Pain has been categorized by duration (acute vs. chronic), location (superficial or deep; cutaneous, bone/joint, muscle, or viscera), and cause or type (inflammatory, neuropathic, cancer). Generally, activation of and/or ongoing activity in specific subpopulations of primary afferent neurons underlies the experience of pain regardless of how it is categorized. Accordingly, primary afferents are key players in understanding mechanisms of, and managing, pain.

Sir Charles Sherrington anticipated by many decades the existence sensory receptors that respond to noxious stimuli, that he called nociceptors and thereby provided for us the operational definition of stimuli that are noxious (i.e., stimuli that damage or threaten damage of tissue). Two considerations are important to this discussion. First, Sherrington functionally defines the nociceptor by its response to a noxious stimulus (e.g., a nociceptive withdrawal reflex, pain). Second, the definition of an applied stimulus as noxious is based on the response to the stimulus applied to skin and subcutaneous structures.

Sherrington’s definition of a nociceptor continues to the present day. However, the term has undergone change and challenge over the past 100 years and may be nearing the end of its utility in the face of the growing understanding of the heterogeneity in the neurons that may not fit so comfortably under this umbrella term. It is therefore important to consider, within the context of our current knowledge, how a nociceptor is defined, identified, and studied, as the interpretation of this information will directly affect the management of pain.

All would agree that a nociceptor is a sensory receptor which, when activated or active, can contribute to the experience of pain. Nociceptors are present in skin, muscle, joints, and viscera, although the density of innervation (i.e., the number and distribution of sensory endings) varies between and within tissues. As originally described by Sherrington, a nociceptor is the peripheral sensory terminal (i.e., the site of energy transduction—see following section), although commonly the term is used to also include the cell body (in a dorsal root, trigeminal, or nodose ganglion) and its central termination in the spinal cord or brainstem. Beyond this, agreement about important features of nociceptors is less uniform. One of the best examples of why this definition is becoming problematic is the observation that injury-induced changes in the central nervous system underlie the emergence of allodynia, pain in response to normally innocuous stimuli. Allodynia is problematic for this definition of nociceptor because pain is mediated by activity in low-threshold afferents that in the absence of tissue injury would, if anything, contribute to the suppression of pain. That is, these afferents would not be considered nociceptors despite the fact that they contribute to the experience of pain.

Furthermore, because stimuli adequate for activation of nociceptors differ between tissues (e.g., tissue damage is not always required), defining a noxious stimulus has become a challenge. For example, some nociceptors in skin and joints and most nociceptors in the viscera have low thresholds for mechanical activation that do not conform to the condition that stimulus intensity must be either damaging or threaten damage. Further, so-called “silent” or “sleeping” nociceptors are unresponsive to intense mechanical stimulation (and are better denoted as mechanically insensitive nociceptors) but develop spontaneous activity and mechanoinsensitivity after exposure to inflammatory and other endogenous mediators. These types of nociceptors—low threshold and sleeping—as well as other subpopulations of sensory neurons that may contribute to the sensation of pain following tissue injury are considered further in the discussion of sensitization in the following section.

Functional Characterization of Nociceptors

As suggested earlier, there are many types of nociceptors, our knowledge of which has been advanced by human psychophysical studies while recording from afferent fibers (Box 3.1). In human skin, for example, there exist nociceptors that respond only to mechanical, only to cold thermal, or only to hot thermal stimuli as well as those that are insensitive to both mechanical and heat stimuli (mechanically insensitive or sleeping nociceptors). The most abundant is the polymodal nociceptor, which responds to mechanical, thermal, and chemical stimuli. In general, nociceptors that innervate skin have the broadest range of modality selectivity, whereas nociceptors innervating deeper structures tend to be less modality-selective and more polymodal in character. For example, mechanical sensitivity is a prominent feature of visceral and joint nociceptors because stimuli adequate for their activation include hollow organ distension and overrotation, respectively. Many of these nociceptors also respond to chemical and/or thermal stimuli as well, although the functional significance of thermal sensitivity in deep tissues is uncertain. An important characteristic of polymodal nociceptors, whether the modalities of stimulation to which they respond are two or all three, is that when sensitized (e.g., by an inflammatory insult), responses to the other modalities of stimuli to which it responds are all increased. That is, it is not only the mechanosensitive modality, for example, that becomes sensitized, but other modalities to which it responds are sensitized as well.

With respect to mechanoinsensitivity, nociceptors at the opposite extremes of sensitivity are most illustrative of the limitations of even a functional definition of a nociceptor. Nociceptors with low mechanical thresholds for response and those with very high mechanical thresholds for response (i.e., sleeping nociceptors) are both clinically important. Mechanoinsensitive sensory neurons with low thresholds for response have long been classified as non-nociceptors (because it was considered that nociceptors had to have response thresholds in the noxious range). Some mechanoinsensitive skin, joint, and many visceral sensory neurons have low thresholds for response (i.e., in the nonnoxious range) but possess characteristics that suggest an important role in pain. First, they encode stimulus intensity well into the noxious range and, moreover, typically give greater responses to all intensities of stimulation than do nociceptors with high mechanical thresholds for response. Second, they respond to tissue insult. Unlike nociceptors with low mechanical thresholds, mechanically insensitive or sleeping nociceptors normally provide no information to the central nervous system but after tissue insult become spontaneously active and mechanoinsensitive.
Identification of Putative Nociceptors

As indicated earlier, nociceptors are defined classically in a functional context. However, in experimental situations where function cannot be assessed, other criteria to classify a neuron as a nociceptor have been advanced. These include the presence or absence of axon myelination, cell size, and/or cell content (e.g., peptide or ion channel) as well as central termination pattern. Sensory neurons commonly identified as nociceptors are those with unmyelinated (C-fiber) axons, small cell body diameters (<20 or 25 μm), that terminate in the superficial layers of the spinal cord dorsal horn. The presence or absence of certain markers (e.g., the tetrodotoxin (TTX)-resistant sodium channel NaV1.8, the transient receptor potential vanilloid receptor TRPV1) have been used to identify subsets of nociceptors. More recently, with the ability to analyze many genes expressed in a single cell, patterns of gene expression have been used to further define subpopulations of nociceptors. 5,6 With respect to cell body size and myelination, it should be appreciated that there exist some large diameter cells with heavily myelinated and rapidly conducting axons that have been documented functionally to be nociceptors. 7 Conversely, many nonnociceptors have unmyelinated axons, and thus, axon myelination or cell diameter cannot be applied as reliable criteria to define a nociceptor. Similarly, identifying nociceptors by content or by what genes they express has limitations. Two examples of this are that (1) cells other than nociceptors express TRPV1 and (2) the features of the subset of nociceptors in the mouse skin that stain positive for the isolectin B4 do not apply to the visceral innervation. 8 And although patterns of gene expression may enable identification of additional subpopulations of nociceptive sensory neurons, 9 the extent to which any particular pattern reliably reveals a neuron’s function remains to be established.

The fact that no single criterion can be used to identify all nociceptors combined with the growing number of examples to the ways in which these neurons are heterogeneous is at the core of a growing concern over the utility of the term. In addition to the anatomical, biochemical, and physiologic heterogeneity in the afferent population, generally referred to as nociceptors, there is functional heterogeneity. As will be discussed later, this

BOX 3.1 Microineurography

The development of a method to record from human nerve fibers in situ, termed microineurography, provided an unparalleled opportunity to expand our knowledge about peripheral sensory receptors, including nociceptors. The method involves percutaneous insertion of the tip of a sharp, insulated metal microelectrode into a nerve (e.g., peroneal or radial nerve) and the application of search stimuli to sites distal to the electrode. In earlier work, mechanical search stimuli (e.g., von Frey filaments) were used, and accordingly, only mechanosensitive afferents were studied. An electrical search stimulus (surface electrode), however, has become popular because the electrical stimulus identifies afferent fibers independent of sensitivity to natural stimulation. After an afferent fiber is isolated, the innervation territory can be drawn on the skin and the adequate, natural stimulus/stimuli determined.

Because microineurography can be easily coupled with a psychophysical approach, human subjects are able to describe stimulus-produced experiences (e.g., pain) while recording from single afferent fibers. Microineurography also has been expanded to include intraneural electrical stimulation of the fiber through the recording electrode, providing additional insight into the qualities of sensation produced, for example, by low- and high-frequency stimulation in addition to qualities associated with natural stimulation. Microineurography has confirmed in psychophysical experiments sensations associated with activation of rapidly adapting (flutter, vibration) and slowly adapting (pressure) cutaneous mechanoreceptors, Aδ-mechan nociceptors (AM), C-polymodal nociceptors (CMH; sharp, pain), C-polymodal nociceptors (CMH; dull, burning [heat] pain), and group IV muscle nociceptors (cramping pain).

Electrical search strategies have revealed a wider range of nociceptors, including A-mechanoheat (AMH), which have similar heat thresholds as CMH (C-polymodal) fibers and also typically respond to chemical stimuli; C-mechanonociceptors (CM); C-heat (CH); C-mechano- and heat-insensitive (CMH, or sleeping nociceptors); and C-mechanoinsensitive-histamine responsive (CMHs-, or itch fibers). Microineurography has also been extended to psycho-physical study of pathologic pain states in humans. In a study of patients suffering from erythromelalgia, a condition characterized by painful, achy, erythematous, red, and hot extremities, a proportion of CMH fibers were found to be spontaneously active or sensitized to mechanical stimuli. Because CMH fibers also mediate the axon flare reflex, their hyperexcitability was considered to contribute to the patients’ ongoing pain and tenderness as well as the redness and warming in this pain syndrome. In patients with painful peripheral neuropathy, Ochoa et al. 10 found that the ratio of CMH to CMH fibers was reduced by about 50%, apparently due to loss of mechanical and heat responsiveness in CMH fibers.

These and future studies will help to understand which nociceptors (and nonnociceptors) in which conditions contribute to spontaneous, ongoing pain as well as stimulus-evoked pain and what therapeutic strategies are most effective.

Functional heterogeneity is manifest both within the context of nociceptive signaling (i.e., subpopulations of nociceptors may underlie distinct “types” of pain such as cold allodynia or thermal hyperalgesia) and in the context of nonnociceptive function (i.e., such as the maintenance of tissue integrity). Even the two functional properties that have been viewed as common to all nociceptors, that they encode stimulus intensity into the noxious range and they sensitize, are, at best, generalities. As noted earlier, the so-called mechanically insensitive afferents shown to play such a prominent role in visceral pain and the burning pain associated with capsaicin do not appear to code stimulus intensity in the noxious range but instead appear to contribute to the patients’ ongoing pain and tenderness as well as the redness and warming in this pain syndrome. Similarly, a variety of nociceptive afferents may be sensitized under the appropriate conditions. The link between the stimulus–response properties of a given subpopulation of afferents and the functional role of these neurons in the context of nociceptive signaling has been complicated by results of selective ablative/ silencing studies in mice, in which sensory specific deficits in nociceptive processing are associated with the silencing of specific subpopulations of afferents despite electrophysiologic data suggesting that the majority of the afferents silenced/ablated are polymodal. For example, the majority of cutaneous neurons in the mouse that express the Mas1-related G protein-coupled receptor D (MrgrD) are polymodal nociceptors, yet mice in which this subpopulation of neurons has been ablated respond normally to changes in temperature, demonstrating, instead, a relatively selective mechanosensitivity deficit. Conversely, the voltage-gated sodium channel (VGSC) NaV1.8 is enriched in nociceptive afferents that are responsive to both noxious thermal (TRPV1-expressing) and mechanical (MrgrD-expressing) stimuli, yet mice in which NaV1.8-expressing neurons have been ablated respond to noxious heat but not noxious mechanical pressure or cold stimuli. Minimally, these results suggest that the behavioral response to noxious stimulation involves a number of factors in addition to the types of afferents that are activated.

All of this heterogeneity does not bode well for the development of broadly acting novel analgesics. That is, given our current understanding of the complexity of peripheral pain mechanisms, it seems unlikely that the range of intensities
within any one modality (thermal, mechanical) that is encoded by a nociceptor and initiates nociceptive transmission involves only a single voltage- or ligand-gated channel. Similarly, even if the ability to sensitize is one means of functionally defining a peripheral neuron as a nociceptor, the endogenous mediators and factors that contribute to an increase in the excitability of nociceptors (i.e., sensitization) are numerous and synergistic and differ in different pain conditions (e.g., inflammation, nerve injury). A sampling of the complexities and contributors to sensitization are discussed in the following section.

Why include in a clinical textbook of pain management a chapter on peripheral pain mechanisms and nociceptors? There are many reasons. First, in most cases, blockage of peripheral nociceptor activity removes the “drive” for the experience of pain. Further, if a primary goal is to develop mechanism-based strategies for pain management, it is critical that characteristics of a key player—the nociceptor—are fully understood. Finally, nociceptor characteristics change as the local environment in which they reside changes (inflammation, nerve injury, etc.). We discuss in the following section how nociceptors are activated, differ in different tissues, and contribute to the experience of pain and how their behavior changes when they become sensitized. Where possible, we have added relevant clinical examples and have inserted text boxes to elaborate on key issues.

**Nociceptor Characteristics**

**ANATOMY OF THE NOCICEPTOR**

As stated earlier, nociceptors are sensory neurons with a cell body located in dorsal root, trigeminal, or nodose ganglia. All sensory neurons arising from these ganglia are pseudo-unipolar neurons with a central process terminating in the central nervous system (e.g., spinal dorsal horn) and a peripheral process terminating in a peripheral target such as the skin, muscle, or viscera. Both central and peripheral processes terminate in a branching pattern referred to as a terminal arbor. The extent of the peripheral arbor depends on theafferent type and site of innervation with the general rule that the higher the spatial resolution for sensory discrimination, the smaller the terminal arbor. In contrast to low-threshold afferents that are responsive to nonnoxious stimuli such as brush or vibration and which terminate in specialized structures, such as Ruffini endings or Merkel disks, nociceptors are said to have “free” (unencapsulated) nerve endings because peripheral terminals of these afferents do not appear to be associated with any specific cell type.

Both light and electron micrographic analyses of peripheral nociceptor terminals reveal complex anatomical structures. As suggested earlier, the structure of the terminal arbor varies with target of innervation. There is also evidence that subpopulations of nociceptive afferents have distinct terminal arbor patterns within the same structure. For example, the terminal arbor morphology of cutaneous C fibers varies according to whether the C fiber is peptidergic (i.e., expresses substance P or calcitonin gene-relative peptide) or expresses the MrgprD. On the other hand, the terminal morphology of a subpopulation of high-threshold Aδ fibers that appear to mediate the pain associated with hairpull is similar to that of a subpopulation of low-threshold mechanosensitive Aβ fibers that appear to underlie the sensation of gentle stroking of the skin. Evidence also suggests that distinct but overlapping subpopulations of nociceptors may signal distinct aspects of the painful experience (i.e., sensory/discriminative vs. emotional/motivational), suggesting that it may someday be possible to selectively treat the suffering associated with chronic pain while still enabling patients to appropriately respond to noxious stimuli in their environment.

Four distinct events are necessary for a nociceptor to convey information to the central nervous system about noxious stimuli impinging on peripheral tissues (Fig. 3.1—the events are discussed fully in the following paragraphs). First, “energy” from the stimulus (mechanical, thermal, or chemical) must be converted into an electrical signal. This process, referred to as signal transduction, results in a generator potential or depolarization of the peripheral terminal. Second, the generator potential must initiate an action potential, the rapid “all or nothing” change in membrane potential that constitutes the basic unit of electrical activity in the nervous system. This process is sometimes referred to as transformation. Third, the action potential must be successfully propagated from the peripheral terminal to the central terminal. And fourth, the propagated action potential invading the central terminal must drive a sufficient increase in intracellular calcium ions to enable release of enough transmitter to initiate the whole process once again in the second-order neuron. Distinct sets of proteins underlie each of these processes and are, therefore, the targets of a wide variety of therapeutic interventions.

**STIMULUS TRANSDUCTION**

An important implication of the fact that nociceptive afferents terminate in “free nerve endings” is that they are not dependent on other cell types for the transduction of a noxious stimulus. That is, proteins responsible for transduction should be intrinsic to the nociceptor. Consistent with this suggestion, isolated sensory neurons are responsive to thermal (both heating and cooling), mechanical, and a wide variety of chemical stimuli, including both endogenous and exogenous compounds that activate nociceptors in vivo. Proteins involved in the transduction of each stimulus modality have been identified (see Gold for review).

With the exception of transient receptor potential (TRP) channels (see the following discussion), two-potassium channels (K2P), and possibly the acid-sensing ion channels (ASICs), chemotransducers are, in general, only activated by chemical stimuli (and not also mechanical or thermal stimuli) and encompass various families of proteins that respond to specific molecules such as adenosine triphosphate (ATP) and protons. There is also compelling evidence to support the suggestion that different members of the TRP superfamily underlie thermal transduction of temperatures ranging from the very cold (TRPA1) to the very hot (TRPV1), with receptors for cool, warm, and hot in between. Subsequent research, however, suggests that in contrast to traditional chemoreceptors, TRP family members are not modality-specific as all are activated by specific chemicals and several contribute to mechanical transduction.

Because the only sensation associated with TRPV1 activation is pain, this receptor has received considerable attention from pain researchers. Data from an array of studies paint a picture of TRPV1 as an excellent example of a polymodal receptor; it is activated by exogenous compounds such as capsaicin and resiniferatoxin, endogenous compounds ranging from protons to lipids, as well as noxious heat. TRPV1 is also present on the central terminals of nociceptive afferents where it also facilitates transmission of noxious mechanical stimuli. Furthermore, excessive activation of the receptor with compounds such as capsaicin results in desensitization of the nociceptive terminal to all modes of stimuli, a process that underlies the therapeutic efficacy of topical capsaicin application. More recently, intrathecal application of resiniferatoxin has been used to selectively ablate the central terminals of TRPV1 containing nerve terminals, resulting in a sustained block of nociceptive transmission.

There is also evidence that TRPV1 receptor antagonists may have some analgesic efficacy, although their therapeutic potential may be limited by a small but significant hyperthermia associated with systemic administration of blood–brain barrier permeable analogs.
**FIGURE 3.1** Nociceptive afferents terminate as free nerve endings in skin and other tissues. A: Their principal sensory functions consist of (1) transduction of external or internal chemical or physical stimuli into generator potentials, (2) transformation of a generator potential into an action potential, (3) propagation of the action potential toward the central nervous system, and (4) release of neurotransmitters and neuromodulators into the superficial dorsal horn of the spinal cord or brainstem. Nociceptive afferents also release transmitters in the periphery, a process that contributes to aberrant or ectopic activity under pathologic conditions. Many of the proteins responsible for each of these processes, both under normal and pathologic conditions, have been identified. Although not a complete list, several lines of data implicate each of the proteins and mediators illustrated in each subpanel. B: Transduction: In naive tissue, proteins thought to play a role in mechanotransduction include transient receptor potential vanilloid type 4 (TRPV4), acid-sensing ion channel type 3 (ASIC-3), and the low-threshold voltage-gated calcium channel (VGCC) CaV3.2. Several different classes of TRP channels are involved in transduction of changes in temperature from noxious cold (TRPA1, ankyrin type 1), cool (TRPM8, melastatin type 8), warm (TRPV4), and hot (TRPV1, vanilloid type 1). Many chemoreceptors are present in nociceptive afferents including those involved in the response to tissue acidosis (TRPV1 and ASIC-3), noxious organic compounds (e.g., aldehydes at TRPA1), and endogenous chemicals (e.g., ATP at P2X3, the ionotropic purine receptor type 3). A wide variety of other receptors for both pro- and anti-inflammatory mediators (not shown) are present on the terminals of nociceptive afferents. These include G protein-coupled receptors (GPCRs) responsive to E-type prostaglandins (EP), bradykinin (B) types 1 and 2, and serotonin (5-HT) types 1A, 2, and 7. Tyrosine receptor kinases (TRK), responsive to trophic factors such as nerve growth factor (NGF) and artemin, are present as are receptors for cytokines such as TNFα and interleukin 1β. Also depicted are transmitters such as ATP stored in epithelial cells. Following tissue insult, there are changes in nociceptive terminals that result in both an increase in sensitivity to noxious stimuli as well as the emergence of membrane depolarization. There are increases in the density of several transducers as well as posttranslational modifications (depicted as phosphorylation, P) that increase channel activity or sensitivity such that the transducers are activated by lower intensity stimuli.
therapeutic agents to block activity arising from peripheral terminals may not provide pain relief. Given the source of much of this activity, therapeutic soma and/or proximal axon may become a source of aberrant or ectopic activity. An important implication of this activity is that local administration of instability (manifesting as oscillatory behavior), and an increase in inflammatory mediators and their receptors. The result of such changes is that the proteins that may make the soma responsive to mechanical, thermal, and chemical stimuli, an increase in sodium channels that may lead to membrane potential generation. In the presence of nerve injury, however, changes in the soma and/or proximal axon can include an increase in transducer proteins, and lipids that will be used throughout the cell. Although the soma is capable of generating action potentials and is likely depolarized in all neurons in the absence of tissue insult, the soma serves as the supply depot for the rest of the neuron, synthesizing and packaging the proteins, transmitters, and lipids that will be used throughout the cell. The dependence of action potential propagation on VGSCs confers the therapeutic efficacy of the therapeutic intervention. A number of ion channels play an important role in determining the threshold for spike initiation and upstroke of the spike. Action potential threshold appears to be critically regulated by potassium (K+) channels, which include voltage-gated K+ channels (KV), inward rectifying K+ channels (KIR), two-pore K+ channels (K2P), large-conductance calcium-modulated K+ channels (BK), and small conductance calcium-dependent K+ channels (SK). The nonselective inward rectifying cation channel (HCN) also contributes to action potential threshold. In some cases, the low-threshold calcium channel (CaV3.2) may contribute to action potential threshold, but when present, CaV3.2 appears to play a more prominent role in mediating burst activity. The voltage-gated sodium channel (VGSC) NaV1.9 may also contribute to establishing action potential threshold. Finally, the channels responsible for the upstroke of the action potential include the VGSCs NaV1.7 and NaV1.8. As with transduction, there are a number of changes in ion channels that affect action potential threshold and spike initiation to increase in the excitability of nociceptive afferents in the presence of insult. These changes include a decrease in K+ channel density and/or current and an increase in CaV3.2, HCN, and NaV channel density and/or activity. These changes are the result of both posttranslational modifications and/or changes in transcription driven by the same inflammatory mediators that influence transduction. Thus, several of the therapeutics listed under transduction may also act by inhibiting changes in channels underlying spike initiation. Other drugs may act via direct inhibition of the ion channels underlying spike initiation (which include local anesthetics, tricyclic antidepressants [TCAs], and several cyclooxygenase inhibitors). K+ channel openers such as retigabine may also have efficacy in increasing the threshold for spike initiation. Action potential propagation: The ion channels underlying action potential propagation are distinct from those underlying spike initiation. The channel most prominently implicated in action potential propagation is the VGSC NaV1.6. In myelinated axons, NaV1.6 is clustered at nodes of Ranvier, whereas in unmyelinated axons, the channel is distributed throughout the axon. Following insult, however, the pattern of channel expression can change, including redistribution of VGSCs NaV1.7 and/or NaV1.8 to the cell membrane. The dependence of action potential propagation on VGSCs confers the therapeutic efficacy of sodium channel blocking compounds such as local anesthetics, TCAs, and some cyclooxygenase (COX) inhibitors. Ectopic activity: As is true for all neurons in the absence of tissue insult, the soma serves as the supply depot for the rest of the neuron, synthesizing and packaging the proteins, transmitters, and lipids that will be used throughout the cell. Although the soma is capable of generating action potentials and is likely depolarized in response to neural activity in the axons, it is not necessary for action potentials to invade the soma for information to propagate from the periphery to the central nervous system. In nociceptive afferents, the VGSC NaV1.8 can be the primary, if not the only sodium channel in the cell underlying action potential generation. In the presence of nerve injury, however, changes in the soma and/or proximal axon can include an increase in transducer proteins that may make the soma responsive to mechanical, thermal, and chemical stimuli, an increase in sodium channels that may lead to membrane instability (manifesting as oscillatory behavior), and an increase in inflammatory mediators and their receptors. The result of such changes is that the soma and/or proximal axon may become a source of aberrant or ectopic activity. An important implication of this activity is that local administration of therapeutic agents to block activity arising from peripheral terminals may not provide pain relief. Given the source of much of this activity, therapeutic interventions designed to block sodium channels and/or inhibit the actions of inflammatory mediators are predicted to have the greatest efficacy.

FIGURE 3.1 (continued) These changes are brought about by actions of inflammatory mediators such as ATP, prostaglandin E2, NGF, and TNF-α that can directly depolarize nociceptive terminals, drive posttranslational changes via the activation of second messenger cascades, and/or alter the expression of transducers via influencing transcription and/or translation. All of these processes may be facilitated as a result of an increase in the release of mediators from epithelial cells, resident (mast) cells, and recruited (macrophages) immune cells. Several therapeutics currently in use or in development act via suppressing the actions of proinflammatory mediators. The specific pattern of changes and mediators depends on many factors, including the type and site of injury, time after injury, and previous history of the injured tissue as well as age and sex, which also influence the relative efficacy of the therapeutic intervention. C: Spike initiation: The appropriate anatomical distribution of ion channels is critical for normal function. A decrease in K+ channel density and/or current and an increase in CaV3.2, HCN, and NaV channel density and/or activity. These changes are the result of both posttranslational modifications and/or changes in transcription driven by the same inflammatory mediators that influence transduction. Thus, several of the therapeutics listed under transduction may also act by inhibiting changes in channels underlying spike initiation. Other drugs may act via direct inhibition of the ion channels underlying spike initiation (which include local anesthetics, tricyclic antidepressants [TCAs], and several cyclooxygenase inhibitors). K+ channel openers such as retigabine may also have efficacy in increasing the threshold for spike initiation. D: Action potential propagation: The ion channels underlying action potential propagation are distinct from those underlying spike initiation. The channel most prominently implicated in action potential propagation is the VGSC NaV1.6. In myelinated axons, NaV1.6 is clustered at nodes of Ranvier, whereas in unmyelinated axons, the channel is distributed throughout the axon. Following insult, however, the pattern of channel expression can change, including redistribution of VGSCs NaV1.7 and/or NaV1.8 to the cell membrane. The dependence of action potential propagation on VGSCs confers the therapeutic efficacy of sodium channel blocking compounds such as local anesthetics, TCAs, and some cyclooxygenase (COX) inhibitors. E: Ectopic activity: As is true for all neurons in the absence of tissue insult, the soma serves as the supply depot for the rest of the neuron, synthesizing and packaging the proteins, transmitters, and lipids that will be used throughout the cell. Although the soma is capable of generating action potentials and is likely depolarized in response to neural activity in the axons, it is not necessary for action potentials to invade the soma for information to propagate from the periphery to the central nervous system. In nociceptive afferents, the VGSC NaV1.8 can be the primary, if not the only sodium channel in the cell underlying action potential generation. In the presence of nerve injury, however, changes in the soma and/or proximal axon can include an increase in transducer proteins that may make the soma responsive to mechanical, thermal, and chemical stimuli, an increase in sodium channels that may lead to membrane instability (manifesting as oscillatory behavior), and an increase in inflammatory mediators and their receptors. The result of such changes is that the soma and/or proximal axon may become a source of aberrant or ectopic activity. An important implication of this activity is that local administration of therapeutic agents to block activity arising from peripheral terminals may not provide pain relief. Given the source of much of this activity, therapeutic interventions designed to block sodium channels and/or inhibit the actions of inflammatory mediators are predicted to have the greatest efficacy.

(continued)
FIGURE 3.1 (continued) F: Transmitter release: The release of transmitter at the central terminals of nociceptive afferents is essential for transmission of nociceptive information to the central nervous system. This process is calcium-dependent—extracellular calcium enters the central terminal generally via high-threshold VGCCs that are activated following invasion of the action potential into the central terminals. N-type channels (CaV2.2) are the most abundant, but P/Q-type (CaV2.1) and L-type (CaV1.3) are also present. CaV2.2 is most readily modulated following activation of inhibitory GPCRs, serving as the primary mechanism for the therapeutic efficacy of intrathecal opioid receptor and alpha adrenergic receptor (α-AR) agonists. Transmitters present in nociceptive afferents are generally packaged in vesicles referred to as small clear vesicles, which generally contain the excitatory amino acid glutamate, and large dense-core vesicles, which contain, among other things, neuropeptides such as substance P and calcitonin gene-related peptide (CGRP). There are a number of excitatory ionotropic receptors, including P2X3 and TRPV1, that appear to facilitate transmitter release from the central terminals. Inhibition of the central terminal may involve activation of voltage-gated (KV) and calcium-modulated (BK) K⁺ channels. Under normal conditions, presynaptic ionotropic γ-aminobutyric acid (GABA) receptors (GABAA) play a major role in mediating presynaptic inhibition of the central terminals of nociceptive afferents. The VGSC that appears to play a major role in enabling spike invasion of the central terminals of nociceptive afferents is NaV1.8. Finally, excitatory GPCRs (e.g., EP and β1, β2 receptors) are also present. As with other steps in the process, a number of changes occur in the central terminals of nociceptive afferents after tissue insult that contribute to the transmission of nociceptive information. These include an increase in the α₁β₂ subunit in VGSCs. This subunit is important for trafficking channels to the membrane and, importantly, is a binding site for gabapentin and pregabalin. There is also an increase in neuronpeptide expression, the emergence of additional excitatory receptors, modulation of ion channels such as NaV1.8, and a decrease in K⁺ currents that facilitate nociceptive signaling. Interestingly, there is a growing body of evidence suggesting that there may be changes in GABA receptor signaling as well such that activation of these receptors may become excitatory secondary to changes in the regulation of intracellular chloride. This issue is complicated by the fact that benzodiazepine receptor agonists may have therapeutic efficacy in the presence of tissue insult which appears to involve, at least in part, activation of presynaptic receptors. From the therapeutic perspective, it is important to note that following tissue insult, in particular that associated with inflammation, there may be an increase in the expression of inhibitory receptors, ultimately facilitating the therapeutic efficacy of opioid and adrenergic receptor agonists. Additional therapeutic interventions may also involve inhibitors of VGCCs, K⁺ channel openers, and inhibitors to inflammatory mediators.

Of the three modalities of noxious stimuli, molecular mechanisms of mechanotransduction remain the most elusive. Many mechanically sensitive proteins have been identified, but none appears to be both necessary and sufficient for mechanotransduction in nociceptive afferents. Data from null mutant mice, where the deletion of a single putative mechanotransducer results in an increase in mechanosensitivity in one population of afferents and a decrease in others, suggests that several different proteins are likely to work together in specific subpopulations of afferents to enable responses to specific forms of mechanical stimuli (e.g., stretch or compression). This picture is complicated further by the observation that some mechanotransducers such as piezo2 contribute to mechanotransduction in specific subsets of sensory neurons. That even these more specialized forms of mechanosensitivity reflect intrinsic properties of afferents are suggested by the emergence of mechanical sensitivity at the severed ends of a subpopulation of axons within hours of transection. Despite the slow progress in this area, identification of a nociceptor specific mechanotransducer blocker remains an active area of investigation because of its therapeutic potential in light of the fact that mechanical hypersensitivity is the primary complaint associated with the vast majority of chronic pain syndromes.

Whereas mechanotransduction is an intrinsic property of many nociceptors, there is evidence that epithelial cells may also be mechanosensitive. Because these cells may store and release transmitters such as ATP, it has been suggested that afferent activity evoked with mechanical stimulation of peripheral structures such as the bladder, gastrointestinal tract, and skin may be secondary to the transduction event that has occurred in the epithelial cell that subsequently releases a chemical mediator capable of activating nearby nociceptor terminals. The implication of this mechanism from a therapeutic perspective is that it may be possible to attenuate mechanical
hypersensitivity in several peripheral tissues with the appropriate antagonist of the responsible chemoreceptors on nociceptive afferents. Consistent with this idea, there is evidence that mechanical hypersensitivity observed in several visceral structures can be attenuated with ATP receptor antagonists. That a similar mechanism may contribute to chemotransduction, at least in the intestine, was recently suggested by the observation that enterochromaffin cells are not only activated by a variety of chemicals but are electrically excitable and form synaptic connections with primary afferents. However, serotonin appears to be the primary transmitter underlying the signaling from these cells to primary afferents, possibly accounting for the therapeutic efficacy of 5-HT3 receptor antagonists for the treatment of visceral pain.

Aberrant expression of transducers may play a significant role in chronic pain associated with tissue injury. The presence of a functional transducer at a site other than the peripheral terminal may underlie the emergence of ectopic activity and contribute to ongoing or spontaneous pain. Such changes have been most extensively detailed following nerve injury, where, as mentioned earlier, mechanical sensitivity is detectable in cut axons within hours of injury and may persist in neurons indefinitely. Similarly, chemosensitivity, particularly to glutamate, develops at noninnervated sites, develops at subpopulations of afferents as well as within the ganglia itself, contributing to ectopic activity arising from both the site of injury and from the ganglia. The emergence of ectopic activity arising from sites distant to the site of injury may explain why interventions targeting the site of injury are unsuccessful.

PASSIVE ELECTROPHYSIOLOGIC PROPERTIES AND THE SPREAD OF THE GENERATOR POTENTIAL

A generator potential at a primary afferent terminal ending is not equivalent to an action potential propagated along that afferent’s axon. The generator potential decays over distance from the point of origin as a function of membrane resistance, membrane capacitance, and internal resistance of the nerve terminal and may or may not be propagated beyond the terminal ending. Generally considered stable properties, there is evidence for dynamic remodeling of the terminal arbor of central nervous system neurons which may influence both membrane capacitance and internal resistance; it remains to be determined whether such changes may also impact passive conduction of generator potentials in peripheral terminals. In contrast, a number of ion channels have been identified that may establish resting membrane resistance and therefore the spread of the generator potential within the terminal arbor. This issue is important to nociceptive signaling because action potential initiation does not always occur at the site of stimulus transduction. Consequently, the magnitude of the generator potential at the site of action potential initiation must be greater than or equal to action potential threshold. Thus, at least in some fiber types, it may be possible to block nociceptor signaling and therefore pain, with manipulations such as the local administration of potassium (K⁺) channels openers that decrease membrane resistance and therefore spread the generator potential.

Although ion channels have been the focus of research into mechanisms controlling neuronal excitability of peripheral neurons, recent evidence suggests that ion pumps and exchangers may contribute as well. Some pumps and exchangers, such as the sodium/potassium ATPase and the sodium/calcium exchanger, are electrogenic; that is, they generate net flux of ions when active and therefore may contribute directly to resting membrane potential and consequently neuronal excitability. They may also contribute indirectly, via an influence on intracellular ion concentrations and consequently the activity and/or actions of other ion channels. For example, the sodium/calcium exchanger, and the calcium ATPase control intracellular calcium levels in sensory neurons and intracellular calcium influence activity of calcium, potassium, and chloride channels in sensory neurons. Conversely, the sodium/potassium/chloride cotransporter and the bicarbonate/chloride exchangers in sensory neurons influence intracellular chloride concentrations, which, in turn, influence the impact of type A γ-aminobutyric acid (GABAA) on sensory neurons.

The results of several recent studies suggest that influencing the magnitude and/or spread of the generator potential may underlie the next generation of therapeutics. Because of the limitations of the nonspecific nerve block produced by local anesthetics, strategies that enable a more selective afferent block are an active area of investigation. In one such strategy, a viral vector was used to drive expression of an ionotropic glycine receptor, an inhibitory ligand-gated ion channel not normally present in sensory neurons. Sensory neurons innervating specific targets such as the skin or the bladder could be selectively infected by injecting the viral vector into the intended target tissue. This strategy enabled the reversal of hypersensitivity with the local administration of glycine. A similar approach involves the use of optogenetics, or light-activated proteins that can be selectively expressed in targeted cell types. Inhibitory light-activated ion pumps, such as halorhodopsin or archeorhodopsin, have been used to study the role of light-sensitive ion pumps and exchangers of afferents in pain, where both viral vector-based and site-specific recombinase technology have been used. Expression of these light-activated proteins can be restricted to specific subpopulations of afferents via a variety of approaches including the site of infection, the virus serotype used, as well as cell-specific promoters. Given the spatial and temporal control of the afferent inhibition afforded by these strategies, combined with the ability to selectively target subpopulations of afferents, the therapeutic potential is tremendous.

ACTION POTENTIAL GENERATION

VGSCs are responsible for the upstroke of the action potential in virtually all excitable tissue. As their name implies, these channels are gated (opened and closed) by changes in membrane potential. VGSCs are generally composed of an α subunit and up to two β subunits. The α subunit is a large molecule (~200 kD) that contains all features necessary for a functional channel including voltage sensor, ion selectivity filter, channel pore, and inactivation gate. Ten α subunits have been identified, none of which form functional channels in heterologous expression systems. The channels encoded by each of these subunits can be distinguished by a combination of pharmacologic and biophysical properties. Eight of these nine α subunits are present in the nervous system, all eight of which are present in nociceptive afferents at one point during development, with six of these detectable in the adult. β Subunits influence channel gating properties as well as trafficking and localization in the plasma membrane. Four β subunits have been identified, at least three of which are present in nociceptive afferents.

VGSC α subunit primarily responsible for action potential initiation in the majority of nociceptors is NaV1.8. This subunit is unique in several ways. First, it is normally only expressed in primary afferents where it is primarily expressed in nociceptors. This unique pattern of distribution, in combination with its primary function in spike initiation, makes it an ideal target for novel therapeutics. Although in no way mitigating the therapeutic potential of this channel, recent evidence suggests that it may contribute to action potential propagation in the distal peripheral axon in addition to its role in action potential initiation. Second, NaV1.8 has a relatively high threshold for activation. Whereas many other VGSCs begin to activate at membrane potentials between ~50 and ~40 mV, a depolarization to ~30 mV or greater is necessary to activate NaV1.8. This feature may explain, at least
in part, why greater intensity stimuli are generally needed for nociceptor activation. Third, NaV1.8 is relatively resistant to steady-state inactivation, a voltage-dependent process whereby channels residing in a closed or resting state transition to an inactive state before they ever get a chance to open. Recovery from the inactivated state requires membrane hyperpolarization; thus, inactivated channels cannot contribute to the upstroke of the action potential. Even a small sustained depolarization to −50 mV cannot activate virtually all other VGSCs. However, NaV1.8 is still fully available for activation at this membrane potential. Fourth, NaV1.8 recovers from inactivation rapidly. These last two features enable the channel to underlie sustained activity in the face of a persistent depolarization that might be observed in the presence of inflammatory mediators. Fifth, NaV1.8 is resistant to cooling-induced inactivation. Other VGSCs are completely inactivated at temperatures at or below 18°C. However, NaV1.8 is still functional at temperatures down to 4°C, enabling the burning pain associated with noxious cold stimuli. Sixth, in contrast to all but one other VGSC α subunit (NaV1.9), NaV1.8 is resistant to TTX and is therefore referred to as a TTX-resistant channel.

Whereas NaV1.8 is critical for action potential initiation in nociceptors, data from the study of rodent sensory neurons suggests that many of these afferents express NaV1.8 in concert with another VGSC α subunit, NaV1.7. The NaV1.7 subunit has unique features enabling it to play a significant role in spike initiation. That this channel may play a critical role in nociceptor activity is suggested by the recent discovery of individuals possessing both gain-of-function and loss-of-function point mutations in this subunit. Strikingly, two distinct pain syndromes—primary erythromelalgia (PE) and paroxysmal extreme pain disorder (PXP)—reveal the specific impact of gain-of-function mutations. PE is associated with burning pain in the hands and feet, and the distribution of pain in the rectum that ultimately progresses to include trigeminal structures including the eye and jaw. The unique distribution of these pain syndromes, in light of the widespread distribution of NaV1.7 in the peripheral nervous system as well as neuroendocrine tissues, highlights the importance of other channels in sculpting the response properties of sensory neurons. Furthermore, in contrast to the impact of the gain-of-function mutations, loss-of-function mutations that result in nonfunctional channels are associated with a complete insensitivity to pain. Although the data from patients with these rare genetic mutations is intriguing, recent evidence suggests the role of NaV1.7 in nociceptive processing may be more complicated than originally anticipated. That is, evidence from null mutant mice suggests that the loss of function phenotype may not only involve sympathetic postganglionic neurons but a compensatory upregulation of the endogenous opioid enkephalin in primary afferents. Furthermore, electrophysiologic analysis of nociceptive afferents in patients with gain-of-function mutations in these channels suggests that at least at rest, the afferents are hypoexcitable. These observations raise the possibility that the pain phenotypes associated with mutations in NaV1.7 reflect developmental processes independent of any ongoing influence of the channel in controlling afferent excitability. Furthermore, in contrast to isolated sensory neurons from the rat, where NaV1.7 appears to contribute to action potential initiation, recent evidence suggests that this may not be the case in human sensory neurons.

Low voltage-activated, or T-type, calcium channels may also contribute to spike initiation in the periphery. Whereas there is compelling evidence that these channels are present in high density in low-threshold D-hair afferents, there is also evidence that they may be present in a subpopulation of nociceptors as well. The biophysical properties of these channels enable them to play a particularly important role in mediating bursting activity, as the channels underlie a sustained membrane depolarization after a single action potential that provides the driving force for the initiation of subsequent action potentials. This feature has led some to speculate that selective T-type channel blockers may be particularly effective for treating paroxysmal pain such as that associated with trigeminal neuralgia.

The focus on NaV1.8 has been on its role in action potential initiation in the periphery. Nevertheless, there is also evidence that the channels are present and functional at central nociceptor terminals. At the central terminal, the channel appears to facilitate the spread of the invading action potential throughout the terminal arbor and consequently the release of neurotransmitter from the primary afferent. There is also a growing body of evidence suggesting that action potentials may also be initiated at the central terminals of nociceptors, where they are conducted antidromically to the periphery. This activity, referred to as the dorsal root reflex, appears to play a significant role in the neurogenic inflammation that develops following tissue injury.

**ACTION POTENTIAL PROPAGATION**

NaV1.8 and NaV1.7 underlie action potential generation and even propagation over the first 5 to 10 mm of peripheral axon. However, a different set of VGSCs underlies action potential propagation into the central nervous system in the absence of tissue injury. NaV1.6 appears to be the subunit primarily responsible for propagation in both myelinated and unmyelinated axons of both nociceptive and nonnociceptive afferents, although data from a small molecule inhibitor of NaV1.7 suggests this channel may contribute to action potential conduction as well (but see Zhang et al. for data suggesting this channel may not be as selective as originally thought). Unfortunately, the distribution of NaV1.6 in the peripheral nervous system in combination with its widespread expression in the central nervous system precludes selective block of propagation in nociceptors via a NaV1.6 specific mechanism. Nevertheless, block of these channels with local anesthetics and/or TTX, or more recently small interfering RNA knockdown, remains an effective means of blocking input into the central nervous system.

**TRANSMITTER RELEASE**

Voltage-gated calcium channels (VGCC) are primarily responsible for the initial influx of calcium necessary for initiation of machinery underlying the release of neurotransmitters. Like VGSCs, these multisubunit channels consist of a large α subunit that contains all of the features necessary for a functional channel as well as a β subunit. Ten α subunits have been identified, encoding channels that are commonly defined by their pharmacologic properties. T-type channels (CaV3.1 to CaV3.3), as mentioned earlier, have a low threshold for activation, whereas all others have a high threshold for activation. The high-threshold channels are further subdivided based on their sensitivity to specific channel blockers: L-type channels (CaV1.1 to CaV1.4) are blocked by dihydropyridines such as nimodipine, N-type channels (CaV2.2) are blocked by the snail toxin ω-conotoxin GVIA, P/Q-type channels (CaV2.1) are blocked by the spider toxin ω-agatoxin IVA, and R-type channels (CaV2.3) are blocked by the spider toxin SNX-482. In contrast to VGSCs, VGCCs are not effectively targeted to the plasma membrane in the absence of the α split subunit complex. A single β subunit also appears to be important for efficient gating. All VGCC subtypes are present in nociceptors. And whereas there is evidence that all high-threshold calcium channels may contribute to transmitter release, N-type channels appear to play a dominant role in the release of transmitter from nociceptors.
The dominant role N-type channels play in mediating transmitter release from nociceptive afferent terminals makes them an ideal target for both endogenous and exogenous analgesics. Opioid and adrenergic receptor agonists act through inhibitory G protein-coupled receptors which enable inhibition of VGCCs via two major intracellular pathways. The first is a rapid, membrane delimited pathway involving G protein βγ-subunit displacement of the VGCC β subunit, resulting in a “sleepy” or “unwilling” channel that requires a larger membrane depolarization for channel opening.\(^2\) The second pathway involves more traditional second messenger kinase dependent signaling with a slower onset and offset.\(^3,\)\(^4\) Interestingly, neither pathway results in complete VGCC block, yet both result in a dramatic inhibition of transmitter release. This amplification effect reflects the fact that there is considerable cooperativity of calcium in mediating vesicle fusion to the cell membrane that is necessary for transmitter release; four to five calcium ions are needed to trigger vesicle fusion.\(^5\) This amplification effect is also likely to facilitate the use of relatively low concentrations of the N-type channel blocker SNX-111 (Prialt), enabling the block of transmitter release from nociceptive afferent terminals in the superficial dorsal horn while minimizing side effects associated with block of channels at more distant sites. Finally, although additional mechanisms likely contribute to the therapeutic efficacy of drugs like gabapentin and pregabalin, that have been shown to bind to the α,δ subunit, the most compelling model involves the inhibition of membrane trafficking of the α subunit. That is, following nerve injury, there is a dramatic increase in α,δ subunit expression in nociceptive afferents.\(^6\) This increase in expression appears to facilitate the trafficking of VGCC α subunits to nociceptive afferent central terminals, which in turn, appears to facilitate transmitter release and consequently the increase in pain associated with nerve injury. These compounds, which are among the most extensively studied inflammatory mediators, act at G protein-coupled receptors and membrane receptors, there are a relatively small number of intracellular pathways that underlie their actions. For example, both prostaglandins and bradykinin, which are among the most extensively studied inflammatory mediators, act at G protein-coupled receptors. Two major G protein-dependent pathways have been implicated. One involves a stimulatory G protein, Gs, which drives activation of adenylyl cyclase, resulting in an increase in cyclic adenosine monophosphate (cAMP) and the activation of protein kinase A (PKA).\(^7\)\(^8\) The other involves a Gq-dependent pathway ultimately resulting in the activation of phospholipase C, the liberation of diacylglycerol (DAG) and IP3, and the subsequent activation of protein kinase C (PKC).\(^9\) PKC-ε isoform appears to play a particularly important role in nociceptor sensitization. Other mediators that appear to play important roles in nociceptor sensitization, such as TNF-α and interleukin (IL) 1β, utilize a mitogen-activated protein kinase (MAPK)-dependent pathway ultimately resulting in the activation of p38.\(^10\) Still other mediators directly activate ion channels. For example, protons (which increase in concentration during inflammation), act at TRPV1 and ASICs. ASIC-3 is important to pain associated with ischemia, such as that which occurs during angina, and deep muscle pain where protons and lactic acid accumulate. In contrast, ATP and its metabolites act at ionotropic P2X and metabotropic P2Y receptors to modulate nociceptor excitability.

**Nociceptor Sensitization**

Sensitization is a characterizing feature of nociceptors; nonnociceptors do not sensitize following tissue insult. Sensitization represents an increase in nociceptor excitability, which is expressed and defined as an increase in response to a noxious stimulus. Sensitization is also typically accompanied by a reduction in the threshold for activation and occasionally by the development of ongoing, spontaneous activity. Nociceptor sensitization is the cause of primary hyperalgesia (i.e., increased pain produced by stimulation at the site of tissue insult) and is important because nociceptor sensitization is the trigger for initiation of an increase in excitability of central neurons in the nociceptive pathway, an event termed central sensitization.\(^11\)

An increase in nociceptor excitability is a reflection of changes in the behavior of nociceptor voltage- and/or ligand-gated ion channels produced by actions of endogenous substances either released or synthesized at the site of tissue insult or attracted there. Endogenous substances considered classically to contribute to sensitization include products of arachidonic acid metabolism (e.g., prostaglandin E2 [PGE]), histamine, serotonin, protons and ATP, but the list has grown quite extensively and now also includes cytokines, chemokines, growth factors, peptides, etc., some of which are released from immune competent cells attracted to the site of insult (e.g., macrophages), from nearby cells (e.g., mast cells), or from nociceptor (and other) nerve terminals (e.g., peptides). Interestingly, despite the variety of mediators capable of producing nociceptor sensitization, several appear to play particularly important roles. This list includes prostanooids, as evidenced by the antihyperalgesic efficacy of nonsteroidal anti-inflammatory drugs (NSAIDs) that act via inhibition of cyclooxygenase and thus prostanooid synthesis.\(^12\)\(^13\) More recently, the importance of tumor necrosis factor alpha (TNF-α) in chronic inflammatory conditions has been highlighted by the antinociceptive efficacy of compounds such as etanercept which are designed to bind and inactivate TNF-α released at sites of inflammation.\(^14\)\(^15\) Finally, nerve growth factor (NGF) appears to play a major role in orchestrating a variety of signaling cascades necessary for an inflammatory response and therefore has also been targeted with antibody-based strategies.\(^16\)\(^17\)

The mechanisms that trigger changes in nociceptor excitability are not fully known. A growing body of evidence suggests that despite what appears to be a heterogeneity of mediators and membrane receptors, there are relatively small number of intracellular pathways that underlie their actions. For example, both prostaglandins and bradykinin, which are among the most extensively studied inflammatory mediators, act at G protein-coupled receptors. Two major G protein-dependent pathways have been implicated. One involves a stimulatory G protein, Gs, which drives activation of adenylyl cyclase, resulting in an increase in cyclic adenosine monophosphate (cAMP) and the activation of protein kinase A (PKA).\(^7\)\(^8\) The other involves a Gq-dependent pathway ultimately resulting in the activation of phospholipase C, the liberation of diacylglycerol (DAG) and IP3, and the subsequent activation of protein kinase C (PKC). PKC-ε isoform appears to play a particularly important role in nociceptor sensitization. Other mediators that appear to play important roles in nociceptor sensitization, such as TNF-α and interleukin (IL) 1β, utilize a mitogen-activated protein kinase (MAPK)-dependent pathway ultimately resulting in the activation of p38.\(^10\) Still other mediators directly activate ion channels. For example, protons (which increase in concentration during inflammation), act at TRPV1 and ASICs. ASIC-3 is important to pain associated with ischemia, such as that which occurs during angina, and deep muscle pain where protons and lactic acid accumulate. In contrast, ATP and its metabolites act at ionotropic P2X and metabotropic P2Y receptors to modulate nociceptor excitability.

The relatively novel, if not paradoxical, mechanism of sensitization involving ionotropic GABAA receptors that we described in the action potential generation section serves as an example of the dynamic interplay between second messenger signaling cascades and the regulation of ion channels. Activation of these receptors are normally inhibitory, even in nociceptive afferents where they drive membrane depolarization, because of the relatively high intracellular chloride concentration maintained in these neurons.\(^1\) The GABAA-mediated depolarization in these neurons is inhibitory, as suggested earlier, because of the decrease in membrane resistance, referred to as shunting, as well as the depolarization-induced activation of low-threshold voltage-gated potassium channels.\(^12\) In the presence of persistent inflammation, there is a constitutive shift in the balance of tyrosine kinase to tyrosine phosphatase activity, such that there is a net increase in protein phosphorylation. This increase results in a net increase in functional GABAA receptors in the plasma membrane because of a decrease in receptor internalization.\(^12\)
The result is an increase in GABA-evoked current and an increase in the magnitude of the GABA-evoked depolarization. These changes are further augmented by a decrease in the density of low-threshold potassium current.15 The result is a shift in GABA signaling, from inhibition to excitation.

This picture gets a little more complicated when one considers the ceramide/sphingosine 1-phosphate (S1P) system because ceramide, a potent proinflammatory sphingolipid-generated de novo from hydrolysis of sphingomyelin or synthesis from serine and palmitoyl CoA, can directly activate protein kinases and phosphatases as well as serve as a precursor to S1P, which acts via G protein-coupled receptors to sensitize nociceptive neurons.153 And although a common set of second messengers, including phosphatidylinositol 3-kinase, phospholipase Cγ, and extracellular signal-regulated kinase (ERK), underlie the acute sensitizing actions of trophic factors such as NGF, these mediators signal via a distinct class of receptors that contain intrinsic tyrosine kinase activity, referred to as tropomyosin receptor kinase, or Trk receptors.154 Channels underlying transduction and spike initiation, in particular TRPV1 and NaV1.8, respectively, appear to be final common targets for this diverse array of mediators and second messenger pathways.155 Phosphorylation of specific residues on the channel or associated proteins results in increases in channel density and, more generally, in increases in channel properties. Importantly, many of the inflammatory mediators including ceramide/S1P and NGF drive long-term increases in nociceptor excitability via changes in gene expression, and mechanisms contributing to long-term changes in gene expression, such as alterations in DNA acetylation and methylation, have emerged as potential therapeutic targets.156,157

Although mechanisms underlying changes in gene expression associated with the emergence of persistent pain have received considerable attention, there is a growing body of evidence suggesting that changes in protein translation are also important. One of the first studies to implicate a change in translation involves the regulation of protein translation per se in manifestation of persistent pain was focused on the role of TRPV1 in persistent inflammatory pain. The authors observed that inflammation is associated with an increase in TRPV1 protein in the absence of a detectable increase in messenger RNA.158 It was subsequently demonstrated that inflammatory mediators such as NGF and IL-6 could rapidly increase protein translation via the phosphorylation of proteins responsible for control over the rate-limiting step of protein translation; that of the initiation of protein synthesis.159 The protein synthesis initiation step involves the assembly of a complex of eukaryotic initiation factors (eIF) eIF4E and G among others, as well as the dissociation of the eIF4E binding protein (4EBP), from eIF4E. At least two distinct signaling pathways have been identified underlying the actions of NGF and IL-6. In addition to the activation of PI3K, NGF activation of TrkA drives the activation of PI3K which subsequently activates Akt (also known as protein kinase B). Akt then activates the mammalian target of rapamycin (mTOR), which facilitates the initiation of translation via facilitating the dissociation of 4E BP from eIF4E, as well as the activation of eIF4G, via the phosphorylation of both proteins. In contrast, IL-6 drives the activation of ERK, which subsequently activates MAP kinase interacting kinase (MNK), which facilitates the initiation of translation via facilitating the phosphorylation of eIF4E (which facilitates the eIF4E/G complex formation).160 Interestingly, this rapid regulation of translation contributes not only to the magnitude and duration of both the mechanical and thermal hypersensitivity associated with acute inflammation and the actions of inflammatory mediators like NGF and IL-6 but also the manifestation of more persistent changes in nociceptive processing, referred to as hyperalgesic priming.161

Hyperalgesic priming is the term used to describe the changes in nociceptive processing associated with an initial inflammatory insult, such as that associated with the subcutaneous injection of carrageenan, or inflammatory mediators such as IL-6.162 These changes were manifest up to weeks after the complete resolution of hypersensitivity associated with the initiating stimulus and were most prominently manifest as a dramatic increase in the duration of the hypersensitivity associated with a subsequent challenge, although an increase in the magnitude of the hypersensitivity has also been described. That is, although the intra-dermal injection of PGE2 into naive skin results in a relatively transient hypersensitivity lasting ~1 hour, the same injection into primed skin results in hypersensitivity detectable for more than 24 hours. This phenomenon has been proposed to contribute to the transition from acute to chronic pain.163 In the context of a discussion of mechanisms underlying nociceptor sensitization, the mechanisms implicated in the manifestation of hyperalgesic priming are notable for several reasons. First, although there are data suggesting changes within the central nervous system, notably involving a descending dopaminergic input to the superficial dorsal horn, contribute to the manifestation of hyperalgesic priming,164 the bulk of data generated so far suggest that changes in nociceptive afferents may play a critical role in the emergence of chronic pain. Second, as further evidence of the differential role of distinct subpopulations of nociceptive afferents in nociceptive signaling, two distinct forms of hyperalgesic priming have been described, where type I depends on a subpopulation of neurons that do not express the neuropeptides calcitonin gene-related peptide (CGRP) or substance P and bind the plant lectin IB4,165 whereas type II depends on a subpopulation of neurons that do express these neuropeptides and do not bind IB4.166 Third, there appear to be sex differences in the mechanisms underlying both the initiation (type I)167 and maintenance (type II)168 of the primed state. And fourth, although a cAMP response element binding protein (CREB)-dependent change in gene expression appears to be necessary for the establishment of the primed state, local translation appears to be critical for the manifestation of the more robust response observed in primed state, at least for type I priming.169 Given the length of some nociceptive afferents, in particular those innervating the distal appendages, local control of translation may prove to be an important therapeutic target for the treatment of pain.

Clinical Implications of Nociceptor Function

As researchers have begun to explore the basis for chronic pain syndromes generally associated with specific body regions and/or organs (e.g., temporomandibular joint disorder [TMJD], inflammatory bowel disease [IBD], irritable bowel syndrome [IBS], or painful bladder syndrome [formerly interstitial cystitis or IC]), a number of common themes have emerged that are likely to impact future treatment approaches. First, specific mechanisms underlying injury-induced sensitization of nociceptors vary as a function of target of innervation. For example, inflammation-induced sensitization of masseter muscle afferents appears to reflect a decrease in a specific subpopulation of voltage-gated potassium channels.167 The same channels do not appear to contribute to the sensitization of TMJ afferents.168 Similarly, inflammation-induced increases in the excitability of bladder sensory neurons appear to reflect one pattern of changes in voltage-gated169 ion channels, whereas the inflammation-induced increase in the excitability of sensory neurons innervating the stomach,170-172 ileum,173,174 or colon175 reflect other patterns. Although tissue-specific patterns of inflammation may contribute to these differences, differences persist when the response to inflammatory mediators is studied in vitro.176,177 These observations imply that it may be possible, if not necessary, to treat
pain arising from a specific structure with a specific intervention. Second, specific mechanisms underlying insult-induced sensitization of nociceptors also vary as a function of the type of insult. For example, acute phosphorylation-dependent modulation of the voltage-gated sodium channel Nav1.8 results in an increase in current which contributes to an inflammation-induced increase in nociceptor excitability.\(^1\)\(^7\)\(^8\)\(^9\) In contrast, following traumatic nerve injury, redistribution of Nav1.8 to the axons of uninjured afferents appears to be necessary for the expression of mechanical hypersensitivity associated with nerve injury.\(^1\) The dynamic allodynia that often develops after nerve injury, however, likely represents more than only a redistribution of the neuropeptide.\(^2\)

With respect to the viscera, because each organ receives innervation from two nerves, the effect of organ insult can be different in the two groups of sensory neurons that innervate the organ (e.g., Wang et al,\(^1\) Traub\(^1\)\(^2\)). These observations underscore the importance of developing diagnostic criteria that enable identification of the factors primarily responsible for ongoing pain. Third, as clearly indicated by the discussion of hyperalgesic priming, the history of the nociceptor influences the response to subsequent challenge. Furthermore, there is evidence of a developmental window within which injury may produce permanent changes in nociceptors.\(^1\)\(^3\)\(^1\)\(^4\) With the development of more specific therapeutic tools, patient history may become a critical factor in the identification of the most appropriate intervention. Fourth, there is evidence for sex differences in both the excitability of different groups of nociceptors\(^1\)\(^5\) as well as the response to tissue injury.\(^1\)\(^6\)\(^1\)\(^7\) These differences appear to be mediated, at least in part, through the actions of gonadal hormones and may contribute to sex differences in the manifestation of a number of chronic pain syndromes. There is also evidence for age-dependent changes in nociceptive function (e.g., Wang et al,\(^1\) Traub\(^1\)\(^2\)). The ever-growing proportion of aging adults, this particular issue is in need of further investigation. Finally, all of these factors interact with what are clearly genetic differences in pain and algiesics mechanisms.\(^1\)\(^9\)

As indicated earlier, the consequences of tissue insult are not limited only to changes in the excitability of nociceptors and the awakening of sleeping nociceptors. Because sensitization leads to an increased response to noxious stimuli and a decrease in response threshold, previously nonnoxious intensities of stimulation also are now able to activate nociceptors. In addition, spontaneous activity may develop. In the aggregate, central nervous system input from sensitized nociceptors, awakened sleeping nociceptors, and spontaneously active nociceptors is significantly increased. For example, approximately 24% of human cutaneous C fibers are sleeping nociceptors,\(^1\)\(^0\) comprising significant new input to the central nervous system if awakened. Consequently, the amount of neurotransmitters (as well as perhaps their relative proportions) released onto central neurons is increased, which in turn alters the excitability of central neurons. The increase in excitability of central neurons is manifest as an increase in the size of the cutaneous receptive field (i.e., secondary hyperalgasia) or area of tenderness referred from deep structures, particularly the visera. Although the principal focus of study of mechanisms of central sensitization has been the spinal cord, it should be appreciated that nociceptor-driven changes in central excitability extend throughout the central nervous system.

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PART ONE: BASIC CONSIDERATIONS


QUERY:
AQ1: Please confirm if 5-HT3 should be defined here. Please reconcile.