There is mounting evidence that the vanilloid (capsaicin) receptor, transient receptor potential channel, vanilloid subfamily member 1 (TRPV1), is subjected to multiple interacting levels of control. The first level is by reversible phosphorylation catalyzed by intrinsic kinases (e.g. protein kinase A and C) and phosphatases (e.g. calcineurin), which plays a pivotal role in receptor sensitization vs. tachyphylaxis. In addition, this mechanism links TRPV1 to intracellular signaling by various important endogenous as well as exogenous substances such as bradykinin, ethanol, nicotin and insulin. It is not clear, however, whether phosphorylation per se is sufficient to liberate TRPV1 under the inhibitory control of phosphatidylinositol-4,5-bisphosphate. The second level of control is by forming TRPV1 heteromers and their association with putative regulatory proteins. The next level of regulation is by subcellular compartmentalization. The membrane form of TRPV1 functions as a nonselective cation channel. On the endoplasmic reticulum, TRPV1 is present in two differentially regulated forms, one of which is inositol triphosphate-dependent whereas the other is not. These three TRPV1 compartments provide a versatile regulation of intracellular Ca\(^{2+}\) levels. Last, there is a complex and poorly understood regulation of TRPV1 activity via control of gene expression. Factors that down-regulate TRPV1 expression include vanilloid treatment and growth factor (notably, nerve growth factor) deprivation. By contrast, TRPV1 appears to be upregulated during inflammatory conditions. Interestingly, following experimental nerve injury and in animal models of diabetic neuropathy TRPV1 is present on neurons that do not normally express TRPV1. Combined, these findings imply an important role for aberrant TRPV1 expression in the development of neuropathic pain and hyperalgesia. In humans, disease-related changes in TRPV1 expression have already been described (e.g. inflammatory bowel disease and irritable bowel syndrome). The mechanisms that regulate TRPV1 gene expression under pathological conditions are unknown but a better understanding of these pathways has obvious implications for rational drug development.

Introduction

Originally described as a receptor for capsaicin and related natural irritants (collectively referred to as vanilloids) on primary sensory neurons, the vanilloid receptor; transient receptor potential channel, vanilloid subfamily member 1 (TRPV1) is now believed to function as a molecular integrator of noxious stimuli (Fig. 1), including heat, acids, pollutants with negative electric charge and endogenous pro-inflammatory substances [1]. In the central nervous system, TRPV1 may be a target for anandamide and related compounds such as N-arachidonoyldopamine (NADA) but the biological consequence of this interaction is unclear [2]. As discussed below, TRPV1 is also expressed in non-neuronal tissues. As in neurons, activation of TRPV1 in these cells results in a rapid increase in intracellular Ca\(^{2+}\) levels. The endogenous ligand(s) that bind to and activate TRPV1 in non-neuronal cells, however, remain to be identified.

The broad and still expanding tissue distribution of TRPV1 was unexpected and remains perplexing. Rats given capsaicin as neonates, a procedure that ablates capsaicin-sensitive neurons, may develop skin ulcers and have megalobladder but are otherwise apparently normal [1]. In accord, mice with homogenous deletion of TRPV1 (\(-/-\)) develop normally and show only the expected changes such as diminished heat response [3] and perturbed micturition [4]. Clearly, rodents can live apparently normal lives without their vanilloid receptors. With several pharmaceutical companies developing high affinity orally active TRPV1 antagonists, the question is gaining utmost practical importance as to what degree the benign phenotype of not having functional vanilloid receptors can be extrapolated from experimental animals to humans.

A further complicating factor of vanilloid pharmacology is the recent recognition that TRPV1 is expressed not only in the cell membrane where it functions as a nonselective cation
channel but also on endoplasmic reticulum [5,6]. Thus, TRPV1 ligands that can pass the cell membrane may have a pharmacological activity different from those who cannot.

Once considered an oddity, TRPV1 is now subject to intense studies and our knowledge of TRPV1 pharmacology and regulation is exponentially expanding. A Medline search shows that the number of papers dealing with the pharmacology of the vanilloid receptor VR1 (TRPV1) has grown from 21 in 1998, the year following the cloning of VR1, to 191 in 2003. Admittedly incomplete, this mini-review highlights only major trends in vanilloid research with particular emphasis on the past year.

**Molecular structure and expression of the capsaicin/vanilloid receptor**

The molecular mediator of vanilloid effects was not described until 1997 when Caterina and coworkers cloned a cDNA termed vanilloid receptor 1 (VR1 now called TRPV1) [7]. TRPV1 is a member of the transient receptor potential (TRP) channel family, which is typified by a predicted six transmembrane domain with intracellular N- and C-termini and a relatively conserved pore domain (Fig. 1). It is likely that functional TRP channels exist as homomeric or heteromeric complexes with four subunits [8,9]; TRPV1 may also be associated with putative regulatory proteins. Of note, the TRPV1 homomer itself appears to contain multiple capsaicin binding domains [10].

The mammalian TRP channel family can be divided into four subfamilies: TRPC (C for classical or canonical), TRPV (V for vanilloid), TRPM (M for melastatin related) and TRPP (PKD-type). The TRPV family currently consists of six members, TRPV1–6. TRPV2 (46%), TRPV3 (43%) and TRPV4 (43%) are the most similar to TRPV1 at the amino acid level. It needs, however, to be emphasized that TRPV1 is the only family member that is activated by vanilloids like capsaicin and its ultrapotent analog, resiniferatoxin (RTX), and thus deserves the name vanilloid receptor. In the brain of TRPV1–/– mice, [3H]RTX binding sites are markedly reduced but not eliminated, implying the existence of other, as yet unidentified, vanilloid receptors [11].

The tissue distribution of TRPV genes is diverse. TRPV1 RNA is expressed at relatively high levels in dorsal root ganglia (DRG) relative to its other sites of expression [12]. In rat, TRPV1 is also found in spinal cord (primarily in sensory efferent fibers), various brain regions such as hypothalamus, hippocampus and substantia nigra, kidney, and urinary bladder [12]. In the brain, TRPV1 RNA is localized to a small number of discrete cells in certain brain regions [13]. For example, TRPV1 colocalizes with a subset of tyrosine hydroxylase neurons in the substantia nigra [13], implying that these neurons are dopaminergic. This hypothesis is consistent with the strong antihyperkinetic activity of capsaicin in 3-nitropropionic acid-lesioned rats, a model of Huntington’s disease [14].

**Fig. 1. TRPV1 is an integrator of inflammatory pain pathways.** The figure shows a schematic of TRPV1 and its activators. TRPV1 is directly activated by a multiplicity of stimuli including vanilloids, such as capsaicin, low pH, elevated temperature, and arachidonic acid metabolites such as anandamide. TRPV1 is indirectly activated by prostaglandin and bradykinin receptors via protein kinase activity. As noted in the text, several amino acids are important for activation of TRPV1 by different mediators. These are shown as filled circles. Red circles correspond to residues important for capsaicin activation, green circles for low pH and blue circles for protein kinase phosphorylation sites.
Within the DRG, TRPV1 is localized mostly in small to medium sized neurons that are neurofilament 200 negative: TRPV1-like immunoreactivity is found in unmyelinated and some thinly myelinated fibers, consistent with the hypothesis that TRPV1 is primarily expressed in nociceptors [15]. TRPV1 expression appears to colocalize to varying extents with Trk A, the nerve growth factor receptor, Trk B, the glial-derived nerve growth factor receptor, P2X3, the lectin IB4 and with neurotrophins involved in nociceptive transmission, calcitonin gene-related peptide and substance P [16,17]. TRPV1-like immunoreactivity is detected in fibers that project primarily to lamina I and II of the spinal cord dorsal horn, although some staining has been observed in the spinal trigeminal nucleus and nucleus of the solitary tract [17]. Organs innervated by fibers with TRPV1-like immunoreactivity include the bladder [18], prostate [19], lung [20], gastrointestinal tract [21] and dental pulp [22], consistent with the distribution of capsaicin-sensitive pathways [1].

Unexpectedly, TRPV1 expression is not restricted to neuronal tissues. At present, notable non-neuronal tissues expressing TRPV1 are the keratinocytes of the epidermis [23], bladder urothelium and smooth muscle [24], liver [25], polymorphonuclear granulocytes [26] and macrophages [27]. This list, however, is expected to grow as scientists continue to challenge the old dogma of TRPV1 expression being a signature of sensory neurons. As in neurons, activation of TRPV1 in non-neuronal tissues results in a rapid rise in intracellular Ca\(^{2+}\) levels. In macrophages, capsaicin downregulates the expression of iNOS and COX-2 genes, which might contribute to the anti-inflammatory action of this drug [27]. The physiological roles of TRPV1 in non-neuronal tissues, however, remain enigmatic.

The expression of TRPV1 in DRG and its ability to mediate pain responses to vanilloids suggested that TRPV1 expression might be altered in models of acute and chronic pain in rats. TRPV1 RNA expression in ipsilateral lumbar DRG in rats given intraplantar carrageenan (4–24 h) or complete Freund’s adjuvant (1–7 d) does not change compared to noninjected controls [12,28]. In contrast, increased TRPV1-like immunoreactivity in rat DRG neurons after inflammation challenge was described [29] and another report suggested that TRPV1 RNA levels increase in sensory afferent and efferent fibers in rats injected with carrageenan [30]. Moreover, increased TRPV1-like immunoreactivity is observed in nerve fiber endings in paws from rats receiving intraplantar carrageenan compared to controls [31]. In a sensory nerve axotomy model, TRPV1 RNA levels decreased in affected DRG [16]. However, in a unilateral L5 spinal nerve ligation model, TRPV1-like immunoreactivity increased in uninjured DRG somata, particularly in A fibers [32]. Moreover, TRPV1 is expressed de novo in A fibers after partial sciatic nerve ligation [33]. Increased numbers of TRPV1-like immunoreactive fibers have been observed in rectal biopsy samples from patients with rectal hypersensitivity and fecal urgency [34] and in the colon of patients with active inflammatory bowel disease [35]. These data suggest that increased TRPV1 expression may be associated with certain types of pathophysologies that result in pain and support the therapeutic potential of TRPV1 antagonists.

Most recently, the TRP channel ANKTM1, originally identified as a cold receptor, was shown to be expressed on capsaicin-sensitive neurons where it is activated by both mustard oil and cannabinoids [36]. This is interesting because these neurons also carry TRPV1 and cannabinoid CB\(_1\) receptors that are also targets for cannabinoids [37]. The physiological implications of this ‘ligand promiscuity’ are unclear.

**In vitro functional analysis of TRPV1**

The cDNA encoding TRPV1 was isolated based on the channel’s sensitivity to capsaicin and the ability of capsaicin to activated calcium influx in DRG neurons [7]. Indeed, the pharmacology of capsaicin, RTX and other vanilloids at recombinantly expressed TRPV1 is comparable to similar studies performed in DRG preparations [38]. TRPV1 is sensitive to low pH solutions and high physiological temperatures [7,39]. Interestingly, low pH potentiates the effects of capsaicin on TRPV1 and lowers the minimum threshold for temperature activation of TRPV1 [39]. TRPV1 is also sensitive to certain arachidonic acid metabolites, including anandamide [2], NADA [40], N-oleoyldopamine (OLDA) [41] and 12-HPETE [42].

Protein kinases impact TRPV1 channel activity. As reviewed recently [43], protein kinase C (PKC) activators potentiate capsaicin-induced activation of TRPV1 in a mechanism that involves direct phosphorylation of the TRPV1 protein and hydrolysis of phosphatidylinositol-4,5-bisphosphate, a negative regulator of TRPV1 channel activity. Activation of intracellular PKC activity results in increased TRPV1 sensitivity to capsaicin, low pH and heat [43]. Studies of cultured DRG cells have implicated PKC alpha as the isoform most important for PKC effects on TRPV1 in vivo [44]. The cyclic AMP dependent protein kinase, protein kinase A, also directly phosphorylates TRPV1 resulting in decreased desensitization and increased sensitivity to anandamide [43]. Recent work suggests that TRPV1 phosphorylation by CAM kinase II may be necessary for capsaicin-mediated activation of TRPV1 [45]. Increased protein kinase activity not only results in TRPV1 activation in the presence of physiological temperature and pH, but is likely to play a role in the activation/sensitization of TRPV1 currents by nerve growth factor and bradykinin [46]. By contrast, dephosphorylation by calmodulin of TRPV1 in response to elevating Ca\(^{2+}\) was shown to inhibit channel activity [47]. This negative feedback may be a major mechanism of desensitization by capsaicin.

Reversible phosphorylation by intrinsic kinases may also link TRPV1 to the anesthetic property of two important substances of use and abuse, ethanol [48] and nicotine [49].

**Molecular pharmacology of TRPV1**

The amino acids necessary for TRPV1 activation by various mediators are beginning to be appreciated. The effects of low pH require acidic residues located in extracellular loops near the pore. Mutation of residues D601, E610 or E648 to neutral or basic residues resulted in reduced low pH-activated currents relative to wild-type TRPV1 [50]. In contrast, mutation of E600 to neutral or basic residues affected an increased sensitivity to vanilloids and higher temperatures [50]. Low pH appears to have several effects
on TRPV1: it increases open probability, in part by stabilizing an open conformation of the channel and it can increase the apparent affinity of capsaicin [51]. Hence, by promoting a more activated state, low pH may induce a conformational change in TRPV1 that is more favorable for interaction with other activators such as anandamide or 12-HPETE.

Targets of protein kinase activity on TRPV1 have also been identified. Serine residues 502 and 800 have been shown to be activated by PKC [52]. Similar experiments demonstrate S116 as a target for protein kinase A [53]. Importantly, phorbol esters such as phorbol 12-myristate 13-acetate can activate TRPV1 directly; that is, in the absence of PKC activity. This hypothesis is supported by the finding that mutation of T704 to alanine results in a loss of direct activation of TRPV1 by phorbol 12-myristate 13-acetate but has no effect on phosphorylation-induced enhancement of the TRPV1 function [54].

Vanilloid sensitivity of TRPV1 has been traced to several amino acids between transmembrane domains 2 and 4. These observations were deduced, in part, from the amino acid sequence of chicken TRPV1, which is insensitive to vanilloids [55]. Two amino acids located in the loop between transmembrane domains 2 and 3, Y511 and S512, may play particularly key roles in mediating the effect of capsaicin on TRPV1 activity. Mutation of these residues to tyrosine or alanine, respectively, results in TRPV1 channels that are still sensitive to pH but not to capsaicin [55].

Other studies have suggested that the cytoplasmic tail is involved not only in functional activation by capsaicin and low pH, but also in the phosphorylation-mediated inhibition of TRPV1 [56,57]. Amino acids in transmembrane domain 6 (N676, M677 and L678) substantially alter TRPV1 responses to capsaicin and low pH [58], and mutation of tyrosine 671 to lysine results in a loss of calcium-dependent desensitization of TRPV1, and also impairs calcium permeability [59].

Concluding remarks

Advances of the past six years have transformed the vanilloid (capsaicin) receptor VR1 from a ‘signature of a specific primary sensory neuron subset’ to TRPV1 that functions as a molecular integrator of diverse noxious stimuli (Fig. 1), both chemical and physical. TRPV1 is not an orphan receptor anymore: at present, putative stimuli (Fig. 1), both chemical and physical. TRPV1 is not a function in mice lacking the vanilloid receptor TRPV1. The wide tissue distribution of TRPV1 from keratinocytes of the skin [23] to smooth muscle of the bladder [24] to liver [25] to hematopoietic cells [26,27] to epithelial cells lining human airways [63] is puzzling and the ligands that operate TRPV1 in these cells remain to be identified. In human airways, activation by airborne particulate matter of TRPV1 is thought to induce apoptosis, implying a protective potential for inhaled vanilloid receptor antagonists [63]. In the central nervous system, TRPV1 has attracted recent interest as a target for the endocannabinoid anandamide [2] and related compounds such as NADA [40] and OLDA [41]. Despite intensive research, the function of TRPV1-expressing brain neurons remains poorly understood.

Furthermore, TRPV1 is an important target for drug development. The observation that TRPV1 –/– mice exhibit reduced hyperalgesia compared to wild-type mice in inflammatory pain models [3] supports the hypothesis that TRPV1 antagonists may be useful analgesics in humans. Although the apparently benign phenotype of TRPV1 –/– mice is encouraging with regard to the safety of TRPV1 antagonists, our limited understanding of vanilloid-sensitive pathways in cells other than primary sensory neurons warrants caution during the upcoming clinical trials.

In summary, recent findings indicate a previously unsuspected level of complexity in TRPV1 regulation. TRPV1 is emerging as a ubiquitously expressed receptor involved in intracellular signaling as an integrator of exogenous and endogenous signals.

Acknowledgements

We thank Marianne Buck for preparing the figure.

References


