Pain hypersensitivity mechanisms at a glance

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Introduction

Pain is an important, evolutionarily conserved physiological phenomenon that is necessary for survival. At the same time, pain is one of the most frequent symptoms of a variety of pathological disorders and represents a major clinical challenge. In recent decades, there has been a dramatic increase in our understanding of molecular and cellular mechanisms underlying pain in physiological, as well as pathophysiological, contexts. A clearer picture is beginning to emerge out of the myriad signaling pathways that have been implicated in disease-related pain hypersensitivity.

Noxious stimuli, including mechanical, chemical and thermal stimuli, are sensed by peripheral nociceptive neurons that are classified as C or A-delta (Aδ) type based on properties of the nerve fiber. A third type, A-beta (Aβ) fibers, are involved in the conduction of non-nociceptive inputs such as light touch, movement or vibration under normal physiological conditions. Morphological, electrophysiological and genetic studies have provided evidence for specificity of peripheral nociceptive and non-nociceptive sensory neurons for distinct sensory modalities. The somata or cell bodies of both nociceptive and non-nociceptive sensory afferents lie in the dorsal root ganglia (DRG), and their central terminals synapse in the superficial spinal dorsal horn.

Spinal circuits further process sensory inputs and relay them to brain centers via diverse pathways, where the perception of pain together with its emotional and aversive components is generated. In this review, we will outline key mechanistic events and attempt to derive common principles and potential therapeutic windows from the abundant literature that is available.

Peripheral signaling pathways involved in acute and chronic pain

Receptors involved in nociception

The diverse range of ion channels that is present on sensory nerve endings mediates the transduction of physicochemical stimuli into changes in membrane potential (see Poster, panel A). Warm and hot temperatures are sensed by transient receptor potential (TRP) channels such as TRPV1 and TRPV2, and also by a calcium-gated chloride (Ca²⁺-gated Cl⁻) channel, ANO1 (Cho et al., 2012; Julius and Basbaum, 2001). Protons are detected by acid-sensing channels (ASICs) and also by TRPV1 (Julius and Basbaum, 2001). TRPM8 is the sensor for cold temperatures, and Nav1.8 (described below) is required for cold-associated pain (Bautista et al., 2007; Zimmermann et al., 2007). Piezo1 and Piezo2 are thought to act as mechanical transducers (Coste et al., 2010), although TRPA1 is required for cold-associated pain (Kwan et al., 2006; Petrus et al., 2007; Tsuda et al., 2000). Activation of these ion channels leads to the generation of a transient potential, which is amplified in the form of a ‘regenerative potential’ by sodium (Na⁺) channels such as Nav1.8 and Nav1.9 (Raouf et al., 2010). At this stage, the signal can be modulated by endogenous inhibition, which occurs via recruitment of potassium (K⁺) channels such as the two-pore channels TREK1 and TRAAK1 (Honréd, 2007). Finally, the activation of other Na⁺ channels, such as Nav1.7, triggers an action potential that carries nociceptive information from the peripheral nervous system into the central nervous system (CNS) (Raouf et al., 2010; Wood et al., 2004). A loss-of-function mutation in the human Nav1.7 gene reportedly leads to complete insensitivity to pain (Cox et al., 2006). Conversely, a gain-of-function Nav1.7 mutation causes congenital paroxysmal extreme pain disorders; for example, erythromelalgia (Fertleman et al., 2006). In line with these clinical observations, nociceptor-specific deletion of Nav1.7 leads to decreased pain hypersensitivity in mice (Nassar et al., 2004). Moreover, a Na⁺-channel blocker has been shown to be effective in relieving spontaneous pain in individuals with erythromelalgia (Goldberg et al., 2012).

Peripheral sensitization

In states of chronic pain, particularly in the context of inflammation and cancer, nociceptive and non-nociceptive sensory afferents are sensitized. Peripheral sensitization represents a reduction in the threshold and/or an increase in magnitude of responsiveness at the peripheral ends of sensory nerve fibers. This occurs in response to...
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that signal via G_9 and G_13 also couple to G_{12} and G_{13} proteins, which are capable of activating the RhoGTPase RhoA and, in turn, a downstream kinase, ROCK. However, the significance of RhoA-ROCK signaling in nociception is not known. Finally, G_7-mediated inhibition constitutes an important checkpoint in determining nociceptor excitability. The anti-nociceptive actions of cannabinoids and opioids, which bind to GPCRs, are also mediated via peripheral mechanisms (Agarwal et al., 2007; Kinsey et al., 2009; Lever et al., 2009; Stein and Lang, 2009).

**Signaling events in nociceptor somata**

In response to persistent nociceptive activity in peripheral tissues, several synapse-to-nucleus messengers are recruited. These include STAT3, MAPKs, e.g. ERK1 or ERK2, and cAMP-PKA, which drive activity-dependent transcription (Woolf and Costigan, 1999). However, the functional role of the somata of sensory neurons goes far beyond sustaining neuronal survival and synthesizing proteins that impart or modulate cell function. The somata of DRG neurons have evolved as the seat of intriguing mechanisms governing aberrant excitation and cell-cell interactions in chronic pain models (see Poster, panel B). For example, they are involved in the generation of ectopic discharges and oscillatory activity in states of neuropathic pain (Devor, 2006), e.g. by recruiting hyperpolarization-activated cyclic nucleotide-gated ion-channels (HCN) channels (Emery et al., 2011). Furthermore, gap junctions on satellite cells surrounding the somata of sensory neurons have also been implicated in the spread of aberrant excitation within the DRG (Zhang et al., 2009). Another intriguing recent finding is that non-neuronal cells, such as neutrophils and T cells, invade the DRG in inflammatory and neuropathic pain states (Kim and Moalem-Taylor, 2011). However, the identity and significance of these cell-cell interactions is not yet clear.

**Central signaling pathways involved in acute and chronic pain**

**Postsynaptic mechanisms**

Excitatory synaptic communication between primary afferents and spinal neurons is mediated primarily by glutamate, with modulatory influences from co-transmitters such as substance P, CGRP and brain-derived growth factor (BDNF). Both ionotropic and metabotropic (mostly G_q- or G_{11}-coupled) glutamatergic receptors play a key role in determining the strength of synaptic transmission and modulations in the spinal cord following persistent nociceptive activity (see Poster, panel C). Activity-dependent changes in spinal function encompass long-term potentiation (LTP) of individual synapses as well as an increase in neuronal and non-neuronal excitation in the spinal dorsal horn, leading to increased pain sensitivity, i.e. central sensitization (Ji et al., 2003; Sandkühler, 2009). A key trigger for both types of change is the activation of spinal postsynaptic NMDARs following persistent nociceptive activity (Woolf and Salter, 2000). The ensuing rise in intracellular Ca^{2+} activates protein kinases, such as CaMKII, that bring about the insertion of greater numbers of AMPA-type glutamate receptors in postsynaptic membranes via recruitment of a variety of AMPAR-interacting proteins, such as GRIP1 (Kuner, 2010). This not only enhances postsynaptic excitation, but also leads to further influx of Ca^{2+} via the recruitment of Ca^{2+}-permeable AMPAR (Galan et al., 2004; Hartmann et al., 2004; Park et al., 2009). Additional Ca^{2+}-
dependent kinases are also activated, such as cyclooxygenases (COX-2) and nitric oxide synthases (NOS), which generate prostaglandin E2 and nitric oxide, respectively. These molecules have been proposed to function as retrograde messengers, facilitating neurotransmitter release from primary afferent terminals in the spinal dorsal horn. A variety of synaptic-interacting proteins come into play to optimally position NMDAR and AMPAR channels in the postsynaptic membrane (Kuner, 2010). Interestingly, persistent nociceptive activity also recruits synaptic proteins that counteract or inhibit central sensitization, either by inhibiting key enzymes, e.g. NOS-interacting protein, or by disassembling complexes of metabotropic glutamate receptors 1 and 5 (mGluR1,5) together with inositol triphosphate receptors (IP3R) that guard intracellular Ca2+ stores. The MAPKs ERK1 and ERK2 are also enhanced AMPAR- and NMDAR-mediated currents in spinal cord neurons in a nociceptive activity-dependent manner, driving a unique genomic program that regulates both functional and structural plasticity in inflammatory pain (Cheng et al., 2002).

**Inhibition and disinhibition of central sensitization**

Persistent nociceptive activity-induced pronociceptive drive and central sensitization are held in check by spinal inhibitory networks, comprising GABAergic and glycnergic neurotransmission (see Poster, panel C, lower left). Endogenously released cannabinoids, opioids and adenosine also play an inhibitory role. For example, local enkephalins (released by enkephalinergic neurons) inhibit neurotransmitter release and depress postsynaptic excitation, via Gi-mediated inhibition of voltage-gated Ca2+ channels and Giri-mediated activation of GIRK-type K+ channels, respectively. These enkephalinergic inhibitory mechanisms are recruited spinally by descending serotonegergic and noradrenergic systems, constituting brainstem control of central sensitization. There are also several molecular signaling events that are associated with disinhibition after nerve injury. For instance, PGE2 inhibits PKA-mediated phosphorylation of the a3 subunit of the glycine receptor, thereby countering glycinergic inhibition (Harvey et al., 2004). Another mechanism for disinhibition of spinal neurons is nerve-injury-induced collapse of the Cl− gradient, brought about by a loss of the postsynaptic potassium chloride (K+ Cl−) exporter KCC2, which ultimately results in reduced generation of GABA-mediated inhibitory postsynaptic currents (Beggs et al., 2012; Coull et al., 2003).

**Signaling events associated with neuro-glia interactions**

In recent years, signaling mechanisms that mediate interactions between spinal neurons and diverse types of glial cells have been uncovered at an amazing pace. Purinergic signaling, involving P2X4 (Beggs et al., 2012), P2X7 (Clark et al., 2010a; Clark et al., 2010b) and P2Y12 (Tozaki-Saitoh et al., 2008) receptors, plays a central role in the recruitment and activation of microglia, which have emerged as key regulators of central sensitization (see Poster, panel C, right-hand side) (Gao and Ji, 2010a; McMahon and Malcangio, 2009). A great deal of interest has been focused on understanding the intracellular signaling pathways in activated microglia. Following nerve injury, chemokines released from the primary afferent terminals, such as CX3CL1, CCL2 and TNFa, as well as ATP, activate their cognate receptors on microglia (Beggs et al., 2012; Clark et al., 2010a; Gao and Ji, 2010a). Activation of these receptors induces the p38 MAPK signaling pathway in microglia, which is believed to underlie the synthesis and release of a variety of molecular mediators, such as BDNF, TNFa, IL-1β, IL-6 and cathepsin S, that alter neuronal function (Clark et al., 2009; Kawasaki et al., 2008). BDNF released from microglia acts on TrkB receptors in postsynaptic neurons to downregulate the expression of the potassium chloride co-transporter KCC2 in neighboring neurons, thereby rendering them more prone to excitation (Coull et al., 2003). Microglia-derived TNFa activates the JNK pathway in astrocytes, leading to the further release of IL-1β, CCL2 and MMP-2, which modulate central sensitization. TNFa was also shown to activate TNFR on presynaptic terminals, leading to the release of glutamate and increased excitatory postsynaptic potential.
(EPSP) via TRPV1 activation (Park et al., 2011). CCL2 and IL-1β released from astrocytes bind to their receptors (CCCR2 and ILR, respectively) at pre- and postsynaptic sites, leading to increased neurotransmitter release and enhanced activation of NMDAR and AMPAR (Gao and Ji, 2010b). MMP-9 and MMP-2 induce cleavage and activation of IL-1β, leading to further activation of microglia and astrocytes, thereby contributing to the development and maintenance of neuropathic pain. Cathepsin S released from microglia cleaves a transmembrane protein, fractalkine (FKN), that is expressed in spinal dorsal horn neurons and leads to the release of soluble FKN (s-FKN) that binds to its receptor, CX3CR1, on the microglia. This again triggers the activation of the p38 MAPK signaling pathway in microglia, establishing positive feed-forward and feedback modulatory loops that probably contribute to the maintenance of chronic pain long after the initial injury is triggered. In chronic pain conditions, astrocyte activation also leads to negative and positive modulation of EAAT and GABA transporters, respectively. This results in increased availability of excitatory amino acids such as glutamate and decreased availability of the inhibitory neurotransmitter GABA, and therefore in increased synaptic transmission.

**Potential therapies to combat pain**

Despite substantial advances in pain research, the barriers in developing novel therapeutics remain enormous. There are several difficulties associated with taking forward a drug target from bench to bedside. First, key mediators of nociceptive processing are not specific and have global functions in normal physiology (e.g. PLC, CREB and MAPK); second, the existence of redundant mechanisms and mediators in pain pathways makes it difficult to select suitable drug targets. To date, the majority of drugs that have been used to treat patients target mechanisms that have been known for several years. For example, traditional anti-inflammatory drugs such as salicylic acid, paracetamol, opioids and non-steroidal anti-inflammatory drugs (NSAIDs) remain the major players for the treatment of pain. Although these drugs are generally effective, the most efficacious ones among them are associated with unpleasant side effects such as nausea, vomiting, and renal and cardiovascular complications. Unfortunately, the newly developed COX-2 inhibitors also failed to be adopted clinically owing to exacerbation of cardiovascular side effects (Mukherjee et al., 2001; McGgettigan and Henry, 2006).

Recently, clinical trials were launched for Tanezumab and other monoclonal antibodies that act against NGF. Results from these trials suggest that anti-NGF therapy could represent an important new class of therapy for pain management in chronic pain conditions. Although promising, this novel approach is not, however, devoid of side effects (McKelvey et al., 2013).

There are also several ion channel inhibitors that provide a novel therapeutic approach for the treatment of pain. Procaine, bupivacaine and lidocaine are voltage-gated Na⁺ channel blockers that are effective anti-nociceptives upon local application, but their efficacy is limited to disorders with ongoing peripheral nociceptive activation (e.g. postherpetic neuralgia), rather than central pain disorders (conditions caused by damage to or dysfunction of the CNS). Other Na⁺ channel blockers, such as carbamazepine and lamotrigin, are efficacious against trigeminal neuralgia pain. The development of subtype-specific Na⁺ channel inhibitors is another strategy being implemented by drug companies, which holds tremendous promise. Ziconotide, a synthetic peptide that blocks presynaptic N-type voltage-gated Ca²⁺ channels and interferes with neurotransmitter release, is highly efficacious in individuals with chronic pain; however, its use is limited by CNS side effects. In addition, the drug must be given intrathecally to circumvent cardiac dysfunction. Lecontotide is an alternative to conopeptides such as Ziconotide, and has been suggested to have fewer CNS side effects (Kolosov et al., 2010). Pregabalin and gabapentin, which are moderately effective in relieving neuropathic pain, have been proposed to target accessory α2δ subunits of Ca²⁺ channels, although the supporting data are highly controversial. Recent studies indicate that gabapentin blocks spine morphogenesis, thereby implicating an alternative mechanism (Eroglu et al., 2009). Newly developed TRPV1 antagonists are also promising, yet they have been found to profoundly affect core body temperature, impeding their use in treating chronic pain disorders (Gavva et al., 2008).

Finally, there has been a substantial amount of interest in developing drugs that interfere with the interaction between non-neuronal populations and pain-processing neurons, based upon recent evidence of the roles played by non-neuronal cells (e.g. microglia and astrocytes) in the development and maintenance of chronic pain. Ongoing clinical trials will shed light on the potential drugability of these interactions, in addition to other newly discovered targets. Given the complexity and diversity of pain conditions, it is clinically very important to develop site-specific delivery tools as well as mechanism-based drugs.

**Summary and outlook**

As outlined in this review, tremendous progress has been made in understanding the neurobiology of peripheral and central sensitization in sensory-afferent–spinal-cord circuits that process nociception. This rich diversity of mediators provides enormous scope for drug discovery in the context of pain therapeutics. By contrast, much remains to be understood about mechanisms driving plasticity and reorganization in cortical circuits, where the perception of pain is generated. This remains a major challenge to tackle in the coming years.

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**COMPETING INTERESTS**

The authors declare that they do not have any competing or financial interests.

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