

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

John P. M. White, LL.D.,* Mario Cibelli, M.D.,† Antonio Rei Fidalgo, M.Sc.,‡
Cleoper C. Paule, B.Sc.,‡ Faruq Noormohamed, M.Sc.,* Laszlo Urban, Ph.D.,§
Mervyn Maze, M.B., B.Ch.,|| Istvan Nagy, Ph.D.#

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-

* Research Scientist, ‡ Ph.D. Student, || Sir Ivan Magill Professor of Anaesthetics, # Senior Lecturer, Section of Anaesthetics, Pain Medicine and Intensive Care, Department of Surgery and Cancer, Imperial College London, Chelsea and Westminster Hospital; † Clinical Fellow, Section of Anaesthetics, Pain Medicine and Intensive Care, Department of Surgery and Cancer, Imperial College London, Chelsea and Westminster Hospital; Department of Cardiothoracic Anaesthesia, The Heart Hospital UCLH; and § Executive Director, Preclinical Safety Profiling, Lead Finding Platform, Novartis Institutes for BioMedical Research, Cambridge, Massachusetts.

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.

Address correspondence to Dr. Nagy: Section of Anaesthetics, Pain Medicine and Intensive Care, Department of Surgery and Cancer, Imperial College London Faculty of Medicine Chelsea and Westminster Hospital, Room G3.45, 369 Fulham Road, London SW10 9NH, United Kingdom. i.nagy@imperial.ac.uk. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

Table 1. Inflammagens as Activators of Ionotropic 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
PGE ₂	Y	—	Y	—	EP ₁₋₄
PGI ₂	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
LTB ₄	—	—	—	—	BLT ₁₋₂
5-HT	—	—	—	Y	5-HT _{1A and 2A} , 5-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; 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.

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 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 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 Ca²⁺ (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-HT₃

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-HT₃ = 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.

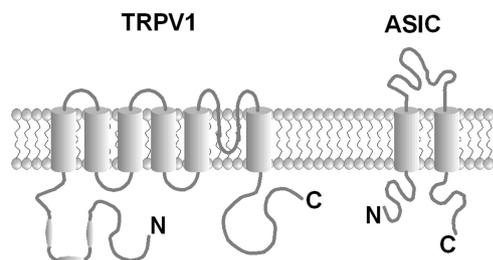


Fig. 1. Predicted membrane structure of TRPV1 and ASIC ion channels. Similar to the other members of the TRP family, TRPV1 has six transmembrane domains. Both the N- and C-termini are intracellular. The hydrophobic loop connecting transmembrane domains five and six is believed to be part of the channel. 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.

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 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 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 expression²³ 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

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.

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 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 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-lipoxygenase 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 PGF2 α , 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-1 β , 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 GFR α 1, GFR α 2, and GFR α 3, 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 cannabinoid 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.

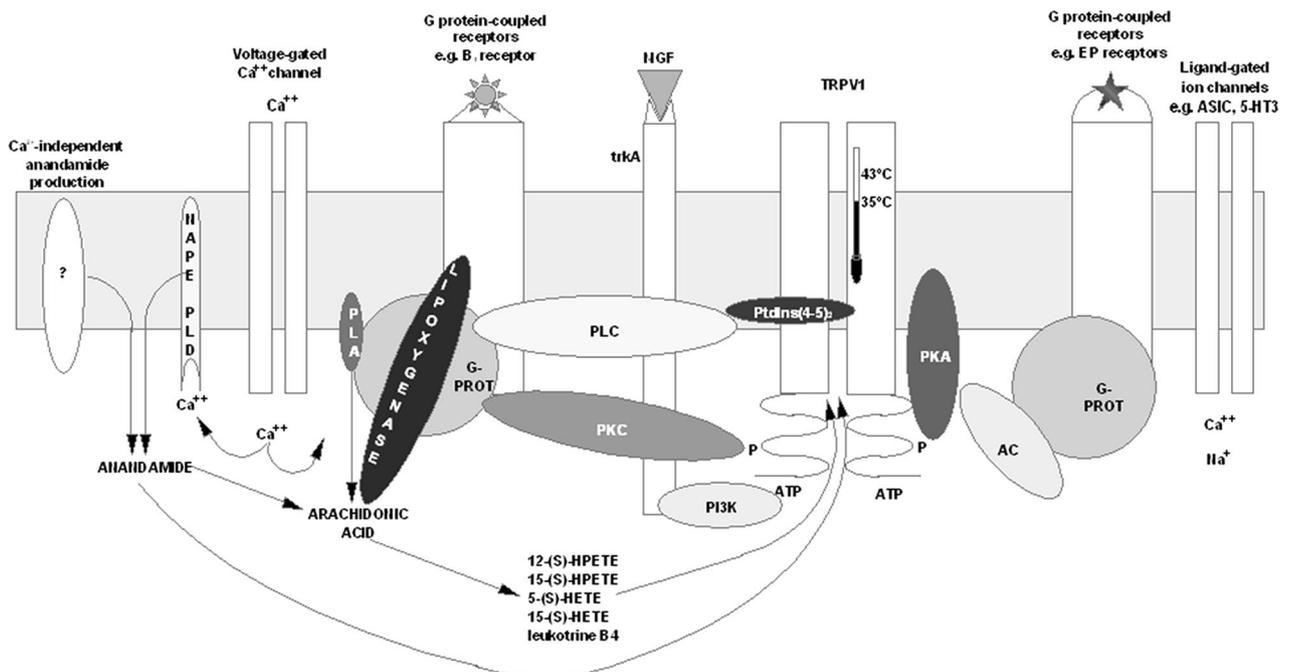


Fig. 2. Molecular mechanisms involved in the functioning of TRPV1 in inflammatory conditions. Reduction of the heat threshold of TRPV1 to well below body temperature may be induced by binding of endovanilloids, protons, anandamide, or lipoxygenase products. Posttranslational modifications (removal of phosphatidylinositol-4,5-bisphosphate [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 Ca²⁺-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⁺, Mg²⁺, and Ca²⁺ can directly gate the TRPV1 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 [Ca²⁺] 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 acti-

vated 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 hyperalgesia 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 pain¹²⁰ 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 MgSO₄ 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 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.¹²⁹ ASIC2b (a splice variant of ASIC2a) is acid insensitive.¹³⁰ There are interesting studies on channel gating in relation to ASIC3 in rat¹³¹ 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 calcitonin 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.

References

- Maingret F, Coste B, Padilla F, Clerc N, Crest M, Korogod SM, Delmas P: Inflammatory mediators increase Nav1.9 current and excitability in nociceptors through a coincident detection mechanism. *J Gen Physiol* 2008; 131: 211-25
- Priest BT, Murphy BA, Lindia JA, Diaz C, Abbadie C, Ritter AM, Liberator P, Iyer LM, Kash SF, Kohler MG, Kaczowski GJ, MacIntyre DE, Martin WJ: Contribution of the tetrodotoxin-resistant voltage-gated sodium channel Nav1.9 to sensory transmission and nociceptive behavior. *Proc Natl Acad Sci U S A* 2005; 102:9382-7
- Amaya F, Wang H, Costigan M, Allchorne AJ, Hatcher JP, Egerton J, Stean T, Morisset V, Grose D, Gunthorpe MJ, Chessell IP, Tate S, Green PJ, Woolf CJ: The voltage-gated sodium channel Na(v) 1.9 is an effector of peripheral inflammatory pain hypersensitivity. *J Neurosci* 2006; 26: 12852-60
- Villarreal CF, Sachs D, Funez MI, Parada CA, de Queiroz Cunha F, Ferreira SH: The peripheral pro-nociceptive state induced by repetitive inflammatory stimuli involves continuous activation of protein kinase A and protein kinase C epsilon and its Na(V)1.8 sodium channel functional regulation in the primary sensory neuron. *Biochem Pharmacol* 2009; 77:867-77
- Joshi SK, Honore P, Hernandez G, Schmidt R, Gomtsyan A, Scania M, Kort M, Jarvis MF: Additive antinociceptive effects of the selective Nav1.8 blocker A-803467 and selective TRPV1 antagonists in rat inflammatory and neuropathic pain models. *J Pain* 2009; 10:306-15
- Strickland IT, Martindale JC, Woodhams PL, Reeves AJ, Chessell IP, McQueen DS: Changes in the expression of Nav1.7, Nav1.8 and Nav1.9 in a distinct population of dorsal root ganglia innervating the rat knee joint in a model of chronic inflammatory joint pain. *Eur J Pain* 2008; 12:564-72
- Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, Koltzenburg M, Basbaum AI, Julius D: Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* 2000; 288:306-13
- Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, Harries MH, Latcham J, Clapham C, Atkinson K, Hughes SA, Rance K, Grau E, Harper AJ, Pugh PL, Rogers DC, Bingham S, Randall A, Sheardown SA: Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature* 2000; 405:183-7
- Yu L, Yang F, Luo H, Liu FY, Han JS, Xing GG, Wan Y: The role of TRPV1 in different subtypes of dorsal root ganglion neurons in rat chronic inflammatory nociception induced by complete Freund's adjuvant. *Mol Pain* 2008; 4:61
- McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, Zhao M, Hayward NJ, Chong JA, Julius D, Moran MM, Fanger CM: TRPA1 mediates formalin-induced pain. *Proc Natl Acad Sci U S A* 2007; 104:13525-30
- Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D: The capsaicin receptor: A heat-activated ion channel in the pain pathway. *Nature* 1997; 389:816-24
- Marsh SJ, Stansfeld CE, Brown DA, Davey R, McCarthy D: The mechanism of action of capsaicin on sensory C-type neurons and their axons in vitro. *Neuroscience* 1987; 23:275-89
- Heyman I, Rang HP: Depolarizing responses to capsaicin in a subpopulation of rat dorsal root ganglion cells. *Neurosci Lett* 1985; 56:69-75
- Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D: Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. *Nature* 2001; 411:957-62
- Sugiura T, Tominaga M, Katsuya H, Mizumura K: Bradykinin lowers the threshold temperature for heat activation of vanilloid receptor 1. *J Neurophysiol* 2002; 88: 544-8
- Nagy I, Paule CC, White JPM, Urban L: Functional molecular biology of the transient receptor potential vanilloid type 1 (TRPV1) ion channel, Cannabinoids and the Brain. Edited by Kofalvi A, New York, Springer, 2008, pp 101-30
- Nagy I, Paule CC, White JPM: Molecular mechanisms of TRPV1-mediated pain, *Neuroimmune Biology, Volume 8, Neurogenic Inflammation in Health and Disease*. Edited by Jancso G. Amsterdam, Elsevier B.V., 2009, pp 75-99
- Guo A, Vulchanova L, Wang J, Li X, Elde R: Immunocytochemical localisation of the vanilloid receptor 1 (VR1): Relationship to neuropeptides, the P2X3 purinoceptor and IB4 binding sites. *Eur J Neurosci* 1999; 11:946-58
- Nagy I: Sensory processing: primary afferents neurons/DRG, *Anesthetic Pharmacology: Physiologic Principles and Clinical Practice*. Edited by Evers AS, and Maze M, Philadelphia, USA, Churchill Livingstone, 2004, pp 191-7
- Mezey E, Tóth ZE, Cortright DN, Arzubi MK, Krause JE, Elde R, Guo A, Blumberg PM, Szallasi A: Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. *Proc Natl Acad Sci U S A* 2000; 97:3655-60
- Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D: The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* 1998; 21:531-43
- Voets T, Droogmans G, Wissenbach U, Janssens A, Flockerzi V, Nilius B: The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. *Nature* 2004; 430:748-54
- Amaya F, Oh-hashi K, Naruse Y, Iijima N, Ueda M, Shimosato G, Tominaga M, Tanaka Y, Tanaka M: Local inflammation increases vanilloid receptor 1 expression within distinct subgroups of DRG neurons. *Brain Res* 2003; 14:190-6
- Amaya F, Shimosato G, Nagano M, Ueda M, Hashimoto S, Tanaka Y, Suzuki H, Tanaka M: NGF and GDNF differentially regulate TRPV1 expression that contributes to development of inflammatory thermal hyperalgesia. *Eur J Neurosci* 2004; 20:2303-10
- Rang HP, Dale MM, Ritter JM, Flower RJ: Local hormones, inflammation and immune reactions, *Pharmacology*, 6th edition. Edited by Rang HP, Dale MM, Ritter JM, Flower RJ. London, Churchill Livingstone, 2006, pp 202-25
- Leeb-Lundberg LMF, Marceau F, Muller-Esterl W, Pettibone DJ, Zuraw BL: International Union of Pharmacology. XLV Classification of kinin receptor family from molecular mechanisms to pathophysiological consequences. *Pharmacol Rev* 2005; 57:27-77
- Steranka LR, Manning DC, DeHaas CJ, Ferkany JW, Borosky SA, Connor JR, Vavrek RJ, Stewart JM, Snyder SH: Bradykinin as a pain mediator: Receptors are localized to sensory neurons, and antagonists have analgesic actions. *Proc Natl Acad Sci U S A* 1988; 85:3245-9
- Faussner A, Bathon JM, Proud D: Comparison of the responses of B1 and B2 kinin receptors to agonist stimulation. *Immunopharmacology* 1999; 45:13-20
- Marceau F, Sabourin T, Houle S, Fortin JP, Petitclerc E, Molinaro G, Adam A: Kinin receptors: Functional aspects. *Int Immunopharmacol* 2002; 2:1729-39
- Calixto JB, Medeiros R, Fernandes ES, Ferreira J, Cabrini DA, Campos MM: Kinin B1 receptors: Key G-protein-

- coupled receptors and their role in inflammatory and painful processes. *Br J Pharmacol* 2004; 143:803-18
31. Marceau F, Larrivee JF, Bouthillier J, Bachvarova M, Houle S, Bachvarov DR: Effect of endogenous kinins, prostanooids, and NO on kinin B1 and B2 receptor expression in the rabbit. *Am J Physiol* 1999; 277 (Regul Integr Comp Physiol 46):R1568-78
 32. Higashi H, Ueda N, Nishi S, Gallagher JP, Shinnick-Gallagher P: Chemoreceptors for serotonin (5-HT), acetylcholine (ACh), bradykinin (BK), histamine (H), and gamma-aminobutyric acid (GABA) on rabbit visceral afferent neurons. *Brain Res Bull* 1982; 8:23-32
 33. Oh EJ, Weinreich D: Bradykinin decreases K(+) and increases Cl(-) conductances in vagal afferent neurones of the guinea pig. *J Physiol* 2004; 558:513-26
 34. Usachev YM, De Marco SJ, Campbell C, Strehler EE, Thayer SA: Bradykinin and ATP accelerate Ca(2+) efflux from rat sensory neurons via protein kinase C and the plasma membrane Ca(2+) pump isoform 4. *Neuron* 2002; 33:113-22
 35. Shin J, Cho H, Hwang SW, Jung J, Shin CY, Lee SY, Kim SH, Lee MG, Choi YH, Kim J, Haber NA, Reichling DB, Khasar S, Levine JD, Oh U: Bradykinin-12-lipoxygenase-VR1 signaling pathway for inflammatory hyperalgesia. *Proc Natl Acad Sci U S A* 2002; 99:10150-5
 36. Cesare P, Dekker LV, Sardini A, Parker PJ, McNaughton PA: Specific involvement of PKC-epsilon in sensitization of the neuronal response to painful heat. *Neuron* 1999; 23:617-24
 37. Oshita K, Inoue A, Tang HB, Nakata Y, Kawamoto, Yuge O: CB(1) cannabinoid receptor stimulation modulates transient receptor potential vanilloid receptor 1 activities in calcium influx and substance P release in cultured rat dorsal root ganglion cells. *J Pharmacol Sci* 2005; 97: 377-85
 38. Tang HB, Inoue A, Iwasa M, Hide I, Nakata Y: Substance P release evoked by capsaicin or potassium from rat cultured dorsal root ganglion neurons is conversely modulated by bradykinin. *J Neurochem* 2006; 97:1412-8
 39. Tang HB, Inoue A, Oshita K, Nakata Y: Sensitization of vanilloid receptor 1 induced by bradykinin via the activation of second messenger signalling cascades in rat primary afferent neurons. *Eur J Pharmacol* 2004; 498:37-43
 40. Inoue A, Iwasa M, Nishikura Y, Ogawa S, Nakasuka A, Nakata Y: The long-term exposure of rat cultured dorsal root ganglion cells to bradykinin induced the release of prostaglandin E2 by the activation of cyclooxygenase-2. *Neurosci Lett* 2006; 401:242-7
 41. Ebersberger A, Grubb BD, Willingale HL, Gardiner NJ, Nebe J, Schaible HG: The intraspinal release of prostaglandin E2 in a model of acute arthritis is accompanied by an up-regulation of cyclooxygenase-2 in the spinal cord. *Neuroscience* 1999; 93:775-81
 42. Claudino RF, Kasssuya CAL, Ferreira J, Calixto JB: Pharmacological and molecular characterization of the mechanisms involved in prostaglandin E2-induced mouse paw edema. *J Pharmacol Exp Ther* 2006; 318:611-8
 43. Narumiya S, Sugimoto Y, Ushikubi F: Prostanoid receptors: Structures, properties, and functions. *Physiol Rev* 1999; 79:1193-226
 44. Murata T, Ushikubi F, Matsuoka T, Hirata M, Yamasaki A, Sugimoto Y, Ichikawa A, Aze Y, Tanaka T, Yoshida N, Ueno A, Oh-ishi S, Narumiya S: Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. *Nature* 1997; 388:678-82
 45. Wienecke T, Olesen J, Oturai PS, Ashina M: Prostacyclin (epoprostenol) induces headache in healthy subjects. *Pain* 2008; 139:1006-16
 46. Pulichino AM, Rowland S, Wu T, Clark P, Xu D, Mathieu MC, Riendeau D, Audoly LP: Prostacyclin antagonism reduces pain and inflammation in rodent models of hyperalgesia and chronic arthritis. *J Pharmacol Exp Ther* 2006; 319:1043-50
 47. Honda T, Segi-Nishida E, Miyahi Y, Narumiya S: Prostacyclin-IP signaling and prostaglandin E2-EP2/EP4 signaling both mediate joint inflammation in mouse collagen-induced arthritis. *J Exp Med* 2006; 203:325-35
 48. Moriyama T, Higashi T, Togashi K, Iida T, Segi E, Sugimoto Y, Tominaga T, Narumiya S, Tominaga M: Sensitization of TRPV1 by EP1 and IP reveals peripheral nociceptive mechanism of prostaglandins. *Molecular Pain* 2005; 1:3
 49. Rost B, Hanf G, Ohnemus U, Otto-Knapp R, Groneberg DA, Kunkel G, Noga O: Monocytes of allergies and non-allergics produce, store and release the neurotrophins NGF, BDNF and NT-3. *Regul Pept* 2005; 124:19-25
 50. Noga O, Englmann C, Hanf G, Grutzkau A, Seybold J, Kunkel G: The production, storage and release of the neurotrophins nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3 by human peripheral eosinophils in allergies and non-allergics. *Clin Exp Allergy* 2003; 33:649-54
 51. Leon A, Buriani A, Dal Toso R, Fabris M, Romanello S, Aloe L, Levi-Montalcini R: Mast cells synthesize, store, and release nerve growth factor. *Proc Natl Acad Sci U S A* 1994; 91:3739-43
 52. Ding WL, Wang WJ: The expressions of NGF and BDNF in Schwann cells cultured in vitro. *Chin J Anat* 2003; 26: 568-71
 53. Lipnik-Stangelj M: Multiple role of histamine H1-receptor-PKC-MAPK signalling pathway in histamine-stimulated nerve growth factor synthesis and secretion. *Biochem Pharmacol* 2006; 72:1375-81
 54. Abe Y, Akeida K, An HS, Aoki Y, Pichika R, Muehleman C, Kimura T, Masuda K: Proinflammatory cytokines stimulate the expression of nerve growth factor by human intervertebral disc cells. *Spine* 2007; 32:635-42
 55. Hattori A, Tanaka E, Murase K, Ishida N, Chatani Y, Tsujimoto M, Hayashi K, Kohno M: Tumor necrosis factor stimulates the synthesis and secretion of biologically active nerve growth factor in non-neuronal cells. *J Biol Chem* 1993; 268:2577-82
 56. Woolf CJ, Allchorne A, Safieh-Garabedian B, Poole S: Cytokines, nerve growth factor and inflammatory hyperalgesia: The contribution of tumour necrosis factor alpha. *Br J Pharmacol* 1997; 121:417-24
 57. Toyomoto M, Ohta M, Okumura K, Yano H, Matsumoto K, Inoue S, Hayashi K, Ikeda K: Prostaglandins are powerful inducers of NGF and BDNF production in mouse astrocyte cultures. *FEBS Lett* 2004; 562:211-5
 58. Wiesmann C, Ullsch MH, Bass SH, de Vos AM: Crystal structure of nerve growth factor in complex with the ligand-binding domain of the TrkA receptor. *Nature* 1999; 401:184-8
 59. Shu X, Mendell LM: Acute sensitization by NGF of the response of small-diameter sensory neurons to capsaicin. *J Neurophysiol* 2001; 86:2931-8
 60. Svensson P, Cairns BE, Wang K, Arendt-Nielsen L: Injection of nerve growth factor into human masseter muscle evokes long-lasting mechanical allodynia and hyperalgesia. *Pain* 2003; 104:241-7
 61. Banik RK, Subieta AR, Wu C, Brennan TJ: Increased nerve growth factor after rat plantar incision contributes to guarding behavior and heat hyperalgesia. *Pain* 2005; 117: 68-76
 62. Wild KD, Bian D, Zhu D, Davis J, Bannon AW, Zhang TJ, Louis JC: Antibodies to nerve growth factor reverse established tactile allodynia in rodent models of neuropathic pain without tolerance. *J Pharmacol Exp Ther* 2007; 322:282-7
 63. Prescott Ed, Julius D: A modular PIP2 binding site as a determinant of capsaicin receptor sensitivity. *Science* 2003; 300:1284-8
 64. Ji RR, Samad TA, Jin SX, Schmolli R, Woolf CJ: P38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. *Neuron* 2002; 26:57-68
 65. Bonnington JK, McNaughton PA: Signalling pathways in

- involved in the sensitization of mouse nociceptive neurons by nerve growth factor. *J Physiol* 2003; 551:433-46
66. Zhuang ZY, Xu H, Clapham DE, Ji RR: Phosphatidylinositol 3-kinase activates ERK in primary sensory neurons and mediates inflammatory heat hyperalgesia through TRPV1 sensitization. *J Neurosci* 2004; 24:8300-9
 67. Zhang X, Huang J, McNaughton PA: NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. *EMBO J* 2005; 24:4211-23
 68. Stein AT, Ufret-Vincenty CA, Hua L, Santana LF, Gordon SE: Phosphoinositide 3-kinase binds to TRPV1 and mediates NGF-stimulated TRPV1 trafficking to the plasma membrane. *J Gen Physiol* 2006; 128:509-22
 69. Zhu W, Oxford GS: Phosphoinositide-3-kinase and mitogen activated protein kinase signaling pathways mediate acute NGF sensitization of TRPV1. *Mol Cell Neurosci* 2007; 24:689-700
 70. Airaksinen MS, Saarma M: The GDNF family: Signalling, biological functions and therapeutic value. *Nature Rev Neurosci* 2002; 3:383-94
 71. Ernsberger U: The role of GDNF family ligand signalling in the differentiation of sympathetic and dorsal root ganglion neurons. *Cell Tissue Res* 2008; 33:353-71
 72. Forrest SL, Keast JR: Expression of receptors for glial cell line-derived neurotrophic factor family ligands in sacral spinal cord reveals separate targets of pelvic afferent fibers. *J Comp Neurol* 2008; 506:989-1002
 73. Malin SA, Molliver DC, Koerber HR, Cornuet P, Frye R, Alberts KM, Davis BM: Glial cell line-derived neurotrophic factor family members sensitize nociceptors in vitro and produce thermal hyperalgesia in vivo. *J Neurosci* 2006; 26:8588-99
 74. Elitt CM, McIlwrath SL, Lawson JJ, Malin SA, Molliver DC, Cornuet PK, Koerber HR, Davis BM, Albers KM: Artemin overexpression in skin enhances expression of TRPV1 and TRPA1 in cutaneous sensory neurons and leads to behavioral sensitivity to heat and cold. *J Neurosci* 2006; 26:8578-87
 75. Elitt CM, Malin SA, Koerber HR, Davis BM, Albers KM: Overexpression of artemin in the tongue increases expression of TRPV1 and TRPA1 in trigeminal afferents and causes oral sensitivity to capsaicin and mustard oil. *Brain Res* 2008; 1230:80-90
 76. Rang HP, Dale MM, Ritter JM, Flower RJ: Other peripheral mediators: 5-Hydroxytryptamine and purines. *Pharmacology*, 6th edition. Edited by Rang HP, Dale MM, Ritter JM, Flower RJ. London, Churchill Livingstone, 2006, 189-201
 77. Yamashita M, Fukui H, Sugama K, Horio Y, Ito S, Mizoguchi H, Wada H: Expression cloning of a cDNA encoding the bovine histamine H1 receptor. *Proc Natl Acad Sci U S A* 1991; 88:11515-9
 78. Gantz I, Schaffer M, DelValle J, Logsdon C, Campbell V, Uhler M, Yamada T: Molecular cloning of a gene encoding the histamine H2 receptor. *Proc Natl Acad Sci U S A* 1991; 88:429-33
 79. Lovenberg TW, Roland BL, Wilson SJ, Jiang X, Pyati J, Huvar A, Jackson MR, Erlander MG: Cloning and functional expression of the human histamine H3 receptor. *Mol Pharmacol* 1999; 55:1101-7
 80. Nguyen T, Shapiro DA, George SR, Setola V, Lee DK, Cheng R, Rauser L, Lee SP, Lynch KR, Roth BL, O'Dowd BF: Discovery of a novel member of the histamine receptor family. *Mol Pharmacol* 2001; 59:427-33
 81. Parada CA, Tambeli CH, Cunha FQ, Ferreira SH: The major role of peripheral release of histamine and 5-hydroxytryptamine in formalin-induced nociception. *Neuroscience* 2001; 102:937-44
 82. Mobarakeh JJ, Sakurada S, Katsuyama S, Kutsuwa M, Kuramasu A, Lin ZY, Watanabe T, Hashimoto Y, Yanai K: Role of histamine H(1) receptor in pain perception: A study of the receptor gene knockout mice. *Eur J Pharmacol* 2000; 391:81-9
 83. Shim WS, Tak MH, Lee MK, Kim M, Kim M, Koo JY, Lee CH, Kim M, Oh U: TRPV1 mediates histamine-induced itching via the activation of phospholipase A2 and 12-lipoxygenase. *J Neurosci* 2007; 27:2331-7
 84. Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sörngård M, Di Marzo V, Julius D, Högestätt ED: Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* 1999; 400:452-7
 85. Olah Z, Karai L, Iadarola MJ: Anandamide activates vanilloid receptor 1 (VR1) at acidic pH in dorsal root ganglia neurons and cells ectopically expressing VR1. *J Biol Chem* 2001; 276:31163-70
 86. Singh Tahim A, Santha P, Nagy I: Inflammatory mediators convert anandamide into a potent activator of the vanilloid type 1 transient receptor potential receptor in nociceptive primary sensory neurons. *Neuroscience* 2005; 136:539-48
 87. Ryu S, Liu B, Qin F: Low pH potentiates both capsaicin binding and channel gating of VR1 receptors. *J Gen Physiol* 2003; 122:45-61
 88. Jordt SE, Tominaga M, Julius D: Acid potentiation of the capsaicin receptor determined by a key extracellular site. *Proc Natl Acad Sci U S A* 2000; 97:8134-9
 89. Silver IA, Murrills RJ, Etherington DJ: Microelectrode studies on the acid microenvironment beneath adherent macrophages and osteoclasts. *Exp Cell Res* 1998; 175:266-76
 90. Ahern GP, Brooks IM, Miyares RL, Wang XB: Extracellular cations sensitize and gate capsaicin receptor TRPV1 modulating pain signaling. *J Neurosci* 2005; 25:5109-16
 91. Kress M, Fetzer S, Reeh PW, Vyklicky L: Low pH facilitates capsaicin responses in isolated sensory neurons of the rat. *Neurosci Lett* 1996; 211:5-8
 92. Liu B, Hui K, Qin F: Thermodynamics of heat activation of single capsaicin ion channels VR1. *Biophys J* 2003; 85:2988-3006
 93. Liang YF, Haake B, Reeh PW: Sustained sensitization and recruitment of rat cutaneous nociceptors by bradykinin and a novel theory of its excitatory action. *J Physiol* 2001; 523:229-39
 94. Susankova K, Tousova K, Vyklicky L, Teisinger J, Vlachova V: Reducing and oxidizing agents sensitize heat-activated vanilloid receptor (TRPV1) current. *Mol Pharmacol* 2006; 70:383-94
 95. Babes A, Amuzescu B, Krause U, Scholz A, Flonta ML, Reid G: Cooling inhibits capsaicin-induced currents in cultured rat dorsal root ganglion neurones. *Neurosci Lett* 2002; 317:131-4
 96. Tai C, Zhu S, Zhou N: TRPA1: The central molecule for chemical sensing in pain pathway? *J Neurosci* 2008; 28:1019-21
 97. Doerner JF, Gisselmann, Hatt H, Wetzel CH: Transient receptor potential channel A1 is directly gated by calcium ions. *J Biol Chem* 2007; 282:13180-9
 98. Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR, Earley TJ, Hergarden AC, Andersson DA, Hwang SW, McIntyre P, Jegia T, Bevan S, Patapoutian A: ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell* 2003; 112:819-29
 99. Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Hogestätt ED, Meng ID, Julius D: Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* 2004; 427:260-5
 100. Kobayashi K, Fukuoka T, Obata K, Yamanaka H, Dai Y, Tokunaga A, Noguchi K: Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with delta/c-fibers and colocalization with trk receptors. *J Comp Neurol* 2005; 493:596-606
 101. Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ, Patapoutian A: Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron* 2004; 41:849-57
 102. Obata K, Katsura H, Mizushima T, Yamanaka H, Kobayashi K, Dai Y, Fukuoka T, Tokunaga A, Tominaga M, Noguchi K: TRPA1 induced in sensory neurons contrib-

- utes to cold hyperalgesia after inflammation and nerve injury. *J Clin Invest* 2005; 115:2393-401
103. Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D: TRPA1 mediates the inflammatory actions of environmental irritants and progalectic agents. *Cell* 2006; 124:1269-82
 104. Macpherson LJ, Dubin AE, Evans MJ, Marr F, Schultz PG, Cravatt BF, Patapoutian A: Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. *Nature* 2007; 445:541-5
 105. Wang S, Dai Y, Fukuoka T, Yamanaka H, Kobayashi K, Obata K, Cui X, Tominaga M, Noguchi K: Phospholipase C and protein kinase A mediate bradykinin sensitization of TRPA1: A molecular mechanism of inflammatory pain. *Brain* 2008; 131:1241-51
 106. Taylor-Clark TE, Udem BJ, Macglashan DW Jr, Ghatta S, Carr MJ, McAlexander MA: Prostaglandin-induced activation of nociceptive neurons via direct interaction with TRPA1. *Mol Pharmacol* 2008; 73:274-81
 107. Andersson DA, Gentry C, Moss S, Bevan S: Transient receptor potential A1 is a sensory receptor for multiple products of oxidative stress. *J Neurosci* 2008; 28:2485-94
 108. Dai Y, Wang S, Tominaga M, Yamamoto S, Fukuoka T, Higashi T, Kobayashi K, Obata K, Yamanaka H, Noguchi K: Sensitization of TRPA1 by PAR2 contributes to the sensation of inflammatory pain. *J Clin Invest* 2007; 117:1979-87
 109. Yang J, Li Y, Zuo X, Zhen Y, Yu Y, Gao L: Transient receptor potential ankyrin-1 participates in visceral hyperalgesia following experimental colitis. *Neurosci Lett* 2008; 440:237-41
 110. Nilius B, Vriens J, Prenen J, Droogmans G, Voets T: TRPV4 calcium entry channel: A paradigm for gating diversity. *Am J Physiol Cell Physiol* 2004; 286:C195-205
 111. Liedtke W: TRPV4 plays an evolutionary conserved role in the transduction of osmotic and mechanical stimuli in live animals. *J Physiol* 2005; 567:53-8
 112. Suzuki M, Mizuno A, Kodaira K, Imai M: Impaired pressure sensation in mice lacking TRPV4. *J Biol Chem* 2003; 278:22664-8
 113. Shibasaki K, Suzuki M, Mizuno A, Tominaga M: Effects of body temperature on neural activity in the hippocampus: regulation of resting membrane potentials by transient receptor potential vanilloid 4. *J Neurosci* 2007; 27:1566-75
 114. Suzuki M, Watanabe Y, Oyama Y, Mizuno A, Kusano E, Hirao A, Ookawara S: Localization of mechanosensitive channel TRPV4 in mouse skin. *Neurosci Lett* 2003; 353:189-92
 115. Alessandri-Haber N, Yeh JJ, Boyd AE, Parada CA, Chen X, Reichling DB, Levine JD: Hypotonicity induces TRPV4-mediated nociception in rat. *Neuron* 2003; 39:497-511
 116. Alessandri-Haber N, Joseph E, Dina OA, Liedtke W, Levine JD: TRPV4 mediates pain-related behaviour induced by mild hypertonic stimuli in the presence of inflammatory mediator. *Pain* 2005; 118:70-9
 117. Alessandri-Haber N, Dina OA, Joseph EK, Reichling D, Levine JD: A transient receptor potential vanilloid 4-dependent mechanism of hyperalgesia is engaged by concerted action of inflammatory mediators. *J Neurosci* 2006; 26:3864-74
 118. Chen X, Alessandri-Haber N, Levine JD: Marked attenuation of inflammatory mediator-induced C-fiber sensitization for mechanical and hypotonic stimuli in TRPV4^{-/-} mice. *Mol Pain* 2007; 3:31
 119. Alessandri-Haber N, Dina OA, Joseph EK, Reichling DB, Levine JD: Interaction of transient receptor potential vanilloid 4, integrin, and SRC tyrosine kinase in mechanical hyperalgesia. *J Neurosci* 2008; 28:1046-57
 120. Brierley SM, Page AJ, Hughes PA, Adam B, Liebrechts T, Cooper NJ, Holtmann G, Liedtke W, Blackshaw LA: Selective role for TRPV4 ion channels in visceral sensory pathways. *Gastroenterology* 2008; 134:2059-69
 121. Sipe WE, Brierley SM, Martin CM, Phillis BD, Cruz FB, Grady EF, Liedtke W, Cohen DM, Vanner S, Blackshaw LA, Bunnett NW: Transient receptor potential vanilloid 4 mediates protease activated receptor 2-induced sensitization of colonic afferent nerves and visceral hyperalgesia. *Am J Physiol Gastrointest Liver Physiol* 2008; 294:G1288-98
 122. Habler C: Über den K⁺ und Ca⁺⁺ gehalt von eiter und exsudaten und seine beziehungen zum entzündungsschmerz. *Klin Wochenschr* 1929; 8:1569-72
 123. von Gaza W, Brandi B: Beziehungen zwischen wasserstoffionenkonzentration und schmerzempfindung. *Klin Wochenschr* 1926; 5:1123-7
 124. Steen KH, Steen AE, Kreysel HW, Reeh PW: Inflammatory mediators potentiate pain induced by experimental tissue acidosis. *Pain* 1996; 66:163-70
 125. Steen KH, Reeh PW, Anton F, Handwerder HO: Protons selectively induce lasting excitation and sensitization to mechanical stimulation of nociceptors in rat skin, in vitro. *J Neurosci* 1992; 12:86-95
 126. Woo YC, Park SS, Subleta AR, Brennan TJ: Changes in tissue pH and temperature after incision indicate acidosis may contribute to postoperative pain. *ANESTHESIOLOGY* 2004; 101:468-75
 127. Steen KH, Reeh PW: Sustained graded pain and hyperalgesia from harmless experimental tissue acidosis in human skin. *Neurosci Lett* 1993; 154:113-6
 128. Waldmann R, Champigny G, Bassilana F, Heurteaux C, Lazdunski M: A proton-gated cation channel involved in acid-sensing. *Nature* 1997; 386:173-7
 129. Bässler EL, Ngo-Anh TJ, Geisler HS, Ruppertsberg JP, Gründer S: Molecular and functional characterization of acid-sensing ion channel (ASIC) 1b. *J Biol Chem* 2001; 276:33782-7
 130. Smith ES, Zhang X, Cadiou H, McNaughton PA: Proton binding sites involved in the activation of acid-sensing ion channel ASIC2a. *Neurosci Lett* 2007; 426:12-7
 131. Immke DC, McCleskey EW: Protons open acid-sensing ion channels by catalyzing relief of Ca⁺⁺ blockade. *Neuron* 2003; 37:75-84
 132. Zhang P, Sigworth FJ, Canessa CM: Gating of acid-sensitive ion channel-1: Release of Ca⁺⁺ block vs. allosteric mechanism. *J Gen Physiol* 2006; 127:109-17
 133. Leffler A, Monter B, Koltzenburg M: The role of the capsaicin receptor TRPV1 and acid-sensing ion channels (ASICs) in proton sensitivity of subpopulations of primary nociceptive neurons in rats and mice. *Neuroscience* 2006; 139:699-709
 134. Sugiura T, Bielefeldt K, Gebhart GF: Mouse colon sensory neurons detect extracellular acidosis via TRPV1. *Am J Physiol Cell Physiol* 2007; 292:C1768-74
 135. Sugiura T, Dang K, Lamb K, Bielefeldt K, Gebhart GF: Acid-sensing properties in rat gastric sensory neurons from normal and ulcerated stomach. *J Neurosci* 2005; 25:2617-27
 136. Jones NG, Slater R, Cadiou H, McNaughton P, McMahon SB: Acid-induced pain and its modulation in humans. *J Neurosci* 2004; 24:10974-9
 137. Mamet J, Baron A, Lazdunski M, Voilley N: Proinflammatory mediators, stimulators of sensory neuron excitability via the expression of acid-sensing ion channels. *J Neurosci* 2002; 22:10662-70
 138. Smith ES, Cadiou H, McNaughton PA: Arachidonic acid potentiates acid-sensing ion channels in rat sensory neurons by a direct action. *Neuroscience* 2007; 145:686-98
 139. Nagae M, Hiraga T, Yoneda T: Acidic microenvironment created by osteoclasts causes bone pain associated with tumor colonization. *J Bone Miner Metab* 2007; 25:99-104
 140. Matta JA, Cornett PM, Miyares RL, Abe K, Sahibzada N, Ahern GP: General anesthetics activate a nociceptive ion channel to enhance pain and inflammation. *Proc Natl Acad Sci U S A* 2008; 105:8784-9
 141. Cornett PM, Matta JA, Ahern GP: General anesthetics

- sensitize the capsaicin receptor TRPV1. *Mol Pharmacol* 2008; 74:1180-2
142. Leffler A, Fischer MJ, Rehner D, Kienel S, Kistner K, Sauer SK, Gavva NR, Reeh PW, Nau C: The vanilloid receptor TRPV1 is activated and sensitized by local anesthetics in rodent sensory neurons. *J Clin Invest* 2008; 118:763-76
143. Suzuki T, Koyama H, Sugimoto M, Uchida I, Mashimo T: The diverse actions of volatile and gaseous anesthetics on human-cloned 5-hydroxytryptamine 3 receptors expressed in xenopus oocytes. *ANESTHESIOLOGY* 2002; 96:699-704
144. Dickinson R, Peterson BK, Banks P, Simillis C, Martin JC, Valenzuela CA, Maze M, Franks NP: Competitive inhibition at the glycine site of the *N*-methyl-D-aspartate receptor by the anesthetics xenon and isoflurane: Evidence from molecular modeling and electrophysiology. *ANESTHESIOLOGY* 2007; 107:694-6
145. Garcia-Martinez C, Fernandez-Carvajal A, Valenzuela B, Gomis A, Van Den Nest W, Ferroni S, Carreno C, Belmonte C, Ferrer-Montiel A: Design and characterization of a noncompetitive antagonist of the transient receptor potential vanilloid subunit 1 channel with in vivo analgesic and anti-inflammatory activity. *J Pain* 2006; 7:735-46
146. Oseguera AJ, Islas LD, Garcia-Villegas R, Rosenbaum T: On the mechanism of TBA block of the TRPV1 channel. *Biophys J* 2007; 92:3901-14
147. Colloc'h N, Sopkova-de Oliveira Santos J, Retailliau P, Vivares D, Bonnete F, Laglois d'Estainto B, Gallois B, Brisson A, Risso JJ, Lemaire M, Prange T, Abraini JH: Protein crystallography under xenon and nitrous oxide pressure: Comparison with in vivo pharmacology studies and implications for the mechanism of inhaled anesthetic action. *Biophys J* 2007; 92:217-24