Neuropathic Pain: Molecular Complexity Underlies Continuing Unmet Medical Need

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Received August 24, 2006

Introduction

Pain is a fundamental and central life experience, a counterbalance to pleasure, a warning of danger, and a reminder to guard injured limbs and tissues while they heal. And yet, however beneficial pain may be to our physiological well-being, it is also the most acutely unpleasant of sensations and one of the primary reasons patients seek medical care. Pain ranges in intensity from annoying, dull aching to throbbing, burning, sharp, or stabbing. At its worst, intractable combinations of these phenotypes are experienced by some cancer patients and others suffering chronic ailments. Scientific research and medical practice have coined a number of terms to describe pain in terms of its origins, manifestations, and chronicity. While attempts to understand the origins and control of pain date from antiquity, over the past 20 years, the expansion of molecular and cellular biology knowledge has led to the creation of tools that have allowed scientists to develop a much more detailed understanding of what causes pain and how it is transmitted, processed, and perceived individually, including our homeostatic control mechanisms. Most importantly, it also has helped identify a large number of potential targets for drug discovery. Unfortunately, despite our present depth of knowledge and a number of key mechanisms identified as intervention points, chronic pain and, in particular, neuropathic pain remain a greatly underserved medical need. Part of the difficulty in developing effective treatments for chronic pain lies in our failure to resolve the complex interplay among mechanisms involved. Unlike many other diseases, pain is not controlled by a "master gene" or target that can dominate the many viable mechanisms influencing pain signal initiation and transmission. In addition, because in many cases the most effective therapeutic agents for particularly neuropathic pain will be those that gain access to the central nervous system (CNS), the field has struggled to develop drugs that provide relief devoid of central side effects such as dizziness and drowsiness that limit the patient's ability to carry out normal activities. The scope of this review is to briefly cover some basic concepts encapsulating our current understanding of pain, including the molecular complexity involved in generating pain. In addition, the distinctions (and overlap) between acute nociceptive, chronic inflammatory, and neuropathic pain will be highlighted with the goal of providing a background for the following articles in this series that focus on a few key mechanistic targets currently being exploited in hopes of developing effective, safe, and tolerable pharmacotherapies for the treatment of neuropathic pain.

Overview of Pain Types and Distinguishing Characteristics

Nociceptive Pain. To gain insight into neuropathic pain and the targets representing potential treatment strategies, this type of pain should be put into perspective relative to other types. Pain is generally classified as either physiological or pathological. Physiological, or nociceptive, pain represents the normal response to noxious insult or injury of tissues, which can include the skin, muscles, organs, joints, tendons, or bones. Nociceptive pain is transduced through high-threshold, small diameter neurons, or nociceptors, present in two types: thinly myelinated Aδ-fibers and unmyelinated C-fibers.[1−5] Aδ-fibers rapidly conduct signals from the periphery to the spinal cord and eventually to higher centers, transducing what is known as "first pain" in response to a stimulus and manifested as a sharp and localized sensation. In contrast, C-fibers transduce "second pain" or pain sensations that are more diffuse and dull and are perceived with a temporal delay relative to the inciting stimulus.[4] Because of the variety of receptors they express, most nociceptors demonstrate polymodal responsiveness; that is, they are capable of generating electrical pulses, or action potentials, in response to activation by a range of noxious stimuli such as heat, acid, cold, and mechanical stimulation. The third major class of sensory neurons is Aβ-fibers, and they transduce sensations of light touch and typically are not involved in transmitting pain-inducing signals except, as described below, in pathological conditions of chronic pain.

Inflammatory Pain. As inflammation often results from, or contributes to, tissue injury, inflammatory pain usually is manifested through activation of the nociceptive pain pathway and is produced when a spectrum of mediators stimulates nociceptors at a site of tissue inflammation.[6−9] Although often described in the literature with the colloquial term "inflammatory soup", the components of this "soup" are complex and vary with the length and type of inflammation. Many of these mediators have been well characterized along with their role in nociceptive pain. Among the mediators that have been implicated as key players are proinflammatory cytokines such as IL-1[a] and TNF-α, chemokines, reactive oxygen species, vasoactive amines, lipids, ATP, acid, and other factors released by infiltrating leukocytes, vascular endothelial cells, or tissue resident mast cells.[10] These mediators either directly activate receptors expressed on nociceptors or recruit and activate additional inflammatory cells, which serves to maintain, and in some cases amplify, inflammation.[11,12] However, in contrast to acute

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[a] Abbreviations: IL-1, interleukin-1; TNF-α, tumor necrosis factor; NSAID, nonsteroidal anti-inflammatory drug; TRP, transient receptor potential; TREK, TWIK-related K channel; DEG/EnaC, degenerin/epithelial sodium channel; ASIC, acid-sensing ion channel; GPCR, G-protein-coupled receptor; TTX, tetrodotoxin; Nav, voltage-gated sodium; Cav, voltage-gated calcium; GIRK, G-protein-coupled inward rectifying potassium; Kv, voltage-gated potassium; KCNQ, voltage-gated potassium 7; CGRP, calcitonin-gene-related peptide; DRG, dorsal root ganglion; GABA, γ-aminobutyric acid; PAG, periaqueductal gray; RVM, rostroventral medulla; PKA, phosphokinase; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, *N*-methyl-D-aspartate; CREB, cyclic adenosine monophosphate response element binding; COX, cyclooxygenase; NOS, nitric oxide synthase; KCC2, K^+/Cl^- co-transporter; BDNF, brain-derived neurotrophic factor; MCP-1, monocyte chemoattractant protein; CCL2, C-C chemokine receptor ligand.

10.1021/jm061023c CCC: $37.00
Published on Web 05/10/2007

Table 1. Diversity of Human Painful Conditions

acute pain	persistent/inflammatory pain	chronic/neuropathic pain	neuropathic pain
-venipuncture/needle procedures	-arthritis (RA/OA)	-osteoarthritis	peripheral neuropathies
-burn	-postoperative pain	-lower back pain	-postherpetic neuralgia
-postoperative immediate	-irritable bowel syndrome	-fibromyalgia	-toxic neuropathies
-posttrauma immediate	-visceral pain	-cancer bone pain	-focal traumatic neuropathies
-acute medical diseases	-burn		-phantom and stump pain
(e.g., pancreatitis, myocardial infarction)	-traumatic tissue injury		central neuropathies
	-migraine		-ischemic cerebrovascular injury (stroke)
-obstretric pain	-lower back pain		-multiple sclerosis
	-endometriosis		-spinal cord injury
	-dysmenorrhea		-Parkinson's disease
	-urologic pain		-amyotrophic lateral sclerosis
			mixed neuropathies
			-diabetic neuropathies
			-sympathetically maintained pain

nociceptive pain signaling, the different receptors brought into play by inflammatory mediators can lower the threshold of nociceptor activation because of the effects of downstream signaling pathways to which these receptors couple.[13−15] Lowered thresholds of nociceptor firing translate into hyperalgesia and allodynia, that is, respectively, the perception of painful stimuli as more painful than they would be if evoked in the absence of inflammation and the perception of normally innocuous stimuli as painful.[4,7,9]

Neuropathic Pain. It is this phenomenon of increased pain sensitivity that lies at the heart of neuropathic pain and has continued to challenge the best efforts of biomedical research to develop effective drugs that combat the physical and emotional burden it places on patients. Neuropathic pain has been defined as abnormal signaling resulting from injury or malfunction in the peripheral or central nervous system leading to pain.[16,17] What makes neuropathic pain abnormal is that it can persist in the absence of visible injury or clinically measurable inflammation. As shown in Table 1, a wide variety of conditions are classified on the basis of presentation of a primarily neuropathic pain phenotype. Neuropathic pain can originate from peripheral, spinal, or supraspinal sites, as exemplified, respectively by conditions such as focal trauma neuropathies or postherpetic neuralgia, chronic lower back pain or other spinal injury, or pain associated with stroke, brain tumors, and AIDS.[16] Other persistent or chronic conditions, such as arthritis, both rheumatoid and osteoarthritis, and lower back pain, while clearly initiated and driven by compression of, damage to, or inflammation of sensory nerves, nonetheless can be present with symptoms of neuropathic pain. This overlap highlights the difficulty of classifying pain simply by etiology. It is well-known that in any number of diseases, pain can arise through a common mechanism, and yet a single mechanism can manifest itself in a variety of pain symptoms. On the other hand, a given pain phenotype can be induced by more than one mechanism, and multiple mechanisms often contribute to a particular pain state.[7,17] As a result, basic research and pain drug discovery have been moving toward a mechanism-based classification system for painful conditions based on the belief that this will ultimately lead to greater chances for treatment success.[18]

Pain of neuropathic origin results from plastic changes to both peripheral and central components of the pain transduction system that usually follow an initial injury, leading to a malfunction of the network and the production of exaggerated pain sensations.[5,7,19] Although the diagnosis of neuropathic pain is usually based on stimulus-induced, that is, evoked, responses, a hallmark of this pain is that it is often spontaneous and stimulus-independent. The types of pain experienced can vary but typically include burning, shooting, or lancinating types. Other common manifestations of neuropathic pain include paresthesias, or tingling, numbness, or pins and needles sensations, and dysesthesia, a poorly discriminating central burning sensation that has been described by some patients as "Dantesque" in that their flesh feels like it is on fire.[20] Neuropathic pain often shares its origins with acute pain resulting from tissue injury or inflammation; however, in contrast to nociceptive pain that subsides once an injury heals, neuropathic pain has lost the ability to be regulated and is now a self-perpetuating, dysregulated sensory phenomenon no longer dependent on external noxious stimuli. As a result, effective treatment of neuropathic pain has become a great medical challenge. Many drugs that effectively treat inflammatory pain, such as NSAIDs, or pain resulting from acute tissue injury, such as opioids, are in general much less effective in relieving neuropathic pain or show efficacy only at high doses or when administered by more invasive (e.g., intraspinal) routes. Moreover, because a large number of receptors and ion channels have been shown experimentally to play key roles in generating and maintaining neuropathic pain, a major, as yet unanswered question is whether any of the many new treatments under development will be able, as a monotherapy, to produce a significant therapeutic benefit for this type of pain.

Pain Mechanisms and Signaling Molecules: Receptors, Ion Channels, and Mediators

Primary nociceptors are designed to respond to high-intensity noxious stimulation that broadly falls into three classes: temperature (hot and cold), mechanical, or chemical.[4] The receptors that respond to these stimulus modalities and transduce them into electrical signals fall into several families: the TRP (transient receptor potential), TREK (TWIK-related K channel), and DEG/ENaC (degenerin/epithelial Na channel) family that includes the acid sensing ion channel (ASIC) family.[4,15,21−23] TRPV1, V2, and V3 respond in a graded fashion as warm temperature sensors. Thus, TRPV3 is activated at warm temperatures ranging between 31 and 39 °C. TRPV1 shows a temperature activation threshold of 43 °C, while TRPV2 is activated at the higher temperature of 52 °C.[24] A number of agonists have been identified for TRPV1, including capsaicin, the ingredient that makes chili peppers hot.[25] In contrast, TRPM8 has been identified as being activated by cold as well as menthol and eucalyptol.[26,27] Another family member, TRPA1, also has been implicated as a cold sensor, although this view is still controversial.[28−30] As the name implies, ASIC channels are activated in response to low pH, although family members, notably ASIC3 (DRASIC in rodents), have been implicated in responses to noxious mechanical stimulation, reflecting the functional overlap observed among these channels.[31−33] Thus,

TRPV1 can be activated in response to both heat and acid, TREK-1 has been shown to be activated by both heat and mechanical stimulation, and TRPA1 can be activated by cold, mechanical, and chemical stimuli. Because these receptors gate cations (Na^+, Ca^{2+}), the intracellular effects of activation are at least 2-fold. Downstream activation of second messengers and kinases sensitizes ion channels and receptors to respond more easily to intracellular or extracellular triggers. In addition, the influx of positively charged ions serves to depolarize the cell membrane, which facilitates the generation of action potentials. A variety of other receptors are also expressed on sensory neurons and come into play in response to inflammatory mediators released by injured neurons, surrounding Schwann cells, vascular endothelial cells, or the several subsets of leukocytes (neutrophils, mast cells, monocytes, lymphocytes) that combine to form the inflammatory nexus at the site of injury. Many of these receptors are heterotrimeric G-protein-coupled (GPCR) and include opioid, cannabinoid, prostaglandin, endothelin, bradykinin, serotonin, adrenergic, chemokine, and metabotropic glutamate receptors. Depending on the G-proteins to which these couple (Gs/Gq vs Gi/o), multiple second messenger pathways are activated, and signals transduced through these receptors can serve to either further activate or inhibit sensory nerve signaling.[34,35]

Pain signals are carried from the periphery to the CNS by electrical signals, or action potentials, generated in sensory neurons by the opening of voltage-gated sodium channels (Nav) in response to membrane depolarization. These channels fall broadly into two main classes, tetrodotoxin (TTX)-sensitive (TTX-S) and -resistant (TTX-R). TTX-S channels, in particular Nav 1.2, 1.6, and 1.7, are expressed in essentially all sensory neurons, while another TTX-S channel, Nav 1.3, normally is expressed at very low levels but is found to be dramatically up-regulated following injury.[36−39] Antisense knockout studies have implicated Nav 1.3 as a potential target for drug intervention.[40,41] Most TTX-S sodium channels show rapid activation and inactivation kinetics in response to membrane depolarization. These channels also recover slowly (reprime) from inactivation, which serves to determine the frequency with which action potentials can be initiated.[42,43] TTX-R channels Nav 1.8 and 1.9 are expressed primarily in small and some medium-sized sensory neurons. In contrast to the TTX-S channels, Nav 1.8 shows a higher threshold of activation and steady-state inactivation, activates and inactivates slowly, but also reprimes quickly and, as a result, can facilitate the repeated trains of neuronal firing frequently observed in injured nerves.[43,44] Although Nav 1.8 is presumably active in inflammatory pain conditions, the role of this channel is less clear following nerve injury leading to neuropathic pain where expression has been shown to decrease, and the pain behaviors in knockout animals are indistinguishable from those of normal mice in models of neuropathic pain.[45−47]

In contrast to the role of Nav channels in transmitting pain signals by opening to let in Na^+ ions that generate action potentials, potassium channels serve an opposite effect, that being to repolarize the cell membrane. This happens by mechanisms whereby potassium channels become activated, that is, open in response to one or a series of action potentials. When potassium channels open, K^+ ions flow out of the cell along a concentration gradient, leaving a net negative charge inside the cell that repolarizes the membrane. There is a wide variety of potassium channel families expressed on sensory neurons, but among them, the G-protein-coupled, inward rectifying potassium (GIRK) channels as well as voltage-gated fast delayed rectifier channels, as exemplified by Kv 1.4, and slow delayed rectifier channels, such as KCNQ (Kv 7.2/7.3), play key roles in maintaining the activation threshold of sensory neurons.[48−50] GIRK channels contribute to both the maintenance of resting membrane potential and pacemaker-like activity, that is, control of the rate at which hyperpolarized cells return to the activation threshold, allowing the initiation of another action potential. These channels are activated by opioids, cannabinoids, and α-2 adrenergic agonists, which leads to inhibition of neuronal excitation.[48] Because of their slow gating, the activation of KCNQ channels (reviewed in a following article) requires several repeated action potentials. As a result, these channels play a significant role in regulating hyperactive neurons that are characterized by repeated firing.

Another ion channel family of great importance to the regulation of nociceptive neuron function and pain signal transmission is the voltage-gated calcium channel (Cav) family, in particular, Cav 2.2.[51,52] A wide variety of Cav channels are expressed in various tissues throughout the body, where they play a critical role in gating neurotransmitter release. In response to membrane depolarization such as in action potentials, Cav 2.2 channels expressed in dorsal root ganglion cell bodies and concentrated in presynaptic terminals open to allow the influx of Ca^{2+} ions, which triggers the release of neuropeptides substance P and calcitonin-gene-related peptide (CGRP) and transmitters, especially glutamate, by mediating the fusion of synaptic vesicles at the synaptic interface.[53,54] Cav 2.2 has been afforded the rare status of being a clinically validated pain target by virtue of the effects seen with the potent cone snail venom, *ω*-conotoxin-MVIIA, also known as Ziconotide or Prialt, a selective Cav 2.2 inhibitor used for the treatment of severe neuropathic pain.[55] However, because of the peptidic nature of this drug, it only can be delivered by the intrathecal route, so much effort is now focused on developing orally active inhibitors of Cav 2.2. In addition, knockout of Cav 2.2 leads to reduced sensitivity to induction of inflammatory and neuropathic pain without other apparent defects, further supporting the targeting of this channel for drug intervention.[56] Together, the coordinated opening and closing of potassium, sodium, and calcium channels tightly regulate neuronal membrane potential, action potentials, and neurotransmitter release, respectively. The complexity of these activities provides many potential means of regulation and, in the case of pathological pain, possible points of dysregulation.

Pain Conduction Pathways

Pain signals initiated through activation of nociceptors travel from the periphery (skin, tissues, and organs), collecting into larger nerve bundles that eventually pass into and form synapses in the spinal cord dorsal horn. As illustrated in Figure 1, the dorsal root ganglia house the cell bodies of these pain-sensing neurons, sending projections both to the periphery and to the spinal cord. As such, much interest has been focused on DRG neurons, the variety of receptors they express, and their physiology, as well as how these neurons interact with second-order neurons in the dorsal horn. Primary nociceptors enter the dorsal horn and make synapses in different regions, or laminas, depending on their nature (C- vs A*δ*-fiber) and phenotype, including the signaling molecules (for example, substance P and CGRP) produced.[1,3,57−59] Within these laminas, DRG neurons may interact with both excitatory and inhibitory interneurons, or short connecting neurons, that help fine-tune the incoming signals as they are relayed to pain transmission neurons. By receiving input from both ascending and descending pathways, interneurons serve to modify stimulus intensity and quality,

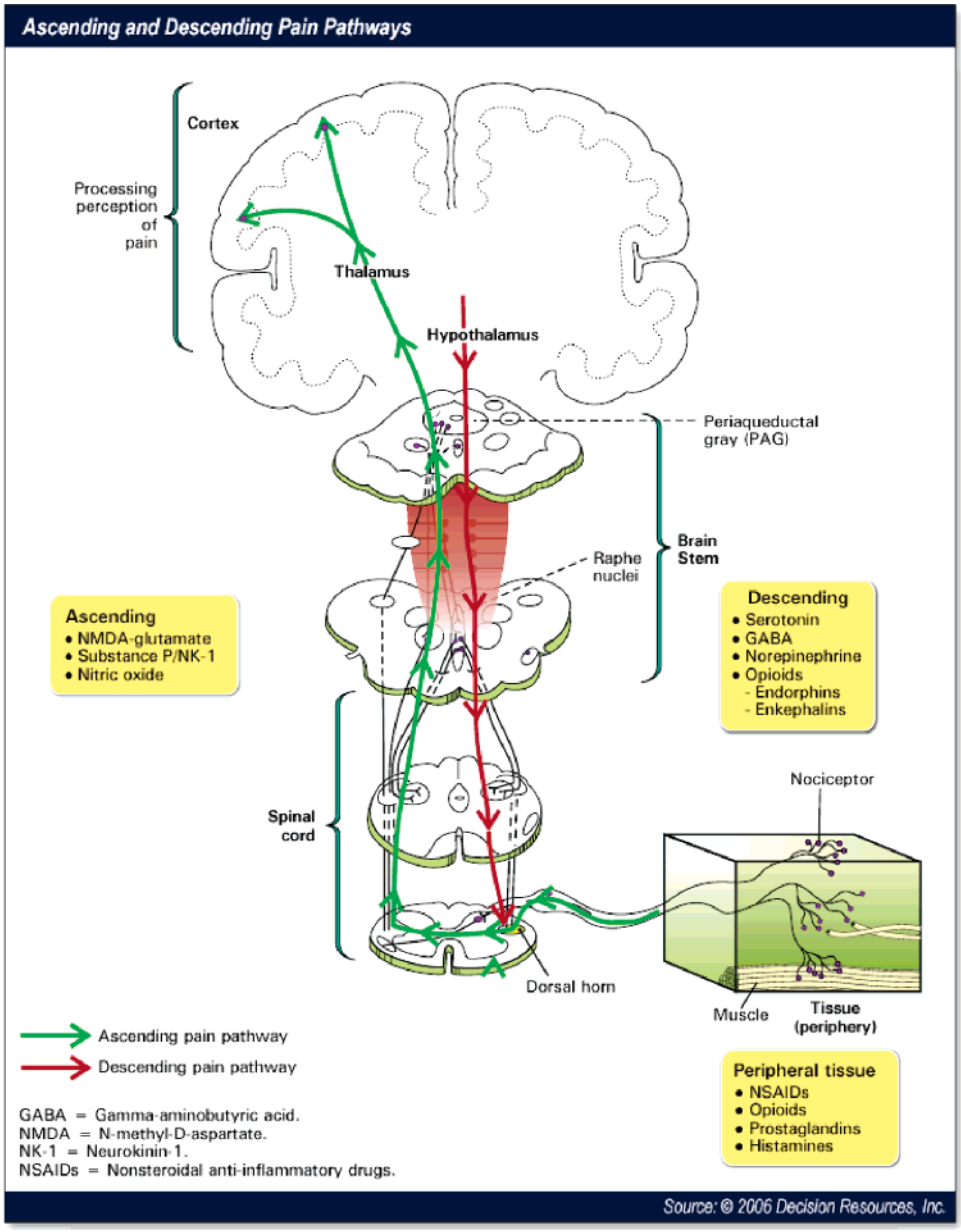

Figure 1. Pain "superhighway". Pain signals originating from the periphery pass through the DRG, the synapse in the spinal cord dorsal horn, and proceed to the higher CNS centers. Several key structures collect and modify signals traveling in both the ascending and descending directions. Highlighted in the boxes are key mediators or drug targets believed to play important roles in pain processing and transmission at different junctures of the pathway. Reprinted with permission from May 2006 *Pharmacor Report* ("Novel Approaches to Pain Therapy" by A. K. Jassen and C. Vasilakis-Scaramozza). Source: Copyright 2006 Decision Resources, Inc.

helping set the overall tone of the pain. As depicted in Figure 2, GABA and glycinergic interneurons, as well as descending noradrenergic neurons, can inhibit pain signaling. In addition to these, microglia present in the dorsal horn are also intimately involved in regulating pain transmission by virtue of the proinflammatory cytokines they produce in response to activation by injured or chronically stimulated afferent and postsynaptic neurons.[60−62] Signals from afferent nociceptors are relayed through the dorsal horn via synapses formed with pain transmission neurons that then pass to the contralateral side of the spinal cord and proceed to supraspinal centers via two main nerve bundles, the spinothalamic and spinoparabrachial tracts.[1,3,63−65] These nerves carry information related to discrimination (location and type) of the pain being transmitted and the affective (emotional or unpleasant) component of pain, respectively. There are several structures and loci along this pathway through which the nerves funnel, and these are involved with modulating the signals in terms of both quality and intensity. Pain signals eventually terminate in cortical regions where they are perceived and where responses are initiated. Important as this ascending pain pathway is to our perception of pain, of equal importance is the descending pain modulatory pathway that originates in the amygdala and hypothalamus and proceeds in tracks parallel to the ascending pathway but traveling distally from the CNS, again passing through a number of structures or relay stations.[66−68] Most notable among these are the periaqueductal gray (PAG) and rostroventral medulla (RVM), which play major roles in determining the quality of the descending signals. As a result, signals proceeding back down the spinal cord, eventually forming synapses with both sensory and motor neurons, can be either inhibitory, that is, serve to lessen the signals generated by the ascending pathway, or facilitatory, increasing the intensity of the pain signals being generated by nociceptors as seen in states of chronic/neuropathic pain where the balance of descend-

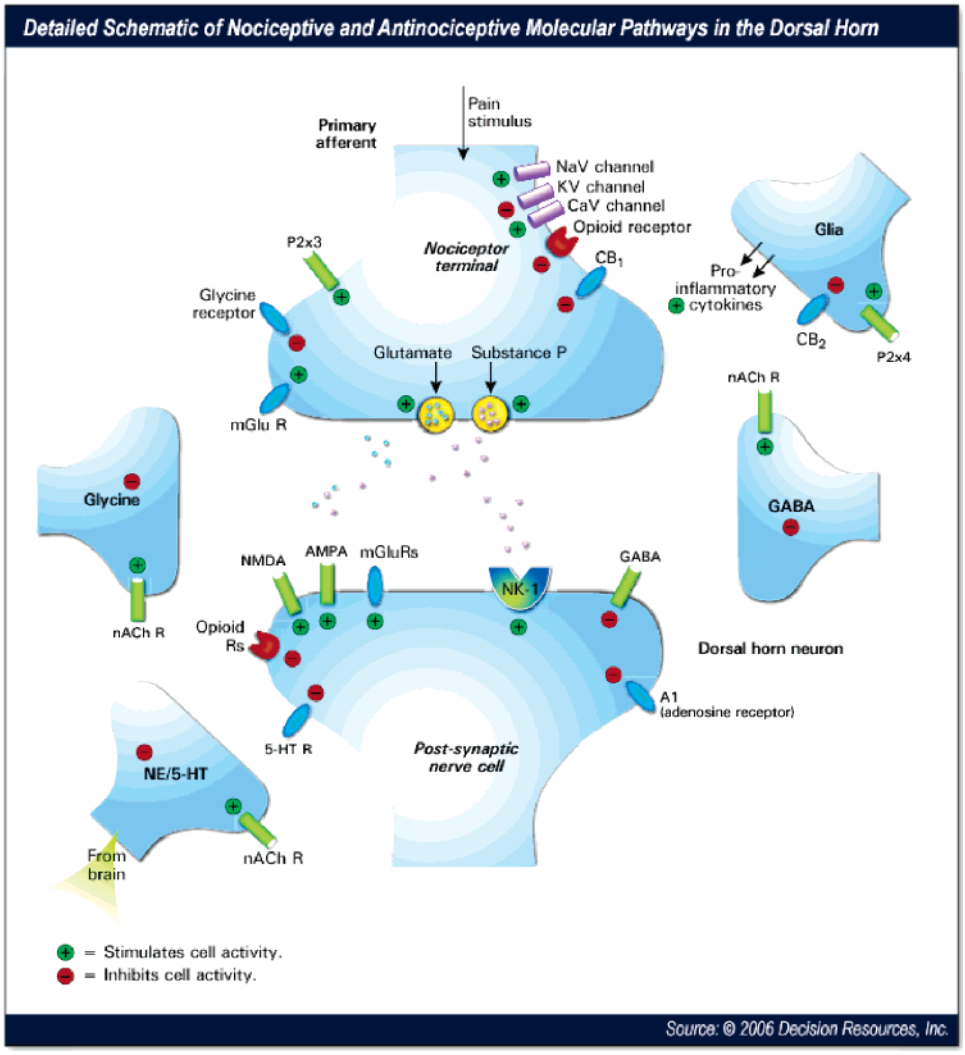

Figure 2. Excitatory and inhibitory neuronal networks that interact in the spinal cord dorsal horn to modify pain signals transmitted from the periphery to the CNS. Reprinted with permission from May 2006 *Pharmacor Report* ("Novel Approaches to Pain Therapy" by A. K. Jassen and C. Vasilakis-Scaramozza). Source: Copyright 2006 Decision Resources, Inc.

ing input is shifted toward facilitation. Though much is known regarding how and under what conditions these conflicting modifications are generated, much is yet to be learned. In general, our understanding is deeper with regard to the peripheral components and mechanisms at play in initiating, propagating, and regulating pain signals in peripheral nociceptors, the DRG, and the spinal cord dorsal horn. Overall, the entire pain conduction pathway put into action resembles in many ways a turnpike superhighway, where smaller roads (nociceptive neurons) feed into the limited-access highway (spinal cord via the DRG and dorsal roots), road construction (nerve damage, compression injury) causes congestion (inflammation), and toll booths (supraspinal relay stations) allow orchestrated additions to and subtractions from the traffic volume in both directions; all are integral parts of the larger antiparallel traffic (signal conduction) flow.

Pathological Pain: Neuronal Plasticity Facilitates Peripheral and Central Sensitization and the Development of Neuropathic Pain

Normal nociceptive pain resulting from injury or noxious insult is perceived through the coordinated actions of a highly regulated and integrated signal recognition, transduction, and transmission system that is difficult to activate and ceases upon removal of the activating stimulus. As such, the pain perception system functions much like the body's other sensory systems to provide basic information about the environment and its status. In contrast, because neuropathic pain is manifested as exaggerated pain sensations that can persist in the absence of visible injury, much research has focused on understanding the mechanisms leading to this inappropriate functioning of the pain signaling pathway. A large body of data supports the concept that nociceptive, inflammatory, and neuropathic pain are manifestations of points along a continuum of activation states that develop and are maintained by plastic changes in the phenotype and functions of both sensory afferent and descending modulatory neurons. These changes have been broadly grouped into two categories: peripheral and central sensitization, hypersensitized states of the pain signaling pathway that are bridged by the phenomenon of windup.[7,15,19,69]

Peripheral sensitization is defined as an increased sensitivity of nociceptive neurons to input stimulus. This can occur by two means: autosensitization and heterosensitization. Autosensitization is an increased responsiveness of nociceptors to intense and/or continued stimulation and is typically mediated by a conformational change in receptors expressed by these neurons, as is seen with the Ca^{2+}-dependent phosphorylation of TRPV1 by PKC and CaMKII.[70] Heterosensitization involves a cross-sensitization of other receptors, bringing them into play and

amplifying the pain signals. Both of these sensitizing processes can be in play simultaneously in the same neuron. In contrast to acute noxious stimuli, the inflammation that accompanies tissue damage results in the release of a variety of mediators that activate neuronal GPCRs, including chemokine and cytokine receptors, and additional ion channels, such as P2X family members, whose activities may not be invoked as part of the primary nociceptive pain signaling pathway. The downstream effect is to activate a number of second messenger pathways, among them kinases such as PKA and PKC, that through phosphorylation (hetero)sensitize ion channels and receptors, thereby increasing their sensitivity to incoming signals, even normally non-noxious stimuli, to which anyone who has ever taken a hot shower following a sunburn can attest.[4,19] Not only does peripheral sensitization increase the sensitivity of sensory neurons, it also activates a feedback/feed-forward loop termed neurogenic inflammation.[9,71,72] The heightened state of sensory neuron activation induces the release of neurotransmitters CGRP and substance P from peripheral terminals, which causes local vasodilation and leakage of plasma and leukocytes from the blood into the tissue and activates these infiltrating cells. The result is edema and the perpetuation of the inflammatory pain response. Finally, the effects of inflammatory mediators and neurogenic inflammation are widespread such that both injured and noninjured nerves are affected, further amplifying the pain signals and spreading hypersensitivity to uninjured tissues peripheral to the injury site. This phenomenon, known as secondary hyperalgesia, is apparent in inflammatory pain, as evidenced by the tenderness of skin adjacent to a cut or surrounding a bee sting, although by definition, secondary hyperalgesia has its basis in central sensitization.[73−75] In the acute setting, signal input to the dorsal horn sensitizes pain-transmitting neurons so that innocuous signals from adjacent, undamaged touch receptors (Aβ-fibers) are now perceived as painful. However, in more chronic and neuropathic conditions where central sensitization is maintained and driven by additional plastic changes to the pain conduction pathway, the recruitment of Aβ-fibers, as discussed below, involves both phenotypic and functional changes.

To this point, the changes described are readily reversible upon resolution of the tissue injury and inflammation. However, if pain signaling continues as a result of chronic inflammation or nerve injury that is slow to heal, additional plastic changes in both the ascending and descending pathways can occur that are less readily reversed and eventually may become self-sustaining.[19,76] A large number of genes are regulated, both up and down, in response to peripheral nerve injury, including a host of peptides and receptors that amplify pain signaling. In addition to these changes in sensory neurons, potassium channels are inactivated and their expression reduced.[77−80] This leads to neuronal hyperexcitability and the manifestation of ectopic, or spontaneous, neuronal firing. In normal pain signaling, AMPA receptors serve as the primary postsynaptic receptors responding to the neurotransmitter glutamate; however, repeated input from the periphery to the dorsal horn leads to increased activity of postsynaptic NMDA glutamate receptors as a result of phosphorylation by Src kinase and loss of the Mg^{2+} block that normally regulates the amount of Ca^{2+} these ligand-gated channels pass.[81−83] Increased activity of NMDA receptors, in combination with activation of NK1 receptors also expressed on dorsal horn neurons, is involved in windup, a phenomenon whereby repeated peripheral C-fiber stimulation leads to the generation of slow excitatory postsynaptic potentials in wide dynamic range neurons that increase over time, leading to increased sensitivity and after-sensations where pain persists for a period following stimulation.[84−86] This reversible plastic change in the pain signaling pathway is easily demonstrated in both animals and humans, for example, as measured by increased sensitivity to repeated tactile stimulation following intradermal capsaicin injection.[86,87]

Though not a prerequisite, windup can be viewed as a forerunner to the development of central sensitization, which involves both input-driven increased activation of dorsal horn neurons and transcriptional changes in these neurons that lead to long-lived phenotypic changes. Among genes showing up-regulated expression are transcription factors NF-κB, c-Myc, CREB, c-Jun, and ATF-3, a number of ion channels, and other cell surface proteins such as prostanoid and chemokine receptors, as well as increased synthesis and activation of COX and NOS, inflammatory cytokines, and chemokines.[15,34,88−92] As important as increased transcriptional activity is to the establishment of central sensitization, of equal importance are other changes that lead to dysregulation of tonic inhibitory influences in the dorsal horn. It is important to keep in mind that central sensitization involves hyperactivity of the entire "superhighway", both afferent and efferent pain pathways, as described earlier.[66−68] Continuous CNS stimulation leads to similar activation and likely plastic changes within both inhibitory and facilitatory descending pathways; in neuropathic pain, the facilitatory pathway dominates. Although the exact mechanisms resulting in the preferential activation of one descending pathway over the other remain to be defined, one intriguing mechanism recently has come to light. The influx of chloride ions in response to activation of GABA-A receptors normally transduces inhibitory signals within dorsal horn neurons; however, reversal of low intracellular chloride concentrations to high, relative to external concentrations, occurs in response to the down-regulation of the chloride transporter KCC2 that is responsible for maintaining a low intracellular chloride concentration. As a result, the opening of GABA-A receptors now leads to a reduced inward chloride flux or, in extreme cases, an outward flux that results in activation, rather than inhibition, of neuronal activity.[93] Recent evidence suggests this phenotype reversal is mediated by the effects of presynaptic BDNF that interacts with up-regulated TrkB receptors expressed on dorsal horn neurons.[94,95] In addition, other potentially permanent changes in pain regulatory mechanisms occur following nerve injury. The greatly increased release of glutamate by injured or chronically stimulated afferent neurons may show selective toxicity toward inhibitory interneurons, leading to the loss of negative regulation of pain signal transmission.[92,96−98] Finally, central sensitization involves the recruitment of normally noninvolved sensory neurons such as very fast-conducting Aβ-fibers. Injury-induced changes in gene expression and mediator release by nearby neurons lead to "bystander" activation of Aβ-fibers, inducing plastic changes in these neurons, including the synthesis of substance P and BDNF.[99−103] This phenotypic change causes the neurons to now behave like afferent nociceptive neurons. In addition, a number of studies reported sprouting of Aβ-fiber terminals following peripheral nerve injury, leading to formation of new and additional synaptic connections from deeper layers of the dorsal horn to pain transmission neurons in the superficial laminas, although this hypothesis remains quite controversial and has been largely discounted as a mechanism contributing to neuropathic pain.[98,104−108] Regardless, it is clear that a physiologic, if not physical, "rewiring" of the pain sensing and conduction pathway is a key part of neuropathic pain pathogenesis and offers another

mechanism for developing allodynia, where sensations of light touch, such as clothing brushing against skin, are now transduced into terrible pain. Moreover, as many of these phenotypic and physical changes in the pain signal transduction pathway may be permanent, the prospects for developing disease-modifying drugs that can effectively reverse the mechanisms leading to the establishment of neuropathic pain, rather than simply controlling its symptoms, become even more challenging.

One additional major contributor that has gained increased attention over the past decade with respect to its role in the development and maintenance of neuropathic pain is the immune system, specifically, microglia. These CNS-resident cells of monocytic lineage, along with astroglial cells, make up approximately 90% of the cell numbers in the CNS. Though for years, neuroscientists and immunologists alike viewed proponents of neuroimmunology as fringe players, if not crackpots, an exploding body of literature has revealed and validated the integral and critical association between glia and neurons and the crosstalk between them that maintains CNS homeostasis.[109,110] However, in response to neuronal injury and inflammation, microglia change their phenotype from protector to aggressor, contributing to neuronal activation through the production of proinflammatory mediators. Among these are chemokines like MCP-1 (CCL2) that function in an endocrine, paracrine, and autocrine fashion to recruit and activate other leukocytes and maintain microglia in an activated state. Peripheral nerve damage leads to the appearance of activated microglia in the spinal cord dorsal horn that in at least one animal pain model of mixed inflammatory and neuropathic phenotype temporally parallels the development and expression of pain behaviors.[111] Of further interest are recent studies showing that injured neurons also produce MCP-1; however, of special note is the observation that in addition to its actions on microglia, this chemokine can facilitate the generation of action potentials in injured, but not uninjured, neurons and can inhibit signaling from inhibitory GABA neurons.[112−115] The intriguing possibility posed by these observations is that central sensitization may be, at least in part, maintained by an MCP-1-mediated communication loop between neurons and microglia.

Conclusions

What can we discover that will break the cycle of central sensitization in neuropathic pain and quiet the overactive neuronal activity that so envelopes and debilitates those in its grip to the point where some prefer death to the prospect of continued suffering? Hope lies in the recognition that not all chronic pain conditions necessarily evolve into a neuropathic state, even while invoking most, if not all, of what are currently believed to be the defining mechanisms. Perhaps the greatest example of this is the pain associated with chronic osteoarthritis, where standard pharmacotherapies provide only modest pain relief and patients will suffer for decades before resigning themselves to joint replacement surgery. Surprisingly, in the vast majority of cases, pain vanishes following the removal of damaged tissue. If biomedical science could understand the key mechanistic differences between this pain and neuropathic conditions, perhaps better drugs could be designed. As it stands today, clinicians employ a polypharmacy approach to the treatment of neuropathic pain and likely will continue to do so until we better understand the roles played by, and relative importance of, individual components in this complex spectrum of disorders. One of the confounding issues with developing new pain drugs is the fact that many potential targets, such as inflammatory mediators and certain ion channels, are also expressed, or express closely related relatives, in other tissues, including the CNS, where inhibition would be expected to cause significant toxicities or intolerable side effects. Though much remains to be learned, a number of targets have shown promise and have reached advanced preclinical study, offering hope as novel, and hopefully safer, pain therapies. In the following articles, several of the most promising targets for the treatment of neuropathic pain are profiled.

Acknowledgment. The author is grateful to Dr. Edward J. Kaftan for comments and critical review of the manuscript provided.

Biography

Jeffrey D. Kennedy received his Ph.D. in Immunology from the University of Iowa under the direction of Professor David Lubaroff. Following postdoctoral training appointments in the laboratory of Dr. Carl W. Pierce at Washington University School of Medicine and The Upjohn Company under the direction of Drs. Ivan Richards and Jia En Chin, he joined the Inflammatory Diseases Group at Wyeth Research in 1993 to pursue asthma research and drug discovery. In 2000, he joined the Neuroscience Department where he became involved with models of neurodegeneration. He has long-standing interests in chronic inflammatory diseases, leukocyte trafficking, and cell activation. Dr. Kennedy currently leads the Pain Research Department within Wyeth Neuroscience Discovery, focusing on the discovery of novel therapeutic agents for the treatment of chronic inflammatory and neuropathic pain conditions.

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JM061023C