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Pain TRPs

Minireview

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Transient receptor potential (TRP) ion channels are molecular gateways in sensory systems, an interface between the environment and the nervous system. Several TRPs transduce thermal, chemical, and mechanical stimuli into inward currents, an essential first step for eliciting thermal and pain sensations. Precise regulation of the expression, localization, and function of the TRP channels is crucial for their sensory role in nociceptor terminals, particularly after inflammation, when they contribute to pain hypersensitivity by undergoing changes in translation and trafficking as well as diverse posttranslational modifications.

Organisms are bombarded with stimuli from their external and internal environments. These include temperature, exposure to photons and protons, and mechanical deformations as well as contact with a diverse range of intrinsic and extrinsic chemicals. Sophisticated sensory systems are essential for detecting environmental changes. A particular requirement is to differentiate potentially harmful from innocuous stimuli. Intense tissue-damaging, or "noxious," stimuli generate a highly unpleasant sensation—pain—which minimizes contact with the injurious stimulus, a key protective response. Inflammation or tissue injury then heightens pain sensitivity so that contact with the wounded body part is limited until healing occurs.

Multiple molecules have been adapted over the course of evolution to act as sensory transducers, each specialized to respond only to particular aspects of the environment, and initiate—directly or indirectly—action potentials in sensory neurons. Collectively, these transducers encode the quality, intensity, duration, and temporal properties of sensory stimuli contributing to the exquisite specificity and differential sensitivity of our sensory experience. Of these, TRP ion channels have emerged as a major sensory transducer family, with many members involved specifically in generating chemically and thermally evoked pain sensations.

Multiple TRP genes have been identified, including 28 in the human genome, and almost all are nonselective cation channels with six transmembrane domains. Based upon their sequence homology, TRPs are classified into six subfamilies. Many TRPs are expressed in the nervous system, particularly in high-threshold nociceptor sensory neurons, where their thermosensory and chemosensory roles are key. TRPV1, TRPV2, TRPV3, TRPV4, and TRPM8 are thermoreceptors (see Table 1); the role of another channel, TRPA1, as a cold pain receptor remains disputed (Bandell et al., 2004; Jordt et al., 2004). Between them, these TRPs are exquisitely tuned to cover a range of temperature sensitivities that encompass cold and hot pain as well as innocuous cold and warmth. A further remarkable characteristic of many TRP channels is their chemosensitivity, e.g., TRPV1's sensitivity to capsaicin (the pungent ingredient in chili peppers), protons, and endocannabinoids; TRPM8's sensitivity to menthol; TRPA1's sensitivity to mustard (Jordt et al., 2004) and cinnamon oil (Bandell et al., 2004); and TRPV3's sensitivity to camphor (Moqrich et al., 2005). For TRPV1, TRPV3, and TRPM8, the sensations elicited by capsaicin, camphor, and menthol correspond to their thermal transduction properties, hot and cool, while TRPA1, a putative cold receptor, generates a burning sensation when activated by mustard oil or wasabi.

TRPV3 and TRPV4, two "innocuous" warm detectors, are highly expressed in keratinocytes as well as in primary sensory dorsal root ganglion (DRG) neurons, and as a result, keratinocytes can detect changes in temperature in the warm range (Chung et al., 2004; Moqrich et al., 2005). These skin cells may release ATP in response to heat activation, which by binding to purinergic ion channel receptors on the peripheral terminals of sensory neurons, could drive sensory inflow in the nervous system. This model expands the sensory apparatus from the nervous system to the skin!

How do noxious temperatures, low or high, activate TRPM8 and TRPV1? It turns out that temperature sensing is closely related to the voltage-dependent gating properties of both channels; changes in temperature produce shifts in their voltage-dependent activation curves (Voets et al., 2004). Interestingly, menthol and capsaicin work on TRPM8 and TRPV1 as gating modifiers, shifting their activation curves toward the resting membrane potential (Voets et al., 2004).

A sensory role for the TRPs in mammals beyond thermo- and chemodetection has been revealed recently: mechanotransduction. TRPA1 appears to be responsible for mechanotransduction in vertebrate hair cells, contributing to hearing and equilibrium (Corey et al., 2004). It is most unlikely to have a chemodetection or a role in cold pain here. This finding raises the possibility that TRPs may be mechanodetectors in the vertebrate somatosensory system. Although the degenerin-homologous family of acid-sensing ion channels (ASICs) has been suggested to be cutaneous mechanotransducers, ASIC knockout studies (ASIC 2 and 3) (Roza et al., 2004) do not show loss of mechanosensitivity. In zebrafish and *Drosophila*, a TRP channel, NompC, is essential for mechanosensation (Walker et al., 2000), and another TRP channel, *painless*, with high homology to TRPA1, is involved in mechanical nociception in *Drosophila* (Tracey et al., 2003). Is TRPA1 also a mechanotransducer in high-threshold mammalian nociceptor sensory neurons, responsible for pinch and pinprick?

In spite of initial expectations that TRPV1 was the

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Table 1. Mammalian Sensory TRPs

Channel Name	Major Tissue Distribution	Sensory Modality	Regulatory Mechanism
TRPV Subfamily			
TRPV1	DRG, trigeminal ganglia (TG), urinary bladder	T $\geq$ 43°C, acid, capsaicin, resiniferatoxin, phorbol ester, N-arachidonyl dopamine, arachidonic acid metabolites, endocannabinoids, 2-aminoethoxydiphenyl borate (2-APB)	(+) PKA, PKC, PI3K, p38, Src, PLC, PLA_2/lipoxygenase, CaMKII, BK, NGF, PGE_2, ATP, ethanol, nicotine, acid, 2-APB
			(–) PIP_2, calmodulin, calcineurin, adenosine
TRPV2	DRG, spinal cord (SC), brain, spleen, intestine	T $\geq$ 52°C, 2-APB	(+) translocation (by IGF-1)
TRPV3	DRG, TG, SC, brain, keratinocytes, tongue	T $\geq$ 30°C–39°C, 2-APB, camphor	(+) 2-APB, camphor
TRPV4	DRG, TG, brain, keratinocytes, kidney, lung, spleen, testis, endothelium, liver, heart, inner-ear hair cells	T $\geq$ 25°C, hypotonicity, noxious mechanical stimulus, acid, phorbol ester, endocannabinoids, arachidonic acid metabolites	(+) PLA2/cytochrome P450, Src, PGE_2
TRPM Subfamily			
TRPM5	taste tissue, small intestine, liver, lung,	taste (sweet, bitter, umami)	(+) $PLC_\beta 2$, intracellular Ca^{++}, PIP_2
TRPM8	DRG, TG, prostate	T $\leq$ 23°C–28°C, menthol, icilin, PIP_2	(+) PIP_2
			(–) intracellular acidification, 2-APB
TRPA Subfamily			
TRPA1	DRG, fibroblasts, hair cells	T $\leq$ 18°C, icilin, cannabinoids, mustard oil, BK, cinnamaldehyde, mechanical stimulus (hair cells)	(+) PLC_β
TRPC Subfamily			
TRPC2 (pseudogene in human)	Vomeronasal organ, testis, spleen, liver, heart, brain	pheromone (mouse only)	(+) DAG

heat pain transducer, TRPV1 knockout mice have minimal defects in noxious heat responses (Woodbury et al., 2004). The TRPV1 null mutants do show, however, a very substantial reduction in inflammatory pain hypersensitivity (Caterina et al., 2000). A major role of this and other TRP channels may therefore be to heighten pain sensitivity in response to tissue injury. How do they do this?

Two major mechanisms contribute to inflammatory pain. One is the phenomenon of peripheral sensitization, involving a reduction in the threshold and an increase in the responsiveness of the peripheral terminals of nociceptors; the other is central sensitization, an augmentation of synaptic transmission in the spinal cord. TRPV1 has a major role in producing peripheral sensitization, acting as the final substrate for multiple inflammatory mediators that operate via distinct intracellular signaling pathways to reduce the heat pain threshold from ~42°C to close to body temperature. This is responsible for the pain experienced in response to a normally warm stimulus, as in a shower after a too-long day at the beach. The contribution of other TRPs to peripheral sensitization remains to be explored.

Changes in the transduction sensitivity and threshold of nociceptors can occur by regulating the number, distribution, and activity of their transducers. Thus, pain sensitivity is potentially influenced by regulation of TRP genes, their messenger RNAs and protein products, as well as multimer organization. Given the coexpression of many TRP channels in nociceptors, there are opportunities for heteromultimerization, but whether this occurs and what functional consequences ensue are not clear. Splice variants can control the activity of the channels by influencing their tetrameric composition. In mouse dorsal root ganglia, there are two alternatively spliced products of TRPV1: $TRPV1_\alpha$ and $TRPV1_\beta$. Although $TRPV1_\beta$ does not function alone, it reduces $TRPV1_\alpha$ activity, acting as dominant-negative channel (Wang et al., 2004). Similar alternative splicing occurs for TRPM1, leading to long and short isoforms. The TRPM1 short isoform cannot form functional ion channels due to lack of transmembrane domains, but when hereologously expressed, it blocks the trafficking of the functional long isoform from the cytoplasm to the membrane. Do TRP splice variants contribute to changes in sensory responsiveness?

TRPV1 levels decrease substantially in injured nociceptor neurons after peripheral axonal injury, but they increase in neighboring noninjured neurons. This includes novel expression in large low-threshold A fiber neurons, a phenotype shift that may contribute to neuropathic pain (Hudson et al., 2001). An increase in TRPV1 expression despite unchanged mRNA levels occurs in primary sensory neurons after peripheral inflammation and requires retrograde transport of nerve growth factor (NGF) and activation of p38 mitogen-acti-

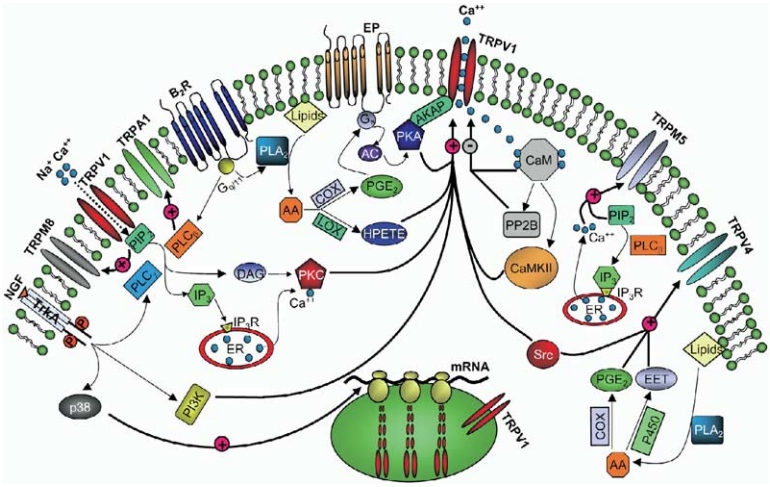

Figure 1. Scheme of the Major Signaling Pathways that Regulate TRP Ion Channels

(+) represents sensitization or activation; (–) represents desensitization. See text for details.

vated protein kinase (MAPK) (Ji et al., 2002). Moreover, protein kinase C (PKC) activation induces rapid delivery of TRPV1 channels to the cell membrane, contributing to the sensitizing effect of this kinase on TRPV1 (Morenilla-Palao et al., 2004). Increases in the trafficking of TRPV1 to the periphery contribute to inflammatory pain hypersensitivity (Ji et al., 2002).

In the early phase of inflammation, increased pain sensitivity originates largely as a result of the local release from inflammatory cells of a number of mediators. Most of these inflammatory mediators do not directly activate nociceptors, but rather act as sensitizers, reducing the threshold of the peripheral nociceptor terminals. Among the major inflammatory mediators are prostanoids, particularly prostaglandin E_2 (PGE_2), bradykinin, and NGF. These chemicals acting through EP prostaglandin and B_1/B_2 bradykinin G protein-coupled receptors and the high-affinity TrkA NGF receptor produce their immediate effects on pain hypersensitivity locally on the nociceptor terminals by phosphorylating TRPV1 as well as the sensory neuron-specific voltage-gated sodium channel Na_v 1.8. Activation of the protease-activated receptor 2 (PAR2) by inflammatory proteases like trypsin has a similar effect. Phosphorylation and dephosphorylation substantially alter TRPV1 ion channel function, and this represents a major means of rapidly and dynamically altering pain sensitivity (Figure 1).

Although NGF directly sensitizes nociceptors by increasing TRPV1 activity, it is not yet clear exactly how it does this. Activation, via TrkA, of phospholipase C_γ (PLC_γ) hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2). PIP_2 serves as a tonic TRPV1 channel inhibitor, and its hydrolysis releases this inhibition (Chuang et al., 2001). Hydrolysis of PIP_2 also releases inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), which activate PKC, leading to TRPV1 phosphorylation. NGF may also potentiate TRPV1 by activating PI3K (Zhuang et al., 2004) either via calmodulin-dependent kinase II (CaMKII), PKC, or ERK (Zhuang et al., 2004). In one study, neither ERK nor PKC but rather PKA coupled NGF to TRPV1 (Shu and Mendell, 2001).

Bradykinin (BK) may, like NGF, release the inhibition of TRPV1 by PIP_2 as well as activate PKC to phosphorylate TRPV1 and in this way sensitize the receptor (Sugiura et al., 2002). The B_2 receptor also couples to phospholipase A_2 (PLA_2), which produces arachidonic acid (AA), which can be converted to 12-hydroperoxyeicosatetraenoic acid (12-HPETE) by 12-lipoxygenase (LOX). HPETE activates TRPV1 (Shin et al., 2002). Inhibition of PLC, PKC, PLA_2, and lipoxygenase reduce peripheral BK-induced nociception (Ferreira et al., 2004). BK also activates TRPA1 in a PLC-dependent manner (Bandell et al., 2004).

PGE_2, another product of arachidonic acid, is catalyzed by cyclooxygenase (COX), the COX-2 isomer of which is highly induced in inflammatory cells. PGE_2 sensitizes TRPV1 via PKA (Hu et al., 2002) and also sensitizes TRPV4. Inhibition of COX-2 at the site of peripheral inflammation is likely to be one of the means by which COX-2 inhibitors produce analgesia, although a central action contributes as well. Epoxyeicosatrienoic acids (EET), arachidonic acid metabolites converted by cytochrome P450 (P450), directly activate TRPV4 (Watanabe et al., 2003).

Diverse studies on different inflammatory mediators report multiple and sometimes contradictory effects on TRPs that occur via different signaling pathways. Although experimental factors may contribute, the apparent diversity in observed effects may be real. Specificity in vivo might rely on a highly-ordered subcellular localization of the TRPs in membrane microdomains in which all components of a particular signaling process are physically confined to a spatially restricted subcompartment of the cell. The physical proximity of the elements of the microdomain is maintained by submembrane scaffolding proteins, preventing signals from diffusing and ensuring a specific outcome to a particular ligand exposed at a particular time to a particular site. Given the multiplicity of pathways that act on TRPV1 to potentiate or desensitize the channel, microdomains may retain order while enabling complexity and flexibility. A kinase anchoring protein (AKAP), an anchoring molecule critical for formation of microdomains, is required for the potentiation of TRPV1 by PKA (Rathee et al., 2002). In addition, in superior

cervical ganglion neurons, the microdomain formed by the B_2 receptor, G proteins, PLC_β, and the IP_3 receptor, along with TRPC1, is delimited by an actin scaffold that supports the IP_3 receptor in a position close to the plasma membrane, allowing specific signaling from the B_2 receptor to TRPC1 and opening of the channel (Delmas et al., 2002).

TRPV1 can be both sensitized and desensitized. Sensitized TRPV1 contributes to heat pain hypersensitivity. However, prolonged application of capsaicin induces a desensitization of TRPV1 that leads to analgesia, and topical capsaicin is used to treat patients with postherpetic neuralgia. TRPV1 desensitization is calcium dependent and may be mediated by calmodulin (CaM), which directly interacts with calmodulin binding sites present on several TRPs (Lambers et al., 2004). However, while one group finds that calmodulin alters open channel probability to promote desensitization and that calmodulin mutants inhibit desensitization (Rosenbaum et al., 2004), another reports that both calmodulin inhibitors and loss-of-function mutants fail to prevent desensitization (Numazaki et al., 2003).

Is sensitization and desensitization of TRPV1 a functional reflection of the balance between phosphorylation and dephosphorylation? Phosphorylation of TRPV1 by CaMKII appears to be critical for its sensitization, and dephosphorylation by calcineurin (phosphatase 2B or PP2B, a calcium-dependent protein phosphatase) appears to be critical for its desensitization (Jung et al., 2004). However, since the calcium/calmodulin complex activates both CaMKII and calcineurin, how does it drive two completely opposite effects? Future work is needed to illuminate whether it is a matter of timing, calcium concentration, or different calcium binding sites that determines facilitation or inactivation of TRPV1.

Conclusions

Progress in TRP sensory biology has been phenomenal since David Julius and Michael Caterina cloned the capsaicin receptor (then known as VR1) and recognized that it was a TRP channel (TRPV1). TRPs clearly play a prime role as molecular sensors for an increasingly varied and complex set of sensory stimuli. Many questions remain, though. Is the reduction in TRPV1 thermal threshold sufficient to drive spontaneous activity in nociceptors in response to body temperature and thereby drive spontaneous pain? Will TRPV1 receptor antagonists be clinically useful analgesics? What is the role of TRPV2, whose temperature threshold (>50°C) is higher than any sensory neuron is ever likely to see? Are any of the TRPs the missing pinch pain detectors? How exactly are channel activities fine-tuned to ensure accurate sensing of a changing environment? Sensory biologists, clinicians, patients, and even gourmands all eagerly await further developments in this exciting pain trip.

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