Pain hypersensitivity mechanisms at a glance

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There are two basic categories of pain: physiological pain, which serves an important protective function, and pathological pain, which can have a major negative impact on quality of life in the context of human disease. Major progress has been made in understanding the molecular mechanisms that drive sensory transduction, amplification and conduction in peripheral pain-sensing neurons, communication of sensory inputs to spinal second-order neurons, and the eventual modulation of sensory signals by spinal and descending circuits. This poster article endeavors to provide an overview of how molecular and cellular mechanisms underlying nociception in a physiological context undergo plasticity in pathophysiological states, leading to pain hypersensitivity and chronic pain.

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.

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 and the ATP-gated purinergic ion-channel P2X3 have also emerged as mediators of mechanical hyperalgesia (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 (Honró, 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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chemically mediated by nociceptors and non-neuronal cells (e.g. mast cells, basophils, platelets, macrophages, neutrophils, endothelial cells, keratinocytes and fibroblasts) at the site of tissue injury or inflammation. A wide range of signaling molecules is involved in mediating peripheral sensitization, including protons, ATP, prostaglandins (PGE2), thromboxanes, leukotrienes, endocannabinoids, growth factors such as neurotrophins [nerve growth factor (NGF)] and granulocyte- or granulocyte-macrophage colony stimulating factors (G-CSF, GM-CSF), cytokines (IL6, IL1β, TNFα), chemokines, neuropeptides [calcitonin gene-related peptide (CGRP)], substance P, bradykinin, histamine, lipids, and diverse proteases (Binshtok et al., 2008; Gold and Gebhart, 2010; Julius and Basbaum, 2001; Schweizerhof et al., 2009). Interestingly, glutamate, which is the major excitatory synaptic transmitter at central synapses, contributes to sensitization at peripheral nerve endings by binding in a non-synaptic manner to AMPA and NMDA receptors (AMPA and NMDAR, respectively) to mediate peripheral cell-cell interactions (e.g. upon release from immune cells) or autocrine regulation (e.g. upon release from sensory endings following TRPV1-mediated Ca2+ influx) (Gangadharan et al., 2011).

In response to signals from chemical mediators, the activity of transmission proteins that control the excitability of nociceptor terminals is modulated at the transcriptional or post-translational level. For example, growth factors (such as NGF acting via the tyrosine kinase receptor TrkA, or GM-CSF acting via tyrosine kinase receptors and JAK-STAT signaling), recruit multiple downstream enzymes, including phospholipase C (PLC), phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK). These enzymes not only directly phosphorylate transducer and ‘amplifier’ molecules, such as TRPV1 and Nav1.8, but also enhance the expression of these molecules via transcriptional regulation (e.g. via recruitment of STAT transcription factors), thus leading to an acute as well as a long-term increase in the excitability of nociceptors (Dib-Hajj et al., 1998; Julius and Basbaum, 2001; Schweizerhof et al., 2009; Zhang et al., 2005).

The role of G-protein coupled receptors

G-protein coupled receptor (GPCR) signaling also plays a role in peripheral sensitization. A diverse set of peptides, metabolic products and bioactive lipids activate GPCRs in sensory neurons. These GPCRs are capable of coupling to different G-proteins: Gq, G11, G0, G12 or G13. The Gq/G11 signaling branch mediates the activation of phospholipase C-β (PLC-β) and protein kinase C (PKC), the release of Ca2+ from intracellular stores, and modulation of extracellular regulated kinases (ERK1, ERK2) (Julius and McCleskey, 2005; Kuner, 2010). The G0 signaling branch, by contrast, is linked to cAMP–protein kinase-A (PKA)-mediated sensitization mechanisms (Hucho and Levine, 2007). Recent results indicate that the functional role of the G0/G13 signaling branch in nociceptors in vivo not only spans sensitization mechanisms in pathological pain states but also covers basal nociception and acute pain (Tappe-Theodor et al., 2012). This includes tonic modulation of TREK channels (Chen et al., 2006), Na+ channels (Tappe-Theodor et al., 2012), TRPV1 (Zhang et al., 2012) and mechanosensory currents (Lechner and Lewin, 2009). Most GPCRs that signal via G0 and G13 also couple to G12 and G13 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, G12-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 transcripition (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 Gq or G11-coupled) glutamate 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 Ca2+ activates protein kinases, such as CaMKIIα, 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 Ca2+ via the recruitment of Ca2+-permeable AMPAR (Galan et al., 2004; Hartmann et al., 2004; Park et al., 2009). Additional Ca2+-
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 found to travel into the nucleus of spinal excitatory neurons (Kohno et al., 2008).

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.
There are 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 lamotrigine, 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 Ca\(^{2+}\) 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 Ca\(^{2+}\) 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

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