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Nociceptor sensitization in pain pathogenesis

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Abstract

The incidence of chronic pain is estimated to be 20–25% worldwide. Few patients with chronic pain obtain complete relief from the drugs that are currently available, and more than half report inadequate relief. Underlying the challenge of developing better drugs to manage chronic pain is incomplete understanding of the heterogeneity of mechanisms that contribute to the transition from acute tissue insult to chronic pain and to pain conditions for which the underlying pathology is not apparent. An intact central nervous system (CNS) is required for the conscious perception of pain, and changes in the CNS are clearly evident in chronic pain states. However, the blockage of nociceptive input into the CNS can effectively relieve or markedly attenuate discomfort and pain, revealing the importance of ongoing peripheral input to the maintenance of chronic pain. Accordingly, we focus here on nociceptors: their excitability, their heterogeneity and their role in initiating and maintaining pain.

Pain is an unpleasant sensory and emotional experience that is commonly associated with actual or potential tissue damage¹. Pain is always subjective¹ and thus is modulated by past experiences and setting, affect, cognitive influences, gender and even cultural expectations. Accordingly, the CNS must be intact for pain to be consciously perceived. Pain usually starts with the activation of sensory receptors in somatic or visceral structures called nociceptors, which convey nociceptive (pain) information to the CNS. Given the obligatory role of the CNS for pain perception and the fact that changes in the CNS are evident in chronic pain states, including ‘functional disorders’ such as irritable bowel syndrome that do not seem to involve tissue injury, shouldn’t a discussion of pain focus on the CNS rather than nociceptors? We choose here to focus on nociceptors because reducing their activity or blocking their input to the CNS can attenuate or relieve the complex sensory and emotional consequences of that input. This is true not only for acute pain associated with tissue injury but also for many types of chronic pain^{2–6}.

Considerable progress has been made in understanding the role of primary afferent (sensory) neurons in nociceptive processing, leading to the development of new therapies that successfully target nociceptors. Some are currently in clinical use⁷ or will be available soon^{8,9}. Space constraints preclude discussion of all recent advances, and instead we focus

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here on three themes. First, through a discussion of the properties of nociceptors, we illustrate their heterogeneity. Second, we provide context to better appreciate why progress in developing new, efficacious drugs for pain management is relatively slow and also highlight what we consider are viable targets for future development. Third, we provide a rationale for an approach tailored to specific aspects of a pain syndrome.

Nociceptor characteristics

Sensitization

Nociceptors are sensory end organs in the skin, muscle, joints and viscera that selectively respond to noxious or potentially tissue-damaging stimuli. An important property of nociceptors is that they sensitize (that is, their excitability can be increased). Sensitization, which typically develops as a consequence of tissue insult and inflammation, is defined as a reduction in the threshold and an increase in the magnitude of a response to noxious stimulation¹⁰. In addition, previously ineffective stimuli may become effective and spontaneous activity may also develop¹¹. Mechanistically, nociceptor sensitization may include a decrease in the response adaptation that is commonly observed with prolonged stimulus application¹². Although it is an important property of nociceptors, sensitization is not necessarily specific to nociceptors, as afferents that encode other sensory modalities can also be sensitized¹³.

Structural attributes

Colloquially, nociceptors are widely considered to include the axon, cell body and central terminals associated with that end organ. Nociceptor endings in tissue are unencapsulated ('free') and are commonly associated with slowly conducting unmyelinated (C) or thinly myelinated (Aδ) axons of generally small-diameter somata in the dorsal root and other sensory ganglia (for example, trigeminal). However, cell size and myelination are not reliable indicators of function. For example, the somata of visceral nociceptors are generally larger than those of nonvisceral nociceptors¹⁴. The inappropriate designation of sensory neurons with slowly conducting axons and small-diameter cell bodies as nociceptors in the absence of evidence for their function has frustrated the efforts of researchers struggling to identify new therapeutic approaches for treating pain.

Threshold and content

Based largely on the appreciation of the difference between painful and non-painful stimuli, it had been assumed that nociceptors had high thresholds for response. However, it is now clear that threshold *per se* is not a distinguishing characteristic of nociceptors. In fact, many cutaneous, muscle, joint and visceral mechanonociceptors have low response thresholds that are indistinguishable from those of non-nociceptor mechanoreceptors. However, unlike low-threshold mechanoreceptors, nociceptors encode stimulus intensity into the noxious range.

Subpopulations of nociceptors are often defined on the basis of cell content of receptor expression. A 'peptidergic' subpopulation is defined by the presence of one or both of the neuropeptides substance P and calcitonin gene-related peptide (CGRP; Fig. 1). This subpopulation is further defined by the expression of the nerve growth factor (NGF) receptor

trkA¹⁵. By contrast, nonpeptidergic nociceptors are defined by the expression of Ret, the receptor for the glial cell line-derived family of neurotrophic factors, most of which also bind isolectin B4 and express the purinergic P2X3 receptor¹⁵. This schema, however, ignores limitations specific to each marker that might incompletely define subpopulations of nociceptors (for example, substance P is found in a subpopulation of CGRP-expressing neurons¹⁶) or have overlapping boundaries (for example, Ret and TrkA are coexpressed in a subpopulation of putative nociceptive afferents¹⁷). More importantly, it also obscures differences between nociceptors that innervate different tissues. For example, a substantially greater proportion of visceral than cutaneous nociceptors is immunoreactive for CGRP and expresses trkA¹⁴. More recently, the presence or absence of additional markers (for example, the voltage-gated sodium channels NaV1.7 and NaV1.8 and the transient receptor potential (TRP) channels A1 and V1) has been advanced to identify additional subsets of nociceptors (Fig. 1). Unfortunately, these additional markers also have limitations, most problematic of which is that the distribution of markers can vary between subpopulations of nociceptors defined by target of innervation. It would undeniably help the study of nociceptor cell biology to be able to identify a nociceptor by some means other than by functional assessment. At present, however, this remains an elusive, if not unobtainable, objective.

Efferent function

Although this review focuses on the role of nociceptors in the transmission of information from the periphery to the CNS, it is important to touch on the fact that at least some afferents, most notably peptidergic nociceptors, can release substances from their peripheral terminals. In the absence of tissue injury, this efferent function contributes to the maintenance of normal tissue integrity, for example, during bone or tooth remodeling¹⁸. In the presence of tissue injury, the vasodilators CGRP and substance P contribute to plasma extravasation and edema. In addition, in sterile inflammation such as that associated with migraine, the peripheral release of afferent transmitters can be markedly increased, resulting in neurogenic inflammation¹⁹. The peripheral release of substances from nociceptors is therefore not only essential for the full manifestation of inflammation but also helps to drive the pain associated with tissue injury²⁰. Local mechanisms, such as backpropagation of afferent activity through collateral branches (the axon reflex²¹), were originally thought to have a dominant role in mediating the peripheral release of afferent peptides. There is evidence that antidromic activity arising from within the spinal cord might also make a substantial contribution to this process²⁰. Given the role of voltage-gated Ca²⁺ channels in the peripheral release of afferent transmitters²², inhibition of these channels in the periphery can attenuate both the neurogenic inflammatory response and pain associated with tissue injury.

Adequate stimuli

Whether a stimulus is adequate to activate a nociceptor depends on the site of application and stimulus modality. The site of application influences the stimulus intensity range over which a nociceptor is responsive (for example, nociceptors that innervate the cornea have a lower threshold for activation than those that innervate the skin), but so does the nature of the stimulus. For example, cutting or crushing stimuli reliably activate most cutaneous nociceptors but do not reliably activate nociceptors in the joints, muscle or viscera. Instead,

rotation and distension are more effective mechanical forces for activating joint and visceral nociceptors, respectively.

Noxious cutaneous stimuli include chemical, thermal (hot and cold) and mechanical modalities. Skin is more densely innervated by nociceptors than are other tissues and also contains a wider variety of nociceptor types. The most common nociceptor in skin is the polymodal nociceptor, which responds to multiple modalities of stimuli (Fig. 1a), but skin also contains the widest variety of modality-selective nociceptors in any tissue (for example, C-heat nociceptors and C-mechano-cold nociceptors (Fig. 1b))²³. The principal stimuli that are noxious in other tissues are mechanical (joint torque, hollow organ distension) or chemical (low pH and lactic acid in muscle, inflammation and ischemia in muscle and viscera, and joint inflammation). Most studies of joint, muscle and visceral nociceptors have used mechanical stimuli, and these have identified subpopulations of mechanically sensitive receptive endings with either low or high stimulus thresholds. Consistent with nociceptor properties, many low-threshold and virtually all high-threshold joint and visceral mechanoreceptors are polymodal, encode stimulus intensity into the noxious range and also sensitize²⁴, which suggests that low-threshold afferents make important contributions to pain when these tissues are inflamed.

Mechanosensitive nociceptors have been well characterized, but there is also a distinct and potentially important population of nociceptors that is relatively insensitive to mechanical stimuli in the absence of tissue injury (Fig. 1c). Originally referred to as ‘silent’ nociceptors, they were first documented in the articular nerve that innervates the knee joint of cats²⁵. When the joint was experimentally inflamed, previously silent afferents became spontaneously active and mechanosensitive. Electrically excitable, mechanically silent afferents were subsequently designated mechanically insensitive afferents (MIAs)²⁶. Many of these afferents are chemosensitive, and perhaps even chemoselective, but MIA is the more appropriate descriptor given their relative insensitivity to mechanical stimulation. MIAs constitute ~15–20% of cutaneous C-fiber afferents in humans, but less is known about MIAs that innervate joints, muscles or the viscera. Because MIAs make up a substantial proportion of the nociceptor population, the acquisition of mechanosensitivity after tissue injury implicates them in the development and maintenance of hyperalgesic or hypersensitive states. Accordingly, their potential contribution to chronic pain states is probably underappreciated. There is virtually no information about the mechanisms of MIA sensitization. It is only assumed that some of the mechanisms and molecules that we discuss below also apply to MIAs.

Nociceptor heterogeneity

Because nociceptors do not consist of a homogeneous group of afferents, no single criterion can reliably identify a nociceptor (Fig. 1). In addition to anatomical, biochemical and physiological heterogeneity, nociceptors are also functionally heterogeneous. There is evidence that subpopulations of visceral nociceptors might underlie different types of visceral pain such as hypersensitivity to organ filling, acidic or burning pain, bloating and gas sensations¹⁴. There is also evidence that various subpopulations of nociceptors underlie different aspects of pain (for example, emotional versus sensory discriminative

components²⁷). The degree of heterogeneity among nociceptive afferents has probably contributed to difficulty in the identification of new therapeutic agents. It has also probably contributed to several of the most notable failures of preclinical data to translate to an effective clinical intervention (for example, substance P receptor antagonists as analgesics²⁸). If the emotional or suffering component of pain is the most troubling for pain patients, as suggested by several studies, and nonpeptidergic nociceptors are responsible for accessing limbic structures in the brain, then one would have predicted—in hindsight—that substance P receptor antagonists would have little therapeutic efficacy, particularly for the management of chronic pain.

Mechanisms of transduction

Nociceptor excitation

The transduction of mechanical, thermal and chemical stimuli in the somatosensory system is initiated by membrane depolarization, referred to as a generator potential. If the generator potential is of sufficient magnitude, spread or both, it is transformed into an action potential. The transduction of chemical stimuli by slower, often second messenger-dependent pathways might be the most common mode of signaling for most nociceptors. However, the contribution of this mode of signaling to the manifestation of pain is poorly defined. In the absence of stimuli there is little spontaneous activity in nociceptors²⁹, owing to a large K⁺ conductance through both voltage-gated³⁰ and voltage-insensitive K⁺ channels³¹. This K⁺ conductance opposes a high membrane permeability to depolarizing Na⁺ and Ca²⁺ ions, at least in cutaneous nociceptors³⁰. The presence of a depolarizing conductance in nociceptors suggests that stimulus transduction might involve the closing of a K⁺ channel. A depolarizing current should also contribute to a relatively depolarized resting membrane potential in nociceptors. Because of the voltage dependence of several transducers, the resting membrane potential may influence both transduction and spike initiation. This suggests that drugs that have ‘state-dependent’ properties, such as local anesthetics that block open channels with a higher potency than closed channels, may be useful for selectively blocking nociceptors.

Intrinsic mechanisms of transduction

There is increasing evidence from molecular biological and biophysical studies that primary afferent neurons contain unique proteins that can subserve the transduction of thermal (heating and cooling), mechanical and chemical stimuli³² (Fig. 1 and Table 1). However, it is proving to be difficult to unravel the mechanisms of transduction *in vivo*. For example, the mere presence of a cold transducer such as TRPM8 or TRPA1 in a nociceptor is not necessarily sufficient to confer cold sensitivity. The cold sensitivity of a subpopulation of putative nociceptors depends on the expression of a voltage-gated K⁺ channel³³; a decrease in K⁺ current results in an increase in the fraction of neurons that respond to cooling. Similarly, most voltage-gated Na⁺ (NaV) channels are inactivated by cold temperatures. This accounts for the loss of low-threshold touch and proprioception in cold tissue. By contrast, NaV1.8, a channel that is enriched in the terminals of nociceptors, is resistant to cooling-induced inactivation. Consequently, NaV1.8 has a prominent role in cold transduction³⁴ and in cold-evoked pain³⁵. The ability of TRPA1 to contribute to cold transduction apparently

requires tissue injury or inflammation³⁶, which probably causes a combination of changes in TRPA1 as well as in associated ion channels. Consistent with the suggestion that a combination of channels underlies cold transduction, a family was recently identified with a gain-of-function mutation in TRPA1; individuals with the mutation suffered from episodes of extreme pain that could be triggered by body cooling, but their cold pain thresholds were normal³⁷. Transduction might also involve indirect mechanisms such as mechanical stimulation–induced release of mediators from epithelial cells that subsequently act on nociceptors (Fig. 2a). These mediators may ultimately converge on transducers such as TRPV1 and TRPA1, which subsequently function as thermo-, chemo- and, ultimately, mechanotransducers. Thus, the transduction of noxious stimuli might involve several cell types and require several specific proteins that are uniquely positioned within the nociceptor membrane. This combinatorial effect can be particularly problematic for drug discovery efforts if multiple distinct mechanisms act in parallel to subserve similar functions³⁸.

Three classes of cell surface proteins that are found on sensory neurons have been studied in the greatest detail with respect to their roles in sensory transduction: ion channels, metabotropic G protein–coupled receptors (GPCRs), and receptors for neurotrophins or cytokines.

Ion channels

Ion channels have received the most attention in efforts to identify mechanisms of thermo- and mechanotransduction. Some ion channels are directly responsible for transduction (TRP channels), whereas others act indirectly (NaV1.8; Table 1). Mechanotransduction is proving to be the most difficult problem to resolve. There are both ion channels with intrinsic mechanosensitivity (two-pore K⁺ channels (K2P), TRPV4) and those without it (T-type Ca²⁺ channels or Ca_v3.2) that seem to have only a modulatory role in mechanotransduction *in vivo*³⁹. Most perplexing in this regard is that the absence of channels such as TRPA1 (ref. 40) or ASIC3 (ref. 41) can result in both decreases and increases in mechanosensitivity in distinct subpopulations of afferents. Most of these seemingly contradictory results were obtained from mutant mice, where the possibility of compensatory changes suggests that these data should be interpreted with caution. The involvement of other cell types in the transduction process (Fig. 2a) might also contribute to these contradictory results. Nevertheless, the presence of multiple mechanotransducers in nociceptors is particularly unfortunate from a drug discovery perspective, given that mechanical hypersensitivity is a predominant, if not the dominant, complaint associated with many chronic pain states. Thus, although it is a tantalizing concept, it might never be possible to selectively block mechanotransduction in nociceptors.

An alternative strategy for identifying ways of selectively blocking transducers would be to target channels that contribute to the transduction process. Three classes of ion channel are essential in the context of rapid signaling, whereby stimulus transduction results in membrane depolarization: those that influence passive membrane properties, those that influence the upstroke of the action potential and those that influence the repolarization or after-polarization phase of the action potential (for example, Kv channels; Fig. 1b). All three

are crucial for establishing the excitability of the neuron and are therefore targets of endogenous modulatory processes.

First, channels that influence passive membrane properties include the K2P channels TREK-1/2 (TWIK-related potassium channel 1 and 2, where TWIK stands for tandem of P domains in a weak inward rectifier potassium channel⁴²) and TRAAK (TWIK-related arachidonic acid-activated potassium channel)⁴³ and one member of the NaV channel family, NaV1.9 (ref. 44).

Second, the upstroke of the action potential depends on rapidly activating NaV channels. NaV channels are composed of α - and β -subunits; eight of the nine α -subunits and all four β -subunits are present in sensory neurons⁴⁵. The α -subunits are differentially distributed between and within sensory neurons. For example, NaV1.7, NaV1.8 and NaV1.9 are preferentially expressed in putative nociceptors, whereas NaV1.1 is preferentially expressed in non-nociceptors. NaV1.8 is normally localized to the cell soma and terminal arbor; by contrast, NaV1.6 is found along axons⁴⁵. Importantly, the expression of both α - and β -subunits is altered after injury, and this probably contributes substantially to the pain and hyperalgesia that are associated with tissue injury (see below). Recent genetic analyses in humans have highlighted a key role for NaV1.7 in nociceptive processing: gain-of-function mutations are associated with pain syndromes such as erythromalgia and paroxysmal extreme pain disorder, whereas loss-of-function mutations are associated with congenital insensitivity to pain⁴⁶. The pattern of channel polymorphisms is associated with the relative severity of pain in a variety of clinical conditions as well as with sensitivity to experimental pain⁴⁷.

Finally, a diverse group of excitatory (depolarizing) and inhibitory (hyperpolarizing) channels contribute to action potential repolarization and the after-potential⁴⁵. These include sustained (delayed rectifier type) voltage-gated K⁺ channels, large-conductance Ca²⁺-modulated K⁺ channels (BK or Maxi-K channels), low-threshold or T-type voltage-gated Ca²⁺ channels, Ca²⁺-dependent Cl⁻ channels⁴⁸, inwardly rectifying K⁺ currents, low-threshold inactivating or A-type voltage-gated K⁺ currents, a hyperpolarization-activated cationic current (*I_h*) carried by a family of non-selective cationic channels called HCN channels, and a slowly activating Ca²⁺-dependent K⁺ current that underlies a slow afterhyperpolarization. The relative density and distribution of these channels and currents influences the pattern of neural activity (bursting versus sustained), the duration of burst activity and the interspike interval. Importantly, each of these channels is targeted by inflammatory mediators, which cause changes in channel distribution, density or both that contribute to long-term changes in excitability associated with tissue injury.

Targeting any one of these ion channels might provide an effective way to reduce nociceptor excitability and thereby to relieve pain. Indeed, preclinical data suggest that the administration of a K_{ATP} channel-activating drug is sufficient to attenuate hyperalgesia induced by inflammatory mediators⁴⁹. However, if such an approach is to be successfully implemented, it will be essential to minimize potential 'off-target' effects. Despite the facts that NaV channels are essential for action potential initiation and propagation in nociceptors, that local anesthetic blockade of NaV channels successfully blocks pain and that alterations

in NaV channels contribute to pain associated with tissue injury, systemic administration of NaV channel blockers often fails to provide effective pain relief. The impact of the off-target effects of these compounds is particularly important because of the widespread distribution of NaV channels and their narrow therapeutic index, which can lead to cardiac and CNS toxicity.

Membrane receptors that indirectly alter nociceptor function

GPCRs

GPCRs are cell surface proteins that are often used to define the functional and histological identity of sensory neurons. Although several of these receptors seem to have important roles in the regulation of nociceptor excitability, there is evidence that dozens of GPCRs that drive increases in excitability are expressed by nociceptors, and this has probably contributed to the relative failure of receptor-specific antagonists to emerge as successful therapeutic interventions. That said, the relatively prominent contribution that CGRP seems to make to migraine has led to the development of CGRP receptor antagonists and successful treatment of migraine pain in early clinical trials⁹. Other excitatory GPCRs that are found on nociceptors include B1 and B2 bradykinin receptors⁵⁰, protease-activated receptors⁵¹, and EP1, EP3C and EP4 receptors for prostaglandin E₂ (ref. 52).

Inhibitory GPCRs are also widely distributed among nociceptors, and several are successfully targeted for pain relief. These include μ , κ and δ opioid receptors⁵³. Interestingly, a class of drugs referred to as triptans are agonists for serotonin 1B and D receptors and are used to treat migraine pain⁵⁴. Despite the widespread distribution of these receptors in the peripheral nervous system⁵⁵, the clinical efficacy of triptans is limited to migraine⁵⁶. Whether this reflects the selective distribution of the receptor in the afferents that underlie migraine⁵⁷, unique signaling in this afferent population or both, the selectivity of these drugs highlights the effect of nociceptor heterogeneity on the therapeutic efficacy of interventions that target nociceptors.

Adding to the complexity of receptor-mediated modulation of nociceptor excitability, there is increasing evidence that a single nociceptor can express receptors for the same endogenous ligand coupled to both excitatory and inhibitory second messenger pathways. For example, some nociceptors express both excitatory and inhibitory glutamatergic⁵⁸ receptors. The coexpression of excitatory and inhibitory receptors on the same neuron means that changes in an array of factors ranging from the concentration of exogenous ligands to the density and distribution of receptor subtypes can have a profound influence on nociceptor excitability. It also raises the possibility that a combination of receptor subtype-selective agonists and antagonists might have the greatest therapeutic efficacy.

The second-messenger pathways that underlie metabotropic receptor signaling are an active area of investigation. A growing array of excitatory pathways has been identified, ranging from the traditional G_s-coupled adenylate cyclase–cAMP–protein kinase A (PKA) and q-coupled phospholipase C β –diacylglycerol–inositol trisphosphate–protein kinase C (PKC) pathways to more complex pathways involving phosphorylated extracellular signal regulated kinase, p38 mitogen activated kinase or both (Fig. 2b). The identification of anchoring

proteins that are responsible for properly positioning activated protein kinases, such as the receptors for activated C-kinases, has proved to be invaluable for teasing apart specific receptor-mediated pathways. Adding to the complexity at a subcellular level are multiple points of cross-talk between pathways. The extent of cross-talk can be illustrated by considering the modulation of TRPV1, which serves as a point of convergence for multiple pathways (Fig. 2b). TRPV1 can be activated or sensitized by the phospholipase C-mediated liberation of inositol trisphosphate from phosphatidylinositol 4,5-bisphosphate, and the combined actions of diacylglycerol and Ca^{2+} released from intracellular stores can activate classical PKC isozymes such as PKC δ . The increase in intracellular Ca^{2+} can also result in the activation of Ca^{2+} -calmodulin-dependent protein kinase II (CaMKII), which can also sensitize TRPV1. Activation of adenylate cyclases by GPCRs can also result in the activation of PKA, which can sensitize TRPV1, or the activation of PKC ϵ by a pathway that depends on cAMP-activated guanine exchange factor (EPAC). This EPAC-dependent pathway not only has a dominant role in nociceptor sensitization in the presence of ongoing inflammation but also is likely to have a key role in tissue 'memory' of a previous injury⁵⁹. Additional second messenger cascades that are initiated after activation of receptor tyrosine kinases include pathways that depend on phosphoinositide 3-kinase, mitogen-activated protein kinase and ceramide; the downstream events for the last two require further characterization. The translocation of TRPV1 to the plasma membrane driven by a number of these second messenger pathways can also contribute to nociceptor sensitization. Finally, given the importance of Ca^{2+} in the activation of both sensitizing pathways and calmodulin, which can inhibit TRPV1, receptors such as the estrogen receptor can indirectly influence TRPV1 function by modulating Ca^{2+} influx pathways. As with approaches designed to target ion channels that control afferent excitability, successful strategies designed to target second messenger pathways that underlie the sensitization of nociceptive afferents will involve the identification of molecules, such as the PKC ϵ isoform, that serve as points of convergence for different signaling cascades and play key parts in increasing nociceptor excitability. Of course, off-target effects will also remain a concern with such approaches.

Neurotrophin receptors

Since the observation nearly 20 years ago that peripheral administration of NGF sensitizes nociceptors and produces hypersensitivity⁶⁰, investigators have appreciated that neurotrophic factors that are essential for survival and axon guidance during development are also important for the pain and sensitivity that are associated with tissue injury in adults. There are two main classes of neurotrophic factors that carry out these functions: the NGF family (NGF, brain-derived neurotrophic factor, neurotrophin-3 and neurotrophin-4) and the glial cell line-derived family (glial cell-derived neurotrophic factor, neurturin, artemin and persephin). Receptors for each of these trophic factors have been used to categorize major classes of nociceptors and non-nociceptors. Because each of these trophic factors has a key role in the maintenance of the afferent phenotype, which includes neurotransmitter expression and the specific composition of ion channels and transducers, both loss of access to trophic factors, which occurs after peripheral nerve injury, or increased production of trophic factors, which occurs in the presence of peripheral inflammation, can markedly increase nociceptor excitability⁶¹. Highlighting the fact that there are multiple ways of achieving nociceptor sensitization, the underlying mechanisms that are associated with a

decrease in trophic factors are distinct from those that are associated with an increase in trophic factors⁶¹. Further underscoring the heterogeneity of nociceptors, there is evidence that the influence of combinations of trophic factors depends in part on the target of innervation.

Although the importance of trophic factors for orchestrating the response to tissue injury has made them enticing targets for therapeutic interventions, the delicate balance between too much and too little trophic factor has proven to be challenging. NGF administration produces long-lasting pain and hypersensitivity⁶², and although there is optimism that trkA receptor antagonists may have analgesic efficacy⁶³, complete block of these receptors is likely to be problematic.

Cytokine modulation of nociception

Although the role of cytokines in nociceptive processing has been addressed in recent reviews^{64–66}, it is important to emphasize that the relative contribution of a particular cytokine to pain associated with tissue injury can change with time. For example, peripheral CCL2 (previously known as MCP1) acting through CCR2 receptors contributes to hypersensitivity in a demyelinating neuropathic pain model after a marked delay, despite the presence of hypersensitivity at earlier time points⁶⁵. Time-dependent changes in the mechanisms that underlie chronic pain suggest that it might be necessary to develop experimental approaches to appropriately ‘stage’ chronic pain so as to tailor interventions to the underlying mechanisms.

There are multiple sources of mediators that can activate the array of receptors on nociceptive afferents (Fig. 2a). These include the blood, sympathetic post-ganglionic neurons, resident (mast cells) and recruited (polymorphonuclear leukocytes) immune cells, epithelial cells, Schwann cells and fibroblasts. Given the efferent function of nociceptors described above, and the fact that each of these sources of mediators is a target (direct or indirect) for the transmitters that are released from nociceptors, it becomes clear why the positive feedback (Fig. 2c) associated with the activation of nociceptor terminals can have such a rapid and profound impact on the pain associated with tissue injury.

Tissue-specific regulation of nociceptor function

In the face of a growing list of ligands, receptors and second messenger pathways that contribute to nociceptor sensitization and pain, two themes have emerged that provide some organizational structure to the apparent chaos. One is the observation that signaling molecules are clustered as a means of facilitating rapid signaling and the appropriate coupling between receptor and downstream target⁶⁷. Clustering in lipid rafts provides a potential target for therapeutic interventions if appropriate clustering is necessary for sensitization induced by inflammatory mediators; breaking up the cluster might be an effective way of preventing or attenuating nociceptor sensitization. Second, there is a considerable degree of convergence on a relatively small number of ion channels that underlie sensitization associated with a wide range of types of injury. If there is to be a ‘silver bullet’ for the treatment of pain, selective block of these ion channels remains the

most viable possibility (see bolded entries in Table 1). Importantly, however, the nature of the contribution of various channels varies as a function of the type of injury and the tissue in which it occurs. For example, acute, phosphorylation-dependent modulation of NaV1.8 results in an increase in current that contributes to an inflammation-induced increase in nociceptor excitability⁶¹. By contrast, after traumatic nerve injury, redistribution of NaV1.8 to the axons of uninjured afferents seems to be necessary for the expression of mechanical hypersensitivity⁶¹. It is important to emphasize that these ‘convergent targets’ are relatively rare, and there is increasing evidence for tissue-specific mechanisms, such as the dominant roles of ASIC channels in the sensitization of muscle afferents⁶⁸, P2X channels in the sensitization of visceral afferents⁶⁹ and Cl⁻ channels in the sensitization of dural afferents⁷⁰, raising the intriguing possibility that it may be possible, if not necessary, to treat pain arising from a specific tissue with a specific intervention.

Changes in nociceptor function after nerve injury

Neuropathic pain is the term used to describe pain after injury to the nervous system, including trauma, metabolic imbalance (for example, diabetes), viral infections (for example, post-herpetic and AIDS neuropathy) and chemotherapeutic agents (for example, Taxol). Consistent with the variety of ways in which the nervous system can be injured, a number of distinct mechanisms seem to underlie neuropathic pain. For example, loss of access to trophic factors after traumatic nerve injury can result in changes in the expression, density and distribution of ion channels that then cause an increase in excitability in injured afferents. Conversely, the loss of competition for trophic factors released from peripheral targets means that there will be a relative increase in trophic factors that are available to the uninjured neighbors of injured afferents. This increase in access to trophic factors can also drive phenotypic changes that mediate an increase in excitability. The relative contributions of injured and uninjured afferents to the pain that is associated with traumatic nerve injury are still hotly debated.

Despite the variety of causes of neuropathic pain, it has several common features, the most obvious and prevalent of which is ongoing pain⁷¹. This feature is distinct from inflammatory pain, where it is often possible to relieve pain if one can eliminate the stimuli that affect the inflamed tissue. Because it is also one of the most troubling aspects of neuropathic pain, the identification of mechanisms that underlie ongoing pain remains an area of active investigation. Investigators continue to focus on the primary afferent as the source of ongoing pain, in large part because ongoing neuropathic pain can be blocked by the administration of a local anesthetic⁶. Two major sources of ongoing afferent activity have been identified (Box 1).

Box 1

Mechanisms of ongoing pain after nerve injury

Nerve injury–induced changes in transduction

Inappropriate activation of transducers may arise from

- emergence of mechanical or thermal transducers (or both) at or near the cut ends of damaged axons⁹⁷ or within the ganglia⁹⁸
- *de novo* expression of transducers in neurons that do not normally express them
- decreases in inhibitory transducers (for example, opioid receptors⁹⁹) and/or increases in excitatory transducers (for example, P2X3 (ref. 100))
- changes in the expression or release of endogenous ligands, receptors or both
- changes in the coupling between transducers and signaling pathways—for example, a change in $\alpha 2$ -adrenergic receptor subtype from one coupled to inhibitory second messenger pathways to one coupled to excitatory pathways underlies the emergence of adrenergic excitation of nociceptors after nerve injury¹⁰¹
- emergence of aberrant sources of nociceptor activation

Nerve injury–induced changes in membrane stability

It may arise from

- changes in the distribution, expression and biophysical properties of ion channels

It is likely that multiple mechanisms contribute to ongoing activity in a single afferent and in different afferents that are affected by a nerve injury. Sources of activity might include the ectopic expression of transducers⁷², aberrant sources of nociceptor activation such as norepinephrine from sympathetic postganglionic terminals that have sprouted into the ganglia⁷³ or alterations in ion channel density resulting in membrane instability and spontaneous activity⁶¹ (Box 1). Many of the sites of activity, such as those within the ganglia, may have limited accessibility to direct manipulation. The underlying mechanisms of activity not only depend on the cause of the injury, but also are likely to change with time. Together, these factors make neuropathic pain one of the most difficult types of pain to manage.

Challenges to developing effective therapeutic approaches

Despite the detailed characterization of the mechanisms that underlie nociceptor excitability, in combination with the identification of several ion channels, receptors and second messenger signaling molecules that serve as points of convergence, the development of effective therapeutic interventions for the management of pain that do not have deleterious side effects has remained elusive. The reasons include differences in mechanism that depend on the target of innervation, the type of injury, time after injury, genetics, sex and history of the injured tissue. In addition, none of the channels or second messengers discussed above functions in isolation. The relative effect of a change in any of these mechanisms depends, therefore, on the context in which any particular mechanism functions.

This is illustrated by the effect of a gain-of-function mutation in NaV1.7 that is associated with erythromalgia⁷⁴. In nociceptors, the increased depolarizing drive that is associated with the NaV1.7 channelopathy results in an increase in nociceptor excitability that depends on the presence of NaV1.8 channels that are resistant to depolarization-induced inactivation. However, in the absence of NaV1.8, as is the case in sympathetic postganglionic neurons, the depolarizing drive that is associated with the NaV1.7 channelopathy results in the steady-state inactivation of the NaV channels that underlie action potential initiation and the result is a decrease in excitability. We recently obtained thematically comparable results in an analysis of dural afferents, in which the primary mechanism underlying sensitization induced by inflammatory mediators was the activation of a Cl⁻ current⁷⁰. However, this current was only excitatory because of the exceptionally depolarized Cl⁻ equilibrium potential maintained in these neurons, the presence of a high density of NaV1.8 channels, and the presence of voltage- and Ca²⁺-dependent K⁺ channels that have a relatively high threshold for activation. Considering that many of the changes that occur after tissue injury seem to be geared toward restoring homeostasis in the insulted tissue, in conjunction with an appreciation of the context in which putative therapeutic targets must function, it becomes clear that the balance between physiology and pathophysiology is precarious. However, appreciation of this context may ultimately facilitate the identification of more effective therapeutic approaches.

Nociceptor sensitization and pain pathogenesis

How acute tissue insult turns into pain that persists after resolution of the initial insult is not known. Most acute insults (for example, sunburn, sprained ankle or a surgical incision) resolve without persisting pain, which emphasizes that the processes of nociceptor sensitization are typically reversible. Many chronic pain states (for example, neuropathic pain) are clearly associated with tissue pathology (for example, nerve transection or amputation) and apparent irreversibility of sensitization. By far the more puzzling chronic pain states are those in which tissue inflammation or pathology is not readily apparent (for example, fibromyalgia, irritable bowel syndrome, painful bladder syndrome or migraine). Despite the absence of a pathobiological explanation, peripheral input has been documented as necessary and sufficient for the persistence of several of these pain states²⁻⁵, implicating unresolved nociceptor sensitization, including contributions from previously mechanically insensitive (silent) afferents (Fig. 1c).

These troubling clinical syndromes raise the possibility that the problem lies not in the particular transducer or ion channel but in the population of afferents that has become hypersensitive to normal stimuli. For example, MIAs innervate the colon and urinary bladder, where they have been estimated to represent 35% of organ innervation⁷⁵. If visceral MIAs are readily recruited to mechanosensitivity by tissue insult associated with the release of a wide range of sensitizing mediators, then MIAs might make a substantial contribution to functional visceral disorders such as irritable bowel syndrome and painful bladder syndrome. MIAs probably include afferents of unknown or unexplored chemoselectivity, which might independently contribute to the discomfort and pain that characterize these chronic and hard to treat functional visceral disorders.

Are different nociceptors involved in different chronic pain states? Although we appreciate that nociceptors are functionally distinct, we do not understand the relative contributions, for example, of sensitized cutaneous C-polymodal (Fig. 1a) as opposed to C-heat nociceptors to the hyperalgesia of sunburned skin. Knowledge of whether and which specific nociceptors contribute to different pain states could decisively inform strategies for the development of new and effective pharmacotherapies.

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Figure 1.

Heterogeneity of nociceptors. (a–c) Nociceptors can be subclassified by an array of anatomical, physiological and biochemical criteria. One common criterion is the response profile of the afferent: afferents that respond to mechanical, thermal and chemical stimuli are referred to as polymodal nociceptors (a), those that respond to mechanical and cold stimuli are referred to as C-MC (mechano-cold) fibers (b), and those that do not respond to mechanical stimuli are referred to as MIAs (c). Even within these classifications there is tremendous heterogeneity, with differences in the particular molecules that underlie transduction, action potential initiation and propagation, as well as in the channels and receptors that can modulate each of these processes. There are also differences in the transmitters that are released, with more recent data pointing to differences in the proteins

that are responsible for vesicle filling. With increasing evidence that MIAs are particularly important in various pain states, the identification of unique patterns of chemosensitivity and the mechanisms that underlie the emergence of mechanosensitivity in these afferents might yield new approaches for the treatment of pain. RTK, receptor tyrosine kinase; SK, small-conductance Ca^{2+} -dependent potassium channel; VGCC, voltage-gated calcium channel.

Figure 2.

Activation and sensitization of nociceptors. **(a)** Transduction can involve both direct and indirect pathways. The ion channel TRPV1, for example, can be directly opened by increases in temperature or by chemicals released from resident (mast) and recruited (polymorphonuclear leukocyte; PMNL) immune cells, epithelial cells, Schwann cells, fibroblasts and sympathetic post-ganglionic neurons (SPGN). **(b)** There are multiple points of interaction between second messenger pathways that are engaged after nociceptor activation, including at the levels of signaling molecules such as Ca²⁺, effector molecules such as PKCε, and common targets, such as TRPV1 and NaV1.8 (not shown) for the pathways activated. For clarity, we have omitted positive modulation of TRPV1 by ceramide, p38, PI3K, PKCε and PKA. Also not shown is the translocation of TRPV1 to the cell surface, which may contribute to injury-induced increases in channel activity. **(c)**

Sensitization of nociceptors also involves positive feedback. Activation of ion channels such as TRPV1 results in membrane depolarization and Ca^{2+} influx through TRPV1 and VGCC. Ca^{2+} influx can drive the release of neuropeptides (stored in dense core vesicles; pink) and glutamate (stored in clear vesicles; yellow), both of which can drive further activation of receptors on nociceptors and release mediators from the sources described in **a**. PGs, prostaglandins; OLAMs, oxidized linoleic acid metabolites; NE, norepinephrine; ER/GPR30, estrogen receptor/G protein receptor-30; 5-HT, serotonin; CaM, calmodulin; PLC, phospholipase C. DAG, diacylglycerol; IP_3 , inositol triphosphate; AC, adenylate cyclase; EPAC, cAMP-activated guanine exchange factor; PI3K, phosphoinositide 3-kinase; ERK1/2, extracellular signal-regulated kinases 1 and 2; TNFR, tumor necrosis factor receptor.

Table 1**Primary afferent transducers**

Transducer	Evidence	Sensitization	Ref.
Mechanical			
ASIC1	Visceral afferents (↑ sensitivity in KO).	No published data regarding sensitization.	76
ASIC2	Visceral afferents (↑, ↓, ↔ sensitivity in KO, afferent type-specific).	No published data.	76
ASIC3	Visceral afferents (↓ sensitivity in KO). Cutaneous afferents (↑, ↓, ↔ sensitivity in KO).	Required for sensitization of colonic, muscle, joint nociceptors.	76
Ca _v 3.2	Role in mechanosensitivity of down (fine) hair.	Target of acute modulation. Knock-down ↓ injury-induced hypersensitivity.	77
TRPV1	Role in response to hollow organ distension (↓ sensitivity in KO).	Block central channels ↓ mechanical hyperalgesia.	78
TRPV4	Direct activation by osmotic challenge, ↑ sensitivity with activation, ↓ sensitivity with block, knock down or KO.	Visceral, cutaneous hypersensitivity in inflammation and nerve injury.	79
TRPA1	Cutaneous afferents (↑, ↓, ↔ sensitivity in KO, ↓ sensitivity with block). Visceral afferents (↓ sensitivity of subpopulations).	↑ with inflammation, ↓ mechanical hypersensitivity with block and KO in somatic and visceral afferents.	40,80
TRAAK	Direct activation by mechanical stimuli.	No published data.	43
TREK1/2	Direct activation by mechanical stimuli, ↑ sensitivity in KO.	↑ inflammatory hyperalgesia, potential role in neuropathic pain.	81,82
P2X3	Indirect activation through ATP release from epithelial cells, ↓ response to hollow organ distension in knock-down and KO.	Pro-nociceptive role in visceral and somatic pain associated with inflammation and nerve injury.	83
Thermal			
TRAAK/TREK-1	Directly activated by heating, closed by cooling; ↑ response to cold in KO.	No published data.	84
NaV1.8	Resistant to cold-induced inactivation. Indirectly essential for cold transduction.	No published data.	34
TRPA1	Direct activation by cold stimulus, ↓ cold sensitivity in some but not all KO studies.	Role in cold sensitivity following sensitization.	85–87
TRPM8	Direct activation by cold stimulus, ↓ cold sensitivity in KO.	Role in increased cold sensitivity after injury (KO studies).	88,89
TRPV3	Direct activation by warm stimulus (presence in afferent is controversial).	‘Selective activation’ with farnesyl pyrophosphate increases nociceptive behavior.	90
TRPV4	Direct activation by warm stimulus.	More prominent role in mechanotransduction.	91
TRPV1	Direct activation by heat stimulus.	Multiple lines of evidence that TRPV1 is essential for thermal hyperalgesia associated with most types of tissue injury.	45
TRPV2	Direct activation by heat stimulus, but role in nociceptive processing remains controversial.	No published data	38
Chemical (ionotropic only)^a			
ASIC1-4	Direct activation by protons.	Role in ischemia-induced pain, muscle hypersensitivity.	45
TRAAK/TREK	Channel activity influenced by a number of endogenous mediators such as protons and acachidonic acid.	No published data.	43,81
TRPV1	Direct activation, modulation by protons, fatty acids, arachidonic acid derivatives, vanilloids.	Most compelling evidence for a role in thermal hypersensitivity.	45

Transducer	Evidence	Sensitization	Ref.
TRPA1	Direct activation by a number of compounds (mustard oil, cinnamaldehyde, acrolein, formalin).	Essential for chemical-induced hypersensitivity.	85
TRPM8	Direct activation by menthol.	Most compelling evidence for role in cold hypersensitivity.	88
P2X1-6	Direct activation by ATP (prominent role for P2X2/3).	Evidence for role in pain and hypersensitivity associated with injury to many different tissue types.	92
5-HT3	Direct activation by serotonin, role in subset of nociceptive afferents.	Most prominent role in visceral pain.	93
nACh (multiple subunits are present in DRG)	Direct activation by acetylcholine (ACh).	Increased subunit expression with injury, increased peripheral ACh with injury, may have pronociceptive role in periphery, antinociceptive role in central terminals.	94
Glutamate (GluR1-5, NR1-2)	Direct activation by glutamate and subtype-specific agonists.	Evidence for peripheral role in pain, but most compelling evidence for role on central terminals.	95
GABA (multiple subunits are present in DRG)	Direct activation by GABA and subunit-specific agonists.	May be inhibitory or excitatory depending on chloride equilibrium potential at receptor.	96

↑, ↓ and ↔ represent, respectively, increase, decrease and no change. KO, knockout.

^aBecause of the large number of metabotropic receptors present in sensory neurons, they have not been included in the table. Bold entries have the most compelling evidence suggesting that selective block of the channel may have therapeutic efficacy in pain.