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 inflammmagens 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, histamine...
pain.7–9 However, inflammatory pain induced by formalin
adjuvant and carrageenan animal models of inflammatory
flammation generated in both the complete Freund’s
solutions. TRPV1 activation is an essential feature of the in-
flamming these ion channels to reduce inflammatory pain sen-
sation of inflammatory pain offers the prospect of manip-
ulating 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 evi-
dence relating to the involvement of the ionotropic recep-
tors’ 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 inflammmagen-induced nociceptive sig-
naling. These ion channels constitute an important com-
ponent 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 manip-
ulating these ion channels to reduce inflammatory pain sens-
ations. TRPV1 activation is an essential feature of the in-
flamation generated in both the complete Freund’s
adjuvant and carrageenan 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 poten-
tially important targets for therapeutic drug intervention
(table 2). In addition, recent important in vitro studies have
demonstrated that certain anesthetic gases—as well as intra-
venous 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 devel-
oped. Here, we introduce the reader to the seminal com-
ponents of that research, which are essential to the understand-
ing 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, cat-
ionic channel with a high permeability for Ca2+ (fig. 1).11–17

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

<table>
<thead>
<tr>
<th>Inflammatory Agent</th>
<th>Direct or Indirect Activation</th>
<th>Activation by Ligand-Binding Cognate (Own) Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinin</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>PGE2</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>PGI2</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>NGF</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Artemin, neurturin, GDNF</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Histamine</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Anandamide</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Protons and cations</td>
<td>Y (pH &lt; 6.5)</td>
<td>Y</td>
</tr>
<tr>
<td>LTB4</td>
<td>—</td>
<td>Y</td>
</tr>
<tr>
<td>5-HT</td>
<td>—</td>
<td>Y</td>
</tr>
<tr>
<td>ATP</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

ASIC = acid-sensing ion channel; ATP = adenosine triphosphate; CB = cannabinoid; GDNF = glial cell-line-derived neurotrophic factor; 5-HT = 5-hydroxytryptamine; LTB4 = leukotriene 4; NGF = nerve growth factor; PGE2 = prostaglandin E2; PGI2 = 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.

### Table 2. TRPV1, TRPA1, TRPV4, and ASICs as Iontropic Receptors

<table>
<thead>
<tr>
<th>Principal Features</th>
<th>Possible inhibiting mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand-gated ion channels</td>
<td>Competitive antagonism</td>
</tr>
<tr>
<td>Have central aqueous pore</td>
<td>Conduction blocker of channel pore</td>
</tr>
<tr>
<td>Channel-gated on ligand-binding</td>
<td>Binding in channel pore to induce failure of “gating”</td>
</tr>
<tr>
<td>Gating results in ionic flux via pore</td>
<td></td>
</tr>
<tr>
<td>For fast synaptic transmission</td>
<td></td>
</tr>
<tr>
<td>Examples: TRPs, ASICs, 5-HT3</td>
<td></td>
</tr>
</tbody>
</table>

ASIC = acid-sensing ion channel; 5-HT = 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; 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.

Heat hyperalgesia secondary to inflammatory tissue injury induced by mustard oil, Complete Freund’s adjuvant, or carrageenan 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 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 sensitization of existing TRPV1 as well as their own receptors.14 Bradykinin-induced thermal hyperalgesia is completely blocked by an inhibitor of 12-lipoxygenase.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 and contribute to enhanced TRPV1 ion channel activity.

**Bradykinin**

Bradykinin is a nonapeptide that is produced at sites of tissue injury25 and mediates its effects through two known types of receptors, denominated B1 and B2, respectively, both of which are G-protein-coupled receptors.26 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. PGF2α contributes to inflamma-

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

<table>
<thead>
<tr>
<th>Activator</th>
<th>TRPV1</th>
<th>TRPA1</th>
<th>TRPV4</th>
<th>ASICs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>—</td>
</tr>
<tr>
<td>Cold</td>
<td>N</td>
<td>Y</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Depolarisation</td>
<td>Y</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hypotonicity</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>Hypertonicity</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>(and other vanilloids)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mustard oil</td>
<td>N</td>
<td>Y</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Garlic</td>
<td>N</td>
<td>Y</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Protons and cations</td>
<td>Y (pH &lt; 6.5)</td>
<td>—</td>
<td>Y</td>
<td>Y</td>
</tr>
</tbody>
</table>

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.

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.

**Prostanoids: PGE2 and PG12**

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-
TRPs and ASICs in Inflammatory Pain

NGF is produced by a variety of cells in the context of inflammation, including monocytes, eosinophils, mast cells, and Schwann cells. Histamine, interleukin-1β, interleukin-6, tumor necrosis factor-α, and certain prostaglandins, including PGD2 and PGE2, also stimulate NGF secretion. TrkA is a receptor with tyrosine kinase activity that forms a high-affinity binding site for NGF. NGF acts on nociceptive afferent neurons, increasing their electrical excitability. Acutely, NGF exerts profound effects on nociceptive transmission and produces pain and hyperalgesia. NGF is known to be capable of sensitizing TRPV1 ion channels, 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.

Artemin, Neurturin, and GDNF

Artemin, neurturin, and GDNF are members of the GDNF family, which are produced in the form of a "prepro" precursor. GDNF, neurturin, and artemin, bind to the alpha receptor subunits GFRα1, GFRα2, and GFRα3, respectively. GFRα2 is linked to the membrane via glycosylphosphatidylinositol anchors. Signal transduction occurs by interaction with the transmembrane receptor ret (c-ret). 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.

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. 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.

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). Histamine has four known histamine receptors (H1, H2, H3, and H4), which are all G-protein-coupled receptors. Histamine contributes to the inflammatory response, with H1 receptors being relatively more important than H2 receptors in mediating formalin-induced nociceptive behaviors. One effect of histamine on a subset of primary afferents is mediated via activation by histamine of phospholipase A2 and 12-lipoxigenase, 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.

Anandamide

Anandamide (N-arachidonylethanolamine) is a member of the group of bioactive lipids known as "long chain C18 N-acylbenzamines.” It is an endogenous ligand of cannabinoid receptors and is one of the several endogenous agents that have been proposed as direct activators of TRPV1. 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 and, hence, a contributor to the pain sensations mediated by inflammmagens. Anandamide and other endogenous activators of TRPV1 may therefore be described as “conditional activators” of this ion channel.
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 TRPV1 ion channel under extreme or pathophyslogic 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 activator sites is coupled in 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 polyomodal 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. 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 Hyperalgesia, Cold Hyperalgesia, and Mechanical Hyperalgesia

TRPA1 is a nonselective ligand-gated cation channel that is directly gated by Ca2+.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.103 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 hyperalgesia after colitis.109

TRPV4 Mediates Inflammatory Mechanical Hyperalgesia

TRPV4 is a nonselective, ligand-gated, cation channel—previously named “vanilloid receptor-related osmotically activ-
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 stimuli-induced nociception. TRPV4 also mediates pain resulting from hypertonicity in rats and the aggravation of that pain, which results from the addition of an inflammatory mediator.

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. 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.

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 pain120 and is required for protease-activated receptor 2-induced mechanical hyperalgesia and excitation of colonic afferent neurons in mice.121

**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).122 Six different members of the ASIC subfamily have been cloned (ASIC1a, ASIC1b, ASIC2a, ASIC3, and ASIC4), which are encoded by four genes. ASIC1b and ASIC2b are splice variants of ASIC1a and ASIC2a.123 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 Ca²⁺ and Mg²⁺, 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.124 ASIC2b (a splice variant of ASIC2a) is acid insensitive.125 There are interesting studies on channel gating in relation to ASIC3 in rat126 and ASIC1 in fish.127

The extent of the respective roles performed by ASICs and TRPV1 ion channels in mediating acid-induced pain varies between species.128 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.130 ASIC expression may also be affected by tissue damage.131 TRPV1 ion channel is modulated by acid at lower pH values than ASICs.121,129 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.\textsuperscript{136}

ASIC expression is increased in inflammatory conditions.\textsuperscript{137} Arachidonic acid potentiates the currents carried by ASIC1a and ASIC3 in rat dorsal root ganglion neurons.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 calcitonin gene-related peptide, which is a key constituent of neurogenic inflammation.\textsuperscript{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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