Molecular Mechanisms of Pain: A Basis for Chronic Pain and Therapeutic Approaches Based on the Cell and the Gene

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INTRODUCTION

The perception of pain serves the adaptive role of providing an affective context for real, impending, or perceived tissue damage. In order to accomplish this critical role, separate classes of peripheral receptors serve to characterize and relay the presence of noxious stimuli. The initial stimuli is processed and filtered in a stepwise fashion in the periphery, throughout the neuraxis, and in the telencephalon. Important molecular events contribute to signal processing and modulation within the periphery, dorsal root ganglion, spinal cord, and brainstem, as well as in specific subcortical nuclei and cortical pathways.

In order for pain to act as an effective motivator, systems must exist to identify the source and location of the pain, as well as to attach a powerful emotional valence to the perception. Even when the direct insult, resulting in a "first pain" has passed, the nociceptive sensation can be prolonged by inflammatory mediators that are released into the surrounding milieu. The result is a more diffuse "second pain," characterized by a burning quality, which plays the physiological role of helping to prevent further insult while a healing response occurs. In addition to physiological *nociception*, which produces sensations in direct or indirect response to noxious stimuli, a pathological form, *neuropathic pain*, results from an underlying dysfunction in the nervous system. Often, neuropathic pain is the result of physical damage to a nerve. In fact, animal models of neuropathic pain focus largely on compression or partial transection. However, neuropathic pain can be temporally and spatially abstracted from the inciting damage, as in the case of phantom limb pain. This abstraction results from neuroplasticity in the periphery and within the central nervous system. These forms of plasticity are known respectively as *peripheral sensitization* and *central sensitization*. Through these processes, pain perception shifts out of proportion with the magnitude of afferent input from the periphery resulting in hyperalgesia or allodynia. Chronic neuropathic pain results when the perception becomes entirely independent of stimulation.

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Because the painful sensation has been at least partially abstracted from the stimuli as a consequence of central plasticity, and because rostral transmission does not seem to be confined to a single fiber tract, neuropathic pain has proven difficult to treat by conventional pharmacological and surgical techniques. Current strategies have been, at best, incompletely successful because they fail to fully account for both the unique anatomic and pharmacological implications of this dynamic, pathological process. Within a given location of the nociceptive processing network, multiple molecular events, including multiple receptors and ligands, encode pain. Thus, surgical therapies that possess only anatomic specificity, inherently lack pharmacological specificity. In contrast, analgesic drugs can target specific neurotransmitter systems, but lack anatomic specificity. The body uses individual transmitter/receptor systems in multiple physiological roles. For this reason, design of more ideal treatments will require achievement of both forms of specificity, to reverse or mask the processes underlying neuropathic pain while allowing retention of the capacity for nociceptive transmission. As such, interest has developed in novel treatments for neuropathic pain that locally manipulate signal transmission at a molecular and cellular level.

This chapter will explore cellular and genetic therapeutic mechanisms designed to reverse or mask neuropathic pain. To provide a context for the discussion of the molecular events that underlie pain, the anatomical and pharmacological basis for nociception and chronic pain will follow. A discussion of nociceptive pain requires an introduction to the functional anatomy with an emphasis placed upon the molecular basis of pain transmission and theorized methods of regulation. An understanding of the pain transmission apparatus, at macroscopic and molecular levels, will provide the background necessary to discuss aspects of peripheral and central sensitization which presage, in pathologic conditions, the development of neuropathic pain. Once the basis for the current understanding of neuropathic pain has been summarized, an exploration of the merits and limitations of current pharmacologic techniques will help to illustrate the need for more advanced treatments.

The development of advanced cell and gene based therapeutic techniques will be introduced as a potential means of circumventing these limitations by providing a platform through which anatomic and pharmacological specificity may simultaneously be achieved. An emphasis will be placed on potential future avenues of research.

FUNCTIONAL ANATOMY OF PAIN TRANSMISSION

Peripheral Stimuli Detection and Transmission[2,3,5,19,22]

Sensation of noxious stimuli requires that the incoming stimuli be converted into a form that can be interpreted by the neural networks of the peripheral nervous system (PNS) and central nervous system (CNS). Afferent fibers are able to preserve the information content of incoming stimuli by using somatotopic fiber organization in tandem with the ability to convert analog afferents into frequency-modulated signals. This provides the ability to convey the location of sensation, the magnitude of stimulation, and the duration of occurrence. In the specialized case of nociception, mechanisms are required to detect temperature, chemicals, and applied force. Nociception is encoded by receptors that are primarily concerned with these other modalities through an activation threshold. That is, pain occurs when mechanical, chemical, and thermal receptor are over-activated (*Table 10.1*).

One of the important detectors of noxious stimuli is the transient receptor potential (TRP) channel. The family of TRP channels is characterized by homology. Each of the TRP channels has six transmembrane subunits, and various subfamilies are known to have disparate functions in different organ systems and across species. With respect to the detection of noxious stimuli, the TRP channels known to be of interest include TRPV1–4, TRPM8, and TRPA1. Each channel is unique, responding to different stimuli. TRPV1–4 all respond to heat and are Ca^{2+} permeable, though to different degrees. Each has a unique threshold for temperature detection. The minimum thresholds for activation have been respectively reported as 43°C, 52°C, 31°C, and 25°C. In contrast, TRPM8 and TRPA1 are cold-sensing receptors with maximum thermal detection thresholds of approximately 25°C and 18°C, respectively.

TABLE 10.1

Peripheral Receptors of Noxious Stimuli		
Mechanical	**Thermal**	**Chemical**
DEG	*Heat Sensing*:	P2X3 (ATP)
DRASIC	TRPV (1-4)	ASIC (H^+)
TREK-1		DRASIC (H^+)
		TRPV1 (H^+)
	Cold Sensing:	
	TRPM8	
	TRPA1	

Thus far, evidence has only linked these specific TRP channels to noxious thermal stimuli in the periphery. Theories for the mechanism of signal transduction are least well developed for encoding thermal energy, when compared with some of the other known sensory roles in which TRP channels function. Some hypotheses include a temperature-dependent change in membrane surface tension, reversible channel denaturation, and cytoplasmic binding of diffusible second messengers. All three of these hypotheses rely upon reversible plasticity of the channel conformation to allow for either a direct or indirect increase in ion flux.

In addition to the TRP channel providing a mechanism for thermal energy transduction, the DEG, DRASIC, and TREK-1 channels are mechanosensors involved with detection of noxious, stimuli. The P2X3, DRASIC, ASIC, and TRPV1 channels are chemoreceptors also known to contribute to nociception. These receptors are categorized in *Table 10.1*.

Peripheral excitability represents a continuum which can be altered in response to exceedingly noxious stimuli, or under conditions present after damage to neural structures. In light of this, it is important to keep in mind that the sensory receptors are responsible for a graded depolarization within dendrites. The excitability of the relay neuron depends on the complement of ion channels responsible for re-encoding incoming analog signals as action potentials, for afferent transmission. These neurons, located within the dorsal root ganglion (DRG), contain a variety of Na^+, Ca^{2+}, and K^+ ion channels, the presence of which can be modulated depending upon several factors, including the duration and magnitude of noxious stimuli applied to the periphery. Briefly, the Na^+ channels are dichotomously considered as being tetrodotoxin (TTX) sensitive or resistant. The role of these channels and the effect of their modulation on pain perception will be reviewed in the later discussion on peripheral sensitization.

Signals generated from the peripheral nociceptors are carried by afferent fibers to specific laminae of the spinal cord dorsal horn. Such afferent nociceptive transmission is thought to occur via a combination of thinly myelinated Aδ fibers and unmyelinated C fibers. The former have conduction velocities on the order of 5–30m/s while the latter transmit at less than 1m/s. Both thermal and mechanical nociceptive fibers use Aδ fibers while thin, unmyelinated C fibers provide a conduit for polymodal nociception, which responds to noxious chemical, mechanical, and thermal stimuli. Under physiologic conditions these nociceptors require high threshold stimuli to induce action potential generation. However, *peripheral sensitization* can lower the threshold to incite nociceptive stimuli transmission, hence allowing trivial stimuli to trigger pain.

CENTRAL TRANSMISSION AND REGULATION[2,3,32,42,43]

Primary sensory (DRG) neurons synapse within specific laminae of the dorsal horn as shown in *Figure 10.1*. The gray matter of the spinal cord is traditionally subdivided into 10 specific laminae based on cytoarchitectural criteria; the dorsal horn contains six of these subdivisions. The predominant sensory layers, beginning dorsally, are: marginal (I), substantia gelatinosa (II), nucleus proprius (III-IV), and deep layers (V–VI). The majority of general sensory afferents synapse in Lamina V, with significant contributions also being made to Laminae III and IV. The marginal layer is almost exclusively comprised of input from nociceptive afferents that project to higher regulatory centers, including both Aδ and C fibers. Aδ fibers also synapse in Lamina V onto wide dynamic rage neurons (WDR), whereas the other primary contribution of C fibers is in Lamina II. A generalized distinction exists between the C fibers innervating Lamina I and the outer portion of Lamina II and those C fibers innervating the inner portion of Lamina II. The former respond more to heat and chemicals, contain neuropeptides, such as Substance P (SP) and calcitonin gene-related peptide (CGRP), and tend to synapse on second order neurons projecting to higher centers. The latter contain a smaller complement of neuropeptides and synapse on interneurons in Lamina II. Lamina II is largely composed of interneurons of both excitatory and inhibitory function. Lamina III to IV receives largely Aβ fibers transmitting non-noxious stimuli while maintaining high spatial resolution. Lamina V, predominantly receive Aβ wide dynamic range (WDR) input and Aδ input. Additionally, WDR neurons are capable of receiving input directly from C fibers, as their dendrites may extend dorsally to superficial laminae, or indirectly, due to the contribution of interneurons.

Glutamate neurotransmission underlies much of peripheral pain transmission. It typically functions through non-NMDA (AMPA kainate) and metabotropic (mGluR) receptors under conditions of low frequency, high threshold stimulation. In contrast, the NMDA glutamate receptor (NMDAR) seems to be a strong contributor to the process of central sensitization. The NMDAR plays a minor role in membrane depolarization under normal circumstances. The primary balance of intracellular phosphatase-kinase activity prevents the receptor from being activated via phosphorylation, except in the presence of mediators promoting a state of sensitization. Additionally, at the resting membrane potential, a Mg^{2+} plug is present, preventing ion flux. This process is depicted in *Figure 10.2*.

Primary inhibitory neurotransmitters acting at the level of the dorsal horn include GABA and glycine. They have both pre- and postsynaptic functions with the majority acting through postsynaptic mechanisms. Both neurotransmitters activate Cl^- ion channels, through $GABA_A$ and specific glycine channels, respectively. GABA is also known to act postsynaptically through the $GABA_B$ metabotropic ion channel, which ultimately achieves the same hyperpolarizing effect through differing modulation of K^+, Ca^+, & Na^+ channels.

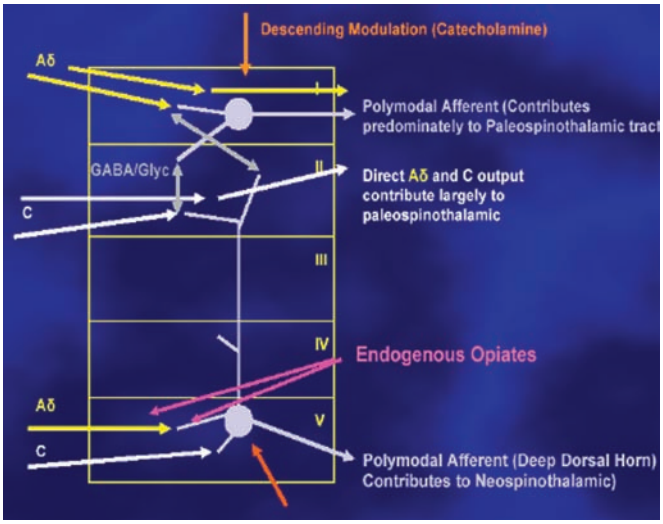

FIGURE 10.1 Rexed's lamina. Input and output synapses use predominantly excitatory amino acid transmission (Glutamate). Inhibitory interneurons (GABA and Glycine) play a role in modulation of the nociceptive signal, as do endogenous opiates. Fibers descending from the brainstem further regulate nociceptive signal transduction through catecholamine transmitters.

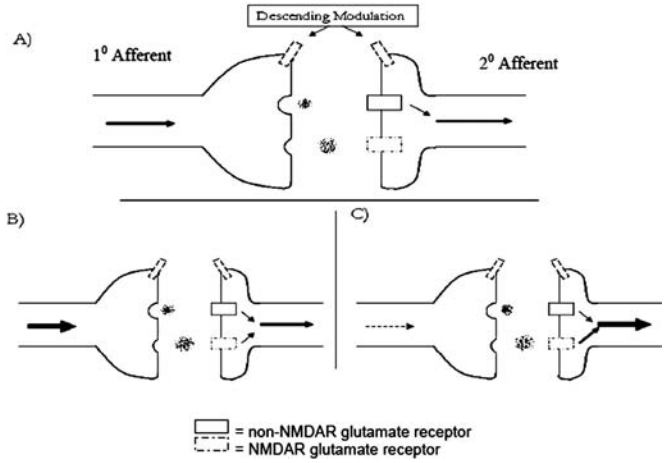

FIGURE 10.2 NMDAR activation in central sensitization. *A*, under normal circumstances, afferent glutamatergic transmission is handled by multiple channel types (e.g., AMPA, kainate, mGluR). Descending modulation seems to be primarily inhibitory and mediated by GABA and glycine under normal conditions. *B*, enhanced afferent signaling to the dorsal horn, in conjunction with changes in descending modulatory tone can promote central wind-up. *C*, after NMDAR activation, second order afferent signalling may be abstracted from peripheral input.

Central sensitization depends on a change in the balance between excitatory and inhibitory transmission within Rexed's lamina. This balance is largely achieved as a result of the post-translational and transcriptional mechanisms that modify the activity and quantity of these receptors. The ultimate result of these changes in activity and expression is an increase in excitability of the afferent tracts responsible for pain transduction. This increased excitability uncouples peripheral receptor activity from the affective sensory experiences of pain.

ASCENDING PATHWAYS[2,43,49]

A minimum of five pathways have been identified as providing conduits for afferent nociceptive neurons. Contributions are provided by the spinothalamic, spinomesencephalic, spinoreticular, spinohypothalamic, and cervicothalamic tracts. These pathways track through either anterolateral or dorsal fasciculi.

The Anterolateral Quadrant

The neospinothalamic tract, or ventral spinothalamic tract, advances to the ventral posterior lateral (VPL) nucleus of the thalamus (alternatively termed ventralis caudalis [Vc]), and is implicated in the discriminatory sensation of pain. Fibers from the neospinothalamic tract are primarily WDR neurons, projecting from deeper dorsal horn laminae (IV–VI). The paleospinothalamic, or dorsal spinothalamic, tracts is implicated in the affective aspect of pain. These tracts are predominately nociceptive neurons projecting from more superficial laminae (I–II). Terminations are largely in medial portions of the thalamus. *Spinoreticular* neurons, largely originating from Laminae VII and VIII of the dorsal horn, terminate within the brainstem. Axons passing to the medulla have been shown to terminate in nucleus retroambiguus, superspinalis, and the dorsal and ventral aspects of the medullae oblongatae centralis. Advancing rostrally, terminations occur in the lateral reticular nuclei, the nucleus gigantocellularis, paragigantocellularis dorsalis and lateralis, and the nuclei pontis caudalis and oralis. This tract affects autonomic regulation and awareness, through the reticular formation and projections to the hypothalamus and thalamus. Contribution is also thought to be made to a "spino-limbic" tract through multisynaptic connections from the midbrain to the hypothalamus, medial thalamus, and limbic system.

The majority of afferents of the *spinomesencephalic* tract originate in Laminae I and IV to VI. Although collaterals to the lateral thalamus exist, the majority of the fibers pass to midbrain nuclei. The afferent fibers exhibit rough somatotopy, with afferents from more inferior fibers terminating caudally within the midbrain. Terminations include the periaqueductal gray matter (PAG), anterior and posterior pretectal nuclei, and nucleus cuneiformis. The PAG is an important modulator of the spinal mechanisms of descending analgesic modulation.

The Dorsolateral Quadrant

A spinocervicothalamic pathway has been noted in multiple species, arising and passing through a contralateral position at the cervical levels C1 and C2. Animal studies have indicated termination in the lateral cervical nucleus, which projects to the lateral thalamic nuclei. This pathway as a conduit for nociception.

Evidence exists to support the conduction of visceral nociceptive activity within tracts of the dorsal column. First, extreme pain is triggered during mechanical probing of the dorsal fasciculus. Second, partial midline myelotomy has been successfully used to treat pelvic cancer pain.

CORTICAL PATHWAYS AND AFFECTIVE ATTACHMENT[13,36,40]

Nociceptive projections to the brainstem and deep brain nuclei either activate descending regulatory mechanisms or relay to cortical structures that mediate our ability to localize pain and motivate protective behavior. This latter activity requires pain to elicit negative reinforcement, which occurs through the limbic structures underlying emotion.

The initial transmission of afferents into subcortical nuclei and to cortical matter seems to contribute to parallel pathways, each constructed of serial connections, which ultimately integrate. Afferents to brainstem structures, such as the reticular formation, largely from the spinoreticular tract, and to subcortical structures, such as the hypothalamus and amygdala, are capable of generating immediate autonomic responses, resulting in arousal, modulation of autonomic tone and sympathetic output, and providing an appropriate emotional context.

Spinothalamic fibers terminate in the thalamus with large contributions to the lateral thalamus (VPL, VPM, VPI) as well as to the medial thalamus (CL, CM, Pf, and VMpo). Projections to the lateral thalamus continue largely to the S_1 somatosensory cortex. Tracts originating in the VPI project to S_2. Fibers terminating in the medial thalamus provide a non- or loosely somatotopic bilateral representation. Medial thalamic fibers from the VMpo pass towards the insular cortex. Thus, lateral thalamic and VMpo serve to spatially and temporally localize pain.

It has been postulated that nociceptive fibers form a long serial tract, coursing from S1 through parietal association areas of S2, to the insular cortex, and ultimately to limbic structures, including the amygdala and hippocampus. This provides a more integrated view in which the discriminative aspect of pain is merged with the affective component by projections between the VPL-S_{1-2}-IC circuit and the limbic system. This converges with direct spinal inputs to the amygdala. A mechanism for marrying the discriminative-affective

components of pain with executive and motivational functions is provided via connections of the insular cortex with the anterior cingulate cortex (ACC). Putatively, this connection merges the ability of the aforementioned serial circuit to maintain integrity of discriminative data regarding the location and magnitude of the stimuli in relation to other, potentially concomitant, stimuli with executive and motivational capabilities, through additional ACC connectivity.

Imaging studies show the ACC to be one of the most consistently active regions in response to nociceptive stimuli, with connectivity to the prefrontal cortex and to premotor areas. Reciprocal projections between the ACC and prefrontal cortex provide a mechanism for additional affective attachment, and for consolidation of decisions regarding avoidance behavior. Those tracts to the premotor cortex could provide the motivational stimuli necessary to drive the appropriate motor response. Additional loops through the prefrontal cortex and limbic system could help to attach long-term affective significance to the inciting event. Thus, affective attachments are integrated with somatosensory localization to allow prefrontal cortex to initiate avoidance behavior (*Fig. 10.3*).

MECHANISMS OF SENSITIZATION

The physiological process of nociception typically consists of the sensation of first and second pains. The former is comprised of a relatively distinct sensation (e.g., sharp, similar to a pin prick) localized to the area of the noxious insult. It is propagated largely via the Aδ fibers. In close temporal approximation to the initial sensation, the second pain is a more diffusely localized sensation with less distinct borders and a more general quality (e.g., dull and burning). Additionally, the second pain outlasts the sensation of the first pain. First pain provides an acute awareness of the presence and location of a noxious stimulant, motivating immediate escape behavior to limit tissue damage. The process of tissue damage provides a context for the development of a second pain. Sustained second pain (peripheral sensitization) depends on the release of multiple mediators due to injury-related cytotoxicity and the downstream effects thereof. The direct and indirect actions of these inflammatory mediators modulate an alteration in membrane excitability of peripheral nerves, particularly the polymodal C fibers, contributing to a lasting, diffuse, and painful sensation. Whereas first pain is largely involved in *escape*, second pain creates a stimulus for learning and hence *avoidance* of future tissue damage.

PERIPHERAL SENSITIZATION[7,37]

Cytotoxicity, presumably due to a noxious insult, results in the release of inflammatory mediators into the surrounding extracellular milieu. A list of mediators contributing to this process is provided in (*Table 10.2*). The process of peripheral sensitization contains several positive feedback loops, whereby nerves respond to local inflammatory mediators by releasing other molecules that serve to further increase peripheral excitability. Prostaglandins, produced by Phospholipase A_2 from membrane bound arachidonic acid, can act to sensitize nociceptors and increase vascular permeability, along with many of the other mediators present. Orthodromic and antidromic transmission within the sensitized fibers can result in the release of the neuropeptide SP at other peripheral endings. SP contributes to the positive feedback cycle through two actions. In one, the presence of SP results in the release of bradykinin from nearby vasculature. The downstream effect of bradykinin results in an increase in vascular permeability and helps to sensitize nociceptors. SP also acts directly on mast cells to induce histamine release and on platelets to cause serotonin release, the presence of which is aided by the effects of bradykinin on vascular perme-

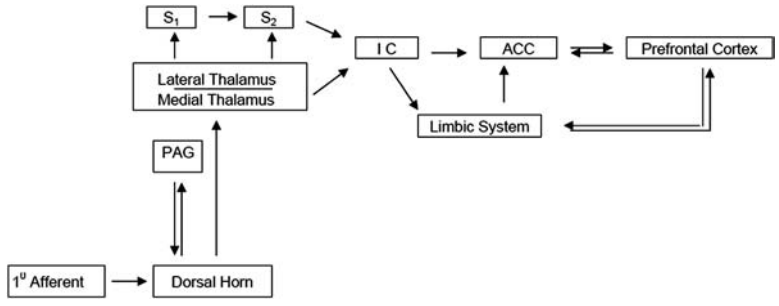

FIGURE 10.3 Descending modulation, largely through the PAG, helps to govern rostral nociceptive transmission. Projections to the medial thalamus continue to the insular cortex updating general bodily awareness. Somatotopy of afferent nociceptive signals is preserved through fibers projecting to S_1, with somatotopy preservation and initial abstraction potentially occurring in S_2. Projections through the insular cortex to the limbic system are thought to be responsible for attaching an affective component to the nociceptive stimuli. Convergence of these signals on the ACC and passage through the prefrontal cortex allows evaluation of the stimuli in context of the affective component attached to the stimuli and location of the insult. As a result, a conscious response to the nociceptive stimuli may be initiated

TABLE 10.2

Mediators of Peripheral Sensitization	
bradykinin	TNF-α
PGE_2	IL-1β
serotonin (5-HT)	ATP
Histamine	Glutamate
H^+	NGF
Endothelin	Substance P (SP)

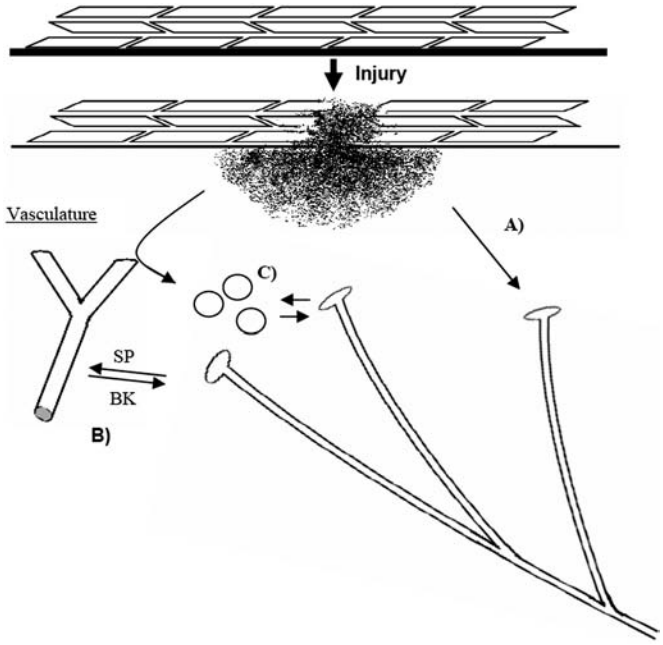

FIGURE 10.4 Mechanistic basis of peripheral sensitization. Upon damage to tissue stroma a series of neuroactive agents are released. Some mediators act directly upon the nociceptive afferents while others act indirectly, through promoting extravasation of inflammatory cells and mediators. *A*, several mediators (e.g., H+, K+, Serotonin, Histamine, Prostaglandin, Bradykinin) are released from damaged tissue. Prolonged presence of these mediators, most notably prostaglandin, aids in nociceptive afferent sensitization as well as in extravasation of inflammatory mediators and cells from adjacent endothelium. *B*, sensitized afferents release Substance P which has dual roles. One of these roles is to cause liberation of Bradykinin from vascular endothelium. This facilitates a positive feedback for continued sensitization by inducing continued sensitization of nociceptive afferents. C, additionally, SP liberated from the terminals of activated nociceptive afferents activates both platelets and mast cells, causing release of serotonin and histamine, respectively. Both contribute to the process of peripheral sensitization.

ability. These actions of SP and those of other mediators serve to utilize the induced inflammatory response to spread the activated receptive field and, thus, to increase non-localized peripheral excitability. In large part, this spread relies on the elevation of vascular permeability which sensitizes neuronal afferents and recruits additional fibers through the influx and subsequent effects of inflammatory mediators. Increased peripheral afferent excitability is accomplished via the effects of these mediators on posttranslational and transcriptional processes resulting in changes to the proteins underlying peripheral nerve activation. These changes affect sensitization along a continuum in physiologic, neurogenic, or neuropathic processes. This process is illustrated in *Figure 10.4*.

Molecular Mechanisms of Peripheral Sensitization

Peripheral sensitization is ultimately mediated by the effects that these inflammatory mediators exert at the molecular level. As excitability is determined by changes in ion flux which modulate the transmembrane voltage, modulation occurs by two primary mechanisms. Ion flux can either be altered in the receptors directly responding to noxious stimuli or in the voltage-gated ion channels that determine parameters such as conduction velocity, activation threshold, and firing inactivation. Proposed mechanisms of altered excitability involve either increased availability or increased activation of these two types of channels.

Post-translational alteration of excitability occurs either through direct modulation of the receptor by allosteric interactions or through intracellular signaling pathways. An example of the former is thought to occur at the TRPV1 receptor, in which the presence of either H^+ or capsaicin is thought to contribute directly to a reduction in the thermal activation threshold. Increased neuronal excitability follows the activation of cytoplasmic signaling cascades triggered via inflammatory mediators. Conserved signaling pathways utilize PKA, PKC, MAPK, PLC, and other intermediaries, often in parallel, to modulate receptor function in response to the presence of extracellular stimuli. Some receptors known to be coupled to PKA through G_s include the B1 receptor (bradykinin), the EP2 (PGE_2), and 5HT-1A (serotonin). Downstream effects of PKA occur on tetrodotoxin sensitive and resistant ($Na_V1.8$ and $Na_V1.9$) Na^+ channels, TRPV1, and specific classifications of Ca^{2+} channels, notably the N-type channel ($Ca_V2.2$), and on particular K^+ channel subtypes. Though candidates are present, the particular K^+ channels involved in modulation of excitability have yet to be definitively isolated. PKC is thought to have similar actions on the same receptors mentioned. $Na_V1.8$ and $Na_V1.9$ modifications require coactivation of the PKC and PKA cascades. Stimu-

latory phosphorylation of the Na^+ channels is thought to directly alter the effects of action potential generation. Stimulatory phosphorylation of Ca^{2+} channels can modulate multiple pathways. Increased intracellular Ca^{2+} can affect further PKC activation and additionally activates CaMK. Both of these activities are known to increase transcriptional activity. Elevated Ca^{2+} levels can also contribute to synaptic vesicle fusion and antidromic release of neuropeptides from the periphery, contributing to the positive feedback involved in peripheral sensitization.

The aforementioned mechanisms provide quick response to tissue damage or peripheral neuronal injury with an increased excitability potentially translating to hyperalgesia and allodynia. This sensitization motivates the individual to protect against further tissue damage. Transcriptional mechanisms largely result in a sustained increase in the complement of nociceptive receptors, providing a mechanism for prolonged peripheral excitability. Members of the MAPK family (ERK, p38, and JNK) act to modulate phosphorylation of the transcription factors CREB, c-Jun, and ATF-3. This altered phosphorylation permits the factors to translocate into the nucleus, upregulating the production of inflammatory mediators, membrane receptors, and ion channels.

A CURRENT THEORY OF CENTRAL SENSITIZATION[1,22,35,41,48]

Peripheral sensitization acts as a complex process in which redundant inflammatory mediators act through a series of processes to both recruit and increase excitability in afferent fibers. Central sensitization seems to be a mechanistically simpler process, largely governed by summation of high frequency afferent signals occurring at synapses within the dorsal horn. Prolonged postsynaptic depolarization results in recruitment of previously inactive membrane receptors that further increase postsynaptic excitability, ultimately resulting in posttranslational modification and contributing to transcriptional upregulation. In concert with changes in descending brainstem modulation, these processes hypersensitize rostral transmission of noxious stimuli to or uncouple transmission from stimulation by peripheral afferents. The following attributes have been suggested to describe the process of central sensitization: lowered discharge threshold present in central nociceptors with consequent increases in the potential for spontaneous and posthumous discharge, a broadening of the peripheral receptor field, and a depressed ability for central inhibitory pathways to counteract the sensitization. Ultimately, this rewiring process and potential for dissociation of perception from peripheral sensation provides a functional underpinning for the development of neuropathic pain.

Molecular Mechanisms of Central Sensitization

The mainstay of nociceptive transmission through the dorsal horn occurs through AMPA, kainate, and mGluR receptors, which are activated by presynaptic glutamate release. Also present, but largely inactive, is the NMDAR. Under conditions of low frequency activation and normal transmembrane voltage, glutamate is not sufficient to result in activation. The channel is held in check by a Mg^{2+} plug that occludes the conducting channel at the resting membrane potential. Additionally, it is held in check through the predominant dephosphorylation of cytoplasmic portions of NMDAR subunits. Sustained depolarization seen in high frequency afferent transmission secondary to peripheral sensitization allows for removal of the plug. Under such circumstances, additional voltage-gated Ca^{2+} channels may also be activated. This leads to the potential for further activation of non-specific cation channels which rely on elevated, intracellular $[Ca^{2+}]$.

Post-translational modification of the NMDAR is mediated by both a receptor bound tyrosine kinase (Src) and a counter-regulator, striatal-enriched phosphatase (STEP). Basal transmission rates favor the activity of STEP. Modulation of Src activation, and thus NMDAR activation, represents a convergence point of several intracellular signaling pathways. Intracellular cascades which impinge upon Src are activated by the presence of pro-inflammatory molecules. The process of NMDAR activation in response to prolonged stimulation, via membrane depolarization or through activation of Src is termed "wind-up." A schematic overview of this process is provided in *Figure 10.5*. In addition to the increased activation of the NMDA receptor, evidence indicates that secondary neurons also become less responsive to inhibitory stimuli, such as GABA and glycine, during sensitization. Recent data correlates activation of the NMDA channel with the postsynaptic presence of superoxides. Putatively, the elevated influx of Ca^{2+} aids in production of superoxides. Evidence has also been provided to indicate that the superoxides react with NO, produced by the neuronal NOS variant, nNos, to produce peroxynitrites. These molecules function to bind and inactivate superoxide dismutase (SOD), in a process that promotes hyperalgesia through downstream effects. Both introduction of SOD mimetics and inhibition of NO production have significantly attenuated this hyperalgesic effect.

Analogous to the mechanisms occurring within the periphery, post-translational and transcriptional modulation occur along a continuum. Kinases, notably one of the MAPK family members, ERK, are responsible for phosphorylating such transcription factors as c-Fos, ELK-1, and CREB. Additionally, inflammatory cytokines contributed from the surrounding milieu, including spinal glia, contribute to activation of the transcriptional processes which increases central excitability. Inflammatory mediators such as SP are upregulated in surrounding Aβ fibers, potentially resulting in allodynia. The presence of reactive oxygen species, mentioned earlier, can also trigger the translocation of some redox sensitive transcription factors, such as $NF\text{-}_KB$ and AP-1,

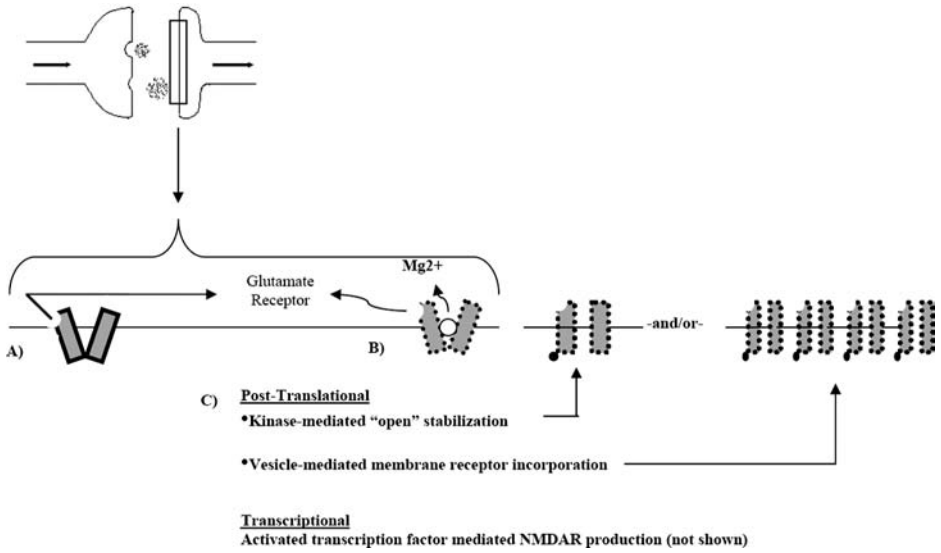

FIGURE 10.5 Mechanisms of central sensitization. Both NMDAR and non-NMDAR postsynaptic receptors are responsive to Glutamate. *A,* under physiological conditions non-NMDAR glutamate receptors produce the depolarization necessary to create APs for afferent signaling. NMDAR channels are not thought to conduct significant current under physiologic conduction frequencies. *B,* high frequency stimulation is thought to remove the Mg2+ plug from the NMDAR channel pore as an artifact of electrostatic repulsion mediated by prolonged membrane depolarization. Glycine binding is also required as a cofactor (not shown).*C,* once activated, a series of intracellular changes occurs to facilitate central "windup" by ensuring continued NMDAR activation and presence. These include both posttranslational and transcriptional events. The end result is that abstraction may occur; peripheral input may not be required, or may be required to a lessened degree, for afferent signaling (not shown).

which signal the production of inflammatory cytokines. Thus, while induction of central sensitization is primarily caused by frequency-dependent depolarization, mediators from the surrounding environment appear to play a large role in aiding the transition from a stimuli-dependent transient excitation to one that persists, functioning independently of inciting stimuli.

CORTICAL AND SPINAL-BRAINSTEM MODULATION[47]

Regulatory modulation of the affect associated with a painful stimulus can occur at each of the levels listed. Intracortical mechanisms of pain modulation could function largely by dissociating an afferent nociceptive stimulus from its affective component. Under situations of extreme physiological stress, this provides clear survival benefits. Such modulation likely explains the initially attenuated affect that soldiers and professional athletes sometimes attach to serious injury, as compared to others in less extreme circumstances. To complement the potential for intracortical modulation, a large body of evidence suggests that both cortical and brainstem structures course caudally to directly converge upon afferent nociceptive signals at the level of individual spinal segments.

Determination of whether or not afferent signals will be sent rostrally via second order neurons in response to peripheral stimulation is thought to be governed by the principles of Hebbian signaling and gate control theory. Nociceptive projection neurons receive projections from first order nociceptive afferents, non-nociceptive afferents, descending modulatory efferents, and inhibitory interneurons. Non-nociceptive afferents serve to decrease afferent nociceptive signaling by increasing tonic signaling through inhibitory interneurons. This is the biological underpinning of the analgesic use of transcutaneous electrode nerve stimulator (TENS). A simpler application of the same principle is to rapidly shake one's hand following a nociceptive insult (e.g., a cut). Descending modulatory fibers can interact either pre- or postsynaptically to modulate neurotransmitter release or excitability. In this way, descending modulation provides an additional means for third party plasticity. Synaptic strength, then, is altered by mediators in the surrounding milieu, local inhibitory interneurons, the presence of non-noxious afferents, frequency of transmission from the primary nociceptive afferent, and the presence of descending modulatory fibers.

Basal Tone: Primary and Secondary Neuronal Pools

Multiple modulatory brainstem nuclei have been demonstrated to contribute to descending modulation. Projections from the PAG to the rostroventral medulla (RVM) have been particularly well studied. Further, the RVM contains other nuclei that are of particular interest in descending modulation. Such nuclei include the nucleus raphe magnus (NRM), nucleus gigantocellularis pars α, and the nucleus paragigan-

tocellularis lateralis. Evidence has accumulated which indicates that PAG receives both cortical efferents and stimuli from ascending tracts. Projections from the PAG to the RVM seem to provide indirect contribution to the descending modulation of nociceptive afferents by coursing through the dorsolateral fasciculus. Work with rat models indicates that under physiological conditions, a net tonic signal seems to be inhibitory and at least partially governed by descending serotonergic output from the NRM, as well as output from the locus coeruleus. In addition to a tonic inhibitory output, the magnitude of inhibition was also shown to increase following noxious insult mimicking that of peripheral inflammation, seemingly as a mechanism to depress a central sensitization. To complement descending inhibition, dorsal horn neurons become more attuned to this signaling, potentially due to an increase of receptors or receptor activation in response to inhibition by opioids and noradrenergic stimulation.

Descending modulation of nociception can be facilitatory as well as inhibitory. The majority of studies that suggest descending mechanisms for facilitation refer to effects on secondary neuronal pools. Antagonists for neurotensin and NMDAR have been shown to prevent secondary facilitation. Furthermore, these receptors have been shown to exist within pathways from the PAG to the RVM. Additionally, antagonists to NO synthesis in this pathway have prevented descending facilitation in animal models, indicating important roles for the NMDAR, neurotensin receptor, and NO.

CHRONIC PAIN: THE NEED FOR TREATMENT

The preponderance of data suggests that neuropathic pain results from aberrant nociceptive signals originating from a damaged pain processing system. Chronic pain is a distinct entity caused by central sensitization secondary either to chronic peripheral tissue damage and inflammation, nerve damage, or a combination of both. The local environment and altered frequency of afferent transmission can cause alterations in the descending modulatory pathways located in the brainstem, which help to facilitate afferent stimulation. The developing cycle of positive feedback instigates the post-translational and transcriptional changes within the second order afferents that result in hyperalgesia and allodynia or serves to dissociate peripheral input from nociceptive transmission. Because the resulting pain is independent of peripheral input, treatments aimed at the periphery are rarely successful. The absence of successful conventional therapies has motivated the development of novel treatment modalities. Thus, the remainder of this article will focus upon current and potential future treatments for intractable chronic pain. A brief introduction to endogenous analgesic mechanisms will provide a basis for description of the classes, rationale for, and limitations of current pharmacologic treatments.

PHARMALOGICAL ANALGESIA[4,38,41]

Endogenous Opioid Actions

Four classes of endogenous opiates have been demonstrated, including enkephalins, dynorphins, endorphins, and endomorphins. The enkephalins are derived from the pre-proenkephalin (PENK) transcript, with two primary splice variants yielding [Leu^5]- and [Met^5]- variants. Multiple dynorphin variants are produced from the prodynorphin (PDYN) transcript (e.g. A & B dynorphin, α- & β- neodynorphin). Analgesic endorphins include α- & β-endorphin, produced as splice variants of the proopiomelanocortin (POMC) transcript. Two tetrapeptide endomorphins are known to exist, endomorphin 1 & 2. Three overarching classes of opioid receptors, at which these agents act, have been cloned: the μ, δ, and $_K$ receptors. A complex receptor subclassification system has been devised to explain the varying pharmacologic effects of the opioid analgesics; however, this system has yet to be substantiated by cloning of the receptors. Based on studies of binding affinity, it is thought that the endomorphins are the endogenous ligands for the μ receptor, that enkephalins serve as endogenous ligands for the δ receptor, and that dynorphins act as endogenous ligands for the $_K$ receptor. The β-endorphin molecule has approximately equivalent affinities for the μ and δ receptor types. Each receptor produces a primary, acute effect through interactions with G proteins that ultimately acts to stabilize membrane excitability. This provides a mechanism for preventing nociceptive transmission.

Briefly, distribution of the endogenous opioids and opioid receptors is not equal throughout the neuraxis. Production of POMC-derivatives occurs primarily within specific hypothalamic nuclei with projections to disparate sections of the brain and spine. PDYN, dynorphin, neurons are present within the limbic system, hypothalamus, and spinal interneurons. Endomorphins, specifically endomorphin-2, are largely localized to the presynaptic terminal of primary afferents within the spinal cord and within areas of the thalamus. Opioid receptors have been located within the peripheral nervous system, brainstem sites of nociceptive regulation, the limbic system, and the spinal cord.

Upregulation of several of the endogenous opioids and associated receptors occurs at the spinal level, in models of peripheral inflammation. PDYN, and thus dynorphin, are particularly upregulated. This opiate is also increased in models of neuropathic pain. In fact, effects of dynorphin on non-opioid receptors may affect the sensitization process and thus the presence of hyperalgesia and allodynia. Among non-opioid receptors, dynorphin affects NMDAR activation, potentially countering the process of sensitization described previously.

Exogenous Treatments

Clinical approaches to pain treatment vary depending on whether the underlying etiology is acute or chronic. Acute pain is commonly treated through the widespread use of NSAIDS, opiates, or a combination of the two. NSAIDS are largely presumed to act in the periphery by reducing the contribution of prostaglandins to the process of peripheral sensitization. The different enantiomers of NSAIDS, R & S, may have a different effect within the spinal cord, with effects on both SP and NMDAR. In contrast, opiates are thought to have a predominantly central effect, at the presynaptic terminal of first order neurons. As mentioned previously, opioid receptors are upregulated in response to tissue damage, in this case at the dorsal root ganglion, with transport to the presynaptic terminal within the dorsal horn.

Opiates are used as a first line treatment for chronic nociceptive pain, but are relatively ineffective at the control of neuropathic pain. Tolerance and the potential for dependence limit application to chronic pain. To address this, and issues of CNS depression, associated with chronic use of high levels of opiates, intrathecal approaches have been used to bathe the spinal cord in analgesic medication. This allows analgesic effects to be achieved, at lowered dosage and with a reduced complement of off-target effects. Nevertheless, escalating tolerance, dependence, inconvenience, and incomplete effectiveness remain issues of importance and should be weighed carefully in considering treatment options for individual patients. The off-target effects of opiate analgesia on consciousness, together with the poor efficacy of opiate therapy for neuropathic pain, provide motivation for development of more anatomically and pharmacologically specific therapies.

In light of the limited efficacy of opiates on neuropathic pain, a variety of pharmacological treatments are used. All of these drugs were initially developed for application to other pathologies. Antidepressants, specifically tricyclic antidepressants, are thought to be useful because of their prevention of norepinephrine uptake in addition to their role in the prevention of serotonin uptake. They have proven to be more effective in treating neuropathic pain than SSRIs, which solely modulate serotonin uptake. Additionally, the effects of the tricyclic antidepressants are almost immediate, as compared with their role in treating depression, which can take weeks. Their mechanism of action may function through stimulation of descending tracts from the Locus Ceruleus and Raphe Magnus, increasing their effects on descending modulation. Evidence also exists to support an NMDAR antagonist mechanism for tricyclics.

Anticonvulsants such as gabapentin, carbamazepine, and clonazepam are also widely used for the treatment of neuropathic pain. Although their mechanisms are incompletely understood, they are known to act as membrane stabilizers. In particular, carbamazepine is known to block pre-synaptic Na^+. Muscle relaxants, such as baclofen and diazepam, are used in the treatment of neuropathic pain. It is speculated that decreased skeletal muscle tone can modify the presence of painful muscle spasms, allowing for better control over pain. At a molecular level, baclofen and diazepam act as GABA agonists, likely serving as muscle relaxants through an effect on spinal inhibitory interneurons. Local anesthetics have been considered due to their ability to block individual ion channels, thus preventing afferent transmission. Agents that mimic the effect of lidocaine such as carbamazepine, mexiletine, and tocainide may reduce chronic pain through specific inhibition of the NMDAR. No antagonists exist that are entirely specific for the NDMAR. However, potential imperfect analogues, such as TCA, ketamine, dextromethorphan, Mg2+, and phencyclidine (PCP), have analgesic properties. Nonetheless, the potential for severe off-target effects exist with NMDA antagonism, as illustrated by PCP.

Although there are currently a number of pharmacological options for the treatment of chronic nociceptive pain, a series of limitations exists for each of the options. Prolonged use of high-dose NSAIDS, especially those not specific for COX-2 can have deleterious effects on homeostatic tissue functions. Furthermore, although some evidence exists for central action, a lack of evidence exists to indicate effectiveness for neuropathic pain. The use of opiates, although effective for acute scenarios, is fraught with drawbacks for the treatment of chronic pain. Issues of tolerance, addiction, physical dependence, depressed quality of life, and a potential need for slow titration to avoid side effects need to be taken into account uniquely for each different drug. The other classes mentioned are primarily indicated for other conditions (e.g. antidepressants, anticonvulsants, muscle relaxants). Thus, off-target effects must be taken into close consideration for individual patients. Local anesthetic analogues can result in CNS depression through their lack of specificity to nociceptive pathways. Considerable effort is being made to pursue an NMDAR specific antagonist to attempt to prevent off-target effects that plague the non-specific interactions of the current NMDAR antagonists. Nonetheless, it is possible that NMDA blockade will have to occur specifically within the nociceptive relay system to avoid the cognitive side effects seen with agents like ketamine and PCP.

Cell- and Gene-Based Approaches: Expanding the Armamentarium[12]

The underlying rationales for considering either cell- or gene-based therapy are similar in that both techniques provide a method to simultaneously achieve anatomic and pharmacological specificity, hence minimizing deleterious off-target effects. Anatomic specificity is achieved through stereotactic implantation. Pharmacological specificity is

achieved by the use of an appropriate cell line or transgene construct. The concept of anatomic and pharmacological specificity is more fully outlined in *Figure 10.6*. Cell lines can be selected or engineered to secrete specific agents, hence creating microscopic biological pumps. Gene transfer can be used to induce local secretion of these agents or to alter the intracellular signaling necessary for nociception.

Cellular "minipumps" provide several possible advantages. Within the context of treating neuropathic pain, cell-based approaches are ideally suited to large scale production and secretion of antinociceptive or trophic peptides. Local concentrations are raised enough to achieve a desired effect, while minimizing the potential for off-target effects. These cell grafts can produce labile peptide products which, by nature, have short half lives in vivo. Release of such labile secretory products, in close apposition to the desired target receptors, increases the likelihood for achieving a therapeutic effect. Gene based therapies can contribute to this process of cell based treatment by programming an implanted cell line to secrete a desired product in a process known as ex vivo gene transfer. The same end may be achieved through a purely gene-based therapy by incorporation of transgenes into neural tissue through the process of in vivo gene transfer. As mentioned, in addition to provoking the release of secreted analgesic molecules, in vivo gene transfer can

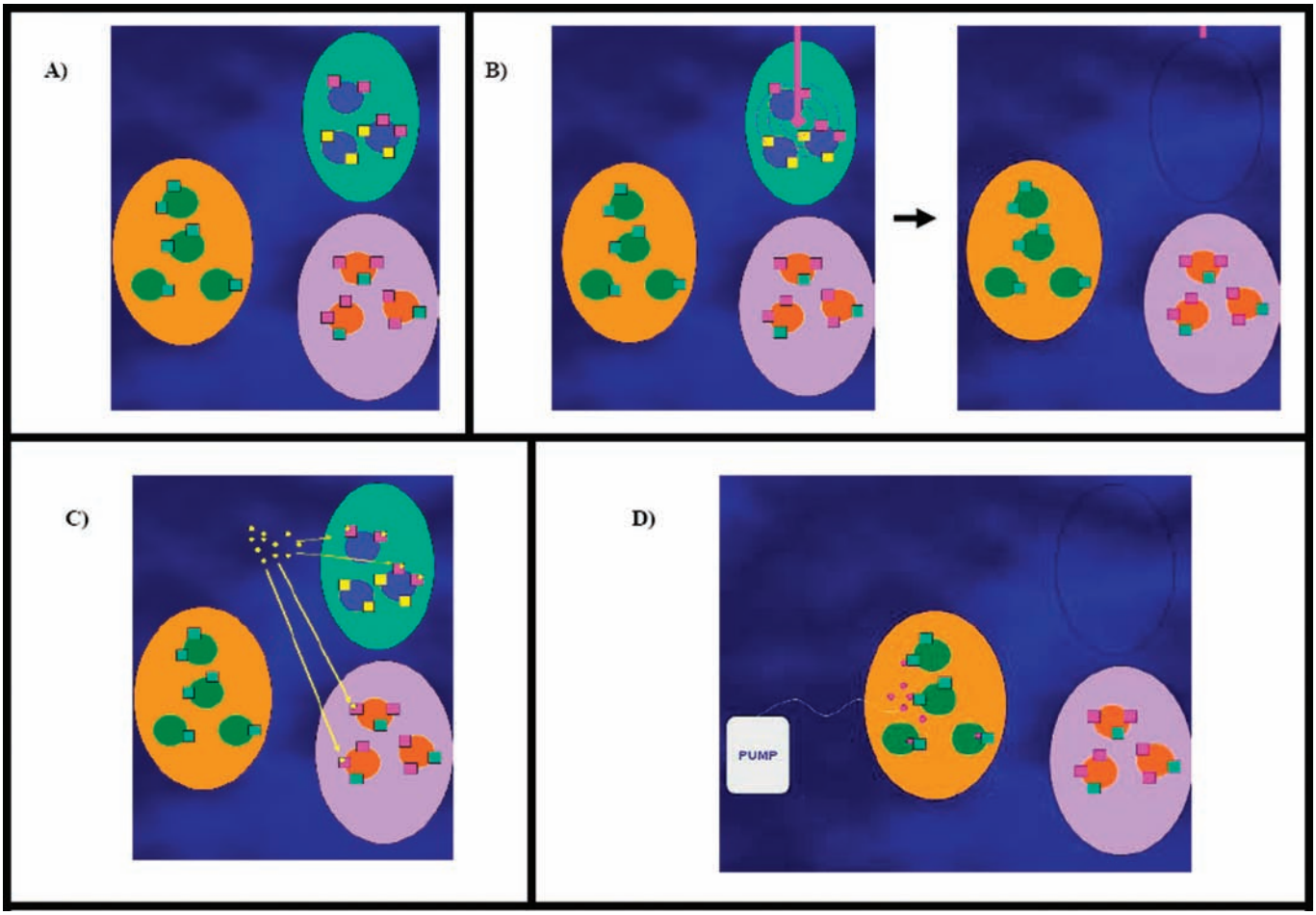

FIGURE 10.6 Specificity (anatomic versus pharmacological). *A*, different tissue types (large ovals) with unique cell types (circles) express a complement of receptors. Although different cell types are present in different tissues, some overlap between receptors exists. *B*, ablation of a tissue locally destroys cells within the range of the instrument, regardless of cell type. This approach is anatomically, but not pharmacologically, specific. *C*, pharmacological therapies may be specific for a given receptor type or subtype. However, this receptor (or subtype) may be present in different tissues, leading to off-target effects. Thus, drug therapies lack anatomic specificity. *D*, local administration of an agent represents an approach to achieve high concentration of an agent in a local area and to prevent off-target effects that result from the activation of receptors outside of the desired field of action. Local pumps created by cellular and gene-based approaches can achieve this balance of anatomical and pharmacological specificity.

also be used to neuromodulate the intracellular signal transduction cascades that provide the architecture for afferent signal transmission.

The individual molecules that underlie nociception, neuropathic pain, central sensitization, and the development of chronic pain provide a host of potential targets for a tailored molecular approach. Most attempts at reversing the neuropathic pain state focus on modulation of transmission at the level of damage within the spinal cord. We will provide a brief background for cell- and gene-based approaches and the current progress reported in the context of spinal, nociceptive neuromodulation.

OVERVIEW OF CELL-BASED THERAPY[8–10,12,14,18,23,51]

Cell-based therapy for neuropathic pain was initially borne of the recognition that adrenal chromaffin cells are capable of secreting a variety of neurotransmitters and anti-nociceptive peptides similar to those released in both descending modulation and by inhibitory interneurons. In particular, chromaffin cells implanted into the spinal subarachnoid space have been demonstrated to raise levels of met-enkephalin, catecholamines, and to reduce morphine cross-tolerance when used in conjunction with morphine. Preclinical testing has shown that molecular alterations within the dorsal horn accompany the changes in CSF composition. Markers associated with central sensitization and neuropathic pain are depressed, including NADP-diaphorase, cGMP, c-fos, and NMDAR-hypersensitivity. Furthermore, these promising molecular alterations within the dorsal horn are accompanied by behavioral alterations, indicating an analgesic effect in a variety of animal models. These include the formalin response, chronic inflammation, central windup, central sensitization, and neuropathic pain. Administration of catecholamine and opioid antagonists during testing has helped to ensure that the behavioral modification occurs, at least partially, as a result of elevated opioid and catecholamine concentration in the CSF. In contrast, recent data demonstrates that simultaneous treatment with NMDAR antagonists augments the antinociceptive effect of the graft. After proof of concept was established in animal models, initial clinical trials were implemented to test the safety and secondarily the efficacy of this treatment modality in patients with intractable, terminal cancer pain.

These Phase I and II trials highlight several critical practical issues in the development of this therapeutic approach. Adrenal chromaffin cells harvested from cadaveric donors were used for these trials as opposed to xenogeneic alternatives in part because use of allogeneic cells mitigates potential for graft rejection. As a primary donor source, however, cadavers are not consistently available and do not provide a homogenous source of graft tissue.

Ex vivo gene transfer to immortalized cell lines has been used to address this issue. In addition to delivering genes for secreted analgesics, ex vivo gene transfer can be used to deliver genes that prevent replication. This strategy has been used to regulate graft proliferation. Controlled proliferation provides large quantities of homogenous graftable cells. Unlike cells harvested from a donor, homogeneous grafts have a more standardized and predictable impact. Cell lines are inherently immortalized, which allows long-term maintenance under in vitro conditions. This characteristic provides the flexibility of prolonged ex vivo storage with little concern for viability. This technology has also allowed incorporation of transgenes into cell lines of interest, providing a mechanism to specifically control peptide production in a desired cell type. Finally, techniques for semipermeable graft encapsulation have been researched to isolate xenogenic cell sources from the immune system and prevent uncontrolled graft migration.

Ex Vivo Gene Transfer: Control of Cell Proliferation

A variety of immortalized cell lines have been used as potential analgesic grafts. To establish proof of principle, immortal cell lines with known secretion of antinociceptive transmitters have been used in preclinical animal models. For example, the PC12 cell line, which is an adrenal medullary tumor line, secretes adrenergic neurotransmitters. Non-adrenal lines have also been used including; B16, NB69, AtT-20, Neuro2A, AtT20/hENK. These have been experimentally used for introduction of hardy, immortal cells in animal model transplant. These immortal cell lines often secrete peptides with potentially analgesic properties.

The ability to passage these cells provides a ready source of homogeneous therapeutic cells. However, immortalized cell lines carry a high risk of tumorigenicity. By introducing a regulated oncogenic element into a cell line, cells will undergo substantial ex vivo proliferation, which can be prevented after transplantation. One such method, conditional immortality, uses cells transfected with a temperature sensitive version of the Tag oncogene. This oncogene is attained from Simian Virus 40 (SV40). When grown at temperatures below approximately 39°C, the cells continue to proliferate in an undifferentiated state. When cultured or implanted in tissue that remains at or above this temperature, differentiation occurs with a resultant increase in peptide output. The still present, though repressed, oncogene does leave a potential for in vivo proliferation. Furthermore, this residual oncogene expression suppresses the secretion of analgesic peptides. Reversible immortalization has been developed to address these shortfalls by entirely removing the oncogenic sequence from the transfected cell once differentiation is desired. In this way, considerable ex vivo passaging can be accomplished while still providing a mechanism to control the potential for graft tissue proliferation. The Cre/lox system provides one method to achieve reversible immortalization. Cre recombinase recognizes the loxP sequence, cut-

ting DNA at this point, recombining it with another loxP site. By flanking the oncogene with loxP sites, Cre can be used to clip out the oncogene. The Cre protein can itself be delivered as a gene under the control of an inducible promoter. Therefore, by inducing Cre expression in the cells containing the foreign genes, the oncogene is removed.

Ex Vivo Gene Transfer: Transgenic Peptides

Many of the cell lines used for grafting do not naturally secrete analgesic peptides, but are chosen for other advantageous qualities. Under these circumstances, the ability to produce neuromodulatory peptides may be conferred through the processes of gene transfer. It is advantageous in these cases to use cell lines with a secretory or neuroendocrine origin to ensure the presence of an appropriate intracellular secretory apparatus. Gene transfer can also be used to augment existing antinociceptive peptide production. For example, β-endorphin is produced by the immortal cell line, AtT20. A variant, AtT20/hENK, has been transfected with the gene for preproenkephalin (PPENK). In this cell line, both enkephalin and β-Endorphin, the natural secretory products of AtT20 cells are produced. Additionally, when neuron-committed progenitor cells of the NT2 cell line were engineered to express PPENK and intrathecally implanted, antinociceptive effects comparable to those attained with chromaffin cell implant were obtained. Secretory transgenes introduced for the treatment of neuropathic pain are not restricted to the classic antinociceptive peptides. A rat neuronal cell line RN33B, was both conditionally immortalized and transfected ex vivo with the gene for brain derived neurotrophic factor (BDNF). In a chronic constriction injury (CCI) model of chronic pain, both immunohistological and behavioral measures indicated that the BDNF served an analgesic role throughout the follow-up period. Recently, it has been demonstrated that both reversible immortalization and the presence of a secretory transgene could be simultaneously incorporated into a transplanted cell line. Vectors for either met-enkephalin or proenkephalin expression caused a reduction in dorsal horn c-fos. These experiments point to the feasibility of creating a homogenous cell line, with controlled replication, capable of secreting desired peptide products. Examples of other transgenes that have been tested in various models for the treatment of neuropathic pain include: galanin, GABA, and POMC.

Methods of Immune Surveillance Protection

Grafts not derived from the host have the potential to provoke an immune response resulting in graft destruction and secondary injury to neural structures. Transplants have largely targeted the subarachnoid space or the spinal parenchyma. In these models, cells are placed into an immunoprivileged site. The subarachnoid space and CNS permit access to fewer circulating immune cells in comparison with other targets for cell or organ transplant. Although immunoprivileged, the potential for rejection remains, prompting the use of immunosuppressants in some experiments. Many of the cell lines studied are xenogeneic, further increasing the likelihood of recognition by the immune system.

Immunoisolation of the transplanted cells is an alternative method to address this issue. Before transplant, grafts are encapsulated in a semi-permeable, polymeric membrane. The porosity of the polymeric membrane is designed such that waste products and the secreted neuromodulatory product may diffuse away from the encapsulated membrane while nutrients can diffuse towards the encapsulated cells. At the same time, this system prevents recognition by antigen presenting cells. Multiple encapsulation constructs are currently used, with different methods of production. Both spherical and cylindrical shapes have been produced. Preclinical testing has provided evidence for prolonged in vivo cell viability and capacity for secretion, extending out beyond 500 days. Furthermore, continued catecholamine and opioid secretion has been demonstrated at pre-implant levels up to 3 months following transplant. Recent clinical trial evidence indicates that immunoisolation of implanted cells will prevent the concomitant need for immunosuppressants and reduce the likelihood of implant rejection.

Immunoisolation can also be achieved by minimizing the antigenicity of implanted graft cells. In the use of primary cell graft implants, this can be partially achieved by ensuring a purified implant. In use of adrenal chromaffin cells, inadvertent incorporation of unnecessary tissue parenchyma will significantly increase the antigenic load of the implanted graft. Another method that has been used to circumvent immune detection is the use of antigenically immature cells as a means to prevent substrate detection. This can be accomplished by using stem cells, progenitors, or fetally-derived cells for transplant.

Neural Stem Cell and Progenitor Cell Contributions

Implantation of neural stem cells represents one newly researched avenue of cell-based therapy. Implantation of neural stem cells into the spinal cord has been used in preclinical experimentation for treatment of spinal cord injuries (SCIs). This approach has been shown to contribute to re-establishing functional connectivity and myelination of damaged fiber tracts. Establishing reconnection of severed fiber tracts is accomplished either directly through differentiation into nerve cells or indirectly through differentiation into supporting, trophic cells (oligodendrocytes) that help to establish an appropriate niche for regeneration. Remyelination is aided by differentiation into oligodendrocytes, allowing direct fiber protection and preservation of signal conduction. The predominant pattern of differentiation is thought to depend upon the trophic milieu surrounding implanted stem

cells. Evidence now indicates that this research may have ramifications for the treatment of neuropathic pain.

A recent study performed by Hoffstetter et al. compared the effects of neural stem cells (NSCs), NSCs pretreated with a transcription factor (Ngn-2), and a control group, to determine which preparation yielded the most functional restoration in a rat model of SCI. The transcription factor, Ngn-2, helps to direct the lineage differentiation of the NSC population. Whereas the untreated NSC population aided in the re-establishment of fiber tract connectivity and in remyelination, a significant degree of allodynia also developed that was not present in either the control group or in the NSC+Ngn-2 group. Additionally, both re-connectivity and sensation was improved to a greater extent in the NSC+Ngn-2 group without evidence to support concomitant development of allodynia. Additional fiber sprouting occurred in the untreated NSC group that was not present in the Ngn-2 + NSC group. This was attributed to greater astrocyte differentiation in the untreated NSC group and mentioned as the likely cause of allodynia development in the NSC-only group. These results have multiple implications for the treatment of neuropathic pain.

Current approaches to treatment of neuropathic pain focus on reversing an already present condition. In contrast, these results provide initial data to support the concept that injuries or interventional procedures associated with development of neuropathic pain could be treated prophylactically with stem cell-based therapies that are aimed at preventing neuroma formation. Appropriate implementation of stem cell-based therapies, after injury, but before development of neuropathic pain, could help to re-establish or improve functional connectivity while minimizing the potential for aberrant axonal sprouting. By helping to prevent a repair response gone awry, and a subsequent over-excitatory input to the dorsal horn, central sensitization could be prevented from occurring. This represents a potential means to treat neuropathic pain of a defined, injury-oriented etiology. Furthermore, to achieve a synergistic effect, stem cell-based therapies could be used in concert with other cell-based, gene transfer-oriented approaches. This could be particularly useful in terms of providing the appropriate chemical, trophic milieu necessary to ensure long-term graft viability with neural stem cells and progenitors. In the present, however, the allodynia associated with naïve NSC implantation suggests a need for greater understanding of the differentiation potential of an implanted cell type so as to prevent exacerbation of neuropathic pain. Furthermore, it indicates a need to more fully understand the factors that drive differentiation towards particular lineages.

CELL-BASED CLINICAL TRIALS[8,12,24,44,51]

As mentioned above, adrenal chromaffin cells were the first and remain the most studied cell type for the treatment of neuropathic pain. To date, chromaffin cell implantation represents the only cell-based therapy to have undergone clinical trial testing. These trials were based on preclinical testing, which largely used grafts in animal models to measure antinociceptive-related effects relating to both biochemical and behavioral markers. Initial clinical trials designed to examine the safety and efficacy of both unencapsulated and encapsulated adrenal chromaffin cells have been completed.

Unencapsulated Chromaffin Cell Trials

The initial trial of cell-based therapy for neuropathic pain consisted of two patients. An analgesic effect was not appreciated. However, in this trial, graft material for individual patients was heterogenous, having been attained from multiple donors for individual grafts. Furthermore, implant was performed on a same-day basis following tissue harvest, with no attempts to ascertain graft tissue viability.

A larger unblinded, uncontrolled trial (n=5) was subsequently conducted by Winne and Sagen between 1991 and 1993. Terminal cancer patients with intractable pain were chosen for this trial. Patients included in this trial showed systemic side effects to opioid treatment, but had not yet been treated by alternative approaches, including intrathecal morphine. Adrenal chromaffin cells were obtained from cadaveric donors. Explants from the adrenal medulla were cultured in vitro for approximately 1 week to ensure explant viability. Patients were given immunosuppressant treatment (cyclosporine) before implantation and for 2 weeks after implant to prevent graft rejection. Within 1 to 1.5 months, four out of the five patients experienced an improvement in pain, as indicated by visual analog pain scores. Additionally, three of these five patients indicated significant reductions in: pain score, analgesic consumption, and increases in activity. Increases in CSF opioid and catecholamine levels over preimplant levels were noted. However, in all cases, large variability was present, preventing the ability to ascertain more than a trend.

A Phase II trial conducted by Lazorthes et al. enrolled 15 individuals, also with terminal, intractable cancer pain. In contrast to the previous trial, inclusion criteria required that patients be refractory to systemic opioid treatment owing to an accumulation of dose-related side effects. In all patients, the pain condition had progressed such that intrathecal opioid administration had been attempted before graft implant in all participants. Of the patients in the trial, five no longer required intrathecal morphine injections, two required a lessened dose, and five had stable morphine requirements. Benefit was not demonstrated for the remaining three patients. The lack of effect was hypothesized to be due to poor participant selection, insufficient quality and quantity of implanted graft tissue, impaired allograft function, immunological graft rejection, and distance between implant engraftment and affected area leading to insufficient local titers of antinociceptive peptides. Autopsies performed on two patients that

survived over a period of 1 year indicated prolonged graft survival. Using the rationale that the CSF is immunoprivileged, grafts were neither encapsulated nor HLA typed to the recipient. Lymphocytosis was noted in 75% of the transplanted allografts. However, no effect was noticed on CSF met-enkephalin levels, which the authors interpreted as providing indication that the grafts were tolerated, at least for the duration of follow-up period for this trial (approximately 4.5 mo). The only reported adverse events were associated with immunosuppressive treatment and intrathecal morphine administration, when required. Ultimately, tolerance of the graft and the lack of observed side effects helped to establish safety and feasibility of this treatment modality. Additionally, the overall reduction in concomitant opioid therapy provided impetus to further explore this and related cell therapies in larger, placebo-controlled trials.

Encapsulated Chromaffin Cell Trials

As mentioned previously, the use of encapsulation resulted from the recognition that primary adrenal cell grafts would require containment and immunoisolation. To date, two trials have been attempted using encapsulation of xenogeneic adrenal medullary chromaffin cells. Concomitant immunosuppressant therapy was not used in either trial.

The initial Phase I trial was designed to test safety of the encapsulated cell transplant intervention. To this end, all symptoms associated with the procedure were minor, self-limited, and were related to the implantation. Further, a need for depressed analgesic usage was noted, providing impetus for a larger, controlled trial. Immunosuppressants were not used in this trial. The second trial was prematurely halted owing to an inability to establish efficacy of the proposed treatment.

CONFLICTING RESULTS: CAUTION FOR A TEMPERED APPROACH[16,28–30]

It should be mentioned that this negative result is not isolated. Several preclinical studies have failed to reproduce antinociceptive results with bovine chromaffin implantation. It has been postulated that the lack of efficacy in the trial and the similar preclinical results may be due to the use of an insufficient number of cells. Thus, recent preclinical studies have examined the use of chromaffin implants when combined with intrathecal administration of an NMDAR antagonist. Results indicate that the analgesic effects of the two modalities in tandem could provide synergistic effects. Augmentation of the analgesic response, using this methodology, could help to produce a clinical effect, even if a sub-therapeutic cell number is implanted.

OVERVIEW OF GENE-BASED THERAPY[14,21,31,39]

As previously described, overlap exists between the therapeutic indications for cell- and gene-based therapies. Furthermore, ex vivo gene transfer represents a hybrid of the two techniques. However, gene transfer provides a wider platform from which to design interventions for the treatment of neuropathic pain. In vivo gene transfer techniques can be further stratified based on the vectors used for gene delivery. In in vivo gene transfer, genes are inserted directly into the cells of parenchymal tissue. As such, different routes have been devised by which to reach these tissues while simultaneously minimizing the invasive nature of the therapy. Vectors may be injected intrathecally, from which point the genetic material of the vectors are incorporated into adjacent parenchyma. Intraparenchymal vector delivery refers to direct injection of vector material into an anatomically specific portion of parenchyma. Finally, remote delivery refers to a specialized form of delivery in which neurotropic viruses are utilized to deliver genetic material to a precise anatomic localization via retrograde axonal transport.

To achieve expression, transgenes must be inserted into an expression cassette. The expression cassette includes a promoter, which drives expression. Expression of the gene also requires a polyadenylation sequence (pA) placed at the 3′ end of the gene. A variety of promoters exist that can drive expression constantly or in a regulated fashion. Some promoters are specific for individual cell types and even neuronal subpopulations, restricting the expression to specific target cells.

Current experimental evidence for the treatment of different facets of neuropathic pain will be presented in terms of the mechanism of vector delivery following a brief introduction to the vector types utilized to mediate in vivo gene transfer, which can be broadly classified as viral or non-viral.

Vector System: Non-viral

In a comparison of vector types, use of nonviral vectors provides the distinct advantage of simplicity, exemplified by a lack of potential for pathogenicity and minimized immunogenicity. The simplest approach is to use naked plasmid DNA, attaining electro-permeabilization with specifically designed intrathecal probes. A primary limitation of this methodology is consistently short expression of the engineered transgene.

In attempts to increase transgene uptake and expression, various attempts at coating the genetic material have been attempted. Both liposome-mediated (lipoplex) transfer, using cationic lipids, and polymer-mediated (polyplex) transfer, using hydrophilic polymers, have been attempted. When used in conjunction, the vector is termed a lipopolyplex. These coatings condense the genetic material partially by isolating the anionic charges present on the structural phosphate moieties. Peptide or protein conjugates with DNA refers to a molecular conjugate. When designing a non-viral vector, the particular coating material and proteins which may be conjugated to this material are carefully chosen to

maximize the processes of vector binding, endosomolysis, cytosolic trafficking, and nuclear entry.

Vector binding can either be receptor mediated or non-receptor mediated. In efforts to achieve receptor mediated binding, multiple ligand types have been coupled to given coating materials. Polyplexes using poly-L-lysine (PLS) have been used to couple a variety of ligands. Additionally, molecular conjugates can and have been used for coupling antibodies designed to recognize specific receptors with vectors. Non-receptor binding is facilitated in both polyplexes and lipoplexes as a result of their positive charges. Evidence is present to suggest that this binding occurs, at least partially, to negatively charged surface proteoglycans. Molecular conjugates have also been shown to use proteoglycans for either primary binding or as co-receptors.

The mechanism by which a particular nonviral vector binds to the cell surface determines whether or not it will be incorporated into a lysosomal or endosomal degradation pathway. Large vectors (approximately 500 nm) seem to be maintained within uncoated vesicles and are ultimately not degraded through endosome fusion with lysosomes whereas coated endosomal vesicles (approximately 80–100 nm) are degraded through this fusion process. Regardless of which category a vector falls under, endosomolytic mechanisms must be present to assure acceptable gene expression. Multiple mechanisms exist whereby nonviral vectors can release DNA; pore formation, membrane flip-flop, or proton sponge mediated osmolysis. Other putative mechanisms take advantage of a combination of these processes. Additionally, gene transfer efficiency can be modulated by the use of agents which alter the stability of cytoskeletal microtubules.

The presence of a nuclear localization sequence (NLS) helps to ensure that the transfected genetic material will actually reach the nucleus. At the nuclear membrane, it promotes aggregation of a pore complex. Several proteins and peptide sequences have been used as putative NLS. In lipoplexes and polyplexes that have not undergone molecular conjugation, cell division seems to significantly increase transgene uptake and expression in at least some cell types. However, dependence on cell division inherently targets gene delivery to non-neuronal populations.

Nonviral vectors, engineered to tightly hold genetic material, may ultimately inhibit expression as noted by an experiment in which both naked plasmid DNA and lipoplex bound DNA were both injected into nuclei. In contrast, however, early exposure of the transfected genetic material within the cytosol can also result in poor transgene expression, due to nuclease dependent degradation.

Vector System: Viral

Viral vectors provide the advantage of increased transduction efficiency. Additionally, some strains have inherent neural tropism. Recognizing a need to minimize the possibility for in vivo proliferation and to minimize the potential for immune recognition, vectors have been attenuated through a process of removing critical replication-specific genes. In addition, inessential accessory genes have been removed in some strains to minimize the potential for immune recognition and to maximize free space for transgene incorporation. Gutting viral vectors of non-essential genes prevents expression of non-self peptides, but requires the presence of helper viruses during incubation to provide the genomic content necessary for replication. In this manner, potential for cell surface expression of foreign peptides via the presence of MHC Class I peptides is minimized.

The general anatomy of the vectors to be considered are similar. An outer lipid bilayer envelope may or may not be present. When present, it is studded with proteins that aid in target recognition and uptake. If the bilayer exists and if proteins necessary for neural tropism are not naturally present, pseudotyping techniques may be used to add these proteins. In the vectors with an outer bilayer, a tegument consisting of necessary enzymes and proteins surrounds a nucleocapsid which may contain nuclear localization signals and contains the genetic material. At minimum, all viral vectors contain a nucleocapsid. If they only possess a nucleocapsid, then receptor binding proteins will be present which can also be pseudotyped to target gene delivery. Viral vectors may be categorized as being either neurotropic or non-neurotropic, a classification which determines suitability for remote viral delivery. Such tropism reflects the presence of viral surface proteins that facilitate cell type specific uptake and retrograde axonal transport.

Herpes Simplex Virus (HSV) and Rabies Virus (RV) are neurotropic viruses. HSV has been extensively engineered for use as a vector. This tropism is, at least in part, conferred by the presence of glycoprotein-mediated binding to neural cell surface proteoglycans. Both are capable of anterograde and retrograde axonal transport, in a strain dependent manner. This process allows subcutaneous administration to result in dermatome-specific DRG expression. Additionally, they are capable of creating latent infections in post-mitotic cells. The HSV-1 genome is 152kb allowing for ease of manipulation in transgene incorporation. Herpes viruses attenuated by 'gutting' are known as amplicons. By virtue of the fact that fewer viral proteins are produced, and thus displayed, on MHC I surface proteins, this process reduces the potential for immune recognition and uncontrolled proliferation, but complicates vector construction. Deletion mutants have been optimized to prevent reversion to wildtype while preserving portions of the original genome.

Lentiviruses (LV) represent a unique subcategory of retroviruses (RV). Like HSV, they are capable of infecting non-dividing cells because they contain enzymes that provide for nuclear docking and energy-dependent transport across the nuclear membrane. HIV is a well known member of this

family, and most lentivectors are derivatives of HIV-1. The extensive understanding of HIV virology motivated the early development of this vector system. Lentiviral particles have been both attenuated and modified to increase the range of tissues susceptible to infection through pseudotyping. Lentiviruses have been modified over successive viral generations to enhance attenuation and safety. The most recent iterations contain only three original genes, while retaining transduction potential for neurons in vivo. Persisting concerns for in vivo reversion to wildtype have been addressed by use of non-human lentiviruses (e.g., EIAV and FIV) and through the use of self-inactivating lentiviruses, attained through deletion of viral genome LTRs.

Adenovirus (AV) is a non-enveloped 36-kb dsDNA virus that is useful as a delivery vector, in part, because of its high transduction efficiency in multiple tissue types, irrespective of cell division potential. An additional positive attribute is a lack of association with any human neoplastic pathologies. Viral uptake is partially mediated through binding to the MHC Class I receptor, followed by subsequent endocytosis. Upon internalization, the genetic material, still partially coated, is localized to the nucleus. However, viral genetic material does not integrate into the cellular nuclear material. A mounted immune response and transgenes driven by predominately short-acting viral promoters have traditionally limited the length of experimental effects to the order of a few weeks. A newer generation of "gutless" AV vectors lack all natural viral sequences except those necessary for DNA packaging. As with other gutless vectors, the likelihood that an immune response will be mounted is significantly reduced, as nearly all potential viral antigens are removed. Furthermore, by gutting the AV vector it gains the ability to package large therapeutic transgenes and complex expression systems.

The adeno-associated virus (AAV) is a non-enveloped 4.7kb ssDNA virus that is not associated with any human pathologies. AAV strains are not naturally pathogenic in humans and are naturally attenuated, requiring the presence of a helper virus to provide the complementary genomic material necessary for replication. In vivo, it incorporates directly into the host genome. This feature may be responsible for the long transgene expression that has been noted to last indefinitely in some cases. In wildtype form, the maximum transgene size that can be incorporated is relatively small. Recent efforts, focusing on heterodimerization of viral particles, have showed promise for potentially doubling the size of transgene incorporation. Like the AV vector, AAV has the capability to infect a wide range of dividing and non-dividing cell types. Pseudotyping has resulted in the creation of multiple serotypes of AAV in an effort to optimize the viruses for different experimental approaches.

In summary, gene transfer can be achieved with viral or nonviral systems. Whereas the nonviral systems have less potential inflammatory side effects, they trigger very transient and limited gene expression. Viral vectors are more efficient but also more complex. First generation vectors, including HSV and AV, have a limited duration of gene expression, whereas LV and AAV vectors induce long lasting expression with minimal inflammatory consequences. Although HSV has inherent tropism to primary sensory cells, pseudotyping promises to deliver enhanced neurotropism to lenti and AAV vectors.

EXPERIMENTAL EVIDENCE FOR IN VIVO GENE TRANSFER [6,11,15,17,20,25–27,33,34,39,45,46,50,52–55]

A variety of approaches have been used to apply gene transfer to models of pain. Approaches differ with respect to the transgene selected, mode of delivery, vector type, and model of pain. Specific therapeutic transgenes have been explored for inflammatory, nociceptive, and neuropathic pain. Much of the work cited in this section is new data and so the results are currently published as abstracts.

Intrathecal Delivery

Intrathecal injection of both non-viral and viral vectors have been used. Lin et al. have previously shown that electroporation following intrathecal incorporation of naked plasmid DNA encoding POMC induced pial transduction in a rat model. Opiate precursor peptides have proven particularly effective in models of nociceptive pain. In this model, they were able to achieve a transient anti-nociceptive effect. More recently, this group has reported success in achieving control of this anti-nociceptive response using a similar POMC plasmid construct modified to incorporate a transrepressor system that is capable of depressing POMC expression in the presence of doxycycline. After characterizing initial β-endorphin expression and markers of antinociception, a reduction in both antinociception and β-endorphin levels was demonstrated to vary in a doxycycline dose-dependent manner.

An early attempt to develop an analgesic viral vector use first generation AV vectors, encoding β-endorphin, injected into the intrathecal space of rats. Following the predominate infection of adjacent pial cells, a temporary reduction in hyperalgesic markers was noted. Subsequent induction of an inflammatory response limited the duration of transgene expression. Through pial delivery of genes for analgesic peptides, these approaches bathe the intrathecal space in anti-nociceptive peptides.

Delivery of anti-inflammatory cytokines seems to effectively inhibit the nociceptive response in models of inflammatory pain. IL-2 is thought to possess both central and peripheral analgesic effects, due in part to interactions at the μ receptor. Yao et al. injected intrathecal plasmid DNA with and without lipofectamine to transfect the spinal cord. mRNA and protein were detected in the pia mater, DRG, dorsal horn, and sciatic nerve. Behavioral measures of analgesia indicated an effect lasting six days. Subsequent use of an adenoviral

vector encoding IL-2 resulted in similar anti-nociceptive effects lasting 3 to 4 weeks. Milligan et al. have used multiple delivery systems to achieve in vivo production of IL-10 and subsequent release into the intrathecal space. IL-10 was chosen for its ability to downregulate the release of TNF-α, IL-1, and IL-6. Furthermore, it has the capability to directly augment other anti-nociceptive processes. Intrathecal injection of an AAV vector caused infection of spinal meningeal cells, with consequent elevation of intrathecal (IL-10). Using a hybrid CMV enhancer/chicken β actin promoter, analgesia was seen for a period of approximately 1 week, transiently preventing and reversing neuropathic pain in rat models. Use of different serotypes and/or longer acting promoters was posited as a potential mechanism for achieving a longer duration of analgesia. In other work, Sloane et al., have experimented with the use of non-viral vectors in an attempt to achieve longer term analgesia. Recently published results have indicated success in use of non-viral vectors to achieve an increased duration of effect. Dual injections, when appropriately spread (between 5 and 72 hrs), were able to maintain long-term behavioral reversal for a period of up to 40 days. Recently, Milligan et al. have examined the use of naked plasmid DNA, plasmid DNA coated with PEI, and plasmids encapsulated within PLGA. Single injections were noted to induce reversal of allodynia for 3 to 6 days while appropriately spaced dual injections resulted in 40 or more days of reversed allodynia in the abovementioned vector systems. Labeled PLGA is currently being used to determine the depth of spinal vector penetration. While these results are promising, they have only been published in abstract form at this time.

The delivery of small inhibitory RNAs (siRNA) has recently emerged as a means to suppress expression of specific genes. As an approach to counteracting inflammatory pain, Lin et al. have recently shown that intrathecal introduction of siRNA specific to a subtype of the PGE2 prostaglandin receptor, the EP4 prostanoid receptor, is capable of reducing inflammatory pain without evoking an immune response. Concurrently, mRNA and protein levels were demonstrated to be significantly diminished within the DRG. As with the IL-10 data, these results have only recently been demonstrated and remain published in abstract form. Additionally, a sole injection of siRNA against the N2B subunit of the NMDA receptor by Tan et al has recently been shown to result in a diminished pain response. The reversal was maximal at 3 days after injection for reduction in mRNA levels and at 7 days for maximal protein subunit reduction.

Remote Delivery

Remote delivery represents a particularly powerful mechanism by which to achieve neuromodulation as it provides a means to deliver genes to the DRG neurons via a subcutaneous injection. This process is largely accomplished by the use of viruses that are either naturally neurotropic or which have been pseudotyped to gain neural tropism. In the wildtype form, subcutaneous inoculation is followed by several rounds of amplification within the adjacent epithelium. Once sufficient titers are present, the viral particles are taken up into primary afferents. Following retrograde transport, a primary infection is established. A latent neural infection is facilitated by alteration of the viral genome, as briefly described below. Vectors derived from neurotropic viruses like HSV, do not replicate, but maintain the capacity for uptake and retrograde delivery to DRG neurons. AV and AAV are capable of remote delivery to a lesser extent. Pseudotyping of lentivectors has achieved levels of remote gene delivery similar to that seen with HSV. Some pseudotyped vectors retain the capability to then undergo anterograde transport into the dorsal horn, participating in trans-synaptic spread.

Pohl et al. initially demonstrated the ability to achieve HSV mediated PPE delivery to the DRG. In this vector, the viral thymidine kinase sequence was replaced by the PPE sequence. Wilson et al. extended this work, determining that behavioral responses (foot withdrawal) were stunted when rats treated with this HSV-based viral vector were exposed to noxious chemical stimuli (capsaicin or dimethyl sulfoxide) as opposed to controls. They further reported that this reversal could be antagonized by naloxone administration. Further examination by Goss et al. has shown elevated production of proenkephalin in the DRG with a reduction in pain behavior noted in the formalin model. Goss et al. also noted that, although the effect had disappeared 4 weeks post-inoculation, re-inoculation at that time resulted in the return of anti-nociceptive behaviors, when tested the following week. More recently, Wolfe et al. have used the HSV vector as a vehicle to drive overexpression of endomorphin-2 via a similar remote approach. Success in achieving multiple markers of anti-nociception, included suppressed mechanical allodynia, thermal hyperalgesia, paw edema, and differential hind paw weight bearing in a model of inflammatory pain. Reduced hind paw withdrawal was noted in both acute and long-term phases of the formalin response. Reduced mechanical allodynia and thermal hyperalgesia were noted in the spinal ligature model of neuropathic pain.

Each of these experiments demonstrate that overexpression of anti-nociceptive peptides modulates network signaling to achieve analgesia. The use of anti-nociceptive peptides provides one means to modulate network activity. Other avenues have been explored focusing at the level of the individual neurotransmitters. Neuropathic pain secondary to partial nerve injury results in a loss of GABAergic transmission. To replace lost GABA transmission, Hao et al. constructed HSV vectors for the delivery of the glutamate decarboxylase (GAD) gene. This vector triggers production of GABA in the DRG. In the neuropathic pain model of L5 spinal nerve ligation, rats exhibited signs of

mechanical allodynia (e.g., reduced latency to withdrawal from mechanical stimuli) and thermal hyperalgesia (e.g., reduced withdrawal to heat stimuli). Subcutaneous inoculation resulted in a reduced mechanical allodynia, beginning 1 week after injection. A peak in mechanical threshold was achieved two weeks post-inoculation with an increased threshold persisting for 5 to 6 weeks. Tolerance was not noted as re-inoculation at 2 months resulted in a similar allodynia recovery profile. Thermal hyperalgesia was alleviated beginning at 1 week after inoculation, peaking between Weeks 2 and 3, and persisting for 6 weeks. Improvement in behavioral responses was mirrored by a block in induction of c-fos or phosphorylation of ERK1/2 that occurred in control rats.

In addition to driving overexpression of either peptides that promote analgesia or that promote inhibitory transmission, other methods are currently being pioneered that attempt to modulate the internal architecture of the network. In one such method, Yeomans et al. attempted to knock down the NaV1.7 Na^+ channel present in primary afferents. The authors attempted to knock down this receptor because it has been noted to be upregulated in inflammatory pain states and could thus be a contributor to the process of central sensitization and the development of chronic pain. After use of remote delivery techniques, the investigators were able to detect a decrease in the prevalence of NaV1.7 Na^+ channels via immunohistochemical techniques and a reduction in inflammatory-related hyperalgesia, providing impetus for further research. Another technique currently under investigation refers to repression of gene expression through the use of Zinc finger Protein Transcription Factors. Jouvenot et al. have reported success in the use of Zinc-Finger protein transcription factors to knockdown the TRPV1 receptor, TRK-A receptor, and Nav1.8 channel. The TrkA receptor has been shown to be upregulated in chronic pain states after spinal cord injury, whereas pharmacological blockade of the TRPV1 channel and reduction in the Nav1.8 channel have been correlated with inhibition of neuropathic pain. They were able to achieve gene repression at the mRNA level and of protein levels as well. More recently, Tan et al. have successfully used an HSV-based vector to drive gene repression of the TrkA receptor and the Nav1.8 channel.

Techniques using remote delivery as a route of administration have also focused on treating the root causes of some specialized types of pain. Fink et al. have previously shown that neurotrophic factors can be used to exert a neuroprotective effect in rodent models of streptozocin-induced diabetic neuropathy. Recently, Chattopadhyay et al. reported that subcutaneous inoculation of HSV vectors carrying the EPO gene can exert a neuroprotective effect. Two weeks after diabetes induction, the animals were inoculated. Markers of neuropathy were tested 1 month later. At this time, the inoculated mice retained sensory nerve amplitude, preservation of thermal nociception, and autonomic function.

SUMMARY AND FUTURE DIRECTIONS

Nociception results from an extremely complex neural processing system that segregates the various components of an adaptive pain response. Roughly, this system includes mechanisms for: spatial and temporal localization, escape motivation, future avoidance of injury through efficient memory formation, and autonomic accompaniment of the escape response. To function effectively as a whole, efficient subsystems exist to regulate the incoming nociceptive signals. Within the periphery and at each synaptic relay point, this regulatory process is subserved by a variety of specific transmitters, ion channels, and receptors that underlie and regulate transmission.

Neuropathic pain results when this complex system is malfunctioning due to aberrant signaling stemming from intrinsic neural pathology. The shift to chronic neuropathic and nociceptive pain results from changes in specific molecular mechanisms that underlie normal nociceptive transmission. The alteration in the function of these molecules, particularly through peripheral and central sensitization, can uncouple pain from peripheral injury or damage. As such, chronic pain often ceases to be amenable to treatment strategies that focus on structural disease of the body. It is critical for neurosurgeons to grasp this mechanism and help their patients to understand it as well so as to prevent the expense and sequelae of unnecessary operations.

Although a variety of distinct pharmacological treatments exist for inflammatory, neuropathic, and nociceptive pain, all are subject to off target effects that can complicate their use. These effects exist because the target receptors may play different functional roles in different anatomical locations. Pain surgery can provide improved specificity by targeting specific anatomical targets in the pain processing system. However, existing approaches lack the specificity to affect individual receptor systems. Because unique sites within the nervous system may have multiple functions encoded by different receptor systems, existing neurosurgical approaches also lack the required specificity. In addition, while the application of implanted stimulators has obfuscated the need for ablation in many cases, this technology requires prosthetics which are subject to failure, infection, and breakage.

Cellular and molecular approaches have the capacity to target specific molecules within specific anatomical locations. Therefore, they have the potential for improved functional specificity over existing approaches. Moreover, as biological interventions, they are not subject to the same mechanisms of failure as implantable prosthetics. Cellular approaches provide a means for the creation of microscopic pumps of specific analgesics that can integrate into

the tissue of a given nociceptive relay. Gene transfer can be applied to control the secretion and replication of these micropumps.

Direct gene transfer can alter the function of existing nociceptive neurons either by inducing the secretion of analgesic mediators or by affecting intracellular function. New technology has made it possible to introduce new genes, increase the expression of existing genes, or silence unwanted genes. A variety of vector types, promoters, vector coats, and delivery strategies exist to tailor gene delivery. Finally, specific therapeutic transgenes have emerged that prove effective at impacting the molecular processes of different pain subtypes, including inflammatory, neuropathic, and nociceptive pain.

Future development will require longer lasting vectors, homogeneous grafts, control of the immune response and gene expression. The vast number of specific molecules implicated in nociception and sensitization provide an encompassing array of potential targets for the development of future molecular therapies.

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