to be fully compatible with existing putative splice donor and acceptor sites (Fig. 1A). We therefore concluded that it was unlikely to represent a cloning artifact. The exon-intron structure of canonical rat TRPV1, NM_031982 (4), is shown for comparison, as is the exon-intron structure of a previously identified TRPV1 splice variant, AF158248 (23). The first three exons of the newly identified TRPV1VAR were identical to exons 2–4 of the canonical mRNA sequence; however, exon 4 of TRPV1VAR...
was comprised of exons 5 and 6 (plus the intervening 101 bp of intronic sequence) of the canonical TRPV1 (Fig. 1A). The remainder of the exon-intron structure of the variant mirrored that of intronic TRPV1 with the exception of exon 13. Exon 13 of TRPV1VAR (corresponding to exon 15 of the canonical TRPV1; Fig. 1A) was absent 11 bp of canonical TRPV1 mRNA sequence (gtcaggccgag) at the 3’ end; this difference is not well shown in the gross map in Fig. 1A but is highlighted in Fig. 1B.

The TRPV1VAR cDNA was then aligned with canonical rat TRPV1 cDNA (NM_031982) and splice variant AF158248 (Ref. 23 and Fig. 1B). The TRPV1VAR cDNA diverged from canonical rat TRPV1 (NM_031982) in the following respects: 1) it included a noncanonical 101-bp cassette toward the 5’ end (Fig. 1B); and 2) it was deleted for an 11-bp stretch at the 3’ end. [In addition, there were 3 insignificant point mutations (see EXPERIMENTAL PROCEDURES).] The 101-bp cassette present in TRPV1VAR but absent from canonical TRPV1 had previously been reported in AF158248 (23). Our newly described variant, TRPV1VAR, also closely resembles AF158248 with the following exceptions: 1) most importantly, the present clone includes an additional 623 bp of upstream cDNA sequence matching canonical TRPV1, which changes the reading frame (Fig. 1B); 2) TRPV1VAR includes a 180-bp cassette toward the middle of the cDNA that is also present in NM_031982 (where it represents exon 7) but absent from AF158248; and 3) TRPV1VAR lacks an 11-bp stretch at the 3’ end that is present in AF158248 (and NM_031982), although this does not influence the reading frame (see below). Inclusion of the 0.6 kb of upstream cDNA sequence preserves the original reading frame of canonical TRPV1, but results in premature termination (see below). The TRPV1VAR cDNA is diagrammed in an expanded view in Fig. 1B. Regions of the cDNA matching either canonical rat TRPV1 or splice variant AF158248, or matching both clones, are readily apparent. Examination of the nucleotide sequence of TRPV1VAR revealed a predicted ORF extending from nucleotides 23 through 781 and encoding 253 amino acids, with six membrane-spanning domains. It features three ankyrin repeat domains within a large 400-amino acid intracellular amino terminus (Fig. 1C). Because of a reading frame shift introduced by inclusion of the 101-bp cassette between exons 5 and 6 of canonical TRPV1, the newly identified TRPV1VAR is predicted to encode a truncated protein of only 253 amino acids (Fig. 1C). The first 248 are identical to canonical TRPV1; the variant diverges thereafter, terminating prematurely after amino acid 253 following the unique sequence Arg-Glu-Ala-Met-Trp. Consequently, the variant lacks two of the three ankyrin domains present in the wild-type. In marked contrast, the splice variant AF158248 uses a nonconsensus start site and is predicted to code for only the COOH-terminal portion of TRPV1. Therefore, although their cDNAs bear many similarities, the conceptual translations of the splice variants TRPV1VAR and AF158248 are predicted to match nonoverlapping regions of the canonical TRPV1 protein (Fig. 1C). For comparison, the predicted protein structure of clone AB015231 is also shown. The veracity of this clone, also known as SIC (24), remains inconclusive (32). Although the NH2 terminus of its conceptual translation matches TRPV1, the COOH terminus is contributed by a gene coding for another TRP channel entirely; the cDNA as originally reported cannot be generated from rat genomic DNA via conventional splicing (28, 32). Therefore, AB015231 is either the product of rare trans-splicing (i.e., intermolecular splicing), as has been observed with several mitochondrial transcripts in plants (29) and with selected tRNAs in a hyperthermophilic archael parasite (18), or it may potentially represent a cloning artifact as some have contended (32).

Confirmation of TRPV1VAR. We used two complementary PCR-based strategies to confirm expression of TRPV1VAR. The only unique sequence at the nucleotide level in TRPV1VAR was the absence of 11 bp at the extreme 3’ end (Fig. 1B). (Although other aspects of the sequence were unique, including its apparent coding for an ORF consistent with a truncated amino-terminal TRPV1 isoform, the remainder of the sequence at the nucleotide level was present in either NM_031982 or AF158248, or in both; Fig. 1B.) We designed PCR primers to bracket the 11-bp deletion and amplified clones from dorsal root ganglion cDNA, or from cDNA prepared from a variety of renal tissues, including cortex, medulla, and papilla. The resultant PCR product was subcloned, and ~10–20 clones were screened/tissue. Of these, ~25% corresponded to the ~11 bp variant, and this clone could be identified in each of the four tissues examined. It appeared that the variant was more prevalent in cDNA prepared from kidney papilla (data not shown).

We sought a second strategy to confirm expression of the TRPV1VAR, taking advantage of its features shared with wild-type TRPV1 and with the previously described splice variant. An upstream PCR primer was designed to hybridize to a region of the TRPV1VAR cDNA that was shared only with clone AF158248, and the downstream primer was designed to match a portion of the variant that was shared only with the canonical TRPV1 (Fig. 1B; see EXPERIMENTAL PROCEDURES). Therefore, PCR product could only arise if template contained both of these elements; neither NM_031982 nor AF158248 cDNA could give rise to PCR product. Abundant product of the appropriate size (~530 bp) was generated from cDNA from all four tissues (Fig. 2). A second primer pair targeting the same regions was similarly effective (see EXPERIMENTAL PROCEDURES). In addition, when the product was excised from the gel, subcloned, and sequenced, it was found to be identical to the novel TRPV1VAR (data not shown).

Identification of a possible TRPV1VAR protein. An effort was made to identify the novel variant at the protein level. Because it includes only five unique amino acids at the COOH terminus (Fig. 1C), this was felt to be insufficient for generating a TRPV1VAR-specific antibody. However, the variant was predicted to encode an NH2-terminal truncation of TRPV1, and this was sought via immunoblotting. Whole cell lysates were prepared from kidney cortex, medulla, and papilla and subjected to immunoblotting with an antibody directed against the COOH terminus of intronic sequence) of the canonical TRPV1 (Fig. 1C). NH2 terminus of the canonical TRPV1 sequence. This antibody recognized TRPV1 (~110 kDa) expression in medulla and papilla (Fig. 3). In papillary tissue only, a band migrating at ~30 kDa was also evident; this is consistent with the predicted molecular mass for TRPV1VAR. The blot was reprobed with an antibody directed against the COOH terminus.
presence or absence of TRPV1VAR (data not shown). When was no clear distinction between the TRPV1 response in the
TRPV1VAR increased both the initial rate and the magnitude of
Specifically, at 0.1 nM resiniferatoxin, the presence of
In some but not all experiments with HEK cells, the
TRPV1VAR exerted no effect on cell surface expression of
To confirm that the calcium transients we were observing were genuinely a consequence of TRPV1-dependent calcium entry, additional control experiments were performed. When HEK cells transfected with TRPV1 + TRPV1VAR were treated with resiniferatoxin in the absence of extracellular calcium, an increase in intracellular calcium was not observed (Fig. 4C). In addition, when the dual-transfected cells were treated with resiniferatoxin in the presence of the TRPV1 antagonist capsazepine, the effect was similarly abolished (Fig. 4D). In cells transfected with TRPV1 alone (in the absence of TRPV1VAR), calcium-free medium and capsazepine also prevented an increase in intracellular calcium in response to resiniferatoxin (data not shown). These data confirmed that the calcium transients observed in the present model were dependent on calcium entry and TRPV1 function.

The effect of cotransfection of TRPV1VAR on expression of wild-type TRPV1 was examined as a possible explanation for the potentiating effect of the variant. Immunoblot analysis of cell lysates with anti-TRPV1 antibody indicated no effect on expression of the transfected TRPV1 (Fig. 4E). In addition, cell-surface biotinylation experiments were performed to determine whether expression of TRPV1VAR influenced trafficking of TRPV1 to the cell membrane. Cotransfection of TRPV1VAR exerted no effect on cell surface expression of TRPV1, based on anti-TRPV1 immunoblotting of avidin-agarose affinity precipitates from cells subjected to surface biotinylation (Fig. 4E). These data suggested that the effect of TRPV1VAR vis-à-vis TRPV1 was potentially mediated via direct interaction.

Fig. 3. Anti-transient receptor potential channel (TRPV1) immunoblot of tissue whole cell lysates prepared from rat renal tissues. Left: blot with an anti-TRPV1 antibody specifically directed against the NH2 terminus of TRPV1. Right: blot with an anti-TRPV1 antibody specifically directed against the COOH terminus of TRPV1. A papillary protein migrating at ~30 kDa (solid arrowhead) was immunodetectable with only the antibody directed against the NH2 terminus and was consistent with the conceptual translation of TRPV1VAR, or perhaps a closely related splice variant. Open arrowhead, TRPV1. The identity of the major immunoreactive species at ~75 kDa is unknown.
Interaction of TRPV1 VAR with TRPV1. The ability of TRPV1 VAR to interact with TRPV1 was tested. HEK cells were transiently transfected with wild-type TRPV1 (TRPV1 WT) plus either empty vector alone or TRPV1 VAR. After transfection (48 h), the ability of the TRPV1 agonist resiniferatoxin (0.1 nM) to increase intracellular calcium was assessed via fura 2 ratio-metry as an index of TRPV1 activity. Cotransfection of TRPV1 VAR potentiated the effect of resiniferatoxin. In the absence of TRPV1 expression, there was no response to resiniferatoxin. TRPV1 VAR, lacking membrane-spanning domains, produced no resiniferatoxin response when transfected in isolation (data not shown). Data in this and similar figures are expressed as fura 2 ratio (340/380, in arbitrary units) as a function of time (see scale bars). B: to demonstrate the effect of TRPV1 VAR on TRPV1 function in HEK 293 cells with greater precision, the increment in fura 2 ratio at 200 s of resiniferatoxin treatment (normalized to the absence of TRPV1 VAR) is shown (n = 4 experiments). C: removal of extracellular calcium from the medium (−Ca²⁺) completely blocked the resiniferatoxin response of HEK 293 cells transfected with TRPV1 + TRPV1 VAR. D: TRPV1 antagonist capsazepine (30 μM × 5 min pretreatment) also completely inhibited the resiniferatoxin response of HEK 293 cells transfected with TRPV1 + TRPV1 VAR. E: potentiation of TRPV1 VAR was not mediated at the level of TRPV1 expression or membrane trafficking. Anti-TRPV1 immunoblot of crude whole cell lysates and avidin-agarose affinity precipitates prepared from HEK cells transfected with TRPV1 WT, in conjunction with either vector alone or with TRPV1 VAR, and subjected to cell surface biotinylation. Neither TRPV1 expression nor TRPV1 trafficking to the cell membrane was upregulated by coexpression of TRPV1 VAR.
cotransfected with FLAG- and epitope-tagged TRPV1VAR (TRPV1VAR-FLAG), both were detectable in whole cell lysates via immunoblotting with an antibody directed against the amino terminus of TRPV1 (Fig. 6). (In whole cell lysates, TRPV1VAR-FLAG comigrated with a nonspecific band, but expression of the tagged variant was clearly more robust than this background.) When lysates were subjected to immunoprecipitation with anti-FLAG antibody to “pull down” TRPV1VAR-FLAG, coprecipitated ΔTRPV1 was clearly detectable via immunoblotting with the anti-TRPV1 antibody (Fig. 6). We speculate that the series of small bands migrating more rapidly than TRPV1VAR-FLAG potentially represent degradation products of this epitope-tagged variant. These data established that TRPV1VAR could interact with TRPV1 via the NH2-terminal cytoplasmic domain of the latter. These data can not, however, fully exclude the requirement for an additional intervening protein or protein complex.

**Functional properties of TRPV1VAR in COS-7 cells.** A second heterologous expression model was used to test the effect of TRPV1VAR vis-à-vis TRPV1 function. COS-7 cells were transfected with either TRPV1 + vector or with TRPV1 + TRPV1VAR. In this epithelial cell model, resiniferatoxin-inducible, TRPV1-dependent calcium entry was partially blocked by cotransfection with TRPV1VAR (Fig. 7A). This effect was reproducible and statistically significant (Fig. 7B). In untransfected COS-7 cells, or in cells transfected with TRPV1VAR (alone, or in conjunction with empty vector), the effect of resiniferatoxin was negligible (data not shown). We speculated that this effect of TRPV1VAR (the opposite of that observed in HEK 293 cells) might be a consequence of TRPV1VAR-dependent decrease in TRPV1 cell surface expression. Cell surface biotinylation experiments in this COS-7 model indicated that coexpression of TRPV1VAR did not decrease TRPV1 surface abundance (Fig. 7C). Specifically, surface expression in the presence of TRPV1VAR was 140 ± 30% of that observed in the absence of the variant (P = 0.15). Taken together, these data indicate that TRPV1VAR may act as either a potentiator or inhibitor of TRPV1 function, depending on the cellular context.

**DISCUSSION**

We believe that TRPV1VAR represents a bona fide expressed splice variant of rat TRPV1 for the following reasons: 1) its cDNA could be predictably and reproducibly identified from multiple isolates and multiple sources of RNA prepared from renal and other tissues; 2) its nucleotide sequence is fully compatible with mRNA splice donor and acceptor sites in the rat TRPV1 gene; and 3) partial cDNAs could be independently amplified using a 5′ (upstream) primer specific for a region of the variant that is not shared with canonical NM_031982, paired with 3′ (downstream) primer specific for a region of the variant not shared with known splice variant AF158248. In addition, although not conclusive, a protein of the appropriate molecular mass is immunodetectable upon anti-NH2-terminal but not anti-COOH-terminal anti-TRPV1 immunoblotting of lysates prepared from the renal tissue (papilla), yielding the variant clone with the greatest frequency. It is possible, however, that this protein represents a different splice variant of TRPV1 or of a related TRP channel.

At least two splice variants of rat TRPV1 have been described. Organization of the rat TRPV1 gene is well established; canonical TRPV1 (NM_031982) is encoded by 16 exons spanning ~25 kb on rat chromosome 10 (32). A TRPV1 5′ splice variant (dubbed 5′sv; AF158248) was detected by RT-PCR analysis in dorsal root ganglion and brain, but not in other tissues (23). Conceptual translation of this variant encodes a TRPV1 devoid of its intracellular NH2 terminus and consisting almost entirely of transmembrane domain (Fig. 1C). When heterologously expressed, the 5′sv variant lacked vanilloid-inducible calcium channel activity (23). We do not believe that TRPV1VAR is a full-length version of clone AF158248 because the latter uniquely lacks exon 7 of the canonical TRPV1 (Fig. 1A) and because of their dissimilar 5′ ends.
ends (Fig. 1B). A second TRPV1 splice variant, cloned as the stretch-inactivated channel, or SIC (AB015231; see Ref. 24), was similarly identified in dorsal root ganglion and the central nervous system; mRNA was also detected in the kidney (14, 23). The fidelity of this clone has recently been questioned (28, 32) because it cannot be generated via conventional splicing from the known TRPV1 genomic sequence. Although it is conceivable that splicing in trans could occur, as has rarely been reported for other gene products (e.g., Refs. 18 and 29), the 3' half of the coding sequence appears to be the product of another TRP channel gene, TRPV4. In addition, the presence of an unidentified rat TRPV1 variant was inferred in a subset of taste receptor cells when an 0.3-kb fragment of TRPV1 could be PCR amplified, yet functional properties of the TRPV1-like activity in these cells were distinct from those of classical TRPV1 (12).

Although rat TRPV1 is perhaps the best studied, alternative splicing has recently been identified in other species as well. Wang et al. (26) characterized murine TRPV1 cDNAs, identifying TRPV1α and TRPV1β variants. TRPV1α was widely expressed and formed functional channels with agonist responsiveness resembling rat TRPV1. TRPV1β, in contrast, which was predicted to lack 10 amino acids immediately upstream of the region bearing the six membrane-spanning domains, was nonfunctional when expressed in isolation; TRPV1β, however, could function in a dominant-negative-acting capacity when coexpressed with TRPV1α (26). This β variant, being nearly full length, bears little relation to rat TRPV1VAR described herein. Lu and colleagues (11) very recently identified a splice variant of human TRPV1, dubbed TRPV1b, that is closely related to murine TRPV1β. The human β variant lacks the same 10 amino acids that are absent from the murine β variant; however, it also abandons an additional 50 amino acids immediately upstream of these 10 (11). Human TRPV1b, unlike human TRPV1, fails to respond to elevated temperature; it is, however, activated by protons and the vanilloid capsaicin (11).

We initially identified TRPV1VAR through the absence of 11 bp in its extreme 3' end encoded by the terminal portion of exon 15 in canonical rat TRPV1 (NM_031982). This feature is absent from any previously reported TRPV1 cDNA and is entirely consistent with putative splice sites in the rat TRPV1 gene; however, because it is far downstream of the ORF in TRPV1VAR, its functional significance remains unclear.

Earlier evidence supported the presence of unique splice variants of TRPV1 in the kidney. Using Northern analysis, Cortright et al. (6) examined mRNA harvested from a panel of human tissues and noted abundant TRPV1 expression only in the kidney. Interestingly, whereas other tissues gave a single distinct band by Northern analysis, hybridization of electrolyzed kidney RNA with a radiolabeled TRPV1 probe yielded a “smear” characteristic of multiple mRNA species. In similar fashion, the original reports of the cloning of TRPV1 suggested the presence of novel renally expressed transcripts, based on Northern blot analysis (4). RNase protection assays from several groups were also suggestive of unidentified kidney-specific splice variants. Sanchez et al. (19) attempted to quantify the relative abundance of canonical TRPV1 mRNA, as well as each of the two putative splice variants [5'sv (AF158248) and SIC (AB015231)], in various tissues. Abundant expression of at least three distinct TRPV1-related transcripts was detected in kidney mRNA; these species were not.
observed in other tissues and were not consistent with expression of either 5'sv or SIC. These data strongly supported the presence of additional TRPV1 splice variants.

In addition to TRPV1, splice variants have been observed for other members of the TRP channel family. At least some have documented functional effects, such as dominantly inhibiting the function of the canonical channel. For example, TRPC4 has both α and β splice variants and α is a dominant-negative-acting modulator of β (21). TRPM2 is activated by ADP-ribose; a splice variant predicted to lack the extreme COOH terminus of the canonical channel fails to respond to this agonist (27), whereas a second splice variant predicted to lack four of six membrane-spanning domains functions as a dominant negative (34). Splice variants of TRPM3 involving the pore region exhibit differences in cation selectivity (15).

The molecular mechanism through which TRPV1 VAR exerts its effect remains unclear. TRP channels are predicted to assemble as aggregates of four subunits, and heteromeric channels have been identified (reviewed in Ref. 5). TRPV1 VAR protein appears to physically interact with canonical TRPV1; it is conceivable that TRPV1 VAR perturbs normal TRPV1 channel architecture, although it would seem unlikely that it could replace an entire subunit because of its lack of membrane-spanning domains. TRPV1 VAR may also serve as a “decoy” to titrate a TRPV1-associated protein or signaling intermediate that inhibits (in HEK 293 cells) or activates (in COS-7 cells) the native channel.

From a physiological perspective, TRPV1 VAR potentiates the vanilloid response of canonical TRPV1 in embryonal kidney cells and inhibits it in an epithelial cell line. These effects are not achieved via alterations in either TRPV1 whole cell expression or TRPV1 trafficking to the cell membrane. TRPV1 VAR interacts with the large intracellular amino terminus of canonical TRPV1, so this effect may be a direct one (Fig. 6). We conclude that the protein product of TRPV1 VAR, and perhaps other related TRPV1 transcripts, may serve to modulate TRPV1 function in some tissues in vivo. For example, with respect to kidney physiology, TRPV1 has been implicated in the renal regulatory response to dietary salt loading. Multiple highly clinically relevant models of systemic cannabinoid anandamide (e.g., see Refs. 1 and 25).

Although much of this effect may be conferred via resultant activation of cannabinoid receptors (1), evidence implicates TRPV1, with which anandamide also interacts (35). Specifically, antagonists of TRPV1 promote vasodilation and TRPV1 agonists prevent it in vitro; this effect may itself be mediated through local release of the vasodilatory peptide calcitonin gene-related peptide (35). A corresponding picture was noted in vivo: the TRPV1 agonist capsaicin decreased blood pressure in rats fed a normal-sodium diet, whereas capsazepine, a pharmacological antagonist of TRPV1 function, potentiated the hypertensive effect of salt loading (10). The presence of TRPV1 splice variants in the kidney exhibiting unique functional properties may afford an additional locus of regulation of this phenomenon.

GRANTS

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