Molecular mechanisms of nociception

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The sensation of pain alerts us to real or impending injury and triggers appropriate protective responses. Unfortunately, pain often outlives its usefulness as a warning system and instead becomes chronic and debilitating. This transition to a chronic phase involves changes within the spinal cord and brain, but there is also remarkable modulation where pain messages are initiated — at the level of the primary sensory neuron. Efforts to determine how these neurons detect pain-producing stimuli of a thermal, mechanical or chemical nature have revealed new signalling mechanisms and brought us closer to understanding the molecular events that facilitate transitions from acute to persistent pain.

Just as beauty is not inherent in a visual image, pain is a complex experience that involves not only the transduction of noxious environmental stimuli, but also cognitive and emotional processing by the brain. Progress has been made in identifying cortical loci that process pain messages, but far greater advances have been made in understanding the molecular mechanisms whereby primary sensory neurons detect pain-producing stimuli, a process referred to as nociception. These insights have arisen predominantly from the analysis of sensory systems in mammals, as well as from studies of invertebrates. Of course, invertebrate organisms do not experience pain per se, but they do have transduction mechanisms that enable them to detect and avoid potentially harmful stimuli in their environment. These signalling pathways can be regarded as the evolutionary precursors of nociceptive processing in vertebrates, and genetic studies have facilitated the identification and functional characterization of molecules and signalling pathways that contribute to the detection of noxious stimuli in animals. Indeed, many of the receptors and ion channels we refer to here are related to molecules highlighted in the accompanying reviews on mechanosensation, vision or olfaction in flies or worms.

The primary afferent nociceptor

Nearly a century ago, Sherrington proposed the existence of the nociceptor, a primary sensory neuron that is activated by stimuli capable of causing tissue damage[1]. According to this model, nociceptors have characteristic thresholds or sensitivities that distinguish them from other sensory nerve fibres. Electrophysiological studies have, in fact, shown the existence of primary sensory neurons that can be excited by noxious heat, intense pressure or irritant chemicals, but not by innocuous stimuli such as warming or light touch[2]. In this respect, acute pain can be regarded as a sensory modality much like vision or olfaction, where stimuli of a certain quality or intensity are detected by cells with appropriately tuned receptive properties.

Many types of nociceptors for many types of pain

Pain is unique among sensory modalities in that electrophysiological recordings of single primary sensory fibres have been made in awake humans, allowing simultaneous measurement of psychophysical responses when regions of the head and body are stimulated[3]. Fibres that innervate regions of the head and body arise from cell bodies in trigeminal and dorsal root ganglia (DRG), respectively, and can be categorized into three main groups based on anatomical and functional criteria (Fig. 1a). Cell bodies with the largest diameters give rise to myelinated, rapidly conducting Aβ primary sensory fibres. Most, but not all[4], Aβ fibres detect innocuous stimuli applied to skin, muscle and joints and thus do not contribute to pain. Indeed, stimulation of large fibres can reduce pain, as occurs when you activate them by rubbing your hand. By contrast, small- and medium-diameter cell bodies give rise to most of the nociceptors, including unmyelinated, slowly conducting C fibres and thinly myelinated, more rapidly conducting Aδ fibres, respectively. It has long been assumed that Aδ and C nociceptors mediate 'first' and 'second' pain, respectively, namely the rapid, acute, sharp pain and the delayed, more diffuse, dull pain evoked by noxious stimuli[5] (Fig. 1b).

There are two main classes of Aδ nociceptor[6]; both respond to intense mechanical stimuli, but can be distinguished by their differential responsiveness to intense heat or how they are affected by tissue injury. Most C-fibre nociceptors are also polymodal, responding to noxious thermal and mechanical stimuli[6]. Others are mechanically insensitive, but respond to noxious heat. Importantly, most C-fibre nociceptors also respond to noxious chemical stimuli, such as acid or capsaicin, the pungent ingredient in hot chilli peppers. Finally, the natural stimulus of some nociceptors is difficult to identify. These so-called 'silent' or 'sleeping' nociceptors are responsive only when sensitized by tissue injury[7].

These nociceptor profiles derive largely from analysis of fibres that innervate skin. But very different features characterize nociceptors in other tissues[6]. For example, although corneal afferents can be activated by capsaicin and sensitized by inflammatory mediators, pain is normally produced by innocuous tactile stimulation. In teeth, almost any stimulus produces pain. Visceral pain is unique in that there are no first (fast) and second (slow) components; instead, pain is often poorly localized, deep and dull[8]. Tissue damage is also not required for visceral pain to occur; it can result from excessive distension (for example, of the colon). And the pain of ischaemia may have unique features that reflect innervation of vasculature by distinct subsets of acid-sensitive primary sensory nociceptors. These features illustrate the difficulty of defining a nociceptor based only on activation threshold or on whether its activation evokes pain.

The neurochemistry of nociceptors

Glutamate is the predominant excitatory neurotransmitter in all nociceptors. Histochemical studies of adult DRG, however,

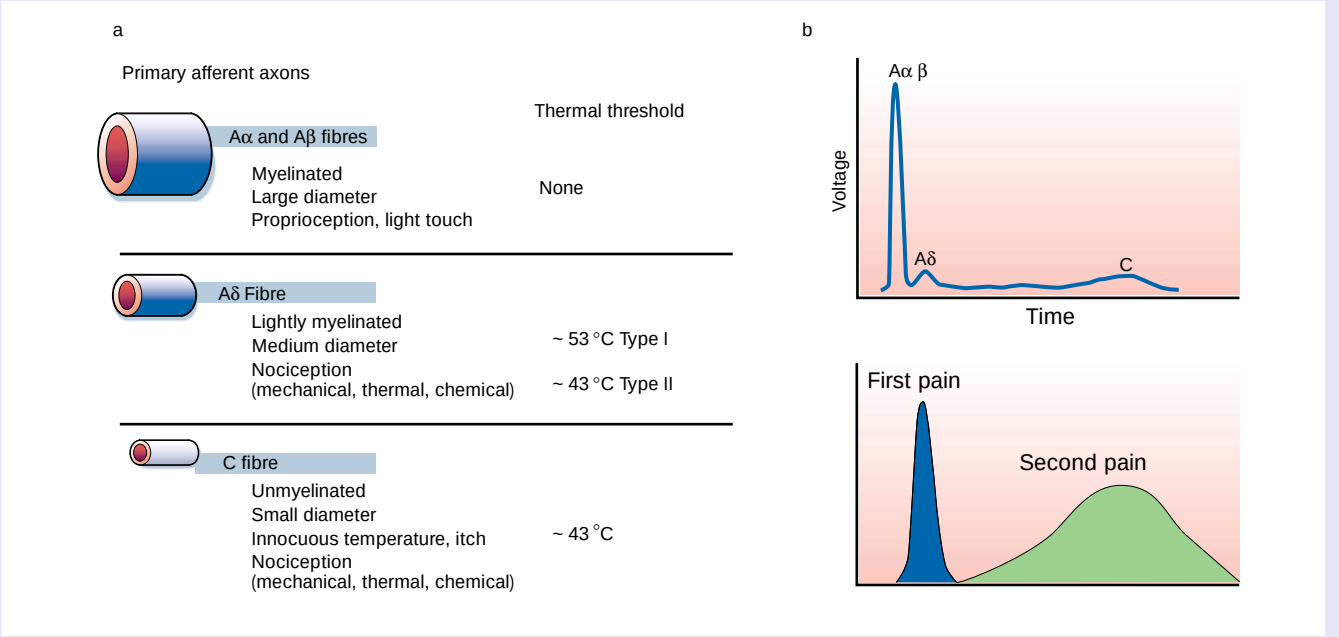

Figure 1 Different nociceptors detect different types of pain. **a**, Peripheral nerves include small-diameter (Aδ) and medium- to large-diameter (Aα,β) myelinated afferent fibres, as well as small-diameter unmyelinated afferent fibres (C). **b**, The fact that conduction velocity is directly related to fibre diameter is highlighted in the compound action potential recording from a peripheral nerve. Most nociceptors are either Aδ or C fibres, and their different conduction velocities (6–25 and ~1.0 m s^{-1}, respectively) account for the first (fast) and second (slow) pain responses to injury. Panel **b** adapted from ref. 75.

reveal two broad classes of unmyelinated C fibre. The so-called peptidergic population contains the peptide neurotransmitter substance P, and expresses TrkA, the high-affinity tyrosine kinase receptor for nerve growth factor (NGF)[9]. The second population does not express substance P or TrkA, but can be labelled selectively with the α-D-galactosyl-binding lectin IB_4, and expresses $P2X_3$ receptors, a specific subtype of ATP-gated ion channel. This categorization is a first approximation at best — as additional molecular markers become available, new subsets are likely to be recognized. It is, however, unclear whether these neurochemically distinct groups represent different functional classes of nociceptor. Moreover, because expression of neurotransmitters, their receptors and other signalling molecules are dramatically altered after tissue or nerve injury[10,11], both the significance and the complexity of nociceptor neurochemistry are increased.

Diversity of nociceptor signalling

All sensory systems must convert environmental stimuli into electrochemical signals. In the case of vision or olfaction, primary sensory neurons need only detect one type of stimulus (light or chemical odorants) and use redundant and convergent biochemical mechanisms to accomplish this goal (Fig. 2a). In this regard, nociception is unique because individual primary sensory neurons of the 'pain pathway' have the remarkable ability to detect a wide range of stimulus modalities, including those of a physical and chemical nature. Compared with sensory neurons of other systems, nociceptors must therefore be equipped with a diverse repertoire of transduction devices (Fig. 2b). At the same time, markedly different stimuli of a chemical (capsaicin and acid) or physical (heat) variety can excite nociceptors by activating a single receptor, enabling the cell to integrate information and respond to complex changes in the physiological environment.

Primary afferent nociceptors are also unique in the extent to which their receptive properties can be modulated. Thus, nociceptors not only signal acute pain, but also contribute to persistent and pathological pain conditions (allodynia) that occur in the setting of injury, wherein pain is produced by innocuous stimuli[5,12]. Allodynia can result from two different conditions: increased responsiveness of spinal cord 'pain' transmission neurons (central sensitization), or lowering of nociceptor activation thresholds (peripheral sensitization). With central sensitization, pain can be produced by activity in non-nociceptive primary sensory fibres. Peripheral sensitization is produced when nociceptor terminals become exposed to products of tissue damage and inflammation, referred to collectively as the 'inflammatory soup' (Fig. 3). Such products include extracellular protons, arachidonic acid and other lipid metabolites, serotonin, bradykinin, nucleotides and NGF, all of which interact with receptors or ion channels on sensory nerve endings. Because nociceptors can release peptides and neurotransmitters (for example, substance P, calcitonin-gene-related peptide and ATP) from their peripheral terminals when activated by noxious stimuli, they are able to facilitate production of the inflammatory soup by promoting the release of factors from neighbouring non-neuronal cells and vascular tissue, a phenomenon known as neurogenic inflammation[5].

In contrast to vision, olfaction or taste, sensory nerve endings that detect painful stimuli are not localized to a particular anatomical structure, but are instead dispersed over the body, innervating skin, muscle, joints and internal organs. Although this has made the biochemical analysis of nociceptive pathways particularly challenging, the combined application of electrophysiological, pharmacological and genetic methods are generating significant progress in understanding the molecular basis of nociceptor signalling. In some respects, the field can be compared to the state of cellular immunology some 25 years ago, when one of the main goals was to define biochemical markers for specific subsets of lymphocytes. The goal for nociceptor research is to elucidate signalling functions for key cell-surface markers and to assign physiological roles to molecularly defined sub-populations of sensory neurons.

Detectors of noxious stimuli

Response to heat

Remarkably, many functional characteristics of nociceptors are retained when sensory ganglia are dissociated and placed into culture[13]. Thus, ~45% of small- to medium-diameter neurons exhibit

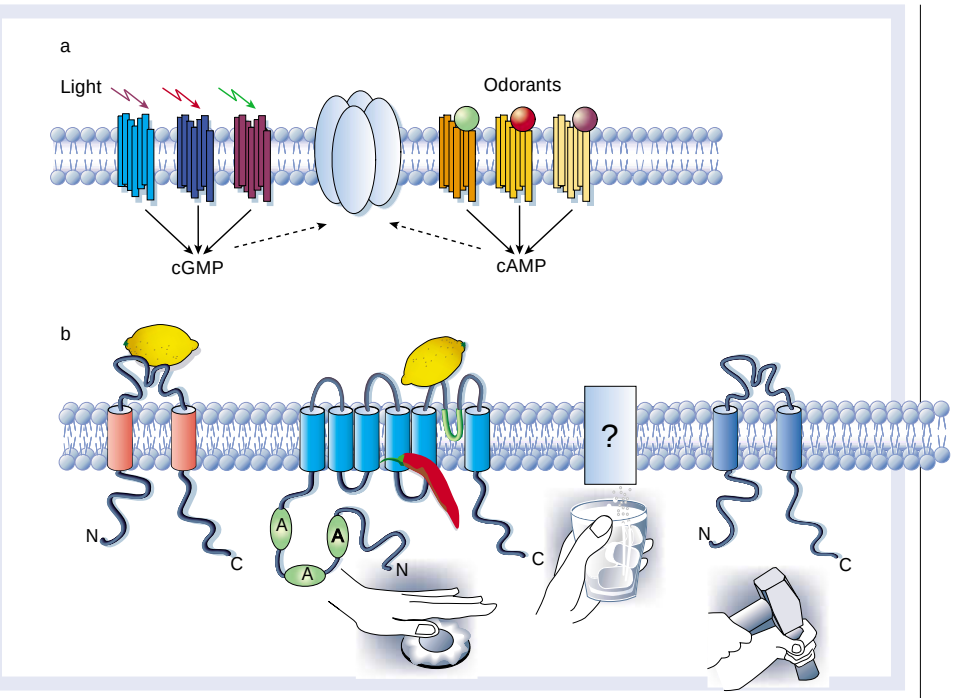

Figure 2 Polymodal nociceptors use a greater diversity of signal-transduction mechanisms to detect physiological stimuli than do primary sensory neurons in other systems. **a**, In mammals, light or odorants are detected by a convergent signalling pathway in which G-protein-coupled receptors modulate the production of cyclic nucleotide second messengers, which then alter sensory neuron excitability by regulating the activity of a single type of cation channel. **b**, In contrast, nociceptors use different signal-transduction mechanisms to detect physical and chemical stimuli. Recent studies suggest that TRP-channel family members (VR1 and VRL-1) detect noxious heat, and that ENaC/DEG-channel family detect mechanical stimuli. Molecular transducers for noxious cold remain enigmatic. Noxious chemicals, such as capsaicin or acid (that is, extracellular protons) may be detected through a common transducer (VR1), illustrating aspects of redundancy in nociception. At the same time, a single type of stimulus can interact with multiple detectors, as shown by the ability of extracellular protons to activate not only VR1, but also ASICs, which are also members of the ENaC/DEG-channel family.

heat-evoked membrane currents with a 'moderate' threshold of ~45 °C, whereas another 5–10% of cells respond with a 'high' threshold of ~52 °C and are insensitive to capsaicin[14,15]. The former corresponds presumably to C and type II Aδ nociceptors, and the latter to type I Aδ nociceptors. What is the molecular basis by which specific thermal thresholds are established in these nociceptor subtypes? The answer came from a well-proven path of success in the pain field, namely identifying the molecular targets through which natural products (for example, morphine from the opium poppy or aspirin from willow bark) produce or modulate our sensation of pain.

In the case of moderate thermal nociception by C and type II Aδ afferents, a transducer was revealed with the cloning and functional characterization of the vanilloid receptor VR1 (Fig. 2b), which is activated by capsaicin and other vanilloid compounds[16]. VR1 is a non-selective plasma-membrane cation channel possessing a very steep temperature dependence ($Q_{10} = 20.6$) and a thermal activation threshold of ~43 °C, characteristics that are shared with native heat-evoked currents in sensory neurons[17,18]. The strong correlation between moderate heat and capsaicin sensitivity, and the similarity of the non-selective cationic currents and pharmacology underlying these responses[14,15] support the hypothesis that heat and capsaicin activate a common transducer. Heat-evoked single-channel currents are observed in membrane patches excised from sensory neurons or VR1-expressing cells, indicating that VR1 is an intrinsically heat-sensitive channel that functions as a molecular thermometer at the cell surface[17].

The high correlation that exists between heat and capsaicin sensitivity in sensory neurons at the whole-cell level is less pronounced at the single-channel level[19]. Although this observation could be explained by different functional states of the channel, it has raised some controversy regarding the link between the activities of native and cloned channels. But studies of mice lacking functional VR1 channels[20,21] clearly show that cultured DRG neurons from VR1-null mice are severely deficient in moderate heat-evoked responses, whereas high-threshold heat responses persist. $VR1^{-/-}$ mice also have significantly fewer (>threefold) heat-sensitive C fibres, and total heat-evoked C-fibre output may be decreased by ~85% compared with wild-type mice. Thus, although VR1 is not the only detector of moderate-threshold heat stimuli, it accounts for the majority of such responses and must contribute significantly to thermal coding in normal animals.

Somewhat paradoxically, $VR1^{-/-}$ mice showed normal behavioural responses at temperatures near the threshold for activation of VR1 and C fibres, but exhibited markedly reduced pain behaviour at higher temperatures (>50 °C). In other words, $VR1^{-/-}$ animals recognize a noxious heat stimulus, but discriminate poorly among stimuli of different noxious intensities. Perhaps the threshold for a behavioural pain response is determined by the thermal threshold of a small number of nociceptors, whereas discrimination among suprathreshold temperatures requires information from a larger cohort of nociceptors that adequately encode stimulus intensity. In some respects, the phenotype of the $VR1^{-/-}$ mouse illustrates the futility of a long-lasting debate as to whether pain is generated by activity in specific nociceptors that are connected to the spinal cord through a dedicated pathway, or whether it is the pattern of activity across afferents that determines the pain response. Both models are likely to be relevant, depending on the magnitude of the stimulus and the context in which it is measured. Moreover, because most nociceptors are polymodal, our ability to distinguish pain sensations resulting from heat, cold or pressure must involve decoding of nociceptive signals within the central nervous system.

Another striking and significant phenotype of the $VR1^{-/-}$ mouse is its failure to develop increased sensitivity to heat in the context of tissue injury[20,21]. This deficit suggests that VR1 is modulated by one or more components of the inflammatory soup, which act on the nociceptor to increase its sensitivity to heat.

What mediates high-threshold heat responses in type I Aδ afferents? One candidate transducer is the vanilloid receptor-like (VRL-1) channel (Fig. 2b) that shares ~50% sequence identity with VR1 (ref. 22). Functional analysis of VRL-1 in transfected mammalian cells and frog oocytes showed that this channel is not responsive to vanilloid compounds, but can be activated by noxious thermal stimuli with a threshold of ~52 °C. VRL-1 is most prominently expressed by a subset of medium- to large-diameter myelinated neurons within the DRG. But because VRL-1 transcripts are also expressed outside the sensory nervous system, this channel probably serves multiple physiological functions[22,23].

VR1 and VRL-1 belong to the larger family of transient receptor potential (TRP) channels, whose core transmembrane structure resembles that of voltage-gated potassium or cyclic nucleotide-gated channels (reviewed in ref. 24). The prototypical TRP channel was discovered in the *Drosophila* phototransduction pathway, where it is activated downstream of phospholipase C (PLC)-coupled rhodopsin (see review in this issue by Hardie and Raghu, pages 186–193). Some

mammalian TRP channels are also activated by G-protein-coupled or tyrosine kinase receptors that stimulate PLC, but as in the fly, the underlying gating mechanism remains enigmatic. Recent studies indicate that PLC-mediated hydrolysis of the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) and the consequent production of lipid second messengers constitute important steps in TRP channel activation[25] (see below).

Detection of noxious cold

The definition of cold sensitivity is less stringent compared with heat sensitivity, in large part because thermal thresholds for activation of cold-sensitive fibres may not be as distinct or precipitous as they are with heat, and because the threshold for cold-evoked pain is not as precise. Whereas noxious heat (47 °C) activates ~50% of C fibres that innervate the hindpaw of a rodent, noxious cold (4 °C) may excite only 10–15% of fibres within the same cutaneous receptive field[20]. However, a significantly greater proportion of C and Aδ fibres are classified as cold-sensitive when the range of stimulus intensities extends below 0 °C (ref. 26). Few, if any, cold-sensitive (4 °C) afferents are heat sensitive, and $VR1^{-/-}$ mice show a normal prevalence of cold-responsive fibres in the hindpaw[20], indicating that noxious heat and cold are detected by distinct mechanisms. It is not known whether noxious cold depolarizes nerve fibres by inhibiting a ($Na^+ + K^+$)ATPase or background potassium current[27], or by promoting calcium and/or sodium influx[28,29].

Pressure and mechanical stress

Nociceptors can be activated by mechanical stress resulting from direct pressure, tissue deformation or changes in osmolarity, enabling the detection of touch, deep pressure, distension of a visceral organ, destruction of bone, or swelling. Functional, mechanically gated channels have yet to be identified at the molecular level in eukaryotes, although model genetic organisms such as bacteria, worms and flies have provided important leads for identifying mechanosensory transducers in mammals (see review in this issue by Gillespie and Walker, pages 194–202). One such ion channel of the degenerin (DEG/ENaC) family, called MDEG (alternatively, brain sodium channel 1 (BNC1) or acid-sensing ion channel 2 (ASIC2); Fig. 2b)[30], has attracted particular interest in the pain field because its messenger RNA is expressed in primary sensory neurons[31]. Responses of primary sensory fibres from BNC1-deficient mice to a range of mechanical stimuli identified a specific class of rapidly adapting mechanoreceptors that showed reduced sensitivity to hair movement[32]. Other types of afferents, including C fibres, showed normal responses in these mutant mice, indicating that BNC1 is involved in some aspects of innocuous mechanical (touch) sensation, but not in the detection of noxious mechanical stimuli.

One way to detect pressure or tissue deformation is through activation of a mechanically gated protein. Another mechanism might involve a 'mechanochemical' process whereby stretch evokes the release of a diffusible chemical messenger that then excites nearby primary sensory nerve terminals. Extracellular ATP is of particular interest because large- and small-diameter sensory neurons express G-protein-coupled ATP receptors or ATP-gated ion channels (P2Y and P2X receptors, respectively), and because extracellular ATP excites primary sensory neurons[33]. Using frog oocytes as a model system, Nakamura and Strittmatter[34] showed that mechanical stimulation can release ATP from the cell, promoting autocrine activation of P2Y receptors on the cell's surface. *In vivo*, mechanical force might therefore promote ATP release from one or more cell types in the periphery, where it could activate purinergic receptors (for example, $P2X_3$) on nearby nociceptor terminals.

In fact, $P2X_3$-deficient mice show reduced urination stemming from a failure to empty a full bladder[35]. Filling and stretching of the urinary bladder promotes the release of ATP from epithelial cells that lie beneath the smooth muscle layer. Once released, ATP may excite sensory nerve endings embedded within the bladder wall to initiate the voiding reflex. The deficit observed in $P2X_3^{-/-}$ mice provides compelling evidence that ATP transduces signals of mechanical tissue distension into depolarization of primary sensory neurons, in this case through direct activation of an ion channel.

Chemical transducers to make the pain worse

As described above, injury heightens our pain experience by increasing the sensitivity of nociceptors to both thermal and mechanical stimuli. This phenomenon results, in part, from the production and release of chemical mediators from the primary sensory terminal and from non-neural cells (for example, fibroblasts, mast cells, neutrophils and platelets) in the environment[36] (Fig. 3). Some components of the inflammatory soup (for example, protons, ATP, serotonin or lipids) can alter neuronal excitability directly by interacting with ion channels on the nociceptor surface, whereas others (for example, bradykinin and NGF) bind to metabotropic receptors and mediate their effects through second-messenger signalling cascades[11]. Considerable progress has been made in understanding the biochemical basis of such modulatory mechanisms.

Extracellular protons and tissue acidosis

Local tissue acidosis is a hallmark physiological response to injury, and the degree of associated pain or discomfort is well correlated with the magnitude of acidification[37]. Application of acid (pH 5) to the skin produces sustained discharges in a third

Stimulus	Representative receptor
NGF	TrkA
Bradykinin	BK_2
Serotonin	5-HT_3
ATP	$P2X_3$
H^+	ASIC3/VR1
Lipids	PGE_2/CB1/VR1
Heat	VR1/VRL-1
Pressure	DEG/ENaC ?

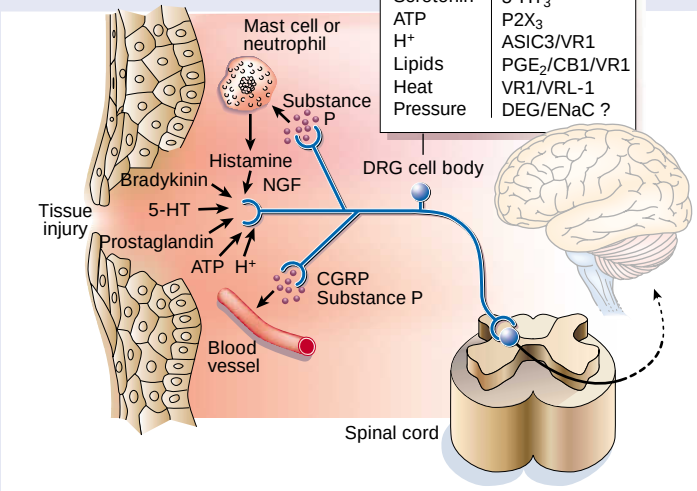

Figure 3 The molecular complexity of the primary afferent nociceptor is illustrated by its response to inflammatory mediators released at the site of tissue injury. Some of the main components of the 'inflammatory soup' are shown, including peptides (bradykinin), lipids (prostaglandins), neurotransmitters (serotonin (5-HT) and ATP) and neurotrophins (NGF). The acidic nature of the inflammatory soup is also indicated. Each of these factors sensitize (lower the threshold) or excite the terminals of the nociceptor by interacting with cell-surface receptors expressed by these neurons. Examples of these factors and representative molecular targets are indicated in the box. Activation of the nociceptor not only transmits afferent messages to the spinal cord dorsal horn (and from there to the brain), but also initiates the process of neurogenic inflammation. This is an efferent function of the nociceptor whereby release of neurotransmitters, notably substance P and calcitonin gene related peptide (CGRP), from the peripheral terminal induces vasodilation and plasma extravasation (leakage of proteins and fluid from postcapillary venules), as well as activation of many non-neuronal cells, including mast cells and neutrophils. These cells in turn contribute additional elements to the inflammatory soup. Figure adapted from refs 75,76.

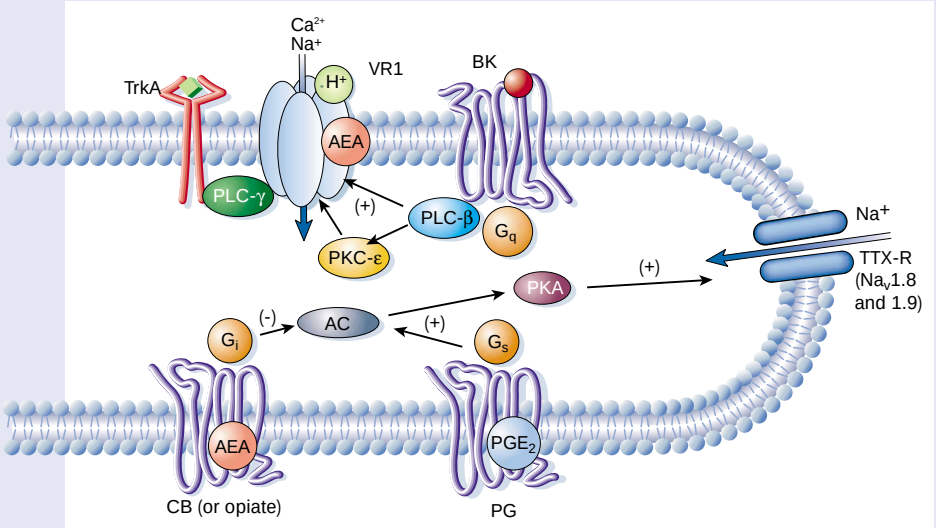

Figure 4 When nociceptors are exposed to products of injury and inflammation, their excitability is altered by a variety of intracellular signalling pathways. The figure highlights the vanilloid receptor (VR1) and tetrodotoxin-resistant (TTX-R) voltage-gated sodium channels ($Na_v1.8$ and 1.9) as downstream targets of modulation. Responses of VR1 to heat can be potentiated by direct interaction of the channel with extracellular protons (H^+) or lipid metabolites, such as anandamide (AEA). VR1 activity can also be heightened by agents such as NGF or bradykinin, which bind to their own cell-surface receptors (TrkA and BK, respectively) to stimulate phospholipase C (PLC-γ or PLC-β) signalling pathways. This, in turn, leads to hydrolysis of plasma membrane lipids and the subsequent stimulation of protein kinase C isoforms, such as PKC-ε. Both of these actions have been proposed to potentiate VR1 function. Prostaglandins (PGE_2) and other inflammatory products that activate adenylyl cyclase (AC) through G_s-coupled receptors also enhance nociceptor excitability. This occurs, in part, by a cyclic AMP-dependent protein kinase (PKA)-dependent phosphorylation of $Na_v1.8$ and/or $Na_v1.9$. By activating G_i-coupled receptors, opiates and cannabinoids can counteract these increases in excitability of the nociceptor, and produce a peripherally mediated analgesia.

or more of polymodal nociceptors that innervate the receptive field[20]. At the cellular level, protons depolarize sensory neurons by directly activating a non-selective cationic current[38,39]. In many DRG neurons, this response consists of a transient, rapidly inactivating current that is carried predominantly by Na^+ ions, followed by a sustained non-selective cationic current. Responses of a sustained nature have been proposed to underlie persistent pain associated with tissue acidosis[37], but this may not occur in all physiological settings. For example, during cardiac ischaemia, DRG neurons that innervate the epicardium show very large, but transient, response to extracellular protons[40]. A number of proton-sensitive channels are found on sensory neurons and thus an important goal has been to determine which, if any, of these molecules contributes to proton sensitivity of nociceptors *in vivo*. Two main candidates have emerged — VR1 and a family of ASICs.

The similarity between native proton (pH 5)-evoked and capsaicin-evoked currents in dissociated DRG neurons[41] is well established, and low extracellular pH can augment responses of cultured DRG neurons to capsaicin[42]. These observations suggested that protons and vanilloid compounds interact with the same ion-channel complex on the nociceptor (perhaps providing a cellular rationale for the culinary appeal of 'hot' and sour soup). Analysis of the cloned vanilloid receptor in heterologous expression systems has substantiated these observations. Protons have two main effects on VR1 function[17]. First, VR1 can be activated at room temperature when the extracellular pH drops below 6, producing currents that resemble the sustained component of proton-evoked responses observed in sensory neurons. Second, protons potentiate responses to capsaicin or heat, and do so over a concentration range (pH 6–8) that matches the extent of local acidosis associated with various forms of tissue injury. These changes in VR1 activity would be expected to increase nociceptor excitability, even at normal body temperature. Structure–function studies have now identified several negatively charged residues within putative extracellular loops of VR1 that are important for mediating these effects[18,43], supporting the idea that protons interact directly with the vanilloid receptor to allosterically modulate channel function.

Although proton-evoked changes in VR1 thermal sensitivity closely resemble those exhibited by nociceptors during inflammation, does VR1 actually contribute to pH sensitivity of nociceptors *in vivo*? DRG neurons or sensory nerve fibres from VR1-deficient mice do indeed show a marked reduction in sustained proton (pH 5)-evoked membrane currents. But proton-evoked responses, particularly those of a transient nature, are not eliminated completely in $VR1^{-/-}$ mice and may be mediated by members of the ASIC channel family.

Lazdunski and colleagues[44] described a family of two-transmembrane-domain proteins that are related to the putative mechanosensory DEG/ENaC channels. These novel cation-channel subunits were named ASICs because of their ability to be gated by reductions in extracellular pH when expressed in heterologous systems (Fig. 2b). Including splice variants, there are at least five ASIC subtypes (1a, 1b, 2a, 2b and 3) in rats, each having a unique profile of pH sensitivity, activation and desensitization rates, ionic permeability and tissue distribution. Most subtypes are expressed in DRG, with ASIC1b and ASIC3 (known also as ASIC-β and DRASIC, respectively) showing exclusive or preferential expression within sensory ganglia. Can ASIC channels account for any aspect of transient or sustained proton-evoked currents observed in DRG neurons? Heterologous expression of most ASIC subunits produces currents consisting of a single transient phase, or one having a sustained component that is Na^+-selective or observed only at non-physiological proton concentrations (≤pH 5). However, co-expression of ASIC3 with ASIC2b (a splice variant of MDEG, also called MDEG2) generates a more native-like current containing a sustained component with non-selective cation permeability[30]. McCleskey and colleagues[40] have provided strong evidence that ASIC3/2b heteromeric channels, rather than VR1, underlie the unusually large and mostly transient proton-evoked currents observed in the relatively small subpopulation (2–3%) of DRG afferents that innervate the heart. This makes good physiological sense because occlusion of a cardiac artery produces modest acidosis (to just below pH 7), but this is sufficient to activate cardiac nociceptors or ASIC3/2b.

By contrast, genetic studies indicate that MDEG (BNC1) gene products (ASIC2a and 2b) do not contribute to acid sensitivity in most non-cardiac nociceptors[32]. Thus, $BNC1^{-/-}$ mice showed no obvious decrement in pH 5-evoked responses among large- or small-diameter DRG neurons. Moreover, acid produced normal sustained discharges in cutaneous C fibres from these animals. Gene knockouts of other members of this family will be needed to clarify the contribution of ASICs to proton detection and nociceptor sensitization at various physiological sites.

Peptides and growth factors

Tissue damage promotes the release or production of bioactive peptides from non-neural cells and plasma proteins at the site of injury[45]. Principal among these is the nonapeptide bradykinin, which, when applied to primary sensory nerve terminals or cultured sensory neurons, produces immediate membrane depolarization as well as sensitization to other noxious, or even innocuous stimuli[46]. Bradykinin activates G-protein-coupled (BK_2) receptors on these cells to stimulate PLC-catalysed hydrolysis of PtdIns(4,5)P_2, consequently releasing Ca^{2+} from intracellular stores and activating protein kinase C (PKC) (Fig. 4). Treatment of cultured DRG neurons with bradykinin augments heat-evoked currents in these cells[47], an effect that may be mediated through direct or indirect modification of VR1 by the ε-isoform of PKC[48] (Fig. 4). PKC-ε-knockout mice exhibit reduced thermal and mechanical hypersensitivity after treatment with adrenaline or acetic acid[49], but effects of bradykinin have not been reported. In any event, molecular validation of this pathway will require biochemical proof that VR1 is phosphorylated in response to BK_2-receptor activation and that mutation of one or more phosphate-accepting residues abrogates channel modulation.

Bradykinin might also heighten VR1 sensitivity through a PKC-independent process[50]. In this mechanism, channel modulation occurs as a direct consequence of PLC-mediated PtdIns(4,5)P_2 hydrolysis, in effect releasing VR1 from PtdIns(4,5)P_2-mediated inhibition. Precedence for such a regulatory mechanism comes from studies of cyclic nucleotide-gated channels[51] and G-protein-gated inwardly rectifying potassium channels[52,53]. Moreover, as mentioned above, several members of the TRP channel family are activated downstream of PLC-coupled receptors, and recent evidence from both vertebrate and invertebrate systems suggests that PtdIns(4,5)P_2 hydrolysis also is important in modulating the activity of these channels[24,25]. Exogenously applied lipids, such as anandamide, arachidonate or diacylglycerol, have been shown to activate VR1 (see below) or other TRP channels (see review by Hardie and Raghu, pages 186–193), raising the possibility that these hydrophobic agonists exert their effects by displacing PtdIns(4,5)P_2 or other membrane lipids from an inhibitory site on the channel complex.

NGF is best known as a survival factor for embryonic neurons and is essential for the development of all primary sensory nociceptors. But in the adult, NGF serves a very different function, being released by mast cells, fibroblasts and other cell types at sites of injury and inflammation, where it acts on primary sensory nerve terminals to promote thermal hypersensitivity[54]. Consistent with this action, Mendell and colleagues[55] showed that exposure of cultured DRG neurons to NGF for several minutes produces acute sensitization of capsaicin-evoked responses. Although NGF elicits long-term changes in gene expression, its ability to promote short-term nociceptor sensitization suggests that post-translational mechanisms also are involved.

NGF binds to TrkA tyrosine kinase receptors on peptidergic sensory neurons to activate mitogen-activated protein (MAP) kinase and PLC-γ signalling pathways[56]. When frog oocytes expressing TrkA and VR1 are treated with NGF for several minutes, proton-, vanilloid- or heat-evoked responses are potentiated, recapitulating the sensitization that occurs in cultured DRG neurons or primary sensory fibres. A TrkA mutant that specifically disrupts coupling of the receptor to PLC-γ abrogates NGF enhancement of VR1, consistent with the potentiation mechanism outlined for bradykinin[50]. The importance of the MAP-kinase pathway in the regulation of gene expression is well appreciated, but the physiological significance of PLC activation to neurotrophin action has been enigmatic. These findings now suggest that PLC signalling contributes to NGF-mediated thermal hypersensitivity and possibly to other forms of short-term, post-translational nociceptor modulation (Fig. 4). $VR1^{-/-}$ mice do not develop thermal hypersensitivity in response to NGF or bradykinin treatment[50], indicating that this channel is a probable downstream target for modulation by these and other pro-algesic agents that activate PLC. TRP channels in the fly eye exist in a complex with other components of the phototransduction machinery (see review by Hardie and Raghu), an arrangement that influences sensitivity and kinetics of the signalling process[57,58]. Interestingly, VR1 forms a signalling complex with TrkA and PLC-γ in heterologous systems[50]. Whether a similar signalling scaffold exists in nociceptors remains to be determined, but phylogenetic comparisons suggest that this might well be the case.

Sensitization and activation by lipids

The efficacy of non-steroidal anti-inflammatory agents, such as aspirin, is attributed generally to blockade of cyclooxygenase (COX) enzymes that convert arachidonic acid, a lipid messenger, into pro-inflammatory prostanoid products, notably prostaglandin E_2 (PGE_2). Most studies indicate that PGE_2 contributes to peripheral sensitization by binding to G-protein-coupled receptors that increase levels of cyclic AMP within nociceptors[45]. However, it now seems likely that cyclooxygenase products are also present in the spinal cord, where they could interact with receptors on the central terminals of nociceptors[59]. This idea has aroused great interest because it argues that COX inhibitors may exert their pain-relieving effects by modulating nociception at both peripheral and central sites[60].

Recent studies have provided important information on a likely molecular target through which PGE_2 sensitizes primary sensory fibres. Nociceptors express a specific subclass of voltage-gated sodium channel that is resistant to blockade by tetrodotoxin[61]. These TTX-R Na^+ channels are believed to contribute significantly to action-potential firing rate and duration in small-diameter sensory neurons. Electrophysiological studies suggest that PGE_2 increases excitability of DRG neurons, in part by shifting the voltage dependence of TTX-R Na^+-channel activation in the hyperpolarizing direction. This reduces the extent of membrane depolarization needed to initiate an action potential and favours repetitive spiking. Pharmacological and biochemical studies indicate that PGE_2-dependent modulation of the TTX-R Na^+ current involves phosphorylation of the channel protein by cAMP-dependent protein kinase A[62–64] (Fig. 4). It is not known which of the two known TTX-R Na^+-channel subtypes within DRG ($Na_v1.8$ or $Na_v1.9$)[65–67] are targeted by lipids, and under what conditions this occurs. Behavioural analysis of $Na_v1.8$-deficient mice revealed modest deficits in acute sensation to noxious stimuli[68]. These animals also showed a delayed onset of inflammatory thermal hypersensitivity, but the maximal response was equivalent to that of wild-type animals. Such observations support the involvement of $Na_v1.8$ in tissue injury-evoked hypersensitivity, but also suggest that there is redundancy among voltage-gated Na^+ channel subtypes in nociceptor function.

Capsaicin is a hydrophobic molecule that bears structural similarity to several lipid second messengers (Fig. 5), leading many to suggest that lipid metabolites might serve as endogenous vanilloid-receptor agonists. The hunt for such ligands has been further advanced by the realization that the vanilloid receptor belongs to the TRP channel family, some members of which can be activated by polyunsaturated fatty acids or other lipid metabolites (see review by Hardie and Raghu, pages 186–193). Indeed, we and others have shown that the endogenous cannabinoid-receptor agonist anandamide (arachidonylethanolamide), or lipoxygenase products of arachidonic-acid metabolism (for example, 12- and 15-(*S*)-hydroperoxyeicosatetraenoic acid), activate native or cloned vanilloid receptors when applied to whole cells or excised membrane patches containing VR1 channels[69–71]. These lipid messengers are admittedly weak as VR1 agonists (EC_{50} ~ 1–10 μM at 25 °C, pH 7.6), at least when compared to capsaicin, and this has fuelled some debate as to whether they should be considered 'endovanilloids'[72]. But the physiological setting in which these molecules are likely to act as VR1 agonists is one involving inflammation, and thus the relevant question should be whether they act synergistically with other

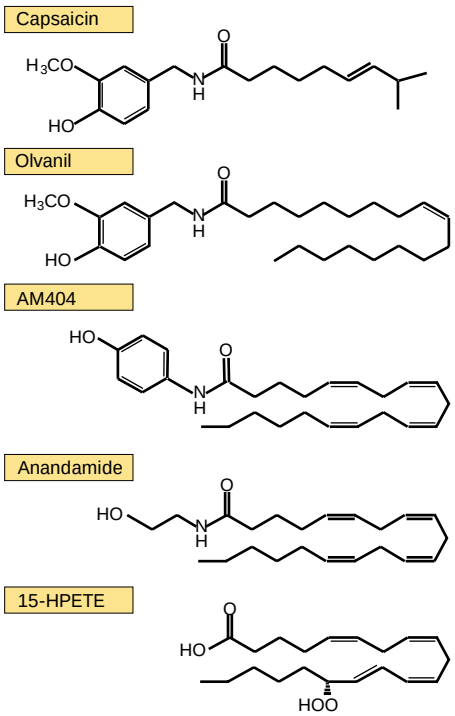

Figure 5 An alignment of natural and synthetic vanilloid receptor agonists illustrates their structural similarity. Olvanil is a synthetic, non-pungent capsaicin analogue that activates VR1 with relatively slow kinetics. Anandamide is an endogenous lipid metabolite (similar in structure to arachidonic acid) that was initially discovered as a ligand for cannabinoid receptors. AM404 is a synthetic drug that blocks cellular re-uptake of anandamide. Both compounds activate native and cloned vanilloid receptors *in vitro* with relatively slow kinetics, similar to olvanil. 15-HPETE and other lipoxygenase products of arachidonic acid metabolism activate VR1 *in vitro* with potencies (1–10 μM) resembling those of anandamide and AM404.

pro-inflammatory agents, such as bradykinin, NGF or protons, to facilitate VR1 gating and promote thermal hypersensitivity at sites of tissue injury. Finally, endogenous ligands with greater potency at VR1 may well exist, but these await discovery.

The central terminal of the primary afferent nociceptor

All primary sensory nociceptors make synaptic connections with neurons in the grey matter (dorsal horn) of the spinal cord (Fig. 3). Subsets of dorsal horn neurons, in turn, project axons and transmit pain messages to higher brain centres, including the reticular formation, thalamus and ultimately the cerebral cortex[5]. Not surprisingly, the neural circuitry within the dorsal horn is incredibly complex, and much interest is focused on understanding whether subclasses of primary sensory nociceptors and the spinal circuits that they engage contribute differentially to the main classes of clinically relevant pains, namely, inflammatory pain resulting from tissue injury and neuropathic pain resulting from nerve injury[9,12].

One of the most interesting questions concerning nociceptors is the extent to which receptors/transducers at the peripheral terminal are also functional at central (presynaptic) terminals in the spinal cord dorsal horn, and if so, whether they serve the same function. For some neurotransmitter receptors (for example, opioid receptors), the source of endogenous ligand is clear — interneurons in the superficial dorsal horn synthesize opioid peptides that can target presynaptic opioid receptors and regulate neurotransmitter release by decreasing Ca^{2+} conductance[5]. As noted above, it is likely that spinal cord-derived PGE_2 targets the central terminal of the nociceptor. Functional presynaptic purinergic receptors have also been described and the source of ATP can be identified[73]. By contrast, presynaptic vanilloid receptors will never be exposed to noxious thermal temperatures or to the magnitude of pH changes that regulate VR1 gating at the peripheral terminal of the nociceptor, and thus other ligands must be considered. Lipid mediators, such as anandamide, may be relevant. There is evidence for presynaptic regulation of neurotransmitter release through an action of anandamide at both CB1 cannabinoid and vanilloid receptors in the dorsal horn[74]. Similarly, the function of central ASICs is unclear, nor is it known whether novel endogenous ligands for these channels exist.

Future directions

One of the main challenges is to understand how both the specific physiological properties of nociceptors and the circuits that they engage in the central nervous system determine pain perception and resultant behaviour. Molecular markers make it possible to identify and manipulate the activity of subsets of nociceptors, so facilitating the mapping of spinal cord and brainstem circuits that are engaged by specific nociceptor populations. It is important to understand the function of the many neurotransmitters, receptors and transducers that are expressed by nociceptors and the significance of their transcriptional and post-translational regulation in the setting of injury. Although opioids and non-steroidal anti-inflammatory agents are analgesic drugs of choice for the treatment of pain, their utility is often limited by unacceptable side-effects due to actions at identical receptors outside of the pain pathway. Because many of the channels and receptors described in this review seem to be unique to the nociceptor (for example, TTX-R Na^+ channels, $P2X_3$ and VR1), they represent promising targets for the development of new and highly selective local anaesthetics and analgesics for treating a wide variety of persistent pain conditions. □

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