Identification and characterization of a novel human vanilloid receptor-like protein, VRL-2

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Sparked initially by a hunt for the capsaicin receptor, the receptor expressed in sensory neurones that reacts to the pungent ingredient in chili peppers to produce a burning pain, an expanding family of related channels has been revealed within the past 3 years. The capsaicin (vanilloid) receptor, denoted VR1, was isolated from rat primary sensory neurones, whose cell bodies are present in the dorsal root ganglia (DRG) (7). The VR1 receptor forms a nonselective cation channel that is activated by both capsaicin and resiniferatoxin (RTX) as well as noxious heat (>43°C), with the evoked responses potentiated by protons. Acid pH is also capable of inducing a slowly inactivating current that resembles the native proton-sensitive current in DRG. Expression of VR1, although predominantly in primary sensory neurones, is also found in various brain nuclei and the spinal cord (20, 23).

Subsequent to the identification of VR1, several related family members have been cloned, all of which share a similar topology to the Osm-9-like transient release potential (TRP) family of store-operated calcium channels (14). The second family member to be isolated was cloned from rat brain and was termed vanillloid receptor-like protein-1 (VRL-1) sharing 49% amino acid identity to VR1 (8). Unlike VR1, VRL-1 is not activated by vanilloids like capsaicin or RTX, nor by acidic pH or moderate heat. Instead, VRL-1 has been proposed to respond to high temperatures, having a threshold of ~52°C (8). In contrast to the specific neuronal localization of VR1, VRL-1 is widely expressed throughout the body, although in the DRG expression is restricted to non-VR1-expressing medium and large-diameter sensory neurones, with myelinated axons (8). A corresponding human VRL-1 ortholog was isolated from a myeloid cell line, followed by an independently identified mouse ortholog defined as a growth factor-regulated channel, or GHR (18).

The VR1 family has recently been extended to include two new members, one of which has been postulated to encode a mechanoresponsive stretch-inhibitable cation (SIC) channel and the other a truncated form of VR1 (VR.5′sv) (28, 24). The SIC channel, although highly homologous to VR1, lacks part of the NH2-terminal do-
main spanning the ankyrin repeat elements and, as well, possesses a different intracellular carboxyl terminal (28). This receptor was detected in both kidney and liver, with the channel being activated by cell shrinkage and inhibited by cell swelling (28). The truncated VR.5 sv clone was detected in DRG, brain, and peripheral blood leukocytes; however, it failed to respond to capsaicin, RTX, or noxious heat, thus implicating involvement of the NH2 terminus of VR1 in these functions (24). The least homologous channels in this family of receptors are known as the calcium transport protein (CaT1) and epithelial calcium channel (ECaC). The former receptor has been detected in the rat duodenum, proximal jejunum, cecum, and colon (22), whereas the latter was expressed in rabbit small intestine, kidney, and placenta (15). Calcium influx via these channels was voltage dependent and pH sensitive, exhibiting inhibition by low pH. Human orthologs for both genes with 80.5% identity to each other have been identified in the EMBL database.

We now describe the cloning, characterization, and tissue distribution of a novel vanilloid-like receptor isolated from human kidney, which we name VRL-2. Localization of VRL-2 to sympathetic nerves as well as nonneuronal cells lining the respiratory tract and kidney distal tubules suggests that vanilloid-like receptors have functions other than as sensory transducers in primary sensory neurons.

MATERIALS AND METHODS
Identification of Human VRL-2

Three proprietary database clones, corresponding to expressed sequence tag (EST)-derived clusters were sequenced by automated DNA sequencing based on the dideoxy chain-termination method using the ABI 373A/377 sequencers (Applied Biosystems). Sequence-specific primers were used with the Big-Dye Terminator Cycle Sequencing kit (Applied Biosystems). The nucleotide sequence was analyzed using programs from the University of Wisconsin Genetics Computer Group to generate a single contig. Identification of the 5’ end was achieved via the isolation of a bacteriophage P1-derived artificial chromosome (PAC) cloning kit (Invitrogen). Resulting clones for each of the three PCR generated VRL-2-fragments were taken for sequence analysis, and separate clones coding a consensus sequence were used in the full-length assembly of the gene into the pBluescript SK(+) vector (Stratagene). A consensus clone was then digested with NotI/XhoI and ligated into the mammalian expression vector pCDNA3.1(+) (Invitrogen).

Chromosomal Mapping by Radiation Hybrid Mapping

PCR primers 5’-ATGGCCACCCAGGGTTCAC (sense) and 5’-TCTGCCAGGGTTCAC (antisense) were designed to amplify a section of VRL-2 spanning part of the 3’ coding region and 3’-UTR. PCR conditions for these primers were optimized using genomic DNA initially as template. PCR was then accomplished using both the somatic hybrid panel from Coriell Cell Repositories (Camden, NJ) and the Stanford G3 radiation hybrid panel from Research Genetics (Huntsville, AL). Amplification products were analyzed on a 2% agarose gel noting the presence or absence of specific

Full-Length Cloning

Human kidney was used as a source of template for the PCR amplification of VRL-2. Primers used for amplification were designed to isolate the gene in three fragments. Primers designed to isolate the 5’ end included a sense primer encoding a NotI site and a strong Kozak motif followed by gene-specific sequence (5’-ATGGCCACCGCCAGCCGCTCAGATCCAGGGTCATGGGAC and antisense primer (5’-CACCTTGGTTGTGACTGTTGACTGTA). The middle fragment, was PCR generated using sense and antisense primers 5’-CAATCTCGCGCATGAAGTCTCCAG and 5’-GCCCGAGAGAATTCAGGGTCATGGT, respectively. Finally the 3’ fragment was amplified with a sense primer 5’-GTTCCATCCCATTCTTCTGTA and an antisense primer 5’-TGACCTCTCCAGAATGATGGCGCAGAGAAGCT encoding a XhoI restriction site. Amplified fragments were cloned into pCRII-TOPO according to the manufacturer’s instructions supplied with the TOPO TA Cloning kit (Invitrogen). Resulting clones for each of the three PCR generated VRL-2-fragments were taken for sequence analysis, and separate clones coding a consensus sequence were used in the full-length assembly of the gene into the pBluescript SK(+) vector (Stratagene). A consensus clone was then digested with NotI/XhoI and ligated into the mammalian expression vector pCDNA3.1(+) (Invitrogen).
bands in each well. Information collected from the G3 radiation hybrid panel was analyzed using the public web server at Stanford University (http://shgc-www.stanford.edu).

Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (FISH) analysis using the VRL-2 PAC clone as a probe was performed according to Lichter et al. (19).

Localization of VRL-2 Expression

The following primers (5’-ACAAGAAGGCGGACATGCGG and 5’-ATCTCGTGGCGGTTCTCAAT) were used to obtain a PCR product from the coding region of VRL-2 to a region located upstream of transmembrane 1. This amplicon was used as a probe on multi-tissue Northern blots and dot blots (Clontech). Membranes were hybridized for 4 h shaking at 60°C in a 10% dextran sulfate, 1% SDS and 1 M NaCl.

Fig. 2. Alignment of VRL-2 with related sequences. Dark-shaded regions represent highly conserved residues; the lighter the shade of the regions, the less conserved the residues become within the family. Grey-lined boxed regions define the ankyrin repeats, whereas black-lined boxed regions highlight the transmembrane spanning regions. CaT1, calcium transport protein 1; ECaC epithelial calcium channel; SIC, stretch-inhibitable cation channel; VR1, vanilloid receptor 1; VRL-1, vanilloid receptor-like protein 1.

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solution. The probe was labeled with [α-32-P]dCTP (Amersham) using the Rediprime DNA labeling system (Amersham), so as to obtain ~500,000 cpm of the labeled probe per ml of prehybridization solution. A quantity of 100 ng of probe was boiled for 3 min (denaturation) and then cooled on ice for 2 min in a total volume of 45 μl. This was added to the labeling tube from the kit together with 3 μl of [32P]dCTP followed by an incubation at 37°C for 30 min. A volume of 400 μl of herring sperm DNA (Sigma) at a concentration of 8 μg/ml was added to the labeled probe and heated at 99°C for 3 min followed by rapid cooling on ice. The labeled probe was added and mixed well in prehybridization solution. The membranes were hybridized overnight at 55°C. The membranes were then washed, first at room temperature in 2× SSC and 1% SDS for 5 min, followed by 2× SSC and 1% SDS for 30 min at 50°C. If necessary, further washes with 1× SSC and 0.5% SDS or 0.1× SSC and 0.1% SDS for 30 min at the same temperature were carried out. The membranes were then exposed to Scientific Imaging Film AR (Kodak) using intensifying screens at −70°C overnight, and the film was developed.

Design and Production of Anti-VRL-2 Antibodies

The peptide CDGHQQGYPRKWRTDDAPL was synthesized by standard solid-phase techniques, purified by gel-filtration chromatography and used to generate antibodies as detailed in Amaya et al. (2).

Immunocytochemical Localization

Wax sections. Lung (n = 1), trachea (n = 1), and kidney (n = 1) sections were fixed in neutral buffered formalin and processed to paraffin wax. Paraffin-embedded wax blocks were sectioned at 5 μm and underwent a microwave antigen retrieval procedure using a citrate buffer at pH 6.0 prior to immunostaining. Endogenous peroxidase was blocked with 3% hydrogen peroxide in water followed by nonspecific protein blocking in 5% milk powder in PBS. Sections were then incubated in the anti-VRL-2 antibody at room temperature for 1 h, followed by goat anti-rabbit (Biogenex) link antibody for 20 min. Finally, horseradish peroxidase-conjugated streptavidin (Biogenex) was applied to all sections and incubated for a further 20 min. Sections were visualized using 3,3'-diaminobenzidine (DAB) and counterstained briefly in Mayer's hematoxylin prior to microscopic examination.

Cryostat sections. Other human tissues tested included intestine, skin, nerve, temporal arteries, DRG, and sympathetic (stellate) ganglion. The tissues were skin from abdomen (n = 3), temporal arteries (n = 2), colon (n = 6), injured peripheral nerve (n = 3), DRG (n = 3), and sympathetic ganglion (n = 1). These tissues were collected at surgery (other than DRG and sympathetic ganglion, which were collected within 48 h postmortem), snap frozen in liquid nitrogen, and then embedded in OCT medium (RA Lamb) or immersed in 4% wt/vol paraformaldehyde in PBS overnight. After washing in PBS containing 15% wt/vol sucrose and 0.1% wt/vol sodium azide as cryopreservative, immersion-fixed sympathetic ganglia were further dissected and embedded in OCT as above. Frozen, 10-μm sections were collected onto poly-L-lysine-coated glass slides. Unfixed sections were postfixed in 4% wt/vol paraformaldehyde in PBS then washed in PBS. All tissue
sections were incubated in 0.3% vol/vol hydrogen peroxide in methanol for 25 min to block endogenous peroxidase activity, then washed in PBS. Primary antibodies were diluted from 1:50 to 1:5,000 (optimum 1:800 for postfixed and 1:100 for prefixed) in PBS containing normal goat serum (1:30), and 0.01% sodium azide was then applied to tissue sections overnight. On the second day, tissues sections were washed in PBS, and secondary biotinylated antibodies were applied for 40 min. Sites of antibody attachment were revealed using avidin-biotin-peroxidase complex (ABC, Vector Laboratories) according to the method of Shu et al. (26). Positive control preparations were immunostained with mouse anti-peripherin (Novocastra Laboratories) to label enteric neurons.

Negative control preparations for all immunolocalization studies included replacement of primary antibodies with nonimmune rabbit serum, incubation in PBS only, or preincubation of primary antibodies with the corresponding immunizing peptide prior to immunostaining.

RESULTS

cDNA Isolation

The rat VR1 amino acid sequence was initially used as a query sequence using the tBlastn alignment program to identify human genes in EST databases (13). Several human ESTs were identified, and those with similarities greater than 50% were selected for clustering to identify overlapping sequences belonging to the same transcript.

Sequencing of the EST clones and further in silico clustering enabled the generation of a 583-amino-acid single contig with 51.5% amino acid identity to rat VR1. To identify the full-length sequence, a genomic clone was isolated by PCR screening of a bacteriophage PAC genomic library. This clone was used to generate a mini-genomic library, which was

![Fig. 4. Tissue distribution of VRL-2. A: Northern blot analysis shows that VRL-2 transcripts are detected, with strongest levels present in the trachea, followed by the kidney, prostate, pancreas, and placenta. B: similarly, dot blot analysis (top; key is at bottom) detects high levels of VRL-2 in trachea, salivary gland, and kidney, with lesser amounts registered in liver, placenta, prostate, lung, fetal kidney, and spleen.](http://physiolgenomics.physiology.org)
sequenced to generate a single contig encoding the genomic sequence of this novel vanilloid-like receptor. The resulting full-length gene, which we have named human vanilloid receptor-like protein-2 (VRL-2), contains an open-reading frame (ORF) of 2,889 nucleotides that encodes a protein of 963 amino acids. The predicted transmembrane topology of this receptor is similar to that encoded by the rat VR1 gene (Fig. 1). The cytoplasmically located NH₂-terminal of VRL-2 is 565 amino acids long and possesses three ankyrin-repeat domains, one possible cAMP/cGMP-dependent protein kinase phosphorylation site, and seven potential protein kinase C phosphorylation sites. This is followed by six transmembrane spanning domains (TMs) with a potential N-linked glycosylation site and an additional short hydrophobic stretch resembling a putative pore loop region present between TM5 and TM6. The cytoplasmically located carboxyl domain consists of 152 amino acids, which is almost identical to the corresponding domain in a related family member cloned from rat kidney, denoted SIC. Interestingly, the transmembrane domains of the SIC clone are identical to rat VR1. An alignment of VRL-2 with six other members of this proposed cation channel family is displayed in Fig. 2. The sequence has been shaded according to similarity across the subunits with topology also highlighted. VRL-2 appears to be most similar to the rat SIC channel, sharing 60% amino acid identity. VR1 and VRL-1 share 46% and 43% amino acid identities with VRL-2, respectively, with the more divergent members of the family, ECaC and CaT1, both sharing 28% identity.

**Chromosome Localization**

Chromosomal assignment of human VRL-2 was carried out by PCR screening of the somatic hybrid panel from Coriell Cell Repositories and the Stanford G3 radiation hybrid panel from Research Genetics. Both panels mapped VRL-2 to chromosome 12, with the latter technique localizing it further to 12q23–24.1, closest to markers D12S177E (lod score = 15) and D12S1893 (lod score = 14), which are both located in a bipolar affective disease locus. This work was also supported by FISH analysis (Fig. 3A). The radiation hybrid panel was also used to ascertain the chromosomal localization of both human VR1 and human VRL-1. Human VR1 mapped to chromosome 17p13 located nearest to marker SHGC-36073 (lod score = 6) whereas hVRL-1 mapped to chromosome 4q25 nearest marker SHGC-10709 (lod score = 7). A summary of the chromosomal localizations and shared domains within the extended human vanilloid receptor gene family is depicted diagrammatically in Fig. 3B.

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**Fig. 5.** Western blot analysis confirms specific anti-VRL-2 antibodies for use in immunolocalization of the receptor. **A:** diagram of VRL-2, with region chosen for peptide designed to the COOH terminus marked in red. **B:** an alignment of the COOH-terminal ends of related receptors with VRL-2 reveals the degree of heterogeneity around this region. Dark-shaded regions represent highly conserved residues; the lighter the shade of the regions, the less conserved the residues become within the family. **C:** Western blot confirming that anti-VRL-2 antibodies are specific to HEK293T cells transiently transfected with VRL-2. Arrow points to a double band of 101–107 kDa. The antibody showed no cross-reaction with HEK cells alone and those transfected with human VRL-1, human VR1, or rat VR1. Corresponding samples exposed to rabbit IgG also revealed the absence of nonspecific binding.
Transcript Distribution

Multiple tissue Northern blot analysis revealed a VRL-2 transcript of ~3.7 kb with highest levels detected in the trachea and kidney, followed by the pancreas, placenta, and prostate (Fig. 4). Dot blots corroborate these findings with a high-stringency signal recorded in trachea and salivary gland followed by kidney and lower levels existing in liver, placenta, fetal kidney, prostate, and lung. We were unable to detect VRL-2 expression in human DRG tissue by RT-PCR (data not shown).

Immunolocalization of VRL-2

A peptide antibody was designed to the COOH terminal of VRL-2 to the sequence CDGHQQGYPRK-WRTDDAPL (Fig. 5, A and B). The resulting antibody was affinity purified, and its specificity to VRL-2 was confirmed by Western blotting using the cell lysates taken from VRL-2-transfected HEK293T cells (Fig. 5C). A doublet band representing possible N-linked glycosylated variants, with a molecular weight of ~101–107 kDa, is detected upon exposure to the anti-VRL-2 antibody. In wells that were loaded with VR1 and VRL-1 transfected HEK cell lysates no antibody cross-reactivity or nonspecific interactions were detected. This anti-VRL-2 antibody was used to examine the VRL-2 expression pattern at a cellular level within the kidney cortex, along the respiratory tract, skin, sweat glands, and sympathetic ganglion. Figure 6 displays various sections taken from the kidney cortex with VRL-2-specific labeling restricted to the cells making up the distal tubules (Fig. 6, A and B). Expression is also detected in neutrophils present in the kidney (Fig. 6B). Immunostaining in trachea and lung sections was localized to the epithelial linings of both trachea and lung airways (Fig. 6, C and D) as well as serous cells of submucosal glands (Fig. 6E) and mononuclear cells (Fig. 6F).

In skin specimens, fine, varicose, VRL-2-immunoreactive fibers were detected, terminating on arrector pili muscle (Fig. 7A) and surrounding sweat glands (Fig. 7B). A sympathetic ganglion showed VRL-2-immunoreactive, varicose fibers throughout, with evidence of VRL-2 immunoreactivity within some neural cell somata (Fig. 7C). Similar fibers were detected close to the adventitia in normal temporal arteries (Fig. 7D). In the intestine, VRL-2-immunoreactive fibers were detected throughout the tissue, with the greatest density in the muscle layers, but with fine caliber fibers extending through the lamina propria to the tips of the villi (Fig. 7E). Neuronal cell bodies were not immunostained with VRL-2 antibody in either submucous or myenteric plexus, although their presence was demonstrated with peripherin antibodies. Sparse VRL-2-immunore-
Active fibers were detected in nerves (Fig. 7F), but no VRL-2 immunostaining was observed in the cell bodies of sensory neurons in the DRG. VRL-2 immunoreactivity was not detected after replacement of primary antibodies with PBS or normal serum or after preincubation of antibodies with $10^{-4}$–$10^{-5}$ mg antigen/ml.

**DISCUSSION**

We have identified a novel human vanilloid-like channel, VRL-2, that has highest sequence similarity within the family to the rat SIC channel, encoding an orthologous cytoplasmic carboxyl terminus, and that is 93% identical at the amino acid level (28). Similar to VR1 and VRL-1, the NH$_2$ terminus of VRL-2 contains three postulated ankyrin repeat domains, the presence of which suggest an association with cytoskeletal proteins at the surface of the cell membrane (4, 25). The highest overall homology between VRL-2 and the expanded vanilloid-like receptor gene family is found within the six transmembrane domains, which are postulated to span the bilayer and form a cation-selective pore (7). The proposed pore loop region of the vanilloid receptor family sits between transmembrane domains V and VI and within VR1 contains residues that are important for proton-mediated potentiation of the response to capsaicin (17). Functional analysis of the VR.5sv channel, which only differs from VR1 in its NH$_2$-terminal domain, indicates that it is likely that it is this domain that is required by VR1 for activation by noxious thermal stimuli and vanilloid agonists (24). The NH$_2$-terminal sequences between receptor subunits across this family of receptors are highly heterogeneous, such that while these channels may all share the ability to act as cation channels, they vary in possessing different regulatory mechanisms which gate the opening of the channels.

Using chromosomal mapping techniques, we have localized VRL-2 to chromosome 12q23–24.1. Significant linkage data exists between this region of chromosome 12 and bipolar affective disorder (11). We also mapped the VR1 gene to chromosome 17p13, and this has been supported by the isolation of a bacterial artificial chromosome (BAC) clone (AF168787) from the same region, which contains genomic sequence encoding human VR1 (29). Sequence homologous to the carboxy terminal of the SIC channel was not found to be present on the AF168787 BAC clone. Extrapolation of these findings would suggest that the SIC channel may be the result of mRNA trans-splicing events from VR1 transcripts produced from chromosome 17 and chromosome 12.

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VRL-2 transcripts from chromosome 12. Precedents for such events are rare in mammalian systems. However, one example includes the trans-splicing of the sensory neuronal-specific sodium channel (SNS) following exposure to nerve growth factor (NGF) (1). Using RT-PCR, we were unable to detect a corresponding human SIC channel in kidney, when using primers designed to amplify a region spanning the junction between VR1 and VRL-2 sequence (primer sites displayed in Fig. 3B). RT-PCR studies in rat kidney to amplify the SIC channel have been previously noted (20). This however, was achieved using primers that spanned the VRL-2 matching sequence; thus the amplification product could represent a novel, rat VRL-2 ortholog.

We carried out a range of experiments on VRL-2 cDNA, recombinantly expressed in HEK293T cells designed to elicit its potential functional role. However, all known gating stimuli for this family of channels (i.e., exposure to vanilloid molecules, noxious heat, acid and alkaline pH) neither generated inward currents or detectable calcium influx (data not shown). Experiments exploring the response to a range of pH values have revealed activation of ECaC (15, 16) and the rat ortholog CaT-1 (22) by alkali conditions and inhibition by acidic conditions. At hyperpolarizing potentials, ECaC is maximally activated, whereas under depolarizing conditions, ECaC-mediated Ca\(^{2+}\) influx rapidly diminishes. In addition, intracellular Ca\(^{2+}\) ions appear to play a direct role in regulation of the channel (16). Similarly, the structurally related TRP family of receptors has been proposed to mediate the entry of extracellular Ca\(^{2+}\) into cells in response to depletion of intracellular Ca\(^{2+}\) stores (5). A mouse TRP-like channel protein, OTRPC4, has recently been reported that responds to changes in extracellular osmolarity (27). This OTRPC4 channel, like VRL-2, did not respond to vanilloid molecules or to high temperature. Because of the inability of VRL-2 to respond to any known vanilloid-like receptor stimuli, it is possible that the protein may act in combination with an auxiliary subunit or indeed may respond optimally when coexpressed as a heteromultimer with another member of the vanilloid receptor family. An example that supports such a hypothesis is the 5-HT\(_{3A}\) subunit, which has to associate with the 5-HT\(_{3A}\) subunit to form a functional serotonin-gated calcium channel (9, 10).

A combination of RNA dot blotting and Northern blotting experiments demonstrated that VRL-2 mRNA is most abundant in trachea, salivary gland, and kidney. We were unable to detect VRL-2 mRNA in either rat or human DRG tissue. To further investigate the localization of VRL-2, we developed polyclonal antibodies directed to the COOH terminus and without any cross-reactivity against recombinantly expressed VR1 and VRL-1 proteins. Immunohistochemical experiments demonstrated localization of VRL-2 to the apical membranes of the trachea and lung airways and to the distal tubule of the kidney. It is interesting that the distal tubule is the only part of the nephron where calcium absorption occurs exclusively through a transcellular pathway, as opposed to paracellular passage, with the latter process occurring throughout the rest of the nephron (12). Localization of this subunit to the distal tubules is also a characteristic shared by rabbit ECaC (15). As ECaC shares a 28% amino acid identity with VRL-2, it is likely that a corresponding ECaC ortholog exists in humans. In support of this suggestion, a homology search using the ECaC sequence with the EMBL database reveals the existence of a human kidney ortholog that shares 84% amino acid identity with rabbit ECaC (21).

Intriguingly, the distribution and character of VRL-2 immunoreactivity in some peripheral organs (skin, blood vessels, intestine, and nerve) suggested its presence in autonomic but not in sensory fibers. The innervation of the arrector pili smooth muscle in skin is sympathetic, with coexpression of noradrenaline and neuropeptide Y (NPY) causing piloerection (6). Postganglionic sympathetic adrenergic fibers also innervate skin blood vessels and cause vasoconstriction (3). The innervation of sweat glands is cholinergic sympathetic; these fibers co-release acetylcholine and vasoactive intestinal peptide (VIP). The pattern of VRL-2 staining in the gut suggests its presence both in sympathetic and parasympathetic fibers but not in intrinsic gut neurons.

The VR1 receptor functions as a peripheral transducer of painful thermal stimuli, which is potentiated by drops in pH, encouraging influx of calcium at reduced temperatures. The structurally related ECaC, VRL-1, and SIC receptors have functional responses that are modified by pH, high heat, and osmotic stretch, respectively, suggesting that this family of receptors may provide mammals with a defense mechanism against diverse physical stimuli. The currently unknown functional roles of VRL-2 in the kidney, airway epithelia, and nerve fibers deserve further investigation to establish whether it, too, participates in a defense mechanism or has some quite different role in regulating ion flux in these diverse tissues.

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