Painful, hot, and toxic secrets of TRP channels

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Abstract
The ability to sense pain, temperature, and natural toxins is required for organismal survival. Prior to our involvement, the underlying neuroanatomical pathways of these senses were understood, but the molecular basis of signaling was unknown. My lab has discovered four sensory receptors in the same transient receptor potential (TRP) family of ion channels that we have found mediates these diverse stimuli. The first channel is TRPV1, which is activated by capsaicin and temperatures exceeding 43°C. On the other hand, the VRL-1 channel is activated by noxious heat exceeding 50°C. The third channel is TRPM8, which contains at least two binding sites activated by menthol and temperatures below 26°C. Finally, TRPA1 is activated by menthol, noxious cold below 20°C, and a large variety of pungent irritants. Interestingly, both TRPV1 and TRPM8 have separate binding sites for temperature and chemical stimuli and produce stronger currents when activated simultaneously by both stimuli. Despite these advancements, future research is required to discover how these signals are transduced to the brain and to develop effective antagonists for natural toxins that bind some of these receptors.

Introduction

Senses connect organisms to the external, physical environment and deliver internal feedback about the world around them. This feedback increases the likelihood of survival. Sensory stimuli excite sensory nerve fibers projecting from the dorsal root ganglia (DRG), where primary afferent neurons convert the stimuli into action potentials and relay sensory information to the central nervous system. The retina allows organisms to see, the cochlea allows organisms to hear, specialized epithelia allow organisms to gustation and olfaction, and the somatosensory system allows organisms to sense touch, temperature, and pain.

This ability to sense pain, temperature, and natural toxins is crucial. Without it, organisms would not be alerted of injury and appropriate protective responses to avoid these injurious stimuli would not be elicited. Sensory disorders include allodynia, where patients experience touch hypersensitivity, and congenital insensitivity to pain with anhidrosis (CIPA), where patients cannot sense pain or temperature. Anatomical pathways involving pain, heat, and natural toxins were well characterized prior to our involvement; however, molecular signaling underlying these pathways remained unknown.

Electrical signaling in neurons requires plasma membranes to maintain concentration gradients for specific ions to experience selective changes in ion permeability. Membrane proteins called ion channels give rise to these selective changes in permeability. Transient receptor potential (TRP) family of ion channels function as store-operated calcium channels and were presumed to be involved in sensory transduction as early as 1989. TRPC was cloned in *Drosophila melanogaster* and found to have an integral photoreceptor response to light.

Our lab began with the question of how capsaicin, the main pungent ingredient in “hot” chili peppers, activates nociceptors, neurons that transmit information regarding tissue damage to pain-processing centers in the brain and spinal

cord7. We hypothesized that capsaicin operated through a receptor mechanism as opposed to direct perturbation of membrane lipids. We found that capsaicin activates TRPV1, a member of the TRP family that is involved in chemosensation and thermosensation. This led to the characterization of three additional receptors including VRL-1, TRPM8, and TRPA1. Natural toxins and venoms also activate some of these receptors and evidence from our lab suggests that these receptors respond to various evolutionary pressures. In addition, mechanisms of TRP channel activation and potentiation have been characterized through our studies.

Fantastic Four

Our lab has contributed to the understanding of four main TRP channels involved in the sensation of pain, temperature, and natural toxins. Mechanisms of activation are described (Fig. 1).

TRPV1: Feel the Burn

Previous studies showed that exposing nociceptor terminals to capsaicin yields neuronal excitation, perception of a “burning sensation,” and local release of inflammatory mediators; however, molecular mechanisms through which capsaicin activates nociceptors remained unclear. We identified a capsaicin receptor through a cDNA library of dorsal root ganglion (DRG) cells, which we named TRPV1, and further characterized this receptor. HEK293 cells transfected with pools of clones from a rodent DRG library were exposed to fluorescent calcium imaging before and during capsaicin injection to measure the amount of calcium influx. HEK293 cells transfected within a certain pool exhibited increases in cytoplasmic calcium, and this pool was subdivided and reassayed until a single clone was isolated. This method was repeated in the characterization of future channels. We named this clone VR1 but later renamed it TRPV1. We found that TRPV1 expression is restricted to sensory neurons in the DRG. Interestingly, TRPV1 is not only activated by capsaicin, but a peak temperature of 45°C. TRPV1 is also activated by decreases in pH from 7.6 to 6.3.

Next, we characterized TRPV1 knock out mice. Using calcium imaging as a functional readout, we found that TRPV1-/- mice do not exhibit calcium influx post-injection of capsaicin, capsaicin agonists, or pH. In addition, TRPV1-/- mice exhibit impaired behavioral and physiological responses to capsaicin in that they ingest water with capsaicin, whereas the control (TRPV1+/+) does not. Primary afferent fibers from TRPV1-deficient mice also demonstrate normal mechanical but reduced thermal nociceptive properties, demonstrating that this receptor does not contribute to mechanosensation, but contributes largely to thermosensation.

Venoms and toxins also activate TRPV1.

Venom from the tarantula, Psalmopoeus cambridgei, contains inhibitor cysteine knot (ICK) peptides that target TRPV1. These venoms function as channel agonists, contrary to previous studies that have attested to their dominant role as channel antagonists. This finding allows for future insights to channel gating9. Furthermore, a peptide toxin from the Earth Tiger tarantula irreversibly activates TRPV1. The double-knot toxin (DkTx) permanently activates TRPV1 by interacting with residues in the pore-forming region of the receptor. This demonstrates the significance of conformational changes in the outer pore region of TRPV1.

VRL-1: Raising the Heat

After TRPV1 characterization, we hypothesized that there were more channels involved in the detection of noxious stimuli. We found that VRL-1 has a similar cDNA sequence to TRPV1; however, VRL-1 is not activated by capsaicin, acid, or moderate heat. Instead, VRL-1 responds to high temperatures exceeding 43°C with a threshold of 53°C. Rat DRG sections stained with anti-TRPV1 and anti-VRL-1 demonstrate that VRL-1 is expressed in a subset of medium to large diameter sensory neurons11. VRL-1 mRNA expression in rat tissues is found in the DRG, spleen, lung, spinal cord, brain, large intestine, and small intestine. This distribution suggests that VRL-1 responds to additional sensory stimuli, other than heat, since it is unlikely that these organs experience noxious heat.

TRPM8: Principal Cold Detector

Menthol was known to evoke a cold sensation, but the molecular mechanisms involved in cold sensation were unknown prior to our involvement. Similar to the paradigm presented by capsaicin, we used the plant chemical, menthol, to elicit a cooling sensation. Through expression cloning and calcium imaging, we identified an excitatory ion channel and named it CMR1, which was later termed TRPM8. We found that TRPM8 is expressed by small diameter neurons of the DRG and trigeminal ganglia. The channel is not only activated by menthol, but also icilin, eucalyptol, and cool to cold temperatures ranging from 8 to 28°C. This finding introduced the implication that TRP channels are capable of detecting stimuli over a broad temperature range.

Soon after characterization of TRPM8, studies from other labs suggested that a favorable blend of excitatory ion channels might have been more critical than TRPM8 to cold transduction, and other studies questioned the contribution of TRPM8 as a whole. We solved this controversy through demonstrating that TRPM8-deficient mice exhibit decreased sensitivity to menthol and cold. TRPM8 deficient mice also lose the ability to discriminate between hot and cold surfaces; however, they are still successful in avoiding contact with temperatures below 10°C. Taken together, our studies indicate that TRPM8 is the principal detector of cold.

TRPA1: Noxious Cold and Pungent Irritants

Outside studies brought a fourth member of the TRP family into the forefront, called ANKTM1. This channel was later renamed TRPA1. It is implicated in the detection of noxious cold. In situ hybridization and immunostaining analyses showed TRPA1 expression in a subset of TRPV1 positive DRG neurons. TRPA1 is similar to TRPM8 in that it is also activated by icilin; however, TRPA1 is activated by noxious cold below 17°C. Our lab found that mustard oil activates TRPA1. Other pungent irritants that activate TRPA1 include capsaicin, garlic, allicin, wasabi, horseradish, and THC, the active ingredient in marijuana. As a result of these findings, TRP channels can also be considered ionotropic cannabinoid receptors. To conclude, our lab has helped characterize four TRP channels involved in the sensation of pain, temperature, natural toxins, and venoms (Fig. 1).

Mechanistic Properties

Our lab has characterized the mechanistic properties of most of the channels described above. Inflammatory responses and sensitivity have been characterized in detail (Fig. 2).

TRPV1 Potentiation, Inflammation, & Hypersensitivity

We asked what effect protons had on the TRPV1 receptor. We found that protons potentiate capsaicin and heat activated TRPV1 currents. Protons lower the threshold and increase the strength of heat-evoked currents in TRPV1-expressing cells. Temperature-response curves in oocytes and HEK293 cells showed this same result. This finding presents a role for TRPV1 in injury-induced hypersensitivity at the level of the sensory neuron. We also found that an extracellular Glu reside, called E600, is a key regulatory site of TRPV1 through setting sensitivity to other noxious stimuli in response to changes in pH or extracellular proton concentration and that another site, called E648, eliminates proton-evoked TRPV1 activation.

Our lab discovered that bradykinin (BK) and nerve growth factor (NGF) sensitize the TRPV1 channel. A stronger influx of calcium occurs when BK and capsaicin are applied together than when capsaicin is applied alone. We also found that phospholipase C (PLC) by itself produces strong TRPV1 activation similar to that produced by BK and NGF. In addition, BK and NGF release TRPV1 from PtdIns(4,5)P2-mediated inhibition through hydrolysis after PLC activation. TRPV1, NGF receptor TrkA, and PLC-γ form a signaling complex, as demonstrated through HEK293 cells transfected with cDNAs where coimmunoprecipitation was visualized through western blot analysis. Then we found a site of TRPV1 that is required for PIP2-mediated inhibition. Mutations that weaken PIP2-TRPV1 interaction reduce thresholds for sensory stimuli thereby increasing sensitivity, whereas TRPV1 channels in which this region is replaced with a lipid-binding domain from PIP2-activated potassium channels, a domain such as IRK, remain inhibited by PIP2 (Fig. 2).

TRPA1 Inflammation & Hypersensitivity

Using TRPA1deficient mice, we show that this channel is the sole target through which mustard oil and garlic activate primary afferent nociceptors to produce inflammatory pain. TRPA1 is targeted by acrolein, an environmental irritant that yields toxic and inflammatory actions. TRPA1deficient mice exhibit normal cold sensitivity and auditory function, which suggests that this channel is not required for the initial detection of noxious cold or sound, although it is implicated in both. However, TRPA1deficient mice exhibit deficits in BK-evoked excitation and pain hypersensitivity. TRPA1 is activated by 4-hydroxy-2-nonenal (HNE), which is produced in response to tissue injury. HNE provokes release of substance P and calcitonin gene-related peptide (CGRP). TRPA1 activation by HNE promotes pain, neuropeptide release, and neurogenic inflammation (Fig. 2). Taken together, we have shown that TRPA1 plays a key role in the inflammatory response.

The formalin test has been used to test the consequences of analgesics, or pain relief drugs, in laboratory animals. Injecting formalin into the hind paw of a mouse elicits a two phase pain response: the first results from activation of sensory neurons and the second results from sensitization. We asked how formalin induces this two phase response and we found that TRPA1 plays an important role in this test. Formalin induces calcium influx in cells expressing TRPA1. In addition, we developed a TRPA1 antagonist, HC-030031, and its application eliminates calcium influx in response to formalin. This finding implicates TRPA1 as integral to pain hypersensitivity.

Next, we aimed to understand the functional role that ankyrin repeats play in TRP channels, specifically TRPA1. TRPA1 is characterized as having a large amount of these

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ankyrin repeats. Chimeric channels allow pharmacological properties to be transplanted across closely related species and offer an answer to this question. Using chimeras across rattlesnakes and humans, we found that ankyrin repeat regions of TRPA1 contain thermal and chemical sensitivity sites at two independent sites. This suggests that ankyrin repeats in TRPA1 regulate channel gating and excitation of nerve fibers.

TRPM8 Assembly & Potentiation

It was thought that TRP channels function as tetramers, but little was known about TRP assembly. Our lab discovered that a coiled coil domain is necessary for TRPM8 assembly. The coiled coil can also act as a dominant inhibitor of channel expression. Furthermore, it was known that TRPM8 exposure to menthol or cold exhibits rapid activation; however, TRPM8 exposure to icilin elicits variable latency. We asked why icilin has this increased latency. We found that icilin requires a simultaneous increase of cytosolic calcium ions. Therefore, two stimuli must be paired together in a sort of coincidence detection in order to experience maximal channel activation. Moreover, the icilin binding site on TRPM8 maps to a region that corresponds to the capsaicin binding site on TRPV1. This implies that evolution has selected for the conservation of this site across both receptors.

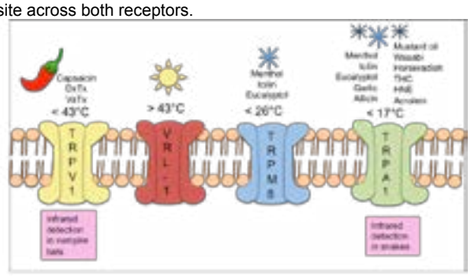

Figure 1: TRP Channels Involved in the Detection of Pain, Temperature, and Natural Toxins: TRPV1 is activated by capsaicin, spider toxins DxTx and VaTx, temperatures below 43C, and required for infrared sensation in vampire bats; VRL-1 is activated by noxious heat above 43C; TRPM8 is activated by menthol, icilin, eucalyptol, temperatures below 26C; TRPA1 is activated by menthol, icilin, eucalyptol, garlic extracts, allicin, wasabi, horseradish, THC, HNE, environmental toxin acrolein, noxious cold below 17C, and required for infrared sensation in snakes.

MitTx & 5-HT3R

We found that a Texas coral snake toxin, called MitTx, targets acid-sensing ion channels to produce pain. Whole-cell patch-clamp recordings showed that this toxin complex yields currents in a subset of trigeminal sensory neurons. We found that the molecular target expressed by this subset of sensory neurons were members of the ASIC family. We expressed ASIC members in *Xenopus* oocytes and measured responses to the toxin. We discovered that the toxin activates both ASIC and TRPV1-expressing oocytes. These findings reveal a surprising contribution of ASIC channels to nociception.

Serotonin is a neurotransmitter belonging to the inflammatory chemical milieu and it was known to be released at the site of tissue damage. It interacts with different receptors; among these receptors is 5-HT3R. We wanted to better understand the relationship between this receptor and inflammation. Using a 5-HT3R knock out mouse model, our lab measured response latencies to a 52.5°C hot-plate. Responses were similar between the mutant and the wild type, indicating that a 5-HT3R does not affect the acute pain response. We achieved similar results when testing the phase 1 period of formalin-induced licking time in the knock out mouse model; there is no significant difference between the wild type and 5-HT3R deficient mice. However, the phase 2 period is different. Phase 2, which is defined as 11-60 minutes after formalin induction, sees a significant decrease in licking time of the 5-HT3R deficient mice. This, along with similar phenotypic tests, indicates that 5-HT3R is involved in the second, more persistent, phase of pain behavior. Furthermore, we found that 5-HT3R deficiencies do not affect edema induced by injury. We also found that subsets of DRG neurons contain 5-HT3R mRNA and that this receptor is found in myelinated Aδ and unmyelinated C fibers. Aδ fibers produce an acute sensation of sharp bursts of pain; C fibers produce slow, burning, and long lasting pain. These two types of fibers had not been characterized prior to this point.

Evolutionary Component

Homeotherms & Poikilotherms

Next, we asked whether warm and cold-blooded, homeotherms and poikilotherms respectively, animals sense temperature differently via TRP channels. We compared TRPM8 in frogs and mammals or birds and found that frogs have a lower thermal activation than rats, while birds have a slightly higher thermal activation than rats. This is due to the temperature range that these species are exposed to on a regular basis. For instance, the chicken is known to have a higher core body temperature than rats and other mammals. To conclude on this point, there is an obvious correlation between body or skin temperature and thermal response range of the TRPM8 channel. Interestingly, varying frog species produced the same temperature range, which demonstrates that cold sensitivity is unique to each type of organism. We also found that activation by icilin appears to be specific to mammalian organisms, where frogs and birds are unresponsive to this chemical; however, frogs, mammals, and birds are all responsive to menthol. These findings suggest that TRP channels have adapted to meet the demands of the particular environment or ecological niche of an organism.

Infrared Sensation in Snakes & Vampire Bats

Snakes use infrared sensation to detect warm-blooded prey and also to avoid predators and regulate thermal behavior. Infrared antenna convey signals regarding infrared sensation through what is called the pit organ, which sits on the sides of a snake's nostrils, and sends the information to the optic tectum of the brain and allows for sensory perception. Sensitivities of the pit organ vary among venomous and nonvenomous species. We aimed to unravel the molecular mechanisms through which the pit organ detects this infrared stimulus. We hypothesized that the pit organ detects infrared signals through thermotransduction. We used a method of transcriptome profiling to find pit-enriched sensory transducers, leading to the discovery that TRPA1 was a candidate detector of infrared sensation. This was surprising since in mammals, TRPA1 is a detector of chemical irritants and inflammatory agents; whereas, in the pit organ of snakes, TRPV1 is an ultra sensitive heat receptor. This study suggests that TRPA1, and potentially other TRP ion channels as well, maintain the capacity to detect different stimuli and that this difference is dependent on the organism or region of the body. This implies that these adaptations have evolved to promote traits that increase organismal survival.

Similarly, vampire bats have leaf pits that are also located near the nostrils. We observed trigeminal ganglia (TG) specialization in these bats that is similar to snakes in that their size distribution was larger in the TG than the average size of other mammals. We found that TRPV1 of vampire bats are activated by a lower threshold of 30°C and that this is the receptor that yields infrared sensation in this organism. We used this finding on vampire bats to support the phylogenetic relationship between bats, horses, dogs, cows, moles, and dolphins as opposed to bats fitting into the same category as humans, monkeys, flying lemurs, mice, rats, and rabbits. These combined findings on infrared sensation of snakes and vampire bats further illustrate the impact of evolution on TRP channels in vertebrates.

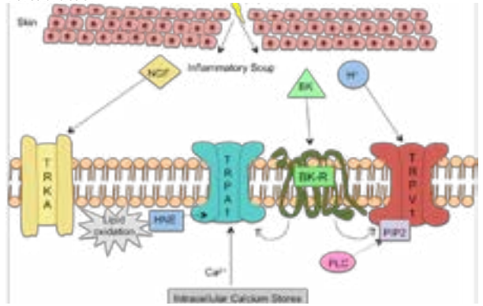

Figure 2: Inflammatory Mechanisms Involving TRPV1, TRPA1, and PIP2 Signaling Pathway: Tissue injury produces an inflammatory soup consisting of nerve growth factor (NGF), bradykinin (BK), and H+ that bind TrkA, BK-R, and TRPV1, respectively. Phospholipase C activation yields PIP2 inhibition and increased sensitivity of TRPV1. Lipid oxidation activates (HNE), which promotes pain, neuropeptide release, and neurogenic inflammation.

TRPV1 & Chickens

It was well established that capsaicin activates what is even nicknamed the "capsaicin receptor," TRPV1. But it was unclear why birds do not experience the "burning sensation" characteristic of capsaicin and can serve as vectors for seed dispersal that includes this pungent ingredient. We found that the chicken TRPV1 can detect noxious heat and exhibits thermal hypersensitivity similar to the rat TRPV1, but does not respond to capsaicin. This explains the bird's indifference to capsaicin and provides insight into the design of bird food. Bird food may contain capsaicin so as to prevent squirrels and other organisms from digesting it instead. This design would ensure that seed dispersal is carried out effectively. Since birds do not respond to capsaicin, findings suggest that capsaicin detection is a more recent development of mammalian vanilloid receptors.

Conclusion

Our lab has deepened the understanding of multiple processes involving somatosensation. Prior to our involvement, the molecular signaling behind the detection of pain, heat, and natural toxins remained unknown. It was unknown that four main TRP channels contribute to the detection and transduction of these senses. We have identified and characterized TRPV1, VRL-1, TRPM8, and TRPA1 in regards to channel activation, as well as various mechanistic properties including gating, assembly, potentiation, inflammation, and sensitization. We have shown that these TRP channels are responsive to evolutionary pressures and contain properties specific to their mode of temperature regulation, their reliance on infrared radiation, or their need to participate in seed dispersal. Properties of TRP channels are also maintained across species, such as the icilin and capsaicin binding sites to TRPM8 and TRPV1, respectively. Despite these advancements, the distribution of nociceptive information is complex. Aspects of pain and temperature pharmacology remain imperfectly understood, and this continues to make nociception an active area of research1. Although we have identified the receptors involved in these specific forms of sensation, future studies are required to unravel intracellular signaling of nociceptors. Furthermore, since we know that some detrimental toxins and venoms activate TRP channels, it is now possible to consider ways in which to design antagonists that inhibit these harmful stimuli.

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