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Molecular Biology of Pain: Should Clinicians Care?

Recent breakthroughs in our understanding of pain mechanisms can be traced to the introduction of molecular biology techniques into pain research. New ion channel proteins involved in generating, modulating, and propagating action potentials along nociceptor axons have been cloned and characterized using molecular biology techniques. These techniques continue to reveal nociceptive mechanisms involving molecules, receptors, and neural networks that underlie neural reorganization ("plasticity") in the spinal cord and brainstem after peripheral tissue damage or nerve injury.

At the latest IASP World Congress, in his presidential address Professor Besson voiced concern that clinical and basic research appear to be diverging, even as dramatic new insights into fundamental mechanisms of nociception promise therapeutic breakthroughs. It is normal for considerable time to elapse between the development of new information from basic research and its application in the clinic.¹⁻² Today, at the dawn of the involvement of molecular biology in clinical pain research and treatment, it is essential that IASP foster the exchange of information between clinicians and basic scientists. Otherwise, this gap may increase under the pressure of ever more rapid accumulation of increasingly specialized information.³ This issue of *Pain: Clinical Updates* describes the contribution of molecular biology to understanding adaptations to nociception in several pain models, including the identification of new molecules involved in this process. We also describe potential goals of molecular pain research, including "translational research" that incorporates preclinical insights into clinical investigations.

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New Molecules Related to Nociception

Neuronal plasticity, an essential feature of the nervous system, is a "buzz word" in current pain research. Fluctuations in gene expression that reflect changes in the functional demands on individual neurons are an everyday occurrence. With sustained

peripheral inflammation, however, prolonged C-fiber activation alters the pattern of gene transcription in dorsal root ganglion (DRG) cells and dorsal horn neurons. Following peripheral nerve injury, changes in neuronal excitability and mRNA levels in sensory neurons offer a substrate for chronic pain. Several mechanisms that contribute to increased excitability in DRG have recently been discovered. One striking example is the capsaicin or vanilloid receptor-1 (VR1), which has been cloned and characterized.⁴ Interestingly, protons, whose concentration increases in an acidic environment (already known to enhance the noxious effect of capsaicin), appear to be endogenous ligands of VR1.⁵ The marked functional similarities between capsaicin-induced and heat-induced activation of VR1 indicate that VR1 is the physiological transducer of noxious heat.

Marked functional similarities between capsaicin-induced and heat-induced nociceptor activation indicate that the capsaicin receptor is the physiological transducer of noxious heat.

Proton-sensitive channels, a family of ion channels triggered by increased local acidity (reduction in pH), have been recently identified.^{6,7} These proteins, named ASICs (acid-sensing ion channels), consist of five subtypes, each with a distinct pattern of activation kinetics, pH dependence, and tissue specificity. Four of these subtypes are expressed in small-diameter sensory neurons, making them candidate mediators of hyperalgesia in inflamed, poorly perfused tissue that becomes acidotic.

Among several other ion channel proteins recently cloned, the tetrodotoxin (TTX)-resistant sodium (Na) channel has attracted the most attention because of its location in the nervous system and its expression only after nerve injury.^{8,9} This channel is found predominantly in unmyelinated small-diameter primary afferent neurons. Electrophysiological and immunohistochemical experiments and studies in "knockout" mice¹⁰ have revealed that a specific TTX-resistant Na channel (termed PN3 or sensory-neuron specific, SNS) may play a key role in persistent pain states, including neuropathic and chronic inflammatory pain.

Another ion channel protein relevant to nociception is the adenosine triphosphate (ATP) receptor. ATP is known to depolarize sensory neurons, and the release of ATP from damaged tissue may augment nociceptor activation.¹¹ Among the several members of the ATP receptor subfamily termed P2X, the P2X3 receptor has been cloned, characterized, and shown by in situ hybridization to be located in small nociceptive neurons. Because of the anatomical location of this channel and the algescic effect of ATP, it has been suggested that the P2X3 channel may mediate ATP-evoked activation of small nociceptive neurons.¹²

Genetic Methods in Pharmacology

Molecular tools for pharmacological investigation include genetic manipulations, particularly null mutations or "knockouts" that delete specific ligands or their receptors. Knockout mice are useful to explore the contributions of particular molecules to nociception. Studies of mice with deletions of opioid receptor genes have shed new light

not only on how opioids work, but also on opioid receptor heterogeneity and interactions, and the involvement of each component of the opioid system.^{13,14} Deletion of nicotinic receptor genes has raised the possibility that these receptors play a role in nociceptive processing.¹⁵ Studies of mice with deletions of genes encoding various neurotrophins and their receptors provide critical information about nociceptor phenotype development and injury-induced neuronal plasticity.¹⁶⁻¹⁸ Mice with deletion of the gene for the second messenger molecule, protein kinase C (PKC) gamma, display intact acute pain responses but reduced thermal and mechanical allodynia after nerve injury. Subnormal allodynia in these knockout mice suggests for the first time that PKC gamma in substantia gelatinosa interneurons is critical for the establishment of central sensitization after nerve injury.¹⁹ These results illustrate the power of genetic manipulation to identify or confirm a role for specific molecules or their receptors in nociception.

In contrast, recent observations in knockout mice lacking either substance P (SP) or its receptor (NK1) have yielded unexpected results.²⁰⁻²² Surprisingly, some SP or NK1 knockout mice showed unchanged mechanical hypersensitivity with inflammation. Other inconsistencies between the SP and NK1 null mutations include differences in hot plate, acetic acid writhing and formalin testing. The knockout approach is susceptible to a number of confounding factors such as masking of the phenotype by compensatory developmental mechanisms in utero and during early postnatal life. Tissue-specific knockout techniques that manipulate gene expression in sensory neurons but not other cells, or inducible knockouts that exert their effects after development is complete, are refinements that minimize these confounding factors.

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Deeper Understanding of Pain Mechanisms

Understanding of pain mechanisms benefits from every level of analysis, not only from the identification of new molecules using techniques described above, but also from new insights into the function of molecules already known to be present after tissue inflammation and nerve injury.²³

1. Transcription factors and second messenger molecules

The most intensively examined transcription factor in dorsal horn neurons is the gene *c-fos*. Since Hunt's finding²⁴ that noxious stimuli induce *c-fos* expression in dorsal horn neurons, many studies have used expression of FOS (the protein product of this gene) as a molecular marker of neuronal activity. Further, phosphorylation of the cyclic cAMP-responsive element binding protein (CREB)²⁵ and extracellular signal-regulated protein kinases (ERK)²⁶ have been observed in dorsal horn neurons within minutes after noxious peripheral stimulation.

2. Neurotrophic factors (neurotrophins)

Many studies provide convincing evidence that neurotrophins in sensory systems have critical roles in the development of sensory systems and in neuronal plasticity. A recent insight is that nociceptors can be divided into two major classes.²⁷ One class of nociceptor neurons, which contain trkA receptors, substance P, and calcitonin gene-related peptide (CGRP), is regulated by nerve growth factor (NGF). A second class of nociceptor contains *c-ret* receptors, has IB4 binding capacity, and is regulated by glial-cell-derived neurotrophic factor (GDNF). The role of GDNF-regulated nociceptors as potential targets for novel analgesics in acute and chronic pain states is now under intensive study.

A surprising finding is that one type of neurotrophin, brain-derived neurotrophic factor (BDNF), is a neuromodulator in small-diameter nociceptive neurons.²⁸ BDNF reportedly is produced in DRG neurons, released from central terminals of primary afferents, and modulates postsynaptic excitability. BDNF can be modulated by target-derived NGF in nociceptors expressing trkA and substance P.

3. Cytokines and inflammatory pain

Pro-inflammatory cytokines including tumor necrosis factors and interleukins (TNF-alpha, IL-1-beta, IL-6, IL-8, etc.) augment activity in nociceptive pathways.²⁹ These substances may indirectly cause sensitization by triggering release of other cytokines and classical mediators of hyperalgesia including prostanoids, sympathomimetic amines, endothelin, or glutamate. Neuronal sensitization by cytokines occurs centrally as well as in the periphery. Anti-inflammatory cytokines (IL-4, IL-10, IL-13, etc.) released from different cell types during inflammation may inhibit release of pro-inflammatory cytokines and decrease cyclooxygenase (COX)-2 expression.

4. Molecular aspects of the opioid system

Molecular biological approaches have provided important new insights into the actions of mu and delta opioids. Recent work has now identified nine exons spanning over 200 kilobases in the mu opioid receptor (MOR)-1 gene.³⁰ Delta receptors are reported to be involved in the production of morphine tolerance.³¹ The pharmacology of orphanin FQ (OFQ)/nociceptin, the endogenous ligand for the opioid-receptor-like substance ORL1, is complex, both potentiating and inhibiting nociceptive input.³² The recently identified active peptides from the OFQ/nociceptin precursor, OFQ/2 and nocistatin, are also of interest because nocistatin counteracts the action of nociceptin.³³

5. Preclinical mechanisms of neuropathic pain

Mechanisms of neuropathic pain after peripheral nerve injury have been vigorously examined.³⁴ Some neuropathic pain models employ partial nerve injury, after which intact afferents transmit sensory input to the spinal cord and divided afferents generate ectopic afferent activity. After clinical nerve injury, A-beta fibers that are normally activated by light touch can transmit nociceptive information. In animal studies,^{35,36} some DRG neurons with A-beta fibers begin to express SP/CGRP after injury. Spared DRG

neurons also show increased expression of SP/CGRP³⁷ that may be regulated by NGF. These compensatory responses in neuropathic pain have been likened to "fail-safe mechanisms".³⁸

6. Structural reorganization in neuropathic pain

In the early 1990s, two important papers demonstrated central nerve sprouting after peripheral nerve injury. One paper reported that A-beta fibers extend from lamina III to lamina II, an area normally occupied only by nociceptive C fibers, and then make synaptic contact with neurons in this region.³⁹ This reorganization is a plausible explanation of allodynia from normally innocuous A-beta stimulation after nerve injury. A second seminal paper described sympathetic sprouting around large neurons in the DRG after peripheral nerve injury.⁴⁰ This finding confirms that sympathetic efferents and sensory neurons may become functionally coupled after nerve injury.

7. Novel pain transmission pathways

Postsynaptic dorsal column neurons and medial lemniscus pathways were recently recognized to carry visceral pain sensations.^{41,42} Noxious input from viscera elicits hyperexcitability in dorsal column neurons that in turn evokes activity of ventroposterolateral (VPL) neurons in the thalamus. Correlative behavioral studies in animals with dorsal column lesions support the concept of the dorsal column as a visceral pain pathway.

Post-injury dorsal column activation has also been described in neuropathic pain models. Nuclei of dorsal column neurons are hyperexcitable and display dramatically altered electrophysiological properties in animals with neuropathic pathology as compared to naive animals.⁴³ Analysis of VPL activity suggests that nociceptive signals from the periphery may shift their afferent pathway from the spinothalamic tract (STT) to the dorsal column.⁴⁴

Goals of Molecular Biology Research: Summary

1. Genes

Molecular biology offers powerful means to clarify how altered gene expression controls neuronal adaptations to inflammation and tissue or nerve injury. Recognition of the importance of newly identified genes depends upon clarification of their function. As the human genome project approaches completion, "new" genes involved in specific pain conditions or chronic pain diseases may be uncovered. Recent insight into the genetic determinants of individual differences in pain and analgesia are pertinent.⁴⁵

Another possible application of molecular biology is pain control through gene transfer. Gene therapy can correct genetic defects by replacing or substituting the defective gene with a new, functional copy. Studies exploring gene therapy in pain control are just beginning.⁴⁶ In the near future, gene therapy using the antisense strategy or insertion of

genetic material into spinal cord neurons by means of viral vectors may be applied clinically.

2. *Gene products*

Many of the molecules and pain mechanisms described in this article have the potential to change clinical therapy. The recent explosion of basic research on ion channels in nociceptors in sensory neurons may allow the development of novel analgesics.³⁸

Receptors for amino acids, neuropeptides, growth factors, catecholamines, cytokines, and so on, will also be targets for novel therapeutic agents. At the most recent IASP World Congress on Pain, a workshop called for a mechanism-based classification of persistent pain. Such a classification may help generate and organize testable hypotheses for selecting targeted pain treatments, including drug design and delivery. As a result, clinicians may be armed with more reliable and appropriate tools for the clinical investigation, diagnosis, and control of pain.

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