Anesthesiology 2010; 112:729–41

Role of Transient Receptor Potential and Acid-sensing Ion Channels in Peripheral Inflammatory Pain

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ABSTRACT

Pain originating in inflammation is the most common pathologic pain condition encountered by the anesthesiologist whether in the context of surgery, its aftermath, or in the practice of pain medicine. Inflammatory agents, released as components of the body's response to peripheral tissue damage or disease, are now known to be collectively capable of activating transient receptor potential vanilloid type 1, transient receptor potential vanilloid type 4, transient receptor potential ankyrin type 1, and acid-sensing ion channels, whereas individual agents may activate only certain of these ion channels. These ionotropic receptors serve many physiologic functions—as, indeed, do many of the inflammagens released in the inflammatory process. Here, we introduce the reader to the role of these ionotropic receptors in mediating peripheral pain in response to inflammation.

INFLAMMATORY pain describes the pain that is generated by the inflammatory response resulting from wounds, surgical incisions, burn injury, arthritis, infarction, infection, allergic reactions, autoimmune diseases, tumor growth, and other forms of tissue injury or disease. Inflammation results in the generation of a plethora of chemical agents that are intended to fight infection and assist in the repair of injured tissue. Unfortunately, the body's inflammatory response to injury, or disease, is ill controlled and is often disproportionate, resulting in pain that is sometimes of such severity that it may hamper recovery or, in the longer term, result in disability. Inflammation commonly results in one, or more, of the three readily recognizable pathologic pain conditions, namely *hyperalgesia* in which an excessive sensation of pain is elicited by a mild noxious stimulus, such as heat (*thermal hyperalgesia*) or mechanical pressure (*mechanical hyperalgesia*); *allodynia* in which pain is elicited by a harmless nonnoxious stimulus; and *spontaneous pain* in which pain is evoked without any precipitating external stimulus. In cases of severe inflammation, these conditions can inhibit necessary active treatment of the tissue damage. In other cases, the inflammation may not subside, or the pain may persist, notwithstanding the fact that its initiating stimulus has abated, leading to a chronic pain condition.

Inflammatory type pain is of immediate concern to anesthesiologists, because it is an inevitable concomitant of every form of open surgery, the only variables residing in its severity and duration from case to case. Pain of inflammatory origin will also dominate the practice of those anesthesiologists whose specialty is in pain medicine because the overwhelming majority of pathologic pain cases have an inflammatory context of origin. The importance of identifying the role of peripheral mechanisms involved in mediating these persistent inflammatory pain conditions resides in the opportunities that such knowledge will provide for facilitating therapeutic interventions to ameliorate these conditions. The mechanisms of nociceptive processing become ever more complex as the signaling, which will ultimately be interpreted as pain by the brain, is conveyed from the nerve terminals of primary afferents in the spinal dorsal horn onward toward the brain. Therefore, the most successful therapeutic interventions are more likely to arise from developing our understanding of the peripheral mechanisms of inflammatory pain.

Inflammation results in the release of a variety of agents that contribute to alter both the firing pattern of nociceptive primary sensory neurons and nociceptive processing in spinal dorsal horn nociceptive neurons. These include bradykinin, eicosanoids, nerve growth factor (NGF), artemin, glial cell-line-derived neurotrophic factor (GDNF), serotonin, hista-

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Received from the Section of Anaesthetics, Pain Medicine and Intensive Care, Department of Surgery and Cancer, Imperial College London, Faculty of Medicine, Chelsea and Westminster Hospital, London, United Kingdom. Submitted for publication November 14, 2008. Accepted for publication October 9, 2009. Support for this work was provided solely from institutional and/or departmental sources.

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Table 1. Inflammagens as Activators of Iontropic and Their Cognate (Own) Receptors in Peripheral Inflammation

Inflammatory Agent	Direct or Indirect Activation				Activation by Ligand-Binding Cognate (Own) Receptors
	TRPV1	TRPA1	TRPV4	ASICs	
Bradykinin	Y	Y	—	Y	$B_{1–2}$
PGE2	Y	—	Y	—	$EP_{1–4}$
PGI2	Y	—	—	—	IP
NGF	Y	—	—	Y	trkA
Artemin, neurturin, GDNF	Y	—	—	—	$GFRalpha_{1–4}$
Histamine	Y	—	—	—	$H_{1–4}$
Anandamide	Y	—	—	—	$CB_{1–2,}$ GPR55
Protons and cations	Y (pH < 6.5)	—	Y	Y	ASICs
LTB4	—	—	—	—	$BLT_{1–2}$
5-HT	—	—	—	Y	$5\text{-}HT_{1A\text{ and }2A,}5\text{-}HT_3$
ATP	Y	—	—	—	$P2X_2$, $P2X_3$, $P2X_{2/3}$

ASIC = acid-sensing ion channel; ATP = adenosine triphosphate; CB = cannabinoid; GDNF = glial cell-line-derived neurotrophic factor; 5-HT = 5-hydroxytryptamine; LBT4 = leukotriene 4; NGF = nerve growth factor; PGE_2 = prostaglandin E_2; PGI_2 = prostacyclin; TRPs = transient receptor potential ion channels; TRPA1 = transient receptor potential ankyrin type 1 ion channel; TRPV1 = transient receptor potential vanilloid type 1 ion channel; TRPV4 = transient receptor potential vanilloid type 4 ion channel; Y = yes; — = not known.

mine, anandamide, adenosine 5′triphosphate, and cations, especially protons. The so-called inflammatory soup constituted by these inflammagens has been a focus of research for many years and, recently, it has been shown that neuronal excitation (leading to nociception) in the context of inflammation may be reduced by elimination of certain of the complex of inflammatory mediators that would otherwise be active.[1] These inflammatory agents act directly on their cognate (own) receptors found on primary afferents (in which case they are specific ligands) to contribute to excitation of these neurons. In addition, certain of these inflammatory agents also activate ion channels (ionotropic receptors) found on these neurons or affect the sensitivity and expression of these ion channels (table 1). Certain voltage-gated sodium channels, including Nav1.9,[1–3] Nav1.8,[4,5] and Nav1.7,[6] are known to have a role in mediating the effects of inflammatory agents. Here, we focus on the growing evidence relating to the involvement of the ionotropic receptors' transient receptor potential vanilloid type 1 ion channel (TRPV1), transient receptor potential ankyrin type 1 ion channel (TRPA1), transient receptor potential vanilloid type 4 ion channel (TRPV4), and acid-sensing ion channels (ASICs) in mediating inflammagen-induced nociceptive signaling. These ion channels constitute an important component of the mechanism whereby inflammatory agents excite primary afferents to result in peripheral pain conditions. Their importance in anesthesiology is severalfold. First, the identification of the role of these ion channels in mediating peripheral inflammatory pain offers the prospect of manipulating these ion channels to reduce inflammatory pain sensations. TRPV1 activation is an essential feature of the inflammation generated in both the complete Freund's adjuvant and carrigeenan animal models of inflammatory pain.[7–9] However, inflammatory pain induced by formalin injection of the animal's hind paw is exclusively mediated by TRPA1.[10] Second, ionotropic channels, by their nature, have a defined mechanism of action and, hence, are potentially important targets for therapeutic drug intervention (table 2). In addition, recent important *in vitro* studies have demonstrated that certain anesthetic gases—as well as intravenous anesthetics such as propofol—may themselves have a role in mediating peripheral inflammatory pain sensation through an action on TRPV1 or TRPA1 receptors.

The subjects of this review have been widely researched during the last decade and a vast literature has been developed. Here, we introduce the reader to the seminal components of that research, which are essential to the understanding of the mechanisms of inflammatory pain.

TRPV1 as a Mediator of Inflammatory Thermal Hyperalgesia and Spontaneous Pain Sensation

The TRPV1 ion channel is a ligand-gated, nonselective, cationic channel with a high permeability for Ca^{2+} (fig. 1).[11–17]

Table 2. TRPV1, TRPA1, TRPV4, and ASICs as Ionotropic Receptors

Principal Features
- Ligand-gated ion channels
- Have central aqueous pore
- Channel-gated on ligand-binding
- Gating results in ionic flux via pore
- For fast synaptic transmission
- Examples: TRPs, ASICs, $5\text{-}HT_3$

Possible inhibiting mechanisms
- Competitive antagonism[143,144]
- Conduction blocker of channel pore[145,146]
- Binding in channel pore to induce failure of "gating"[147]

ASIC = acid-sensing ion channel; $5\text{-}HT_3$ = 5-hydroxytryptamine receptor type 3; TRPs = transient receptor potential ion channels; TRPA1 = transient receptor potential ankyrin type 1 ion channel; TRPV1 = transient receptor potential vanilloid type 1 ion channel; TRPV4 = transient receptor potential vanilloid type 4 ion channel.

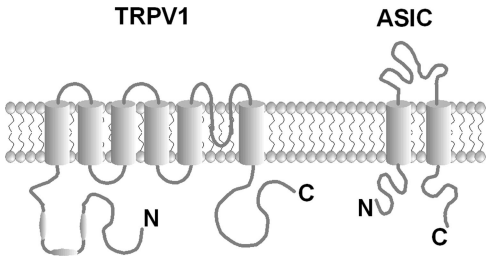

Fig. 1. Predicted membrane structure of TRPV1 and ASIC ion channels. Similar to the other members of the TRP family, TRPV1 has six transmembrane domains. Both the N- and C-termini are intracellular. The hydrophobic loop connecting transmembrane domains five and six is believed to be part of the channel. In contrast, ASIC ion channels have only two transmembrane domains. Both the N- and C-termini are again intracellular. ASIC = acid-sensing ion channel; C = C terminus; N = N terminus; TRPV1 = transient receptor potential vanilloid type 1 ion channel.

Table 3. Known Activators of TRPV1, TRPA1, TRPV4, and ASICs in the Absence of Inflammation

Activator	TRPV1	TRPA1	TRPV4	ASICs
Heat	Y	N	N	—
Cold	N	Y	—	—
Depolarisation	Y	—	—	—
Hypotonicity	N	N	Y	N
Hypertonicity	N	N	Y	N
Capsaicin (and other vanilloids)	Y	N	N	N
Mustard oil	N	Y	—	—
Garlic	N	Y	—	—
Protons and cations	Y (pH < 6.5)	—	Y	Y

ASIC = acid-sensing ion channel; N = no; TRPs = transient receptor potential ion channels; TRPA1 = transient receptor potential ankyrin type 1 ion channel; TRPV1 = transient receptor potential vanilloid type 1 ion channel; TRPV4 = transient receptor potential vanilloid type 4 ion channel; Y = yes; — = not known.

Such cation-selective ligand-gated ion channels produce, on activation, a net inward current that depolarizes the neuronal membrane and increases the probability of action potential generation. About 40% of the total neuronal population of primary sensory neurons express TRPV1. TRPV1 is expressed in the perikarya, as well as in both the central and peripheral terminals, of primary sensory neurons; it also has a wide-spread distribution in both peripheral tissues and in the central nervous system.[18–20]

Heat hyperalgesia secondary to inflammatory tissue injury induced by mustard oil, Complete Freund's adjuvant, or carrigeenan fails to develop in TRPV1 null mice to the same extent as in wild-type mice. On the other hand, pathologic thermal sensations after peripheral nerve injury constituted by partial sciatic nerve ligation remain the same in both wild-type and TRPV1 null animals.[7,8] These findings led to the suggestion that TRPV1 is an important component in the development of pathologic thermal hyperalgesia resulting from inflammation in certain contexts but not from nerve injury as such. TRPV1 is responsive to a variety of activators. Ligands (such as capsaicin and related vanilloids), heat, protons, and depolarization induce TRPV1 opening directly[11,21,22] (table 3). Gating of this ion channel can also be evoked indirectly. These indirect activators include various inflammatory mediators, which are produced and released during inflammation in tissues. These agents, through activating their own target receptors, which are also expressed on the nociceptive primary sensory neuron expressing TRPV1, induce activity in the intracellular second messenger system of the neuron. This, in turn, results in posttranslational modification of TRPV1 in a process called "sensitization," which increases the responsiveness of TRPV1, and also results in increased expression of TRPV1. The development of thermal hyperalgesia after inflammation involves an increased level of TRPV1 expression[23] as well as the sensitization of existing TRPV1 channels. Levels of both NGF and GDNF increase after inflammation and contribute to inflammatory thermal hyperalgesia *via* an increase in TRPV1 expression. The increases in the levels of NGF and GDNF, respectively, follow different time courses and they act on distinct populations of dorsal root ganglion neurons.[24] Inflammatory agents, such as bradykinin and NGF, increase the temperature and proton sensitivity of TRPV1 and contribute to enhanced TRPV1 ion channel activity.

Bradykinin

Bradykinin is a nonapeptide that is produced at sites of tissue injury[25] and mediates its effects through two known types of receptors, denominated B1 and B2, respectively, both of which are G-protein-coupled receptors.[26] These receptors are found in primary sensory neurons, as well as in the spinal cord.[19,27] Bradykinin is a potent inflammatory agent.[28–34] Several features of the involvement of bradykinin with the activation of TRPV1 ion channels are now known.[14,15,35–39] Importantly, bradykinin activation of B2 receptors results in activation of an intracellular second messenger pathway involving mobilization of arachidonic acid by phospholipase A2, and generation of the 12-lypoxygenase product, 12-hydroperoxyeicosatetraenoic acid, which is capable of activating TRPV1.[35] Bradykinin-induced thermal hyperalgesia is completely blocked by an inhibitor of 12-lipoxygenase.[35] Moreover, bradykinin lowers the threshold temperature for heat activation of TRPV1 to well below physiologic body temperature.[15] Both bradykinin and NGF each activate TRPV1 as well as their own receptors.[14]

Prostanoids: PGE2 and PGI2

The prostanoids are a major group of bioactive lipids, which work as local mediators, exerting their actions on other cells near their cell of synthesis. Prostaglandin-E2 (PGE2) and prostaglandin-I2 (PGI2), also known as prostacyclin, are the prostanoids whose functions have been most clearly defined. The function of other members of this group, including PGF2α, PGD2, PGJ2, PGG2, PGH2, and thromboxane A2, are less well understood. PGE2 contributes to inflamma-

tory pain[40–42] and mediates its effects by binding to four G-protein-coupled receptors, denominated EP1, EP2, EP3, and EP4.[43] PGI2 also contributes to inflammatory pain and mediates its effects by binding to IP receptors.[44–47] Prostaglandins released in the dorsal root ganglia excite those neurons by lowering the threshold for activation by heat of TRPV1 ion channels. PGE2 and PGI2 each, individually, increase TRPV1 responses through their respective EP1 or IP receptors, predominantly in a protein kinase C-dependent manner in both human embryonic kidney 293 (HEK293) cells expressing TRPV1 and in mouse dorsal root ganglion neurons. In the presence of PGE2 or PGI2, the temperature threshold for TRPV1 activation is reduced below 35°C so that body temperature itself is sufficient to activate TRPV1, resulting in the phenomenon of spontaneous pain sensations.[48] In TRPV1 and EP1 null mice, both PGE2-induced thermal hyperalgesia and inflammatory nociceptive responses are diminished. Moreover, PGI2-induced thermal hyperalgesia observed in wild-type mice is almost completely absent in both TRPV1 and IP null mice.[48]

Nerve Growth Factor

NGF is produced by a variety of cells in the context of inflammation, including monocytes,[49] eosinophils,[50] mast cells,[51] and Schwann cells.[52] Histamine, interleukin-1β, interleukin-6, tumor necrosis factor-α, and certain prostaglandins, including PGD2 and PGE2, also stimulate NGF secretion.[53–57] TrkA is a receptor with tyrosine kinase activity that forms a high-affinity binding site for NGF.[58] NGF acts on nociceptive afferent neurons, increasing their electrical excitability.[59] Acutely, NGF exerts profound effects on nociceptive transmission and produces pain and hyperalgesia.[60–62] NGF is known to be capable of sensitizing TRPV1 ion channels.[53] NGF, by binding to, and activating, its TrkA receptor, sets in motion a biochemical chain of events that, if sustained, results in the sensitization of TRPV1. As a cell membrane surface receptor, TrkA relies on the further activation of intracellular messengers to mediate this effect. The issue which is most discussed in relation to the sensitization of TRPV1 by NGF relates to which one, or more, of the several intracellular signaling pathways perform this function. These pathways are those denominated: phospholipase C (PLC), phosphatidylinositol-3-kinase (PI3K), and mitogen-activated protein kinase (MAPK), respectively.[14,63–69]

Artemin, Neurturin, and GDNF

Artemin, neurturin, and GDNF are members of the *GDNF* family, which are produced in the form of a "prepro" precursor.[70] GDNF, neurturin, and artemin, bind to the alpha receptor subunits GFRα1, GFRα2, and GFRα3, respectively. GFRalphas are linked to the membrane *via* glycosyl phosphatidylinositol anchors. Signal transduction occurs by interaction with the transmembrane receptor ret (c-ret).[71,72] Artemin, neurturin, and GDNF, each individually potentiate capsaicin-evoked TRPV1 signaling in isolated mouse dorsal root ganglion neurons and cause thermal hyperalgesia when injected into mouse hind paw *in vivo*. Artemin mRNA (but not neurturin or GDNF) is upregulated during cutaneous inflammation evoked by hind paw injection of complete Freund's adjuvant, suggesting that artemin, in particular, enhances TRPV1 signaling in response to inflammatory injury. Hind paw injection of artemin, neurturin, GDNF, or NGF produces acute thermal hyperalgesia that lasts up to 4 h. Moreover, a single combined injection of artemin and NGF produces hyperalgesia that persists for 6 days.[73] Overexpression of artemin in the skin of mice enhances expression of TRPV1 and TRPA1 in cutaneous sensory neurons and results in increased behavioral sensitivity to heat and cold.[74] In addition, overexpression of artemin in the tongue increases the expression of TRPV1 and TRPA1 in trigeminal afferents and causes oral sensitivity to capsaicin and mustard oil in mice.[75]

Histamine

Histamine is a basic amine and is an important neurotransmitter in both the central and peripheral nervous systems. In the periphery, histamine is found in mast cells and basophils and is secreted when complement components C3a and C5a interact with specific membrane receptors or when antigen interacts with cell-fixed immunoglobulin E (IgE).[76] Histamine has four known histamine receptors (H1, H2, H3, and H4), which are all G-protein-coupled receptors.[77–80] Histamine contributes to the inflammatory response, with H1 receptors being relatively more important than H2 receptors in mediating formalin-induced nociceptive behaviors.[81,82] One effect of histamine on a subset of primary afferents is mediated *via* activation by histamine of phospholipase A2 and 12-lipoxygenase, which leads to the production of 12-hydroperoxyeicosatetraenoic acid and activation of TRPV1 ion channels. Activation of TRPV1 then leads to excitation of the primary sensory neurons on which they are expressed. Histamine-induced itching is proposed to be mediated, in part, by this pathway.[83]

Anandamide

Anandamide (*N*-arachidonylethanolamine) is a member of the group of bioactive lipids known as "long chain C18 *N*-acylethanolamines." It is an endogenous ligand of canabinoid receptors and is one of the several endogenous agents that have been proposed as direct activators of TRPV1.[84] The capacity of anandamide to activate TRPV1 in normal physiologic conditions is limited. This limitation is essential to prevent unnecessary activity of TRPV1, thereby signaling pain, in the absence of a relevant pain-inducing stimulus. However, when TRPV1 is activated by other stimuli, such as inflammatory mediators, anandamide becomes a powerful activator of TRPV1,[85,86] and, hence, a contributor to the pain sensations mediated by inflammagens. Anandamide and other endogenous activators of TRPV1 may therefore be described as "conditional activators" of this ion channel.

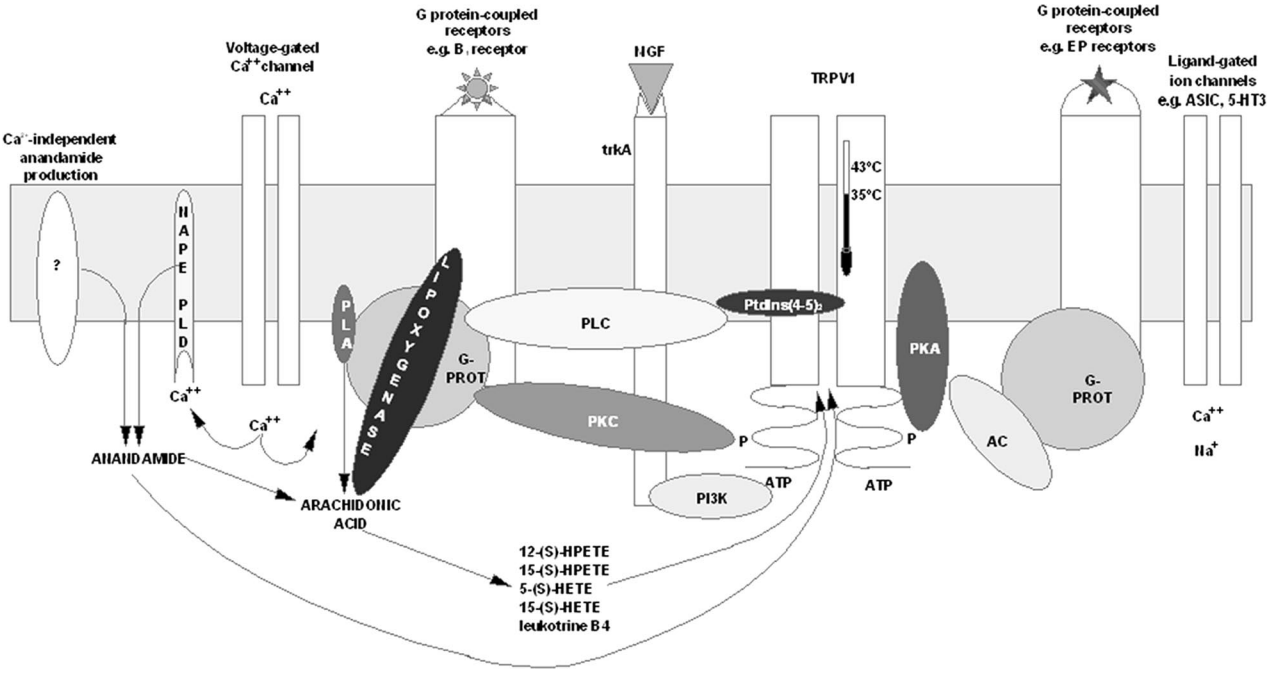

Fig. 2. Molecular mechanisms involved in the functioning of TRPV1 in inflammatory conditions. Reduction of the heat threshold of TRPV1 to well below body temperature may be induced by binding of endovanilloids, protons, anandamide, or lipoxygenase products. Posttranslational modifications (removal of phosphatidylinositol-4,5-biphosphate [PtdIns(4,5)P2] and protein kinase C [PKC]-mediated phosphorylation) can also produce this effect. Protein kinase A (PKA)-mediated phosphorylation also sensitizes TRPV1. Phosphatidylinositol 3-kinase (PI3K) activity induced by nerve growth factor (NGF) and ATP-binding also potentiates responses evoked by endovanilloids. The molecular mechanisms involved in the functioning of TRPA1 and TRPV4 remain to be elucidated. AC = adenylyl cyclase; ASIC = acid-sensing ion channel; ATP = adenosine triphosphate; EP receptors = receptors for prostaglandin E2; G prot = G protein; 5(*S*)-HETE = 5-hydroxyeicosatetraenoic acid; 15(*S*)-HETE = 15-hydroxyeicosatetraenoic acid; 12(*S*)-HPETE = 12-hydroperoxyeicosatetraenoic acid; 15(*S*)-HPETE = 15-hydroperoxyeicosatetraenoic acid; NAPE-PLD = *N*-acylphosphotidylethanolamine phospholipase D (involved in anandamide production in primary sensory neurons); PLA = phospholipase A; PLC = phospholipase C; trkA = neurotrophic tyrosine kinase receptor type 1; TRPV1 = transient receptor potential vanilloid type 1 ion channel; *question mark* = an unidentified enzyme producing anandamide in a Ca^{2+}-independent manner.

Proton Activation of TRPV1

Protons are able to activate the TRPV1 ion channel at pH less than 6.5.[87] Hence, minor reductions in pH less than (neutral) 7.4 do not, as such, activate this ion channel. Ligand binding, the temperature threshold for activation of the ion channel, and channel gating are all affected by pH. Thus, lowering pH enhances the apparent binding affinity of capsaicin and reduces the heat threshold for activation of the channel. It also promotes the occurrence of long openings and short closures and stabilizes at least one of the open conformations of the channel.[87] Jordt *et al.*[88] believed that protons modulate TRPV1 activity by interacting with specific amino acid residues on the extracellular surface of the channel protein. The response of TRPV1 to protons may be the result of at least two different mechanisms, namely, first, activation of the channel and, second, potentiation of the currents generated by an already activated channel.[87] These mechanisms seem to be distinct and separate, although originating at the site where protonation is initiated. It has been suggested that this site could set the sensitivity to other noxious stimuli in response to changes in extracellular proton concentration.[88]

Cation Activation of TRPV1

In addition to protons, excess positive charges carried by various other ions are also able to activate TRPV1. Ahern *et al.* showed that extracellular Na^+, Mg^{2+}, and Ca^{2+} can directly gate the TPRV1 ion channel under extreme or pathophysiologic concentrations. However, even discrete pathology may result in the generation of extracellular microenvironments that readily achieve concentrations of cations that are sufficient to directly gate TRPV1. For example, in bone, the [Ca^{2+}] surrounding resorbing osteoclasts approaches 40 mM.[89] A yet more significant effect of extracellular cations may occur under normal physiologic conditions at which millimolar increases in cation concentrations (1–5 mM) are sufficient to sensitize TRPV1 to various ligands, including capsaicin and anandamide.[90] It is also proposed that divalent cations are more potent agonists than protons, because they impart a greater net positive charge at their binding sites.[90]

TRPV1 as a Stimulus Integrator. An important feature of TRPV1, in the context of the many agents generated by the inflammatory response, is its ability to act as a receptor that integrates its respective effects (fig. 2). Various activators of TRPV1 can increase the effect evoked by another, or others, of its activators. It seems that this cooperation of various ligands is synergistic, rather than additive, in activating TRPV1.[11,21,91] The findings that various ligands potentiate each other's effect on TRPV1 indicate that the different activator sites are coupled in TRPV1. It has been suggested that

the TRPV1 ion channel acts as a "stimulus integrator" of exogenous stimuli, given the polymodal nature of its activation and the potentiating effect of each of the activating stimuli on the effect produced by another activator of TRPV1.[21] In fact, TRPV1 acts similarly in relation to many endogenous agents, which makes it of particular relevance in the context of inflammation given the wide variety of inflammatory agents generated as components of the inflammatory response.

TRPV1 and Spontaneous Pain Sensation. The fact that TRPV1 is synergistically responsive to at least two accompaniments of inflammation, namely, local decreases in pH and increases in temperature, strongly supports a role of TRPV1 as a mediator of spontaneous inflammatory pain sensations. The pH threshold for proton-evoked TRPV1 activation is approximately 6.5. At lower pH values, protons can themselves activate TRPV1. Both the temperature threshold for activation and channel gating are affected by pH. Lowering pH reduces the heat threshold for activation of the channel. Moreover, ligand binding and protonation of the channel interact allosterically, where each of these agonists can increase the effect of the other.[87] Sensitivity to noxious heat is the cardinal feature of TRPV1. Heat contributes to TRPV1 activation in two distinct ways. First, heat reduces the threshold for the activation of TRPV1 by all other TRPV1 activators. Second, heat more than approximately 43°C independently activates TRPV1.[21] At less than 43°C, TRPV1 openings are few and brief. However, raising the ambient temperature rapidly increases the frequency of channel openings.[92] The crucial point is that, if the temperature activation threshold for TRPV1 is reduced to 37°C by reduced pH, or otherwise, the result is spontaneous activation of TRPV1, leading to spontaneous pain sensations as a result of normal body temperature alone. Finally, in this context, it may be noted that yet a further component of the inflammatory response, namely, bradykinin, has been identified as being capable of reducing the heat threshold for activation of TRPV1 well below body temperature.[15,93] There is also some evidence from studies on heterologously expressed human TRPV1 that the presence of either reducing or oxidising agents results in an increased response to heat by TRPV1 channels.[94] Temperature clearly plays a crucial role in the activation of TRPV1 as evidenced by the finding that cooling inhibits capsaicin-induced currents in primary sensory neurons.[95]

TRPA1 as a Mediator of Inflammatory Thermal Hyperalgesia, Cold Hyperalgesia, and Mechanical Hyperalgesia

TRPA1 is a nonselective ligand-gated cation channel that is directly gated by Ca^{2+}.[96–99] TRPA1 is found in a subset of nociceptive sensory neurons of dorsal root and trigeminal ganglia that coexpress TRPV1.[98–100] However, not all TRPV1-expressing primary afferents also express TRPA1. TRPA1 is a noxious cold-sensitive channel that is activated by cold at approximately 17°C.[98,101,102] However, TRPA1-deficient mice display normal cold sensitivity,[103] suggesting that the association between TRPA1 activation and noxious cold is qualified. The full range of activators of TRPA1 has yet to be identified, but it is clear that TRPA1 is activated by a diverse group of ligands and conditions. Most compounds known to activate TRPA1 are able to covalently bind cysteine residues. Covalent modification of reactive cysteines within TRPA1 can cause channel activation, rapidly signaling potential tissue damage through the pain pathway.[104]

Many of the exogenous activators of TRPA1 produce an inflammatory response when applied to the body. More importantly, in this context, TRPA1 is activated at least by certain of the chemical agents generated by the inflammatory response. Thus, TRPA1 is responsible for the pain, inflammation, and robust hypersensitivity to thermal and mechanical stimuli that results from the topical application of mustard oil (allyl isothiocyanate) to the skin.[99] Studies using TRPA1 null mice show that this channel is the sole target through which mustard oil and garlic activate primary afferent nociceptors to produce inflammatory pain.[103] Formalin excites sensory neurons by directly activating TRPA1. It induces a robust calcium influx in cells expressing TRPA1, which is attenuated by a TRPA1-selective antagonist. Sensory neurons from TRPA1 null mice lack sensitivity to formalin, whereas pharmacologic blockade or genetic ablation of TRPA1 in mice produces marked attenuation of the characteristic flinching, licking, and lifting responses resulting from intraplantar injection of formalin.[10]

Bradykinin is an indirect activator of TRPA1.[101] TRPA1-deficient mice exhibit pronounced deficits in bradykinin-evoked nociceptor excitation and pain hypersensitivity.[103] Phospholipase C is an important signaling component for TRPA1 activation.[101] Bradykinin potentiates the activation of TRPA1 by other agonists. Bradykinin increases the TRPA1-mediated currents evoked by allyl isothiocyanate or cinnamaldehyde in HEK293 cells that express TRPA1 and the bradykinin B2 receptor. This potentiation is inhibited by a phospholipase C inhibitor or protein kinase A inhibitor and is mimicked by a phospholipase C activator or protein kinase A activator. Bradykinin, released in response to tissue inflammation, may mediate the sensation of pain by sensitizing TRPA1.[105]

A PGD2 metabolite seems to be capable of directly activating TRPA1.[106] Multiple agents produced during episodes of oxidative stress can activate TRPA1 expressed in sensory neurons.[107] A functional interaction of protease-activated receptor 2 and TRPA1 in dorsal root ganglion neurons may contribute to the sensation of inflammatory pain.[108] TRPA1 may also be involved in contributing to visceral hypearalgesia after colitis.[109]

TRPV4 Mediates Inflammatory Mechanical Hyperalgesia

TRPV4 is a nonselective, ligand-gated, cation channel—previously named "vanilloid receptor-related osmotically acti-

vated channel"—which functions in the transduction of osmotic and mechanical stimuli.[110] Studies in TRPV4 null mice show that TRPV4 is necessary for the maintenance of systemic osmotic equilibrium and for normal thresholds in response to noxious mechanical stimuli.[111] Disrupting the *TRPV4* gene in mice markedly reduces the sensitivity of the tail to pressure and acidic nociception. The TRPV4 channel expressed *in vitro* in Chinese hamster ovary cells is opened by low pH, citrate, and inflation but not by heat or capsaicin.[112] In addition to its expression on peripheral sensory neurons, TRPV4 is found in the central nervous system where hippocampal neurons express functional TRPV4, which are constitutively active at physiologic temperature.[113]

TRPV4 is expressed by both small (nociceptive) and large (nonnociceptive) dorsal root ganglia neurons in mice.[114] TRPV4 protein is transported in sensory nerves distally toward the peripheral nerve endings. *In vivo* single-fiber recordings in rat show that hypotonic solution activates 54% of C-fibers, an effect enhanced by PGE2. This osmotransduction causes nociception, and the channel is required for hypotonic stimulus-induced nociception.[115] TRPV4 also mediates pain resulting from hypertonicity in rat and the aggravation of that pain, which results from the addition of an inflammatory mediator.[116]

TRPV4 mediates mechanical hyperalgesia occasioned by agents produced in the inflammatory process. Thus, intradermal injection of carrageenan, or of a soup of inflammatory mediators, enhances the nocifensive paw-withdrawal reflex elicited by hypotonic or mechanical stimuli in rat. Spinal administration of TRPV4 antisense oligodeoxynucleotide blocks enhancement, without altering baseline nociceptive threshold. Similarly, in TRPV4 null mice, inflammatory soup fails to induce any significant mechanical or osmotic hyperalgesia.[117] Again, when the mechanical receptive fields of C-fibers in $TRPV4^{+/+}$ and $TRPV4^{-/-}$ mice are injected *in vivo* with PGE2 and serotonin, the percentage of C-fibers responding to a hypotonic stimulus and the magnitude of the response is significantly greater in $TRPV4^{+/+}$ mice compared with $TRPV4^{-/-}$ mice. Only C-fibers from $TRPV4^{+/+}$ mice exhibit increased spontaneous activity and decreased mechanical threshold in response to PGE2 and serotonin, demonstrating that TRPV4 is crucial in mediating mechanical hyperalgesia.[118] Levine *et al.*[119] showed that mechanical hyperalgesia is reduced in TRPV4-deficient mice in various models of painful peripheral neuropathy, which exhibit mechanical hyperalgesia. TRPV4 contributes to mechanically evoked visceral pain[120] and is required for protease-activated receptor 2-induced mechanical hyperalgesia and excitation of colonic afferent neurons in mouse.[121]

ASICs (and at Lesser pH Levels, TRPV1) Mediate Inflammatory Pain Resulting from High Tissue Proton Concentration

ASICs, on exposure to local tissue acidosis, excite the neurons on which they are expressed. ASICs are the primary acid sensors as they are activated by protons at considerably smaller reductions in pH than are TRPV1. Reduction in pH and local acidosis in inflamed tissues are potent contributors to pain and hyperalgesia. An increase in the local hydrogen ion concentration is a common accompaniment of inflammation consequent on tissue damage or disease. It has long been known that low pH (down to 4.7) is commonly found in inflamed tissues and that acidic solutions are particularly painful when injected into the skin.[122–127] More generally, high cation concentrations may result in pain, mediated by ASICs and TRPV1, with acute pain-related behavior being evoked by elevated ionic strength. Intraperitoneal injection of $MgSO_4$ evokes writhing responses in mice, whereas salt (NaCl), when applied to injured tissue, evokes a burning pain sensation similar to that evoked by capsaicin, heat, or extracellular protons. Therefore, extracellular cations can result in acute burning pain sensation.[90]

ASICs Mediate Pain Sensations from Both Minor and TRPV1-sensitive Reductions in pH

ASICs are activated by extracellular protons. In the periphery, they contribute to the excitation of primary sensory neurons when exposed to an acid solution, including that comprised in an acidic microenvironment. ASICs are H^+-gated Na^+ channels that belong to the degenerin/epithelial sodium (Deg/ENaC) superfamily of ion channels (fig. 1).[128] Six different members of the ASIC subfamily have been cloned (ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4), which are encoded by four genes. ASIC1b and ASIC2b are splice variants of ASIC1a and ASIC2a.[129] All ASICs—with the exception of ASIC4—are expressed in sensory neurons of the dorsal root ganglion. Homomeric ASIC1 can be activated by extracellular H^+ in the physiologic pH range. Extracellular, divalent cations, such as Ca^{2+} and Mg^{2+}, and the polyvalent cation spermine, shift the steady-state inactivation of ASIC1a and ASIC1b to more acidic values. This leads to a potentiation of the channel response and is due to a stabilization of the resting state. ASIC1b is an effective sensor of transient H^+ signals during slight acidosis and, in addition to alternative splicing, interaction with divalent and polyvalent cations extends the dynamic range of ASIC H^+ sensors.[129] ASIC2b (a splice variant of ASIC2a) is acid insensitive.[130] There are interesting studies on channel gating in relation to ASIC3 in rat[131] and ASIC1 in fish.[132]

The extent of the respective roles performed by ASICs and TRPV1 ion channels in mediating acid-induced pain varies between species.[133] It may also be the case that the extent of the respective roles performed by ASICs and TRPV1 in mediating acid-induced pain varies between tissues and, indeed, within tissues.[134] ASIC expression may also be affected by tissue damage.[135]

TRPV1 ion channel is modulated by acid at lower pH values than ASICs.[21,136] There is evidence that, in humans, TRPV1 plays a relatively minor role in signaling cutaneous acid-induced pain of moderate intensity, with ASICs being the main mediators of pain in that context. However, it may

well be that TRPV1 plays a more prominent role in more acidic conditions.[136]

ASIC expression is increased in inflammatory conditions.[137] Arachidonic acid potentiates the currents carried by ASIC1a and ASIC3 in rat dorsal root ganglion neurons.[138] Acidic microenvironments may be created by osteoclasts in bone disorders with increased osteoclastic bone resorption. The resulting hyperalgesia is mediated, in part at least, by upregulation of the expression of ASICs.[139]

Anesthetic Gases Affecting Activation of TRPV1 and TRPA1 Ion Channels

Certain inhalational anesthetics that result in unconsciousness and, therefore, induce the absence of sensibility to pain may preclude the mental appreciation of pain solely as a result of inducing unconsciousness and not as a result of any concomitant analgesic effect on nociceptive processing. Based on this, surgery may occasionally be painful, of which the patient is unaware by reason of being unconscious under a general anesthetic, but such surgery may nevertheless result in the excitation of primary nociceptive afferents and the excitation of spinal dorsal horn neurons. This process may cause an alteration of nociceptive processing in the dorsal horn of the spinal cord, resulting in a pathologic pain condition. Appreciation of the risk of the occurrence of this phenomenon has resulted in the concomitant administration of analgesics intended to reduce nociceptive processing in spinal dorsal horn neurons. However, it has become clear in more recent times that the general anesthetic itself may not only fail to inhibit nociceptive afferents but may also excite nociceptive primary afferents, thereby contributing to intraoperative excitation of spinal dorsal horn neurons. Ahern *et al.*[140] found that the pungent general anesthetic isoflurane produces inward currents in voltage-clamped TRPA1 expressing HEK293 cells and in cultured mouse dorsal root ganglion neurons. Both isoflurane and desflurane were found to robustly activate TRPA1. In addition, the intravenous general anesthetics, propofol and etomidate, were found to produce a robust activation of TRPA1 in voltage-clamped HEK293 cells. On the basis of this finding, these authors suggest that selective TRPA1 antagonists may represent an effective treatment strategy for preventing the pronociceptive effects of pungent general anesthetics that may otherwise sensitize primary nociceptive afferents during the maintenance of anesthesia.[140]

Ahern's laboratory has also made important findings in *in vitro* experiments in relation to a sensitizing effect on TRPV1 by not only pungent but also by nonpungent inhalational anesthetics. Clinically relevant concentrations of isoflurane, sevoflurane, enflurane, and desflurane have been found to sensitize TRPV1 to capsaicin and protons and to reduce the threshold for heat activation of this ion channel. Although these volatile general anesthetics were found not to directly activate TRPV1, they were nonetheless found to sensitize this ion channel to certain of the many endogenous and exogenous stimuli that can activate it. This has led the learned authors to suggest that their findings support an hypothesis that, in the clinical context, volatile general anesthetics may augment nociceptive signaling arising from surgical insults.[141] However, we believe that these data from *in vitro* experiments—although itself no doubt correct—are too remote from the complexities of the *in vivo* processing by the human body of general anesthetics to justify this suggestion. Moreover, the suggestion may also be considered to be intuitively unjustified given the generally manageable outcome as regards postoperative pain for the vast majority of patients who undergo countless surgical procedures under general anesthesia. Hence, the importance of obtaining further evidence is that it clarifies the clinical implications of the use of these general anesthetics and, particularly, the use of sevoflurane.

A role for TRPV1 has also been suggested in the pain resulting from administration of the local anesthetic lidocaine. Thus, lidocaine activates TRPV1 and, to a lesser extent, TRPA1 in rodent dorsal root ganglion sensory neurons, as well as in HEK293t cells expressing TRPV1 or TRPA1. In addition, lidocaine has also been shown to induce the release, from isolated skin and peripheral nerve, in a TRPV1-dependent manner, of calcitionin gene-related peptide, which is a key constituent of neurogenic inflammation.[142]

Conclusion

This article was intended to introduce the reader to the role of transient receptor potential and ASIC, ionotropic receptors as mediators of peripheral inflammatory pain—a subject that is in the course of rapid development. TRPV1 initially seemed to hold out the promise of explaining much of the mystery of peripheral inflammatory pain and of providing a target for therapeutic drug intervention to relieve the pain. However, TRPA1 has newly emerged as a potent contributor to primary afferent excitation in inflammation, indicating that the ionotropic receptors involved in mediating peripheral inflammatory pain are likely to be several. Although the distribution of TRPA1 throughout the body has yet to be determined, the already known extent of the distribution of TRPV1 throughout the body, and its involvement in multiple physiologic functions, suggests that TRPV1 antagonists, unless sufficiently specific, are likely to result in damaging side-effects in addition to any analgesic effect that they may provide. The several ion channels that have already been identified as contributors to inflammagen-induced primary afferent excitation—TRPV1, TRPA1, TRPV4, ASICs and, of course, the sodium Nav1.7, Nav1.8, and Nav1.9 channels suggest that multiple pathways exist, whereby inflammagens may affect the excitation of primary afferents. On the basis of this hypothesis, drugs that inhibit the activity of several, rather than a single, ion channel will be required. A recent important study shows that neuronal excitation in the context of inflammation may be reduced by eliminating certain of the complex of inflammatory mediators that

would otherwise be active,[1] thus suggesting that it may suffice to negate the effect of only some of these agents to achieve an analgesic effect. Therefore, research that is directed toward negating the effects of these inflammatory agents provides an alternative avenue toward possibly successful therapeutic intervention.

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