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REVIEW —*Current Perspective*—

Capsaicin Receptor in the Pain Pathway

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ABSTRACT—Capsaicin, the main pungent ingredient in 'hot' chili peppers, elicits burning pain by activating specific (vanilloid) receptors on sensory nerve endings. The cloned capsaicin receptor (VR1) is a nonselective cation channel with six transmembrane domains that is structurally related to a member of the TRP (transient receptor potential) channel family. VR1 is activated not only by capsaicin but also by increases in temperature that reach the noxious range (>43°C). Protons potentiate the effects of capsaicin or heat on VR1 activity by markedly decreasing the capsaicin concentration or temperature at which the channel is activated. Furthermore, a significant increase in proton concentration (pH <5.9) can evoke channel activity at room temperature. The analysis of single-channel currents in excised membrane patches suggests that capsaicin, heat or protons gate VR1 directly. VR1 can therefore be viewed as a molecular integrator of chemical and physical stimuli that elicit pain. VRL-1, a VR1 homologue, is not activated by vanilloids or protons, but can be activated by elevation in ambient temperature exceeding 52°C. These findings indicate that related ion channels may account for thermal responsiveness over a range of noxious temperature.

Keywords: Capsaicin receptor, Ion channel, Heat, Proton, Pain

Introduction

Pain is initiated upon activation of specifically tuned sensory nerves (nociceptors), most of which are small-diameter, unmyelinated C-fibers. These fibers transmit this information to the central nervous systems, leading to the sensation of pain (1, 2). Nociceptors are also characterized by their sensitivity to capsaicin (Fig. 1a), the main active ingredient of 'hot' chili peppers. Exposure of nociceptors to capsaicin not only causes excitation of these neurons but also induces release of several inflammatory mediators, suggesting that the target of capsaicin action plays an important role in pain detection and tissue inflammation (3, 4). Several lines of evidence, including the development of a competitive vanilloid antagonist (capsazepine), have suggested that capsaicin acts on a specific membrane protein to increase transmembrane (TM) cation conductance. Recently, the gene encoding the capsaicin receptor (VR1) was isolated, and the pharmacological and electrophysiological properties of the cloned receptor were examined in heterologous expression systems (5).

Cloning of capsaicin receptor gene and its basic characterization

It has been recognized for several years that capsaicin depolarizes nociceptors and increases their cytosolic free-Ca^{2+} concentration (6). This property allowed Caterina and colleagues to isolate the gene encoding the capsaicin receptor by using a Ca^{2+} imaging-based expression strategy (5). The cloned capsaicin receptor was designated vanilloid receptor subtype 1 (VR1) because a vanilloid moiety constitutes an essential chemical component of both capsaicin and its ultra potent analogue, resiniferatoxin (7). VR1 messenger RNA was detected only in sensory neurons by Northern blot analysis. Moreover, within dorsal root and trigeminal sensory ganglia, *in situ* hybridization revealed VR1 transcripts predominantly in small diamerter cell bodies, most of which are probably unmyelinated C-fibers. These findings strongly suggest that VR1 mediates the pain-producing action of capsaicin. From its deduced amino acid sequence, VR1 is predicted to have six TM domains and a short, pore-forming hydrophobic stretch between the

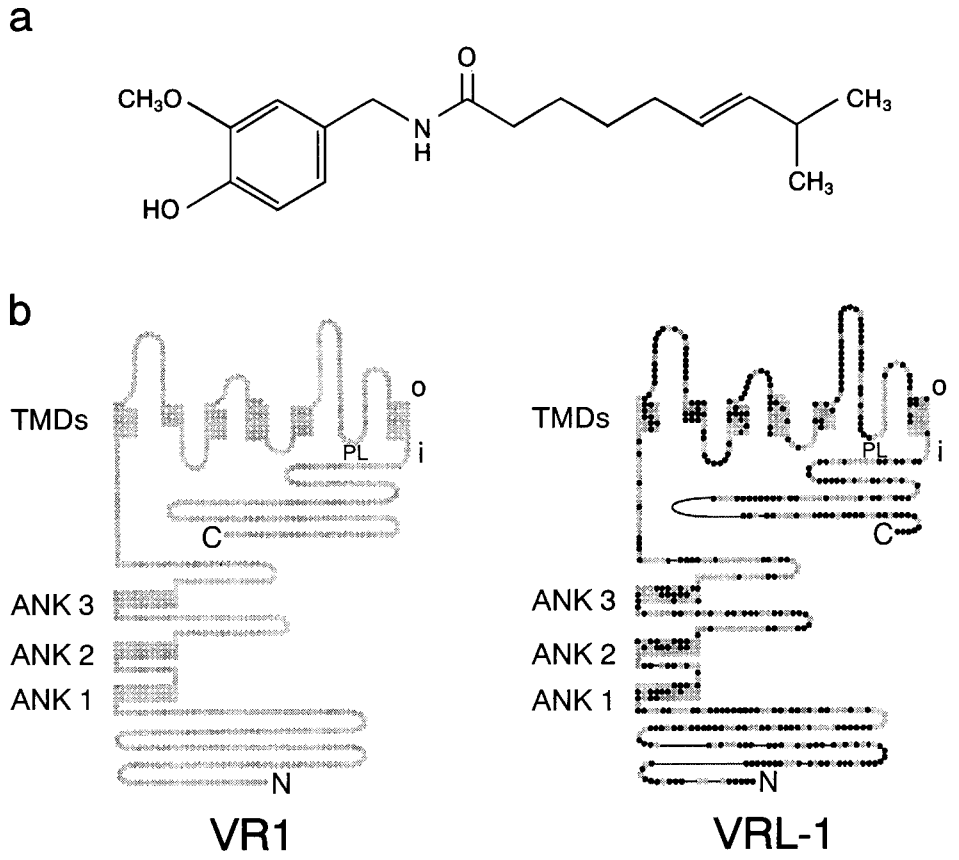

Fig. 1. Structures of capsaicin, VR1 and VRL-1. a: Structure of capsaicin: *N*-(4-hydroxy-3-methoxybenzyl)-8-methylnon-6-enamide. b: Predicted membrane topology and domain structure of VR1. Shaded symbols in VRL-1 indicate the same amino acid as in VR1. o: outside of the cell, i: inside of the cell, TMDs: transmembrane domains, ANK 1 – 3: ankyrin repeats, PL: pore loop.

fifth and sixth TM domains (Fig. 1b). A similar topological structure has been reported for many ion channels, including cyclic nucleotide-gated channels involved in phototransduction and olfactory transduction. Although VR1 exhibits little primary sequence similarity with most of these channels, it is slightly more similar (approx. 20% homology) to members of the so-called TRP (transient receptor potential) family of ion channels (see below).

A functional analysis of VR1 was carried out using two systems, patch-clamp recordings from human embryonic kidney-derived HEK293 cells transfected with VR1 cDNA and two-electrode voltage-clamp recordings in *Xenopus* oocytes injected with VR1 cRNA. At negative holding potentials, robust inward currents were observed upon application of capsaicin or resiniferatoxin in both systems. The currents were reversibly and dose-dependently inhibited by the competitive antagonist capsazepine or by the non-competitive antagonist ruthenium red. It also became clear that capsaicin-activated currents showed non-selective cation permeability with an outwardly rectifying current-voltage (I-V) relationship. A relatively high Ca^{2+} permeability ($P_{Ca}/P_{Na} = 9.6$) may be one of the reasons why the Ca^{2+}-imaging strategy was successfully applied in the cloning process. Upon application of capsaicin to membrane patches excised from HEK cells expressing VR1, clear single-channel openings were observed (conductance of approx. 77 pS for Na^+), indicating that no cytosolic second messengers are necessary for VR1 activation.

Capsaicin not only causes pain, but it also exhibits analgesic properties, particularly in the treatment of pain associated with diabetic neuropathies or rheumatoid arthritis (8). This paradoxical effect relates to the ability of capsaicin to desensitize nociceptive terminals to capsaicin, as well as to other noxious stimuli following prolonged exposure. At the molecular level, an extracellular Ca^{2+}-dependent reduction of VR1 responsiveness with continuous vanilloid exposure (electrophysiological desensitization) may partially underlie this phenomenon, although physical damage to the nerve terminal probably contributes to this effect as well.

Structural similarity to TRP family

In the invertebrate eye, light evokes membrane depolarization by activating the phosphatidylinositol signaling pathway (9, 10). One of the proteins responsible for this depolarization is TRP, a Ca^{2+}-permeable channel whose absence leads to an aberrant light-evoked photoreceptor potential (11). As described above, TRP and VR1 share an overall topology that includes six putative TM domains. Moreover, both proteins have, at their amino termini, three so-called ankyrin repeat domains that are thought to play a role in protein-protein interactions. Many TRP-related proteins have been identified in both invertebrate and vertebrate systems. At the primary sequence level, VR1 shares up to 23% sequence identity with some members of the TRP family. TRP-related molecules have recently been proposed to act as store-operated Ca^{2+} channels (SOCs) (10). These channels admit Ca^{2+} into the cell in response to depletion of intracellular Ca^{2+} stores within the endoplasmic reticulum (ER). At present, it is unclear whether or how these channels sense Ca^{2+} depletion: Is a soluble signaling molecules involved or is there a physical interaction between SOCs and ER, analogous to that between ryanodine receptors and dihydropyridine receptors of skeletal muscle? While capsaicin is an exogenous ligand for VR1, it remains possible that there exist endogenous, pain-producing, chemical regulators for this channel. If so, they might be structurally similar to unknown molecular regulators of TRP family members.

Activation of VR1 by heat

The burning quality of capsaicin-induced pain suggests that capsaicin and heat may evoke painful responses through a common molecular pathway. Therefore, the effects of elevated temperature on VR1 activity were examined in both HEK cells and oocytes (5, 12). VR1 was, in fact, found to be activated by heat at >43°C, a temperature threshold that is similar to that at which heat evokes pain in vivo (13, 14), suggesting that VR1 is involved in the detection of painful heat by primary sensory neurons. VR1-mediated, heat-evoked currents showed high Ca^{2+} permeability, an outwardly rectifying I-V relation and sensitivity to capsazepine and ruthenium red, properties similar to those of capsaicin-activated currents. Single-channel openings recorded in excised membrane patches suggested that VR1 is, itself, a heat sensor. VR1 might therefore constitute the heat-activated ion channels that have been characterized electrophysiologically in culture sensory neurons (15, 16).

Activation of VR1 by proton

Tissue acidification is induced in pathological conditions such as ischemia or inflammation (17 – 19). Such acidification exacerbates or causes pain (18, 19). In HEK 293 cells and *Xenopus* oocytes expressing VR1, currents evoked by either capsaicin or heat could be potentiated by bath acidification from pH 7.4 to 6.4 (12), despite the fact that pH 6.4 bath media alone evoked no currents in these cells. In both systems, acidification shifted the capsaicin dose-dependent curve to the left without changing the maximum response or the Hill coefficient. Acidification also shifted the temperature-response curve to the left so that the channel was activated at lower temperatures and the response was bigger at a given suprathreshold temperature. Indeed, the channels were activated by 37°C (body temperature) at pH 6.4. In fact, VR1 could be activated at room temperature when proton concentration was increased another tenfold (pH 5.4). These proton-activated currents have the same properties as capsaicin- or heat-activated currents, and single-channel openings were observed in excised membrane patches, indicating again that no second messengers are required for the activation by protons. Interestingly, protons could open the channels in outside-out configuration but not in inside-out configuration with acidified bath solution, suggesting that protons interact with extracellular regions of the receptor.

In sensory neurons, proton-evoked currents consist of two major components: one is rapidly inactivating and Na^+-selective with a linear I-V relation (20, 21); the other is a more sustained, non-selective cation conductance with an outwardly rectifying I-V profile (19, 22). The latter is believed to underlie the prolonged sensation of pain, and VR1 may represent a responsible molecular entity for this component. Indeed, proton concentrations that activate VR1 at body temperature (pH 6.4) are certainly attainable in the context of infection or ischemia.

Distribution of VR1 protein

Capsaicin sensitivity is probably the best functional marker of C-fiber nociceptors (23). Indeed, expression of VR1 protein was observed in the terminals of afferent fibers projecting to the superficial layers of the spinal cord dorsal horn and the trigeminal nucleus caudalis (12). The densest staining was found in laminae I and II in the spinal cord, to which unmyelinated C-fibers project. Dense VR1 immunoreactivity was also observed in the nucleus of the solitary tract and area postrema, which receive vagal projections from visceral organs through the nodose ganglion. These observations, plus the fact that VR1 protein is detected in nerve terminals of the bladder, indicates that VR1 is expressed in both central and peripheral termini of small-diameter sensory neurons.

Primary afferent nociceptors have been histochemically divided into two distinct classes in the adult rodent: one expresses neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP); the other expresses specific enzyme markers such as fluoride-resistant acid

phosphatase and binds the lectin IB4 (24). These two classes of neurons are sensitive to the neurotrophic factors, nerve growth factor (NGF) and glial cell-line derived neurotrophic factor (GDNF), respectively. Both subpopulations of sensory neurons respond to capsaicin and colocalization studies of VR1 with IB4 and SP probes were carried out to determine if VR1 can account for the vanilloid sensitivity of these cells. Many of SP immunoreactive cells or IB4-positive cells costained with VR1, although approx. 10% of the VR1-positive neurons stained with neither SP nor IB4. Extensive double labeling of SP and VR1 was found in lamina I and the outer portion of lamina II, whereas colocalization of dense VR1 immunoreactivity and IB4 binding was found predominantly in the medial part of the inner portion of lamina II, indicating that VR1 is expressed in both classes of nociceptors and that there is heterogeneity of nociceptors within the inner portion of lamina II.

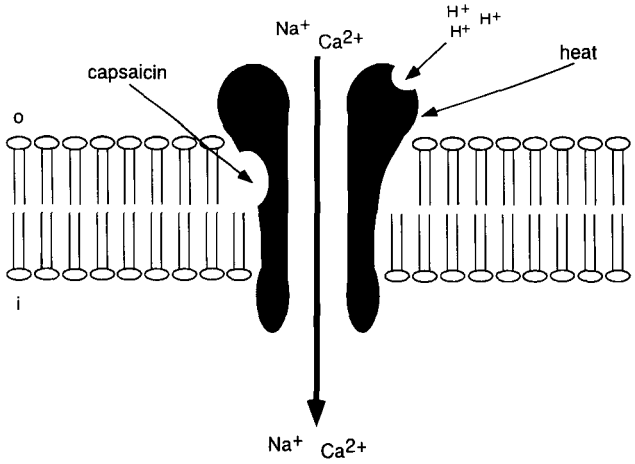

Fig. 2. Proposed model for VR1 activation by different stimuli. Capsaicin, protons or heat opens the channel leading to influx of Ca^{2+} and Na^{+} (a thick arrow). o: outside of the cell, i: inside of the cell.

VR1 as a polymodal signal detector

VR1 can be activated by three different pain-producing stimuli: capsaicin, heat (>43°C), or protons (Fig. 2). These stimuli are likely to work in concert to regulate the activity of VR1 in vivo, especially under pathological conditions where tissue acidosis and elevated temperature may come into play. Moreover, endogenous ligands for this receptor such as membrane derived lipids may exist and contribute to the modulation of channel activity in vivo (25).

It is not yet known how VR1 detects or integrates these different stimuli. However, it appears that the ability of VR1 to respond to these agonists represents an intrinsic property of the channel protein, since activation or modulation of the channel was observed in excised membrane patches and in different cellular environments.

Cloning of a VR1 homologue, VRL-1

A protein with 49% identity to VR1 was recently cloned and designated vanilloid-receptor-like protein 1 (VRL-1) (26) (Fig. 1b). VRL-1 was not activated by vanilloids, protons or moderate thermal stimuli, but could be activated by high temperatures with a threshold of approx. 52°C. VRL-1 current properties were similar to those of VR1, such as an outwardly rectifying I-V relation at positive potentials, inhibition by ruthenium red and relatively high Ca^{2+} permeability.

Aδ mechano- and heat-sensitive (AMH) neurons are medium- to large-diameter, lightly myelinated neurons that fall into two groups: type I AMHs have a heat threshold of around 53°C, and type II AMHs are activated at 43°C (27, 28). Intense VRL-1 immunoreactivity was observed in medium to large diameter cells (approx. 29 μm) in dorsal root ganglion (DRG) neurons. Very few of the VRL-1-positive cells stained with IB4 or SP antibody. However, about one-third of the VRL-1-positive cells costained with antibody against CGRP. Many of VRL-1 immunoreactive cells in the primary cultures of rat DRG stained with the anti-neurofilament antibody RT97, a marker for myelinated neurons. Together with the observation that the most intense VRL-1 staining in the spinal cord was observed within the marginal zone, lamina I and lamina II/III border, these data suggest that intense VRL-1 expression might account for the high thermal threshold ascribed to type I AMH nociceptors (26).

VRL-1 transcripts were found not only in sensory neurons but also in many non-neuronal tissues where they are unlikely to be exposed to a temperature above 50°C. VRL-1 may be regulated by other physiological stimuli in those tissues. Interestingly, it has recently been reported that the mouse Ca^{2+}-permeable cation channel induced by insulin-like growth factor-I showed 79% identity to rat VRL-1, suggesting that the channel is a mouse VRL-1 orthologue (29).

Conclusion

VR1 is the first candidate receptor molecule for detecting the pain-producing signals at sensory nerve endings. Other channels and receptors clearly participate in the detection of painful chemical and physical stimuli by the nociceptor. Gene cloning and gene knockout studies will help to elucidate the contribution of these molecules to pain generation in vivo.

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