Pain physiology: basic science

Allan I. Basbaum MD

ALTHOUGH there is considerable information about the mechanisms through which injury stimuli produce acute pain, recent studies have established that there are significant long-term consequences of persistent injury. Pain is exacerbated, in part, because of a reorganization of spinal cord circuitry in the setting of persistent injury. In this brief review I will address some of the mechanisms that have been identified, focusing on issues of interest to our own laboratory, and indicate how they are likely to operate in the production of the intense pain associated with tissue or nerve injury.

A major interest of our laboratory is in the contribution of the primary afferent neurotransmitter, substance P (SP), to these changes. By following internalization of the SP receptor in spinal cord dorsal horn neurons, we have identified the stimuli that evoke SP release and the neurons that respond to these stimuli. Importantly, based on the intensities of stimuli required to evoke internalization, we conclude that SP, in the normal animal, is only released under conditions in which severe pain would be produced.¹ Furthermore, the release of SP can be evoked by intense stimulation of somatic and visceral tissue, and multiple stimulus modalities are effective. We also found that the numbers of neurons that are influenced increase dramatically in the setting of inflammation.² Although intense stimuli were still the most effective at inducing the internalization in the setting of injury, it was also possible to induce changes with non-noxious stimuli. This raised the important possibility that severe injury alters the means through which SP-containing afferents activate spinal cord "pain" transmission neurons.

This possibility has been dramatically demonstrated in recent studies by Mantyh and colleagues in a model of bone cancer pain in the mouse.³ The cancer was induced in the femur and within 21 days led to a profound mechanical allodynia. Even light palpation of the hindlimb provoked immediate withdrawal. Importantly, the bone cancer was associated with dramatic changes in the neurochemistry of the spinal

cord. For example, there was a profound induction of glial cells throughout the ipsilateral dorsal horn. This indicates that the neuroanatomical and neurophysiological consequences of the developing cancer were not limited to the target of the primary afferents that entered the dorsal horn. Furthermore, the authors found that light palpation produced a dramatic increase in the numbers of neurons that internalized the SP receptor. This presumably could underlie the mechanical allodynia that characterized this condition. Future studies must determine the source of the SP that induced internalization in the setting of bone cancer. It could have derived from peripherally-sensitized, SP-containing nociceptors. Alternatively, because there is evidence that large diameter afferents begin to synthesize SP in the setting of profound inflammation,⁴ the possibility that a different population of afferents has been recruited must be considered. Finally, because invasion of peripheral nerves by tumour is common, the likely development of associated neuropathic pain conditions must be taken into account. Thus, recent studies on the neurochemical basis of neuropathic pain conditions are undoubtedly relevant to the development of intractable cancer pain.

Among the other interesting molecules that our laboratory has implicated in persistent injury conditions is the vanilloid receptor (VR-1), which is targeted, by capsaicin, the active ingredient in hot peppers. It has long been known that a capsaicin receptor is expressed, perhaps exclusively, by primary afferent nociceptors, and that the pain that is produced by capsaicin (an exogenous molecule) is mediated by activation of nociceptors through this receptor. When the receptor was cloned⁵ it was further determined that the receptor responds to noxious thermal temperatures, which suggested that the receptor is also involved in the generation of thermal pain. To address these questions, we have been studying mice with a deletion of the gene that encodes VR-1.⁶ Consistent with the *in vitro* characterization of the channel, the mice demonstrated reduced responsiveness to noxious heat, but they were not completely unresponsive, which indicates that other high thresh-

From the Departments of Anatomy and Physiology and W.M. Keck Foundation Center for Integrative Neuroscience, University of California San Francisco, San Francisco, California, USA.

Address correspondence to: Dr. Allan I. Basbaum, Department of Anatomy, University of California San Francisco, Box 0452, 513 Parnassus Avenue, San Francisco, California 94143, USA. Phone: 415-476-5270; Fax: 415-476-4845; E-mail: aib@phy.ucsf.edu

olds, thermally-responsive channels must exist. More importantly perhaps, we found that the thermal hyperalgesia/allodynia that occurs in the setting of tissue inflammation was completely prevented in the VR-1 mutant mice. Mechanical allodynia in the same condition was preserved and the thermal and mechanical changes produced in the setting of nerve injury were not altered. Because VR-1 is regulated by pH,⁷ and because pH is likely to be significantly lowered when there is tissue injury, we suggest that thermal hyperalgesia in the setting of tissue injury involves pH sensitization of VR-1. Importantly, if there is a significant pH drop when there is visceral inflammation, it is possible that visceral pain may, in part, be generated by VR-1 responsiveness at temperatures approaching body temperature.

Finally, our laboratory has made progress in understanding some of the molecular contributors to nerve-injury induced neuropathic pain, which is a major persistent pain condition for which treatment is not satisfactory. Although there is some evidence that opioids are effective for some types of neuropathic pain, in general the therapeutic value of opioids is limited. Use of non-selective uptake inhibitors clearly has value, although their mechanism of action is unclear. Whether their effectiveness reflects their action on the uptake of monoamines or on Na⁺ channels (like local anesthetics) remains to be established.

There is much evidence for a contribution of the NMDA type of glutamate receptor channel.⁸ This channel is triggered by intense stimuli, which would include nerve injury. Opening of the NMDA channel leads to the entry of Ca²⁺ into postsynaptic dorsal horn neurons. This, in turn triggers a variety of long-term changes that establish a central sensitization/hyperexcitability of dorsal horn neurons.⁹ The clinical problem in addressing the contribution of the NMDA receptor is that most NMDA receptor antagonists have an unsatisfactory side-effect profile. Recent studies, however, have turned to antagonists that block subcomponents of the NMDA receptor complex (e.g., the NR2B subtype). The goal is to increase the therapeutic window of the drugs.

Another potential important contributor to nerve-injury induced persistent pain is an anatomical reorganization of primary afferents. Following peripheral nerve injury, a profound sprouting of axons occurs. Sprouting has been demonstrated in the periphery¹⁰ and in the spinal cord dorsal horn.¹¹ Each of these sites may contribute to abnormal transmission of impulses that could contribute to the development of neuropathic pain. For example, in the periphery there is evidence for sprouting of sympathetic efferents, par-

ticularly in the region of the neuroma.¹² This could lead to an abnormal activation of nociceptive primary afferents. Concurrent upregulation of adrenergic receptors by nociceptors would exacerbate this condition. There is also evidence for a dramatic sprouting of sympathetic efferents around the cell bodies of large diameter dorsal root ganglion neurons.¹⁰ This could contribute to the A-beta-mediated mechanical allodynia that characterizes many nerve injury-induced pain conditions.¹³ The sprouting of large diameter afferents in the spinal cord dorsal horn is likely to inappropriately activate dorsal horn nociresponsive neurons, and could lead to pain being produced by innocuous mechanical stimulation. Of interest is the possibility of regulating/reducing such abnormal sprouting by manipulating the levels of growth factors at the level of the spinal cord.¹⁴

The next question concerns the contribution of specific molecules to the development and persistence of neuropathic pain conditions. Although traditional approaches to addressing this question have relied on pharmacological antagonism, there are limitations to those studies. Most importantly, selectivity of the antagonists is often called into question. Furthermore, it is often difficult to deliver antagonists for extended times, as is necessary in the study of the pain produced by persistent injury. For this reason, we have turned to the use of mice with deletion of genes that code for proteins that may be involved in the development of the long-term changes that underlie neuropathic pain conditions. In particular we have studied mice with a deletion of the gamma isoform of protein kinase C (PKC γ), a major second messenger molecule that is exclusively found in a population of interneurons in the superficial part of the dorsal horn.¹⁵ Acute pain processing is completely normal in these mice, however they do not develop thermal or mechanical allodynia following partial peripheral nerve injury (a model of neuropathic pain). Of interest, we have recently demonstrated that despite the inability to establish a long-term allodynia, there is a short-term sensitization that occurs following injury in the PKC γ mutant mice. Within two hours of injury, however, the sensitization is lost. This indicates that PKC γ is required for the transition from short- to long-term sensitization. Importantly, our studies and those of other laboratories, indicate that multiple transduction pathways underlie different components of the persistent pain response. For this reason, therapeutic interventions targeted at individual second messenger molecules are unlikely to have comparable and global effects on persistent pain. A multifaceted approach to the problem is likely to be required.

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